ความน่าเชื่อถือได้ของการวัดมวลไขมันในร่างกาย โดยวิธีไบโออิเล็คทริคอลอิมพิด๊านซ์เปรียบเทียบกับวิธีการชั่งน้ำหนักใต้น้ำ

นางสาวจรินทิพย์ คงศักดิ์พรชัย

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THE VALIDATION OF MEASUREMENT OF BODY FAT BY BIOELECTRICAL IMPEDANCE METHOD COMPARED WITH UNDERWATER WEIGHING METHOD

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จรินทิพย์ คงศักดิ์พรชัย : ความน่าเชื่อถือได้ของการวัดมวลไขมันในร่างกายโดยวิธีไบโออิเลคทริคอลอิมพิด๊านซ์เปรียบเทียบกับวิธีการชั่งน้ำหนักใต้น้ำ (THE VALIDATION OF MEASUREMENT OF BODY FAT BY BIOELECTRICAL IMPEDANCE METHOD COMPARED WITH UNDERWATER WEIGHING METHOD) อ. ที่ปรึกษา : ผศ. นพ. มนต์ชัย ชาลาประวรรตน์, อ. ที่ปรึกษาร่วม : รศ. นพ. ชาญวิทย์ โคธีรานุรักษ์ ; 83 หน้า. ISBN 974-332-964-1.

การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาความเชื่อถือได้ของวิธีไบโออิเลคทริคอลอิมพิด๊านซ์ในการวัดมวลไขมันและร้อยละของไขมันในร่างกายเปรียบเทียบกับวิธีมาตรฐานคือวิธีการชั่งน้ำหนักใต้น้ำ ได้ทำการศึกษาในคนไทยที่มีสุขภาพดี 33 คน (ชาย 19 คนและหญิง 14 คน) มีอายุระหว่าง 19-41 ปี ร้อยละของไขมันในร่างกายสามารถคำนวณได้จากความหนาแน่นร่างกายที่ได้วิธีการชั่งน้ำหนักใต้น้ำโดยใช้สมการของซีรีและคำนวณเป็นค่ามวลไขมันอ้างอิง ค่ามวลไขมันและร้อยละของไขมันในร่างกาย โดยวิธีไบโออิเลคทริคอลอิมพิด๊านซ์วัดได้จากเครื่องไบโออิเลคทริคอลอิมพิด๊านซ์ยี่ห้อ Bodystat 1500 นำข้อมูลที่ได้จากการวิจัยมาวิเคราะห์ผลทางสถิติโดยการคำนวณค่าความคลาดเคลื่อนคงที่ (constant error; CE), ค่าความคลาดเคลื่อนมาตรฐานของการประมาณ (standard error of estimate; SEE), ค่าสหสัมพันธ์ (r) และค่าความคลาดเคลื่อนทั้งหมด (total error; TE) จากการทดลองพบว่า ค่ามวลไขมันและร้อยละของไขมันในร่างกายที่วัดได้จากได้วิธีการชั่งน้ำหนักใต้และวิธีไบโออิเลคทริคอลอิมพิด๊านซ์มีสหสัมพันธ์กันอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ค่าความคลาดเคลื่อนคงที่คำนวณได้มีค่าสูงบ่งชี้ว่าวิธีไบโออิเลคทริคอลอิมพิด๊านซ์วัดค่ามวลไขมันและ ร้อยละของไขมันในร่างกายต่ำกว่าวิธีการชั่งน้ำหนักใต้น้ำอย่างมีนัยสำคัญทางสถิติ ($p<0.005$) นอกจากนี้ค่าความคลาดเคลื่อนในการทำนาย (ค่าความคลาดเคลื่อนมาตรฐานของการประมาณและค่าความคลาดเคลื่อนทั้งหมด)ที่คำนวณได้มีค่าสูงเช่นกัน ค่าร้อยละของไขมันในร่างกายในแต่ละบุคคลที่วัดได้จากวิธีไบโออิเลคทริคอลอิมพิด๊านซ์ที่ความคลาดเคลื่อนร้อยละ 4 มีค่าเท่ากับร้อยละ 30 ของกลุ่มตัวอย่างทั้งหมด สรุปผลการวิจัยพบว่า วิธีไบโออิเลคทริคอลอิมพิด๊านซ์ไม่สามารถวัดหรือประมาณองค์ประกอบร่างกายได้อย่างถูกต้องในคนไทยที่มีสุขภาพดีเมื่อใช้สมการของเครื่องไบโออิเลคทริคอลอิมพิด๊านซ์ในการวัด

ภาควิชา .....
สาขาวิชา เวชศาสตร์การกีฬา
ปีการศึกษา ๒๕๔๒

ลายมือชื่อนิสิต จรินทิพย์ คงศักดิ์พรชัย
ลายมือชื่ออาจารย์ที่ปรึกษา มนต์ชัย ชาลาประวรรตน์
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

พิมพ์ต้นฉบับบทคัดย่อวิทยานิพนธ์ภายในกรอบสีเขียวนี้เพียงแผ่นเดียว

#1970228030 : MAJOR SPORTS MEDICINE
KEY WORD: VALIDATION / UNDERWATER WEIGHING / BIOELECTRICAL IMPEDANCE / BODY FAT
CHARINTIP KHONGSAKPORNCHAI : THE VALIDATION OF MEASUREMENT OF BODY FAT BY BIOELECTRICAL IMPEDANCE METHOD COMPARED WITH UNDERWATER WEIGHING METHOD. THESIS ADVISOR : ASSIST. PROF. MONTCHAI CHALAPRAWATT, M.D. THESSIS CO-ADVISOR : ASSO. PROF. CHARNVIT KOTHEERANURAK, M.D., M.B.A. 83pp. ISBN 974-332-964-1.

This study examined the validity of bioelectrical impedance analysis (BIA) for estimating fat mass (FM) and percent body fat (%BF). Thirty-three healthy Thai people (19 males and 14 females) ranging in age from 19 to 41 yr participated in this study. The reference method was underwater weighing (UW) at residual lung volume. Body density was converted to percent body fat using Siri equation. $\%BF_{HW}$ used to calculated reference fat mass. FM_{BIA} and $\%BF_{BIA}$ were measured using a Bodystat 1500 bioelectrical impedance analyzer. The cross-validation statistical analysis included examination of the constant error (CE), standard error of estimate (SEE), r, and total error (TE). There were significant correlations of FM and %BF between HW and BIA (0.56-0.77 and 0.61-0.85, respectively; $p<0.05$). The BIA resulted in high CE values, indicating that BIA estimates significantly underestimated both FM and %BF (3.48-4.22 and 6.71-6.91, respectively; $p<0.005$). In addition, the prediction errors (SEE and TE) estimated by BIA were also high values (SEE = 2.07-3.31 of FM, SEE = 3.84-5.23 of %BF, TE = 4.02-5.32 of FM, and TE = 7.68-8.63 of %BF). For individual, the $\%BF_{BIA}$ within ± 4% BF was 30% of the subjects. In conclusion, BIA technique does not accurately estimate body composition in healthy Thai populations when BIA manufacturer's equation are used.

ภาควิชา..... ลายมือชื่อนิสิต ชรินทิพย์ คงศักดิ์พร

สาขาวิชา เวชศาสตร์การกีฬา ลายมือชื่ออาจารย์ที่ปรึกษา มนต์ชัย ชลาประวรรัตน์

ปีการศึกษา ๒๕๔๒ ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

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A	cross-sectional area
%BF	percent body fat
BIA	bioelectrical impedance analysis
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
°C	degree in centigrade
CAS	brussele cadaver analysis study
CE	constant error
cm	centimeter
Cl^-	chloride ion
CT	computed tomography
D_b	body density
D_{fm}	density of fat mass
D_{ffm}	density of fat-free mass
D_w	density of water
DEXA	dual energy x-ray absorptiometry
DPA	dual-photon absorptiomrtry
ECF	intracellular fluid
FM	fat mass
FFM	fat-free mass
g	gram
g/cm^3	gram per cubic centimeter
Gd	galdolinium
GI-tract	gastrointestinal tract
GI gas	gastrointestinal tract
HCO^-_3	hydrogencarbonate

HPO^{2-}_4	hydrogenphosphate
Ht	height
HT^2/R	impedance index
ECF	extracellular fluid
IVNA	in vivo neutron activation
K^+	potassium ion
kcal	kilocalorie
kHz	kilohertz
L	liter
mm	millimeter
Na^+	sodium ion
r	correlation coefficient
R	resistance
RV	residual volume
SD	standard deviation
SEE	standard error of estimate
SKF	skinfold measurement
TBW	total body water
TE	total error
W_a	body weight out of water
W_w	weight in water
X_c	reactance
Z	impedance
ρ	specific resistivity
μA	microampere

CHAPTER I

INTRODUCTION

The study of human body composition spans over 100 years and continues to be an active area of basic sciences and clinical research. Nearly every aspect of clinical nutrition, selected areas within many medical specialties, and components of exercise science are touched on by the study of body composition. More than 30 recognized major components comprise body weight at atomic, molecular, tissue system, and whole-body levels of body composition (Wang et al., 1992). Of these components, the most actively investigated is total body fat. Fat is a molecular level component, not to be confused with fat cells or adipose tissue, which are components at the cellular and tissue system levels of body composition, respectively (Wang et al., 1992). Evidence supports the notion that being overweight (excess body fat) is related to musculoskeletal injury, nonadherence to an exercise training, reduced athletic performance, and many health problems (Cureton et al., 1978). The excess body fat has been shown to be associated with such health problems as hypertension, diabetes mellitus, depression, hyperlipidemia, and coronary heart disease. Thus the measurement of the total body fat provides useful information in clinical studies and exercise science. Many indirect methods for assessing total body fat utilized in both laboratory and field setting. They are:

Hydrostatic or Underwater Weighing (UWW)

Hydrostatic or underwater weighing is the most widely used laboratory procedure for measuring body density and often serves as a reference method for other indirect techniques (Behnke et al., 1974; Pollock et al., 1990). This method utilizes Archimedes ' principle that a body immersed in a fluid is acted on by a

buoyancy force up that is evidenced by a loss of weight equal to the weight of the displaced fluid and this is based on the assumption that the body can be considered to consist of two distinctly chemical compartments, the fat mass (FM) and the fat-free mass (FFM) (Keys, 1953; Brozek et al.,1963). The density of these two compartments are constant density of 0.90 g/cm^3 (Fidanza et al., 1953) and 1.10 g/cm^3, respectively (Behnke, 1942; Brozek et al., 1963). The measured weight from underwater weighing is calculated to estimate body density (body mass / body volume). When the body density is known, the body fat percentage can be calculated by Siri's equation (Siri, 1969):

$$\text{Percent body fat} = \left(\frac{4.95}{\text{body density}} - 4.50 \right) \times 100$$

Bioelectrical impedance analysis (BIA)

Bioelectrical impedance analysis (BIA) is a relatively new technique for estimating body composition in humans (Lukaski, 1985). Salient features of the technique are that it is portable, rapid, noninvasive, and requires only the placement of four electrodes on well-defined landmarks. For these reasons, it is gaining popularity as a measurement technique for epidemiological studies (National Center for Health Statistics, 1994). BIA is based on the principle that the impedance through an applied electrical current is inversely related to the amount of the FFM contained within the body (Nybour, 1972). This relationship exists because FFM has a greater water and electrolyte content, readily conducts the applied electrical current, and therefore has a greater conductivity than fat, which acts as an insulator and conducts little of the current. The greater the FFM, the greater the conductivity, and the lower the impedance. Then the measured impedance leads to the prediction of total body water (TBW), which is highly correlated with FFM (Hoffer et al., 1969), by being one part of impedance index (the ratio between height squared and impedance; Ht^2 / Z), which is assumed to be a good predictor of volume in the determination of TBW (Lukaski et al, 1986;

Kushner and Scholler, 1986). Consequently, relative body fat can be easily calculated from FFM by this formula: % body fat = (body weight - FFM) / body weight x 100.

As previously mentioned, hydrostatic weighing is widely accepted to be a criteria method, but it is a complex technique, time-consuming, needs many equipment and requires much cooperation by the individual and, therefore may not be easily applied to obese or elderly and physically disabled individual. Thus the body composition methods which are rapid, noninvasive, easy to use, and appropriate for use in field setting are necessary. BIA method are new alternative body composition methods used in clinical and field settings. It is rapid, requires only the placement of four electrodes on well-defined landmarks. Unfortunately, the overwhelming majority of BIA method has been developed and cross-validated only white populations. There are only a few studies applying this method in Asian people including Thai. The usefulness and validity of BIA method should be evaluated in Thai people. This is because ethnicity may affect the body composition. Thus the validation study of measurement of body composition, especially body fat mass by BIA method compared with hydrostatic weighing is important and may lead to applications in other studies.

The objective of this study

To examine the validity of BIA instrument (Bodystat-1500, TM) for estimating fat mass and percent body fat by comparing the estimates with values obtained from UWW in Thai people

Research Question

Can the BIA instrument (Bosystat-1500, TM) be used to estimate fat mass and percent body fat as accurately as UWW, considered as gold standard method?

Operational Definitions

1. Air weight - The weight is measured in air.
2. Underwater weigh - The weight is measured in underwater.
3. Body density (D_b) - The overall density of the fat, water, mineral, and protein components of the human body; total body mass expressed relative to total body volume.
4. Percent body fat (%BF) - The fat mass expressed as a percentage of total body mass.
5. Body fat mass - The weight of all extractable lipids from adipose and other tissues in the body.
6. Impedance (Z) - A measure of the opposition to the flow of electrical current through the body; composed of two vectors – resistance and reactance.
7. Resistance (R) - A measure of pure opposition to the flow of electrical current flowing through the body; a vector of impedance; the lesser the resistance, the greater the current flow.
8. Reactance (X_c) - A measure of the opposition to current flow through the body due to the capacitance of cell membrane; a vector of impedance.
9. Capacitance - The storage of voltage for a brief moment in time.
10. Residual volume - The volume of air remaining in the lungs following a maximal expiration.

Expected Benefit of the study

The results of this study might be useful for other studies related to estimating fat mass using BIA method. In addition, if BIA is valid, it will be useful for assessing fat mass and percent body fat in hospital and health clubs.

CHAPTER II

REVIEW OF THE LITERATURE

1. Role of fat in the body and related diseases

Fat is the most compact of food, since it supplies 9.3 kcal/g. Excessive fat intake (more than energy demand of body) is stored in as forming triglyceride which served as the body's largest store of potential energy. It is accumulated within visceral organs and under subcutaneous tissue. These fats serves as a cushion for the protection of vital organs and provides insulation from the thermal stress of a cold environment. However, the intake of dietary fat, especially high animal-fat diet, is associated with obesity. Obesity, which may predispose and complicate numerous serious health problems (Stanfield, 1992) include:

a) impaired cardiac function as a result of increased mechanical work and autonomic and left ventricular dysfunction (Rossi et al., 1989)
b) hypertension and stroke (Stamler et al., 1978)
c) adult-onset diabetes, as about 80% of these patients are overweight (Pi-Sunyer, 1994; Stern et al., 1975)
d) renal disease (Weisinger et al., 1974)
e) pulmonary disease and impaired function resulting from the added effort needed to move the chest wall (Barlett and Buskirk, 1983)
f) problems in administering anesthetics during surgery (Warner, 1968)
g) osteoarthritis, degenerative joint disease, and gout (Felson et al., 1992)
h) several types of cancers, some studies reported that cancers of breast, colon and possibly prostate were closely related to the uptake of high animal-fat diets (Simopolous, 1987)
i) abnormal plasma lipid and lipoprotein levels (Rossner and Hallberg, 1978)

j) an enormous psychological burden (Bray, 1986).

2. Five-level model of body composition

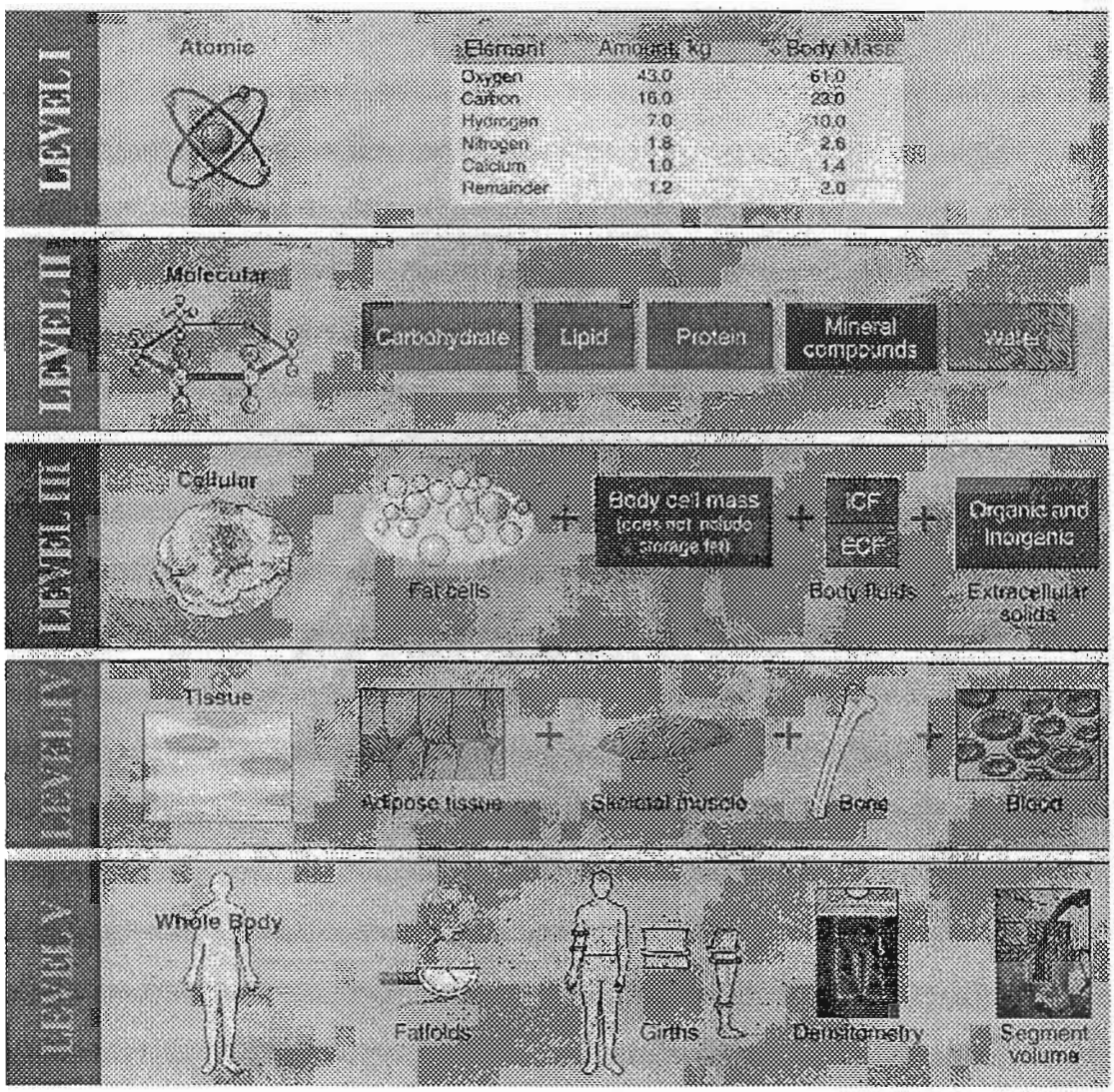

Figure 1. The new proposed five-level component model for quantifying body composition (Wang et al., 1992). Each level of the model becomes more complex with increases in the body' s levels of biological organization. Note that there are subdivisions within each of the five levels. One of the aims in developing the model was to identify and then attempt to quantify the various components of each of the levels. An essential feature of the model in each level is separate and distinct, and that either direct or indirect methods of

measurement procedures in relation to each classification level includes the following:

2.1 Atomic level.

The atomic level is the foundation of body composition analysis and is the starting point for the five-level component model. This level mainly considers that the body mass is the sum of all elements in the body. Four elements - oxygen, carbon, hydrogen, and nitrogen - account for over 95% of body mass and with an additional seven elements - sodium, potassium, phosphorus, chlorine, calcium, magnesium, and sulfur - comprise over 99.5% of body mass (Snyder et al., 1975; Wang et al., 1992). Element analysis of human is traditionally carried out in cadaver or in biopsy specimens from selected tissues and organs. In addition, the whole-body content of most major elements can now be measured directly in vivo: total body potassium can be determined by whole-body ^{40}K counting (Myhre and Kessler, 1966): total body sodium, chlorine, phosphorus, and calcium can be determined by delayed-gamma neutron activation (Cohn and Dombrowski, 1971): total body nitrogen can be determined by prompt-gamma neutron activation (Cohn et al., 1983) and total body carbon can be determined by inelastic neutron scattering (Kehayias et al., 1990). More than 98% of body weight can now be reconstructed from elements that can be estimated in vivo, largely by neutron activation techniques.

2.2 Molecular level.

The 11 principal elements in atomic level are incorporated into molecules that form over 100,000 chemical compounds found in human body (Wang et al., 1992). This level, however, can be classified into five main groups: water, lipid, protein, carbohydrate, and mineral compounds.

Water. Water accounts for approximately 55-60% of the body weight in adult. The total body water is distributed between the major two compartments, which are divided by cell membrane. The intracellular fluid (ICF) compartment is the larger compartment; it contains approximately two-third of total body water. The extracellular fluid (ECF) compartment contains approximately one-third of total body water and includes five subcompartments: interstitial, plasma, connective tissue, bone, and gastrointestinal tract (Forbes, 1987; Wang, 1995).

Lipids. Lipids or fat are defined as organic compounds which include many chemical substances. They have the same specific properties, that is, they are soluble in organic solvents such as diethyl ether, benzene, and chloroform (Diem, 1962; Gurr and Harwood, 1991). It has often been confusing to use the term fat in reference to total lipids. In chemical study, lipids can be mainly classified two categories: essential lipids and nonessential lipids (Snyder et al., 1984). Essential lipids, such as sphingomyelin, phospholipid, and steroid, serves important functions such as forming cell membranes and including the various steroid hormone and cholesterol. Nonessential lipids, largely in the form of triglyceride, generically referred to as fats. In humans triglyceride provides the thermal insulation and a storage depot mobilizable fuel. About 10% of total lipid is essential and 90% is nonessential in the reference man (Snyder et al., 1984).

Protein. The term of protein in body composition research usually includes almost all compounds containing nitrogen, ranging from simple amino acids to complex nucleoproteins (Wang et al., 1992). However, noninvasive body composition methods are available only for estimation of total protein (Cunningham, 1994) and muscle and non-muscle protein (Cohn, 1981).

Glycogen. Glycogen is the primary storage form of carbohydrate, which is found in the cytoplasm of most cells. There is less than 1 kg of

glycogen in healthy adults; the remaining carbohydrates are considered negligible (Diem, 1962; Snyder et al., 1975). The two main intracellular glycogen pools are in liver (approximately 1% wet weight) and skeletal muscle (approximately 2% wet weight) (Snyder et al., 1975).

Minerals. Minerals comprise about 5% of body weight in healthy adults and are distributed in two main compartments: bone minerals (osseous) and soft-tissue minerals (nonosseous). Calcium hydroxyapatite is the primary bone mineral and contains over 99% of total body calcium and approximately 86% of total body phosphorus (Heymsfield et al., 1991). Soft-tissue minerals include well-known ions such as Na^+, K^+, Cl^-, HPO_4^{2-}, and HCO_3^-.

Although the molecular level can be divided into five main components, the development of the body composition method requires the combinations of components together. For example, the molecular level can be described as any of the following combinations: a two-compartment model in which body weight (BW) = fat + fat-free body mass; a three-compartment model in which BW = fat + water + residual (i.e. the sum of glycogen, minerals, and protein) (Wang et al., 1995), and BW = fat + bone mineral + lean soft tissue; and a four-compartment model in which BW = fat + water + mineral + residual (i.e. the sum of glycogen and protein).

The model combined more than two components is defined as multicompartment models (Heymsfield et al, 1997). There are many published multicompartment models, and more are possible. All multicompartment models share in common their development from simultaneous equations which may include two or more unknown components. As a general rule, for each unknown component estimated there must be one independent equation that includes the unknown component, the known component, and the measurable property (Heymsfield et al., 1997). Measurable molecular level components include total body water by isotope dilution technique (Forbes, 1987) and

bioelectrical impedance analysis, total body protein by neutron activation of total body nitrogen (Cohn, 1981), glycogen (regional) by nuclear magnetic resonance spectroscopy (Jue et al., 1989), and bone and soft-tissue minerals by activation of body calcium (Rossner et al., 1990; Dutton, 1991), whole-body ^{40}K counting (Forbes, 1987), and dual energy X-ray absorptiometry (Mazess et al., 1990), and total body fat by hydrostatic weighing and skinfold measurements.

2.3 Cellular level.

The cellular level's importance is centered primarily on the protoplasmic or intracellular compartment, which is the site of most metabolic processes. The human body is composed of three main compartments on the cellular level: cells mass, extracellular fluids ,and extracellular solids.

Cells. The cell mass component is of interest primarily for the metabolically active protoplasm that includes cytoplasmic organelles and mitochondria found within the intracellular space (Heymsfield et al., 1997). Adipocytes, or fat cells, are a type of loose connective cell in which fat or triglyceride is stored.

Extracellular fluid. Extracellular fluid, which is about 94% water by volume, is distributed into two main compartments: plasma in the intravascular space and interstitial fluid in the extravascular space (Wang, 1992). It is slightly larger that extracellular water, also a molecular level component, as it is includes dissolved electrolytes and proteins.

Extracellular solids. Extracelllular solids are also a non-metabolizing portion of the human body that consists of organic chemical compounds. The organic extracellular solids include three types of fiber: collagen, reticular, and elastic (Jocob et al., 1987). The inorganic extracellular solids represent about 65% of the dry bone matrix in the reference man (Snyder,

1984). Calcium, phosphorus, and oxygen in bone are the main elements of the organic extracellular solids that are incorporated into calcium hydroxyapatite (Heymsfield et al., 1991). However, the extracellular solids are not much clinical interest, as they consist mainly of bone minerals and collagen, reticular, and elastic fibers (Wang et al., 1992).

Nowadays the most widely used cellular level model is BW = fat + extracellular fluid + extracellular solids + body cell mass. Isotope dilution procedure is available for estimating extracellular fluid component (Forbes, 1987).

2.4 Tissue level.

The body is composed of eleven subsystems (circulatory, respiratory, nervous, integumentary, muscular, endocrine, respiratory, lymphatic, digestive, skeletal, and reproductive), but for body composition evaluation, the following four tissue-system can be more easily grouped to represent total body mass (adipose tissue, skeletal muscle, bone, and blood). Computerized tomography, magnetic resonance imaging, and ultrasound procedures can be used to estimated the volumes of subcutaneous fat, visceral adipose tissue, and segmental muscle mass (Kvist et al., 1988)

2.5 Whole body level.

The whole-body level concerns body size, shape, and exterior and physical characteristic. The common anthropometric procedures include skinfolds thickness, body breadths, girths, bone diameters, body mass, stature, body mass index, body surface area, segment lengths, segmental and body volume, and body density.

3. Measurement of body composition

Two general procedures are used to evaluate body composition:

3.1 Direct assessment. This is done by chemical analysis of the animal carcass or human cadaver. It involves the physically dissection of a variety of body components such as fat, fat-free adipose tissue, muscle and bone, and therefore it is difficult to practice because it must be only done in human cadavers.

3.2 Indirect assessment. Unfortunately, due to short of cadaver analyses, there is no means of directly measuring fat and muscle mass. Thus many indirect assessments are commonly used to evaluate body composition. Body composition measurement techniques can be divided into those utilized in the laboratory settings, such as underwater weighing, neutron activation analysis, dual energy X-ray absorptiometry (DEXA), computed tomography (CT), magnetic resonance imaging (MRI), and those in field situation, including measurement of skinfold measurement, body mass index (BMI), and bioelectrical impedance analysis (BIA).

3.3.1 Hydrostatic Weighing

Hydrostatic weighing or underwater weighing (UWW) is the body composition method in the molecular level. It utilizes a two-compartment model as proposed by Brosek et al., (1963) and Siri (1961) which are based on the assumption that body composition is made up of fat mass (FM) and fat-free mass (FFM). The FFM includes mineral, protein, carbohydrate, and water. This system assumes that the composition of FM and FFM is constant for all individuals, that is, the density of FM is 0.90 g/cm^3 and FFM is 1.10 g/cm^3 (Behnke et al, 1942; Siri 1961; Brozek et al, 1963). In addition, UWW is based on Archimedes' principle which stated that a body immersed in a water is acted on by a buoyancy force that is evidenced by a loss of weight equal to the weight

of displaced water. Thus there are two ways to measure body density; (a) by measuring the displaced water volume, or (b) by measuring the change in body weight underwater. The latter method is widely used and is the method suited to discussion in this sections. Based on Archimedes' principle, body density can be calculated as follows:

$$\text{Body density} = \text{Body mass} / \text{Body volume} \quad (1)$$

As body mass is weight out of water and body volume is weight in water (1 g of water = 1 cm^3 in volume)

From Formula (1), it can be rewritten as Goldman and Buskirk's equation (1961)

$$D_b = \frac{W_a}{\frac{W_a - W_w}{D_w} - (RV + 100 \text{ ml})} \quad (2)$$

Where :

D_b = body density (g/ml)

W_a = body weight in air (g)

W_w = weight in water (g)

D_w = density of water (g/ml)

RV = residual volume (ml) can be measured from wet spirometer

One hundred ml is estimated because air remaining in the lung and the volume of air trapped in GI tract contribute to buoyancy at the time of UWW, this volume must be considered. Although GI gas volume can vary, Goldman and Buskirk (1961) has proposed the use of the constant correction value of 100 ml. The error in calculation of body density associated with variation in GI gas is relatively small, so that the constant of 100 ml has been routinely used. This is in contrast to the residual volume (RV), the amount of air remaining in the lungs at the end of a maximal expiration, that is, it is large and variable which can result a sizable effect on the final calculation of body density (Wilmore,

1969; Pollock and Wilmore, 1990). Thus, it must be measured and subtracted from the total body volume.

Whereas the RV tends to be slightly less when measured while the subject is in water rather than air. It is probably the result of the compressing force of water against the thoracic cavity, the effect on computed body fat is small (Robertson et al., 1978; Ostrove and Vaccaro, 1982). RV can therefore be measured in air before UWW without a loss in accuracy compared to the simultaneous measurement of RV with UWW (Hsieh et al., 1985). In the determination of RV value, it can be estimated as a constant fraction of vital capacity, which is more readily measured than RV, The following equation developed by Willmore (1969b) are recommended:

Male : RV = 0.24 x vital capacity

Female : RV = 0.28 x vital capacity

Alternatively, RV value can be estimated from the averaged population values based on age, sex, and height using the equations (Goldman and Becklake, 1959; Pollock and Willmore, 1990):

Male : RV = 0.017(age in years) + 0.06858 (height in inches) -3.477

Female: RV = 0.009(age in years) + 0.08128 (height in inches) -3.900

Computing percent body fat

The percentage of fat in the body can be determined using an equation that incorporates whole body density. As early as 1961, the simplified equation derived by Berkley scientist William Siri is obtained by substituting 0.90 g/cm^3 for the density of FM and 1.10 g/cm^3 for the density of FFM. When the density of the total body is known, the percent body fat can be calculated by the formulas:

Body volume = FM volume + FFM volume (3)

Substituting volume by mass / density, the equation becomes :

$$\frac{100}{D_b} = \frac{FM}{D_{fm}} + \frac{FFM}{D_{ffm}} \quad (4)$$

As $\%\,FM = 100 - \%\,FFM$ (5)

The equation 5 can be rewritten as :

$$\%\,FM = \frac{1}{D_b} \times \frac{D_{fm} \times D_{ffm}}{D_{ffm} - D_{fm}} - \frac{D_{fm}}{D_{ffm} - D_{fm}} \quad (6)$$

Where : D_{ffm} = density of fat-free mass

D_{fm} = density of fat mass

Its final derivation referred to as the " Siri equation " (Siri, 1961) is :

$$\text{Percent body fat} = \left(\frac{4.95}{\text{Body density}} - 4.50 \right) \times 100 \quad (7)$$

The potential source of error in estimating body composition.

1. Although underwater weighing has been served as the reference method for determination of body composition, it does not mean that there is no error attached. This is due to the underwater weighing assumption that a constant densities of FM and FFM are 0.90 and 1.10 g/cm^3, respectively. In fact, FFM, which includes water, mineral, protein, and carbohydrate, is different among in individuals. On the basis of the cadaver data summarized by Brozek et al., (1963), water, mineral, and protein average 73.8%, 6.8%, and 19.4%, respectively, with their densities accepted at 36°C as being 0.9937, 3.038, and 1.34 g/cm^3, respectively. Deviation from these assumed constant results is an error in the estimating percent body fat. Variability in density of FM between individuals or among depots within the body is small (standard error = 0.0007 g/cm^3) (Fidanza, 1953), and its effect on estimating percent body fat from body density is considered negligible (Martin and Drinkwater, 1991). In contrast, variability in the density and composition of the FFM is though to be substantial and act as the primary limiting factor for the accuracy of underwater weighing for estimating body composition (Siri, 1961; Lohman, 1984; Martin and

Drinkwater, 1991). Siri (1961) estimated that the standard deviation (SD) of body density due to variability in body water, mineral, and protein in the general population was approximately 0.01 g/cm^3, which is equivalent to a SD in estimating percent body fat of approximately 4% body mass. Lohman (1984) estimated that in a more restricted sample, such as young white men, variability in density of FFM would be approximately one-half that in the entire population and equivalent to error of approximately 2.7% body mass.

2. In addition to the error from variability in the FFM, the determination of residual volume value is also a potential source of error. Indeed, residual volume value should determine from actual measurement i.e. helium-dilution technique or oxygen-dilution technique or nitrogen washout. However, in Wilmore's study (1969) demonstrated the comparison of the actual residual volume and two estimation techniques for measuring residual volume, as mention above, in the assessment of body composition by underwater weighing for college-aged males and females. The results indicated a close agreement (over 0.001 g/cm^3) between measures of body density, percent body fat, and FFM, using actual measurement of residual volume, and two techniques used for estimating residual volume (Wilmore, 1969). Thus, estimated residual volume can be used satisfactorily for screening purposes in large populations. Nevertheless, because the variability of residual volume estimates based on age, height, and weight, or vital capacity in large population, only the actual measures are valid for use with individuals with small number of subjects.

3. The amount of trials is another factor of underwater weighing which need to be considered. Katch (1968) conducted a study to determine the minimum of trails necessary to established "true" underwater weight during body density measurements. He demonstrated that there is a learning curve associated with successive trials of underwater weighing and concluded that 9 to 10 trials were necessary to obtain the most representative underwater weight for an individual and recommended that the average of the last three trails be used

to calculate body density. Later, Bonge and Donnelly (1989) suggested that the averaging of three highest trials that are within 100 g is equally accurate and therefore save time and prevent undue stress on the subjects.

As mentioned previously, UWW is commonly considered as the criterion method for determining body composition against which other indirect techniques are judged. However, UWW is limited in accuracy and usefulness, because the assumption, which they rely may not be valid in certain groups such as children, pregnancy women, and elderly diseased individuals. In addition, the major drawbacks of underwater weighing procedure are as follows:

1. The cost and time-consuming to conduct a test.
2. The need for subjects to tolerate water submersion at residual volume.
3. The constraints by limited production-specific equation that convert body density to percent of body fat.

3.3.2 Bioelectrical impedance analysis (BIA)

Bioelectrical impedance analysis (BIA) is a rapid, noninvasive, and relatively inexpensive method for evaluating body composition in clinical and field settings. Initially, Nyboer (1959) outlined the principle of BIA application: biological conductivity is primarily within fat-free tissue because it contains virtually all the water and conducting electrolytes in the body, impedance inversely related to the volume of this tissue, and volume change produce impedance changes. Later, Thomasett (1962) began to investigate the clinical use of nonpulsatile impedance and proposed using the value of whole body impedance measured between the left hand and right foot for determining ionic concentration and electrolyte balance of the body. Hoffer et al. (1969) introduced the hypothesis that measurement of whole body impedance can be further extended to obtain total body water (TBW) on the basis of the principle of volume conduction: the impedance of a geometrical system is related to conductor length and configuration, its cross-sectional area, and signal

frequency. Using a constant signal frequency and a relatively constant conductor configuration, impedance (Z) to the flow of current can be related to the volume of the conductor:

$$Z = \rho L / A \quad (1)$$

where Z is impedance in ohm, ρ is volume resistivity of FFM in ohm-cm, L is conductor length in cm, and A is conductor cross-sectional area in cm^2.

Multiplying equation 1 by L / L yields:

$$Z = \rho L^2 / AL \quad (2)$$

where AL is equal to volume of the FFM (V). Substituting gives:

$$Z = \rho L^2 / V \quad (3)$$

In biological systems, electrical conduction is related to water and ionic distribution in the conductor. Because FFM contains large amounts of water (about 73.2%) and conducting electrolytes, readily conducts the applied electrical current whereas fat acts as an insulator and conducts little of the current. Thus the conductivity is far greater in the FFM than the fat (Pethig, 1979). Equation 3 was proposed by Nyboer (1959), as mentioned above, who demonstrated that electrically determined biological volumes were inversely related to impedance (Z), resistance (R) and reactance (X_c), where $Z = (R^2 + X_c^2)^{0.5}$. Rearrangement of equation 3 yields:

$$V = \rho L^2 / Z \quad (4)$$

Thus, if ρ is constant, then L^2 / Z will be directly proportional to, and hence a measure of, the volume of the FFM. Furthermore, because the major component in the FFM is water, there should be a strong correlation between L^2 / Z and TBW. Based on the observation of Lukaski et al. (1985) that the magnitude of reactance (X_c) was small relative to resistance and can a negligible effect, and resistance (R) is a better predictor of impedance than is reactance, thus using resistance (R) to approximate impedance, thus:

$$V = \rho L^2 / R \quad (5)$$

Application of this equation to human subject requires the assumption that the human body physically approximates a cylinder where L equals standing height (Ht) or stature in cm and that the body water space had uniform

resistivity. The basic relationship therefore becomes TBW is approximately equal to Ht^2 / R. It based on Hoffer's research (1969), which demonstrated a high level of correlation (r = 0.92) between whole body electrical impedance and total body water (TBW) and further demonstrated that TBW and FFM were strongly correlated with Ht^2 / R, where body resistivity or impedance as measured with a tetrapolar electrode configurations.

Bioelectrical Impedance and Frequency

Impedance measures in a biological system or in the human body are a function of the current frequency. This function relationship is expressed in the reactance measure of impedance. At low frequencies, the biological current flows primarily through extracellular fluids and reactance is minimized since the capacitant properties are though to be bypassed, although this assumption has been questioned by Settle et al.(1980). As the frequency increases, the capacitant aspects of the body (cell membranes, tissue interfaces, and the like) start to retard the current, producing an increase in reactance. The capacitant effects reach a peak with a maximum reactance value, but as the frequency continues to increase the capacitant effects are reduced and reactance decrease. At high frequencies, the current penetrates all body tissues completely, and again reactance is minimized as the high frequency prevents capacitant effect from occurring in body tissues. Electrical circuit diagrams have been used to describe characteristics of these body fluid relationships (Schwan and Li, 1953; Nybore, 1959,).

The procedure of BIA measurement

A known amount of current (I), of about 800 μA and most often at a frequency of 50 kHz is introduced into the body. The current passes between two electrodes, often source and detector electrode, and generates voltages between different points in the body volume according to Ohm's law [current =

voltage / resistance; I = V / R). In present practice, the electrodes are usually located on the wrist and ankle. The current flows through all conducting materials present in the body in the path between the source and detector electrodes. Because living tissue constitutes a volume conductor, the physical carriers of the current are predominantly charged ions, such as sodium or potassium ions, which are able to move within the volume. Conductivity within such materials as blood and urine is high, whereas that of muscle is intermediate, and that of such materials as bone, fat, or air is low. Current will flow predominantly through materials with higher conductivity. There is a lower resistance to current flow in regions where the conductor has a larger cross-sectional area, such as the body trunk, and a higher resistance in regions with a smaller cross-sectional area, such as the forearm. Finally, there is less resistance to current where there are fewer obstacles, such as cell membranes, that form barriers to charge movements.

As mentioned above, BIA method is also based upon the nature of the conduction of an applied electrical current in an organism. In biological structures application of a constant, low level alternating current produces an impedance to the spread of the current that is frequency dependent. The living organizing contains intracellular fluid and extracellular fluid that behaves as electrical conductors and cell membrane that act as imperfect reactive elements. At low frequencies (approximately 1 kHz), the current passes through the extracellular fluids only; at higher frequencies (500-800 kHz), it penetrates cell membrane and passes through the intracellular fluid and extracellular fluid (Lukaski, 1987). Given that fat is anhydrous and a poor conductor of electrical current, the total body impedance, measured at the constant frequency of the 50 kHz, primarily reflects the volume of the water and muscle compartments comprising FFM and the extracellular water volume (Kushner, 1992).

Nevertheless, the application of the equation $V = \rho L^2 / R$ to estimate body composition in humans has limitations. It is assumed that conductor is

homogeneous with respect to composition, shape (cross-sectional area), and current density distribution. This is not the case in the human body (Smith, 1987). Height and not the actual length of the conductor (although height and conductor length are highly correlated) is used as a measure of conductor length; and the specific resistivity varies among individuals, based upon the amount and distribution of tissues and fluids within the body (Lukaski , 1987). These limitations indicate that Ht^2 / R (the impedance index) does not have the same relationship to TBW or FFM in all individuals. In spite of these limitations, high correlations between Ht^2 / R, TBW, and FFM indicate that Ht^2 / R can be used as a reasonable predictor of TBW and FFM (Lukaski et al., 1986; Kushner and Schoeller, 1986; Chumlea et al.,1988). After this, relative body fat can be easily calculated from FFM by this equation: % body fat = [(bodyweight - FFM) / body weight] x 100.

Although the relative predictive accuracy of the BIA method is similar to SKF measurement, BIA may preferable in some settings because (a) it does not need a high degree of technician skill (b) it is generally more comfortable and does not intrude as much upon the subject's privacy and (c) it can also be satisfactory used to estimate body composition of obese individuals (Segal, 1988).

3.3.3 Skinfold measurements (SKF)

The most widely used method of indirectly estimating percent body fat in clinical settings is to measure skinfold thickness (the thickness of a double fold of skin and compressed subcutaneous tissue as shown in Figure 2 (Pollock and Jackson, 1984; Pollock et al., 1984; Nieman, 1990). Although more accurate method for measuring percent body fat exists, skinfold measurement has three advantages; the equipment needed is inexpensive and requires little space; the measurements are easily and quickly obtained; and when correctly done, skinfold measurement provides the estimation of body composition that

correlate well with those derived from hydrostatic weighing, the most widely used laboratory method for determining body composition (Katch and McArdle , 1973; Pollock and Jackson, 1984; Nieman, 1990).

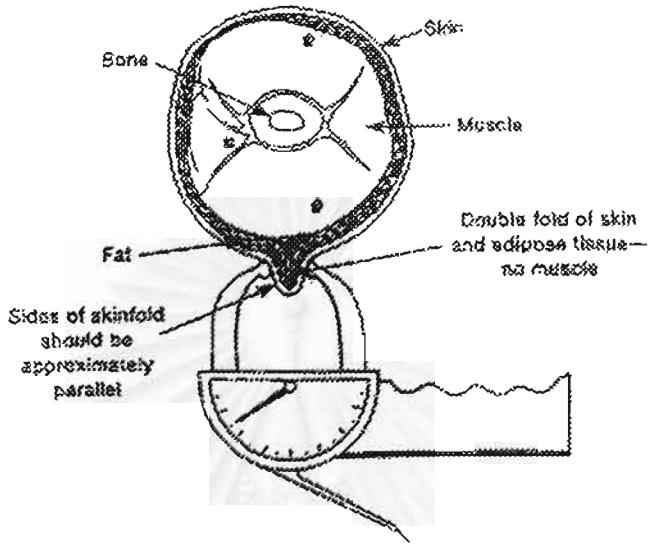

Figure 2. The double fold of skin and subcutaneous adipose tissue grasped by the thumb and index finger of the left hand should be large enough to form approximately parallel sides. Care should be taken to elevate only skin and adipose tissue.

Assumption in Using Skinfold Measurements

Estimating body fat from skinfold thickness measurement involves several assumptions, which are not always true (Martin et al.,1985). When a caliper is initially applied to skinfold, the caliper reading decreases as its tips compress the fold of skin and subcutaneous adipose tissue. Thus, it is recommended to read the caliper dial about 2-4 sec after the caliper tips have been applied to skinfold (Harrison et al., 1988). Research has shown significant differences in skinfold compressibility at a particular site among individuals (interindividual variation) and at different sites on one individuals (intraindividual variation) (Himes et al., 1979; Martin et al., 1985). Even after

the timing of caliper readings has been standardized, similar thickness of adipose tissue may yield different caliper readings because of differences in compressibility. Of all the assumptions considered in this section, this probably has the greatest potential for being a significant source of error in estimating body composition from skinfold measurement. (Martin et al., 1985).

Assumptions involved in using skinfold measurement to predict body fat

1. The double thickness of skin and subcutaneous adipose tissue has a constant compressibility.
2. Thickness of the skin is negligible or a constant fraction of the skinfold.
3. The thickness of subcutaneous adipose tissue is constant or predictable within and between individuals.
4. The fat content of adipose tissue is constant.
5. The proportion of internal to subcutaneous fat is constant

Thickness of subcutaneous adipose tissue varies widely among different skinfold sites within individuals and for the same skinfold sites between individuals. (Himes et al., 1979; Siervogel et al., 1982; Clays et al., 1987). Consequently, overall subcutaneous adipose tissue is best assessed by measuring multiple skinfold sites. A minimum of three is recommended. Proper site selection is critical because subcutaneous fat layer thickness can vary significantly within a 2- to 3-cm proximity of certain sites (Lohman,1981; Lukaski, 1987). Research has shown that certain skinfold sites are highly correlated to subcutaneous or total body fat and that sites from the lower limbs should be included in body composition prediction formulas (Martin et al., 1985; Lohman; 1988).

Adipose tissue can be divided into subcutaneous (what lies directly under the skin) and internal portions (that within and around muscle and surrounding

organs) (Lohman, 1981). The only direct data on the relationship of external and internal adipose tissue comes from the Brussels Cadaver Analysis Study (CAS). These data show that each kilogram of subcutaneous adipose tissue is associated with approximately 200 g of internal adipose tissue and that skinfolds are significantly correlated with total adiposity.(Clarys et al., 1987).

Although estimating body composition by skinfold measurement fails to meet all assumptions , it is more preferable compared to the use of other anthropometric variables and is certainly more meaningful than height-weight indices alone (Jackson and Pollock, 1985). Thus, skinfold measurement is the most widely used method indirectly estimating percent body fat in clinical setting.

General Technique

In all selected sites for skinfold measurements should be marked with a water-soluble first pen. All measurements should be taken on in the right side of the body. After all the sites have been marked, each fold is measured by grasping (drawing up) a layer of skin (skinfold) with the thumb and index finger of the nondominant hand about 1 cm or 1/2 inch above (proximal) the skinfold site and pull it away. For example, a right-handed measurer should hold the caliper in the right and grasp the skin with the left hand. The measurer should avoid pinching the subject. The proper technique is to pull the skin together and slightly away from the body but not so hard that the skinfold grasp is compressed. With the skinfold site isolated, the caliper should be placed perpendicular to the long axis of the skinfold and with the caliper's dial facing up and easily readable. The caliper tips should be placed on approximately 1 cm or 1/2 inch below (distal) to the finger holding the skinfold, so that pressure from the fingers will not affect the measured value. The caliper should not be placed too deeply into the skinfold or too close to tip of the skinfold. The dial is read approximately 2 sec after the pressure from the measurer's hand has been

released on the caliper handle, and recorded in millimeters. A minimum of three measurements should be taken at each site. This average of value is used as the skinfold scores. Measurement should be at least 15 sec apart to allow the skinfold site to return normal.

When measuring the obese, it may be impossible to elevate a skinfold with parallel sides, particularly over the abdomen. In this situation, the measurer should use both hands to pull the skinfold while a partner attempts to measure the width. If the skinfold is too wide for the calipers, underwater weighing or another technique have to be used.

Site Selection

The following description of eight of the most commonly used skinfold sites is taken from the Airline Consensus Conference protocol as outlined in the Anthropometric Standardization Reference Manual (Lohman, 1988).

Chest or pectoral fold: a diagonal taken one half the distance between anterior axillary and the nipple for men and one third of the distance between the anterior axillary line and the nipple for women.

Triceps: A vertical fold on the posterior midline of the upper right arm, over the triceps muscle, halfway between the acromion and the olecranon process tip of the shoulder to the tip of the elbow; the arm should be relaxed and fully extended.

Biceps: A vertical fold on the anterior midline of the upper right arm over the belly of the biceps muscle.

Abdominal: A horizontal fold taken at a distance of 3 cm to the right of and 1 cm below the midpoint of the umbilicus.

Subscapular: An angular fold taken at a 45-degree angle 1 cm below inferior angle of the scapula

Suprailiac: An oblique fold in line with the natural angle of the iliac crest taken in the anterior axillary line immediately superior to the iliac crest. **Thigh**: A vertical fold on the anterior midline of the thigh, midway between the proximal border of the patella and the inguinal crease (knee joint and the hip); the midpoint should be marked while the subject is seated.

Medial calf: A vertical fold at a level of the maximum circumference of the calf on the midline of the medial (inner) border.

3.3.4 Neutron Activation Analysis

Neutron activation analysis can quantify all of the main elements found in vivo, including total body hydrogen, carbon, nitrogen, oxygen, sodium, calcium, phosphorus, and chloride (Ma et al., 1993). Total body potassium can be measured with ^{40}K whole-body counting, a procedure included as part of some IVNA protocols. Also know as In Vivo Neutron Activation (IVNA) analysis, the method delivers a beam of neutrons to the subject, which interests with the body's elements in several characteristic ways. In the most important of these (in terms of neutron activation analysis), atoms of target elements are activated, creating unstable isotopes such as calcium-49 (^{49}Ca), nitrogen-15 (^{15}N), and sodium-24(^{24}Na) (Beddoe and Hill, 1985). As these unstable isotopes revert back to their forms, gamma radiation of a characteristic energy is emitted from each. This is received by the system's detectors and analyzes by its computers. The unique energy level of gamma radiation identities the element, and the radiation's level of activity represents its abundance (Lukaski, 1987; Cohn et al, 1974). Gamma-ray emission can occur almost immediately (in which case the technique is refereed to as prompt gamma analysis, used to measure total body hydrogen and nitrogen (Dilmanian et al., 1990; Ma et al., 1993) or over a several-minute period (in which case it is known as delayed gamma analysis, used to measure total calcium, phosphorus, sodium, and chloride (Dilmanian et al., 1990; Ma et al., 1993).

Because a major component of muscle is nitrogen, the ability to measure nitrogen using neutron activation analysis allow the body's muscle and nonmuscle mass to be estimated (Lukaski, 1987; Cohn et al, 1980). The precision of repeated nitrogen determinations in healthy humans has been 2 to 3 % (Lukaski, 1987).

Bone mineral content is of considerable interest to those studying osteoporosis, and neutron activation analysis is useful in measuring total body calcium, which is then used to quantify total body calcium, which is then used to quantify total bone mineral. The precision of repeated calcium measurement in healthy adults using neutron activation analysis has been 2.5%, making the method suitable for longitudinal studies of bone mass (Lukaski, 1987). A more recent study of calcium content in phantoms (representations of the human body used for precision and accuracy testing) as measured by neutron activation analysis resulted in values within 3.6 % of their known composition (Ryde et al., 1990).

The importance of neutron activation analysis is that element analysis allows reliable and reproducible reconstruction of molecular level components such as total body fat, protein, and mineral (Heymsfield et al, 1991). This important characteristic of in vivo neutron activation methods positions them as uniquely qualified to serve as reference methods, particularly in conditions in which unstable component associations render many currently available methods inaccurate (Heymsfield et al, 1997).

However, Neutron activation system has several drawbacks. It exposes subject to ionizing radiation, time-consuming, and studies in healthy children and young women are not usually recommended. It is highly cost and require a skilled group of investigators to maintain and operate. In addition, the units are not mobile and are not widely available (Lukaski, 1987).

3.3.5 Computed Tomography (CT)

Computed Tomography (CT) can quantify all major tissue-system level components. It is an imaging technique producing high detailed cross-sectional images of the body due to differences in the transmission of an x-ray beam (ionizing radiation) through body tissues of different densities (Brodile, 1988). Although its role in medicine is primarily for diagnostic purposes, it has proven to be a valuable research tool in assessing body composition and nutritional status. The computed tomography (CT) system consists of a rotating x-ray source aligned opposite an array of radiation detectors. As the CT x-ray beam pass through the subject, it is attenuated by the body's tissues and eventually picked up by the detectors. The response of the detectors is then transmitted to a computer, which also considers the spatial arrangement of the subject and x-ray beam. From this data, the computer reconstructs the subject's cross-sectional anatomy using mathematic equations adapted for computer processing (Bushong, 1988).

Computed tomography has been particularly useful in studying the relative deposition of subcutaneous and intra-abdominal fat (Grauer et al., 1984; Weits et al., 1989; Seidell et al., 1990). Estimates of subcutaneous and intra-abdominal fat from CT have been shown to compare very closely with direct measurements in cadaver (Rossner et al., 1990) and laboratory animals (Weing et al., 1988). CT scans at three sites - lower chest, abdomen, and midthigh - have been shown effective in estimating body fat mass in premenopausal obese women (Ferland et al., 1989).

The potential for using CT in assessing body composition and nutritional status is limited by problems of radiation exposure and high cost and limited availabilty of the instrument (Lukaski, 1987; Heymsfield et al., 1987). Multiple scans of the same individual, whole-body scans, and use of CT with children, women and women of childbearing age are not encouraged because of the

exposure to ionizing radiation. The use of the technology in nutrition assessment is restricted, for the most part, to special research application.

3.3.6 Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging (MRI) has been proposed as an alternative technique of determining body composition on the tissue-system level components (Heymsfield, 1997). The fundamental MRI concept is based on interaction between nuclei of abundant hydrogen atoms and magnetic fields produced and controlled by the system's instrumentation. Proton, the hydrogen nucleus, have a magnetic moment that causes them to function as small magnets. Under usual conditions in the earth's weak magnetic field, these magnetic moments are random oriented, and they tent to cancel each other. When a subject is placed in a large bore magnet generating a very strong magnetic field, the magnetic dipoles become aligned in relation to magnetic field. If a radio-frequency wave is then directed into the subject's body, some of the nuclei will absorb energy from the radio wave and change their orientation with respect to the magnetic field. When the radio discontinued, the nuclei gradually return to their equilibrium state (relaxation) and emit a signal that can be received by the system. This signal generated by energy release is used to create MRIs. These data then processed by computer to generate an image much the same way as in computed tomography (Lukaski, 1987; Heymsfield et al., 1987; Brodile, 1988; Bushong, 1988).

MRI provides valuable, noninvasive method and has been effectively used measure total and subcutaneous adipose tissue in individuals of varying degree of body fatness (Staten et al., 1989). There has been shown that MRI can be used to measure different body fat depots in human subjects (Fowler et al., 1991; Sohlstrom et al., 1993). Agreement between MRI and hydrostatic weighing of body fat was good ($r = 0.84$) (Sohlstrom et al., 1993). In contrast to images produced by computed tomography, MRI dose not use ionizing

radiation. Because no radiation hazards are involved, examinations may be carried out repeatedly in the same subjects, which is valuable when the effect of factors such as reproduction, growth, training, and dietary regimens on the amount and distribution of adipose tissue is to studied. However, a drawback with the MRI technique is the high cost of the equipment.

3.3.7 Dual-Energy X-ray Absorptiometry (DEXA)

Dual-energy projection methods have been used for over a decade to measure bone and soft-tissue composition in vivo (Mazess et al., 1990). There are two types of dual-energy projection methods. Dual-photon absorptiometry (DPA) is based on the differential attenuation by tissues of transmitted photons at two energy levels (Witt and Mazess, 1978). DPA uses a gadolinium radionuclide (^{153}Gd) and has been widely used for the measurement of regional bone mineral density (BMD) and bone mineral content (BMC), particularly of the spine and proximal femur (Mazess et al., 1990).

Dual-energy X-ray absorptiometry (DEXA) is recently introduced noninvasive radiologic projection technique. The energy source for DEXA is x-ray rather than the galdolinium used in DPA (Mazess et al., 1989). This technological change has improved the ability to quantify various parameters of body composition; radiation exposure is minimal (the radiation does for a whole-body scan is < 5 mrem), evaluation time is reduced, and precision is improved by enhanced resolution. DPA and DEXA instruments differentiate body weight into three chemical compartments-bone mineral content, fat soft tissue, and lean soft tissue (Heymsfield and Waki, 1991) and the computer software permits measures of the whole body as well as individual segments, i.e. limbs or abdominal regions (Mazess et al., 1990). DEXA is an accepted clinical tool for assessing spinal osteoporosis and other related bone disorders. Preliminary results suggest that DEXA can be used to accurately estimate soft-tissue composition with better precision (about 1-1.5%) than was possible with

DPA (2-3%) (Russell-Aulet et al., 1991; Johnson and Dawson-Hughes, 1991). The underlying principle of DEXA is that the bone and soft tissue areas can be penetrated to a depth of about 30 cm by two distinct energy peaks from an x-ray source. The penetration is analyzed by a scintillation detector. With the subject lying supine on a table, the source and detector are passed across the body at a relatively slow speed of 1 cm/s. A DEXA scan of the whole body takes about 12 minutes. Specialized computer software reconstructs an image of bone mineral content, total fat mass, and fat-free body mass. Selected trunk and limbs regions also can be pinpointed for a more on-depth analysis of tissue composition.

Unlike underwater weighing, DEXA is not limited by the assumptions associated with the two-compartment constant-density model. Tissue densities are measured directly and are differentiated. This is a significant advantage over underwater weighing for the determination of body composition in humans.

Reliability and validity of DPA (Peppler and Mazess, 1981) and DEXA (Haarbo et al., 1991; Mazess et al., 1989) are well established. Precision errors are less than 3% for fat and 1.11 kg and 30 g for muscle and bone, respectively (Haarbo et al., 1991). Correlations between body composition parameters as measured by DPA and other methods such as hydrostatic weighing and neutron activation analysis are typically greater than $r = 0.90$ (Heymsfield et al., 1989; Heymsfield et al., 1990; Lichtman et al., 1990). The advantages of DPA and DEXA are safe, rapid, require minimal cooperation by the individual, and account for individual variability in bone mineral. Unfortunately, DPA and DEXA are expensive (cost can exceed $60,000) and often require trained radiology personnel. This limits the applicability of DPA and DEXA to clinical and laboratory settings. As the cost of DEXA becomes less prohibitive, it will likely replace underwater weighing as a criterion measure of body composition in many studies.

Related Literature

Because the salient features of BIA technique are that it is safe, portable, rapid, noninvasive, and required only the placement of four electrodes on well-defined landmarks. Thus, if it is yield valid results, it is appreciable for many clinical and field setting. Many previous studies investigated the validity of BIA method for estimating the relative body fat in various ethnic groups.

Eckerson et al. (1992) studied to compare the validity of selected BIA prediction equation for estimating the FFM estimating in lean males. UWW was used as the reference method. Sixty-one Caucasian males (19-40 yr) participated as subjects for this study. The results indicated that FFM was more highly correlated with body weight. Body weights alone estimated FFM as accurately as any of the BIA equation in lean males.

Stolarczyk et al. (1995) investigated to assess the predictive accuracy of various BIA equations in estimating the FFM of Hispanic populations, high levels of body fat have been associated with risk for cardiovascular and metabolic diseases. The reference method was UWW at residual volume. Thirty-one Hispanic women (20-39 yr) volunteered for this study. The result indicated that the fatness-specific BIA equation or age-specific BIA equation might have potential for assessing the body composition of healthy, Hispanic women.

Ainworth et al. (1997) had placed the purpose of their study as same as the purpose of Stolarczyk et al. (1995) but target populations of their study were African-American women, an obese (BMI>27.3) and the highest rates of obesity in the United States. The reference method was UWW at residual volume. One hundred thirty-five African-American women (18-40 yr) volunteered for this study. The result indicated that the fatness-specific BIA

equation might have potential for assessing the body composition of premenopausal in this group.

4. Method selection in this study

Only by analyzing cadavers can direct measurement of body composition be made (Brodile, 1988). This is obviously not a practical approach in human. Such analysis are high cost, time-consuming, require specialized laboratory equipment and involve many ethical and legal problems in obtaining cadavers and human tissue for research purposes. For these reasons, many indirect methods are developed and cross-validated against the gold standard method. Often, neutron activation analyses, DEXA, MRI, and underwater weighing are cited as in vivo reference standards. DEXA, MRI, and neutron activation analyses are highly accurate but costly and require specially trained personnel. Particularly, there are only a few center in the world that have resources similar to those at Brookhaven National Laboratory, Long Island, provide good examples of the main in vivo neutron activation analyses methods in current use (Dilmanian et al., 1990; Ma et al., 1993). For these reasons, underwater weighing is the most common criterion method of body composition analysis.

Besides above these reasons, the selection of a reference method considers the appropriateness of the body composition level and compartment under study (Heymsfield et al, 1997). For example, fat is a molecular level component and adipose tissue is a tissue-system level component. Adipose tissue measured by MRI would therefore not be expected to serve as a reference method against which fat estimates by a method such as underwater weighing are compared.

The final consideration is that reference body composition method must be available to investigators if they are useful. There are thus many factors to

consider when choosing a references body composition method (Heymsfield et al, 1997).

As described earlier, many body composition methods are valid and highly accurate. Nevertheless, most of these methods require cumbersome procedures and are less suitable for field studies. SKF and BIA are alternative body composition techniques used in clinical and field settings. The drawbacks of SKF, however, are measurement error (site selected and tester reliability) (Pollock and Jackson, 1984). BIA, a safe, rapid, noninvasive method, is more suitable to measure body composition, if it is valid. Due to UWW is the reference method in the molecular level and is more available than other reference methods for assessing the body fat. Thus UWW is served as criterion method to examine the validation of BIA method estimated body composition based on the molecular level.

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CHAPTER III

MATERIALS AND METHODS

Materials and Equipment

Underwater weighing

1. A 2500 liters capacity of fiberglass tank
2. A chair seat
3. A 15-kg capacity of load cell for underwater weighing (15-kg UWE HS-15K, the nearest 10 g)
4. A weighted belt (if necessary)
5. A wet spirometer
5. A thermometer

Bioelectrical impedance analyses

1. A hand-held bioelectrical impedance machine(Bodystat-1500, Bodystat Ltd, Isle of Man, UK)
2. A weighing scale (Yamato DP-6100GP)
3. A scale for height
4. Alcohol
5. Cotton

Subjects and Methods

Subjects

Thirty-three Thai people (19 males and 14 females) aged 18-41 years volunteered to participate in this study. Inclusion criteria were (1) self-identified as Thai people. (2) ranging of age between 18-41 years. (3) free of illness that could

affect body weight and body composition (e.g. anorexia, digestive disorder, chronic diseases. (4) free of heart diseases and related pulmonary diseases. (5) for female, non pregnant. (6) body mass index (BMI) not exceed 25.0.

All body composition determinations (Underwater weighing and Bioelectrical impedance analyses) were carried out on the same day. Before testing, all subjects need to prepare themselves adequately. Such pretest requirements include the followings:

1. refrain from eating, drinking and exercise for 4 hours
2. no alcohol consumption within 48 hours
3. no diuretics within 7 days of the test
4. no smoking for 2 hours
5. avoidance of any condition that would alter the hydration of the body such as physical activity, saunas etc.
6. urinate and defecate within 30 minutes of the test
7. for females, avoidance of testing the menstruation period as well as 3 days before an after the period

Body weight and height

Subjects were weighed in shorts and T-shirt without shoes. Body weight was measured to the nearest 0.02 kg on digital platform scale (Yamato DP-6100GP). Height was measured using a wall scale with subject standing upright and arms hanging freely at the sides. Height was measured to the nearest 0.5 cm. A body mass index (BMI) was computed as weight devided by squared height (Wt / Ht squared)

Hydrodensitometry

Total body density (D_b) was assessed from underwater weighing at residual volume (RV) using approximated of vital capacity (Wilmore, 1969a). RV was determined on land with subject seated in a position similar to that assumed during underwater weighing. The subject exhales as fully as possible and bend forward slowly via the pipe of wet spirometer at any water temperature. Two closest reading within 100 ml from three trials were averaged and used to calculate RV value for correcting body volume estimated from underwater weight. Also, a constant of 100 ml was used to correct body volume for gastrointestinal volume.

A load cell system (the 15-kg capacity UWE HS-15K scale) was used to measure underwater weight to the nearest 10 g. This procedure can be explained as follows:

For males, he wore a shorts or swimming suit, and for females, she wore a T-shirt and shorts. Before entering the tank, subjects should have urinated and defecated. The subjects cloth were then weighed for the correction of the dry weight or weight in air (W_a) to the nearest 20 g. Later, the subject enters the water tank, stands still, gets completely wet, and rubs the hands over the entire body to remove any trapped air bubbles from skin, hair, and clothing. Once the subject is seated, water is added or drained from tank so that water level is adjusted below the subject's chin. At this point, the test procedure is explained. The subject is instructed to hyperventilated two to three times after a deep breath, and start a full expiration while the head is still above the water.

When the subjects were familiar with the procedure, he or she exhales as fully as possible and bend forward slowly until the head is completely under the water, as shown in Appendix B. If the head only partially submerged, the tester should tap the subject's head until it goes completely under the water. The tester

encouraged the subject to continuing to exhale underwater until no more air can be expelled. After exhaling fully, the subjects remain motionless and count for 5 to 7 seconds before coming up for air. This will allow the tester time to read the scale and record underwater weight. After that, the testes taps on the side of the tank to signal for the subject to surface. While in the water, the subject should move slowly to avoid water turbulence that can make scale reading difficult.

The procedures was repeated 10 times, because there is a learning effect associated with the technique (Katch, 1968). It is important that the tester be patient and make the subject feel as comfortable as possible. The tester should also critique each trial, giving the subject feedback so that the next trial they could be perform better.

Once the subject's weight levels off after repeated trials, the procedure is completed. The water temperature should be recorded, and water density should be determined from the standard tables. The tare weight is determinated after the subjects gets out of the tank. Tare weight is combined weight of chair, chains taken at the same level at which the under weight was observed. The net weight in water is the gross weight in water minus the tare weight. Using the equation of Goldman and Buskirk (1961), the average of three highest weightings within 100 g was used as the underwater weigh for the calculation of body density (D_b). D_b was convert to percent body fat (%BF) using the Siri two-compartment equation: percent body fat = $(4.95 / D_b - 4.50) \times 100$. And then, fat mass (FM) can be easily calculated from %BF by this equation: fat mass = total body mass x %BF.

Bioelectrical impedance analysis (BIA)

BIA was performed with a hand-held bioelectrical impedance machine (Bodystat-1500) which is programmed to run on the manufacturer's prediction equation. The exact prediction equation used were not disclosed by manufacturer

(Bodystat Ltd, Isle of Man, UK). Subjects lied in a supine position on a nonconductive bed with arms and legs slightly abducted from the middle line of the body to ensure that no parts of the body were in contact with each other. Shoes and socks were removed, and each electrode site was prepared by swabbing with alcohol and then allowing the site to dry before electrode replacement. On the right side, two adhesive aluminum foil signal introducing electrodes were placed on the middle of the dorsal surface of the hand and foot at the third metacarpal and matatarsal phalangeal joint, respectively, and two detecting electrodes were placed between styloid processes of radius and ulna and between the medial and lateral malleoli of the ankle, as shown in Appendix C. A low-level electrical current (typically 800 μA at a frequency of 50 kHz) was applied to distal electrodes and voltage drop was detect by proximal electrodes.

Statistical analysis

Data is presented as mean ± SD. Difference between predicted values (FM and %BF of BIA method) and criterion value (UWW) were tested by dependent t-test. The validity of the BIA were based on an evaluation of predicted FM and %BF (and FFM) versus the criterion %BF and FM from UWW via calculation of the constant error (CE = estimated value minus actual value), correlation coefficient (r), standard error of estimate ($SEE = SD\sqrt{1-r^2}$), total error ($TE = \sqrt{\Sigma[\text{predicted} - \text{actual}]^2 / n}$) and the similarity between the standard deviation of the predicted and actual FM and %BF values. The percentage of the sample that are predicted within ±4%BF for each method was also determined since this value is the conventionally accepted error for predicted %BF within a specific population (Lohman, 1981). Table 3. has shown the Standards for evaluating the SEE for %BF which was developed by Lohman (1992). These values are based on empirical derived measurement errors associated with the reference method (underwater weighing).

Table 3. Standards for evaluating the SEE

SEE %BF Males and females	Subjective rating
2.0	Ideal
2.5	Excellent
3.0	Very good
3.5	Good
4.0	Fairly Good
4.5	Fair
5.0	Poor

Note. Data from Lohman (1992)

CHAPTER IV

RESULTS

The description of the total sample, males, and females are provided in the Table 4.1. Cross-validation results for BIA of FM and %BF are presented in Table 4.2 and 4.3, respectively. The dependent t-test determined that BIA estimates significantly underestimated both FM and %BF compared with UWW ($p<0.005$). The correlation of FM and %BF between UWW and BIA were met in ranging 0.56-0.77 and 0.61-0.85, respectively. The estimates of FM and %BF by BIA were the lowest predictive accuracy in males ($r=0.56$, SEE=3.31, TE=5.32 and $r=0.61$, SEE=5.23, TE=8.63, respectively) and the highest predictive accuracy in females ($r=0.76$, SEE=2.07, TE=4.02 and $r=0.62$, SEE=3.84, TE=7.68, respectively). As seen in Table 3.1, which was shown the SEE values of %BF and developed by Lohman (1992), the prediction error of BIA in males were poor in males and fairly good in females. The proportions of subjects whose mean BIA estimate of %BF fell within various ranges of criterion measure appear in Table 4.4. BIA accounted only for 30.3%BF.

Table 4.1 Descriptive characteristic of the study subjects.

Variables	Total samples (n=33)	Males (n=19)	Females (n=14)
Age (yr)	25.55 ± 5.33 (18-41)	24.37 ± 5.22 (18-41)	27.14 ± 5.23 (19-38)
Height (cm)	164.27 ± 7.90 (150-182)	169.42 ± 5.82 (160-182)	157.29 ± 3.91 (150-164)
Weight (kg)	57.12 ± 7.55 (44.1-73.9)	61.42 ± 6.74 (49.78-73.9)	51.27 ± 3.68 (44.1-57)
Body density (g/ml)	1.05 ± 0.02 (1.0161-1.0821)	1.06 ± 0.02 (1.0333-1.0820)	1.03 ± 0.01 (1.0161-1.0587)
Impedance (ohm)	546.52 ± 82.38 (410-729)	490.42 ± 46.05 (410-570)	622.64 ± 54.11 (549-729)
Body mass index (kg/m^2)	21.10 ± 1.65 (18.1-25.1)	21.35 ± 1.50 (18.3-23.3)	20.76 ± 1.84 (18.1-25.1)

Values are mean ± SD; range in parentheses.

Table 4.2 Cross-validation analysis of fat mass (FM) in kg for BIA.

	Mean ± SD	CE	*r*	SEE	TE
Total sample					
(n=33)					
UWW	12.88+4.47				
BIA	8.98+3.56	3.9*	0.77##	2.85	4.81
Males (n=19)					
UWW	10.65+3.99				
BIA	6.43+1.77	4.22*	0.56#	3.31	5.32
Females (n=14)					
UWW	15.91+3.18				
BIA	12.43+2.15	3.48*	0.76##	2.07	4.02

CE, constant error

r, correlaton coefficient between criterion (UWW) and predicted %BF

SEE, standard error of estimate

TE, total error

* significantly different from criterion values, $p<0.005$

\# significant correlation with criterion values, $p<0.05$

\## significant correlation with criterion values, $p<0.01$

Table 4.3 Cross-validation analysis of percent body fat (%BF) for BIA.

	Mean ± SD	CE	*r*	SEE	TE
Total sample (n=33)					
UWW	23.20 ± 8.9				
BIA	16.38 ± 7.52	6.82*	0.85##	4.69	8.24
Males (n=19)					
UWW	17.54 ± 6.60				
BIA	10.63 ± 3.17	6.91*	0.61##	5.23	8.63
Females (n=14)					
UWW	30.89 ± 4.90				
BIA	24.18 ± 3.39	6.71*	0.62#	3.84	7.68

CE, constant error

r, correlation coefficient between criterion (UWW) and predicted %BF

SEE, standard error of estimate

TE, total error

* significantly different from criterion values, $p<0.005$

\# significant correlation with criterion values, $p<0.05$

\## significant correlation with criterion values, $p<0.01$

Table 4.4 Percentage of mean BIA assessment falling with various of the criterion measure in total subjects.

	BIA
±1%BF	6.06%
±2%BF	6.06%
±4%BF	30.30%
±6%BF	33.33%
±8%BF	63.64%
±10%BF	66.67%

Table 4.5 Percentage of mean BIA assessment falling with various of the criterion measure in male subjects.

	BIA
±1%BF	10.53%
±2%BF	10.53%
±4%BF	36.84%
±6%BF	36.84%
±8%BF	63.16%
±10%BF	68.42%

Table 4.6 Percentage of mean BIA assessment falling with various of the criterion measure in female subjects.

	BIA
±1%BF	-
±2%BF	-
±4%BF	21.43%
±6%BF	28.57%
±8%BF	64.29%
±10%BF	85.71%

Table 4.7 Descriptive characteristic of total subjects.

No.	Sex	Age (yr)	Height (cm)	Weight (kg)	Body density (g/ml)	Vital capacity (ml)	Residual volume (ml)	Impedance (ohm)	Body mass index
1	F	25	156	46.66	1.0385	2160	604.80	623	19.20
2	F	32	159	52.28	1.0312	1300	364.00	595	20.70
3	F	26	164	55.02	1.0307	2380	666.40	652	20.40
4	F	28	156	48.58	1.0587	2350	652.40	563	20.00
5	F	30	155	49.30	1.024	1970	551.60	624	20.50
6	M	28	170	61.24	1.0402	3450	828.00	513	21.20
7	F	27	155	52.60	1.0214	2410	674.80	641	21.90
8	F	26	163	50.16	1.0288	2760	772.80	667	18.90
9	M	26	160	52.70	1.0333	2660	638.40	512	20.60
10	M	18	163	49.78	1.0487	2595	622.30	530	18.70
11	M	20	168	65.80	1.0694	2810	674.40	448	23.30
12	M	18	180	66.45	1.0778	3390	813.60	501	20.50
13	M	23	177	69.95	1.0792	3620	868.80	447	22.30
14	M	26	165	62.35	1.0637	2870	423.00	423	22.90
15	M	21	168	62.30	1.0708	3200	768.00	438	22.10
16	M	20	171	66.60	1.069	2890	693.30	410	22.80
17	M	21	169	62.90	1.0821	3150	756.00	484	21.90
18	M	24	166	59.35	1.0764	3440	825.60	439	21.60
19	M	23	167	55.85	1.0719	3390	813.60	547	20.00
20	F	21	156	57.00	1.0161	1450	406.00	562	23.40
21	M	27	166	51.80	1.0536	2320	556.80	532	18.80
22	M	29	168	60.30	1.0495	2650	636.00	536	21.40
23	F	22	150	56.40	1.0217	2200	616.00	549	25.10
24	M	23	177	68.70	1.0441	3680	883.20	500	21.90
25	M	41	167	50.90	1.0479	2130	511.20	531	18.30
26	M	28	164	61.82	1.0456	3080	739.20	475	23.00
27	M	22	182	73.90	1.0443	3380	811.20	482	22.30

No.	Sex	Age (yr)	Height (cm)	Weight (kg)	Body density (g/ml)	Vital capacity (ml)	Residual volume (ml)	Impedance (ohm)	Body Mass Index
28	F	19	159	52.50	1.0177	2090	501.60	687	20.80
29	F	38	154	52.14	1.0320	2540	711.2	573	22.00
30	M	25	171	64.34	1.0570	3160	758.4	570	22.00
31	F	35	156	48.15	1.0253	2540	711.2	729	19.80
32	F	26	156	44.10	1.0311	2100	588.0	668	18.10
33	F	25	163	52.88	1.038	2580	722.4	584	19.90
Mean		25.55	164.27	57.12	1.05	2688	671.64	546.52	21.10
S.D.		5.33	7.90	7.55	0.02	597.0	132.94	82.38	1.65
Min		18	150	44.1	1.0161	1300	364	410	18.1
Max		41	182	73.9	1.0821	3680	883.2	729	25.1

Table 4.8 Descriptive characteristic of male subjects.

No.	Sex	Age (yr)	Height (cm)	Weight (kg)	Body density (g/ml)	Vital capacity (ml)	Residual volume (ml)	Impedance (ohm)	Body Mass Index
1	M	28	170	61.24	1.0402	3450	828.00	513	21.20
2	M	26	160	52.70	1.0333	2660	638.40	512	20.60
3	M	18	163	49.78	1.0487	2595	622.30	530	18.70
4	M	20	168	65.80	1.0694	2810	674.40	448	23.30
5	M	18	180	66.45	1.0778	3390	813.60	501	20.50
6	M	23	177	69.95	1.0792	3620	868.80	447	22.30
7	M	26	165	62.35	1.0637	2870	423.00	423	22.90
8	M	21	168	62.30	1.0708	3200	768.00	438	22.10
9	M	20	171	66.60	1.069	2890	693.30	410	22.80
10	M	21	169	62.90	1.0821	3150	756.00	484	21.90
11	M	24	166	59.35	1.0764	3440	825.60	439	21.60
12	M	23	167	55.85	1.0719	3390	813.60	547	20.00
13	M	27	166	51.80	1.0536	2320	556.80	532	18.80
14	M	29	168	60.30	1.0495	2650	636.00	536	21.40
15	M	23	177	68.70	1.0441	3680	883.20	500	21.90
16	M	41	167	50.90	1.0479	2130	511.20	531	18.30
17	M	28	164	61.82	1.0456	3080	739.20	475	23.00
18	M	22	182	73.90	1.0443	3380	811.20	482	22.30
19	M	25	171	64.34	1.0570	3160	758.4	570	22.00
Mean		24.37	169.42	61.42	1.06	3046	716.89	490.42	21.35
S.D.		5.22	5.82	6.74	0.02	436.7	126.31	46.05	1.50
Min		18	160	49.78	1.0333	2130	423	410	18.3
Max		41	182	73.9	1.0821	3680	883.2	570	23.3

Table 4.9 Descriptive characteristic of male subjects.

No.	Sex	Age (yr)	Height (cm)	Weight (kg)	Body density (g/ml)	Vital capacity (ml)	Residual volume (ml)	Impedance (ohm)	Body Mass Index
1	F	25	156	46.66	1.0385	2160	604.80	623	19.20
2	F	32	159	52.28	1.0312	1300	364.00	595	20.70
3	F	26	164	55.02	1.0307	2380	666.40	652	20.40
4	F	28	156	48.58	1.0587	2550	652.40	563	20.00
5	F	30	155	49.30	1.024	1970	551.60	624	20.50
6	F	27	155	52.60	1.0214	2410	674.80	641	21.90
7	F	26	163	50.16	1.0288	2760	772.80	667	18.90
8	F	21	156	57.00	1.0161	1450	406.00	562	23.40
9	F	22	150	56.40	1.0217	2200	616.00	549	25.10
10	F	19	159	52.50	1.0177	2090	501.60	687	20.80
11	F	38	154	52.14	1.0320	2540	711.2	573	22.00
12	F	35	156	48.15	1.0253	2540	711.2	729	19.80
13	F	26	156	44.10	1.0311	2100	588.0	668	18.10
14	F	25	163	52.88	1.038	2580	722.4	584	19.90
Mean		27.14	157.29	51.27	1.03	2216	610.23	622.64	20.76
S.D.		5.23	3.91	3.68	0.01	423.7	119.82	54.11	1.84
Min		19	150	44.1	1.0161	1300	364	549	18.1
Max		38	164	57	1.0587	2760	772.8	729	25.1

Table 4.10 Collective data of fat mass and %body fat from UWW and BIA of total subjects.

No.	Underwater weighing		Bioelectrical impedance	
	% body fat	Fat mass (kg)	% body fat	Fat mass (kg)
1	26.65	12.43	21.00	9.80
2	30.00	15.68	23.70	12.40
3	30.26	16.64	23.60	13.00
4	17.55	8.53	19.80	9.60
5	33.40	16.47	25.40	12.50
6	25.87	15.84	12.40	7.60
7	34.63	18.22	27.80	14.60
8	31.03	15.57	21.50	10.80
9	29.05	15.31	13.10	6.90
10	22.01	10.96	10.00	5.00
11	12.89	8.48	9.30	6.10
12	9.27	6.16	7.10	4.70
13	8.67	6.06	7.90	5.50
14	15.35	9.57	8.50	5.30
15	14.32	8.92	6.70	4.20
16	13.03	8.68	5.00	3.30
17	7.45	4.66	9.90	6.20
18	9.88	5.87	7.10	4.20
19	11.81	6.60	12.30	6.90
20	37.18	21.19	24.70	14.10
21	19.80	10.25	12.20	6.30
22	21.64	13.05	14.60	8.80
23	34.47	19.44	28.20	15.90
24	24.10	16.55	10.80	7.40
25	22.36	11.38	16.10	8.20
26	23.41	14.47	13.10	8.10
27	24.01	17.81	9.90	7.30

No.	Underwater weighing		Bioelectrical impedance	
	% body fat	Fat mass (kg)	% body fat	Fat mass (kg)
28	36.39	19.11	24.80	13.00
29	29.66	15.46	27.40	14.30
30	18.30	11.77	15.90	10.20
31	32.78	15.78	30.10	14.50
32	30.07	13.26	21.80	9.60
33	28.33	14.98	18.70	9.90
Mean	23.20	12.88	16.38	8.98
S.D.	8.90	4.47	7.52	3.56
Min	7.45	4.66	5.00	3.30
Max	37.18	21.19	30.10	15.90

Table 4.11 Collective data of fat mass and %body fat from UWW and BIA of male subjects.

No.	Underwater weighing		Bioelectrical impedance	
	% body fat	Fat mass (kg)	% body fat	Fat mass (kg)
1	25.87	15.84	12.40	7.60
2	29.05	15.31	13.10	6.90
3	22.01	10.96	10.00	5.00
4	12.89	8.48	9.30	6.10
5	9.27	6.16	7.10	4.70
6	8.67	6.06	7.90	5.50
7	15.35	9.57	8.50	5.30
8	14.32	8.92	6.70	4.20
9	13.03	8.68	5.00	3.30
10	7.45	4.66	9.90	6.20
11	9.88	5.87	7.10	4.20
12	11.81	6.60	12.30	6.90
13	19.80	10.25	12.20	6.30
14	21.64	13.05	14.60	8.80
15	24.10	16.55	10.80	7.40
16	22.36	11.38	16.10	8.20
17	23.41	14.47	13.10	8.10
18	24.01	17.81	9.90	7.30
19	18.30	11.77	15.90	10.20
Mean	17.54	10.65	10.63	6.43
S.D.	6.60	3.99	3.17	1.77
Min	7.45	4.66	5.00	3.30
Max	29.05	17.81	16.10	10.20

Table 4.12 Collective data of fat mass and %body fat from UWW and BIA of female subjects.

No.	Underwater weighing		Bioelectrical impedance	
	% body fat	Fat mass (kg)	% body fat	Fat mass (kg)
1	26.65	12.43	21.00	9.80
2	30.00	15.68	23.70	12.40
3	30.26	16.64	23.60	13.00
4	17.55	8.53	19.80	9.60
5	33.40	16.47	25.40	12.50
6	34.63	18.22	27.80	14.60
7	31.03	15.57	21.50	10.80
8	37.18	21.19	24.70	14.10
9	34.47	19.44	28.20	15.90
10	36.39	19.11	24.80	13.00
11	29.66	15.46	27.40	14.30
12	32.78	15.78	30.10	14.50
13	30.07	13.26	21.80	9.60
14	28.33	14.98	18.70	9.90
Mean	30.89	15.91	24.18	12.43
S.D.	4.90	3.18	3.39	2.15
Min	17.55	8.53	18.70	9.60
Max	37.18	21.19	30.10	15.90

CHAPTER V

DISCUSSION AND CONCLUSION

Discussion

The result of analyses were evaluated based on the following criteria (Lohman, 1981):

(a) The mean values for actual and predicted FM and %BF should be comparable.

(b) A low SEE value is desirable and is preferred over the correlation coefficient since the correlation is likely to be affected by intersample variability in FM and %BF.

(c) TE should be calculated since it reflects the true difference between the actual and predicted FM and %BF, whereas SEE only reflects error associated with the regression between the variables.

(d) There should be a close similarity between TE and SEE because it reflects the relationship between the regression of actual and predicted FM and %BF.

Although all of these criteria deserve attention, the TE is the best single criterion for determining the difference between the actual and predicted FM and %BF (Sinning and Wilson, 1984).

Based upon the above criteria, the results of the cross-validation analyses for BIA manufacturer equation (Bodystat 1500) were high prediction error in normal subjects. This results is similar to the results of some previous studies which reported that the equations supplied by the BIA manufacturer are

unacceptable when compared with UWW. Segal et al. (1985) and Jackson et al. (1988) who report that the BIA manufacturer equation perform unsatisfactorily as it underestimated the densitometrically determined percentage of body fat by 2.9%BF and 2.4%BF, respectively. Stout et al. (1994) and Pierson et al. (1991) reported that the BIA manufacturer equation significantly overestimated the criterion measure of percentage of body fat by 2.8%BF and 3.6%BF, respectively. The standard error of estimate by Segal et al. (1985) was 6.1%BF, while the SEE term reported by Jackson et al. (1988) and Stout et al. (1994) were 4.6%BF and 4.2 %BF, respectively. The total error of 5%BF reported by Stout et al. was less than the total error reported in this study. Pearson product moment correlations (r) between BIA estimations of %BF and densitometrically determine body fat have been moderate, ranging between 0.72–0.74 (Stout et al, 1994; Pierson et al., 1991; Jackson et al., 1988), which are less than the correlation report in this study. With BIA estimating only 30.3% of the participants within ±4%BF of UWW, the results from this study are lower than with the others (approximately 50%) (Stout et al, 1994; Jackson et al., 1988; Segal et al., 1985) who have concluded that the BIA manufacturer equation performs poorly and may not be suitable for estimating %BF.

As mentioned the results in this study previously, it has been shown that the BIA technique, a simple, portable, and expensive method, does not accurately estimate percentage of body fat and fat mass in Thai populations when the BIA manufacturer's equation are used. Possibly, the causes of high prediction error of BIA in Thai populations result from the limitations of both UWW and BIA methods in this study.

The limitations of UWW

1. One problem encountered in the evaluation of human body composition techniques is that no benchmark method exists inherently that is error free. In

other words, none of these direct body composition methods yields a "true" value. Even though hydrodensitometry (underwater weighing) is often criterion measure in applied research, the error of UWW in estimating percent body fat has been estimated to be ~2.5% for special population (Lohman, 1981). Thus, some degree of inaccuracy in predicting body composition may be a result of error in the reference method.

2. In addition, UWW is based on the two-component model and used to assess the body density by using Siri two-compartment model to derive estimates of criterion %BF. The Siri equation assumes constant proportions and densities for the components of the FFM (1.10 g/cm^3) between individuals and among population subgroups, regardless of many factors, i.e. gender, age, ethnicity. Many studies have reported that FFM density varies with these factors, depending mainly on the relative proportion of water and mineral comprising the FFM (Williams et al., 1993; Buamgartner et al., 1991; Wang et al., 1989). The biological variability in FFM densities may contribute to the violation of the basic assumption. For example, the FFM densities of African-American women (1.106 g/cm^3) and African-American men (1.113 g/cm^3) are greater than (1.10 g/cm^3) because of their higher mineral content (about 7.3%FFM) and bone density (Deck-Cote and Adams, 1993; Ortiz et al., 1992; Schutte et al., 1984). Because of this difference in FFM density, the body fat of African-Americans will be systematically underestimated when two-component model equations are used to estimates %BF. Likewise, the FFM density of children is estimated to be only 1.084 g/cm^3 because of their relative lower mineral (5.2%FFM) and high body water (76.6%FFM) compared to reference body (Lohman, 1984). Also, The average density of FFM of elderly men and women is 1.096 g/ml because of the relatively low body mineral value (6.2%FFM) in this population (Heymsfield et al., 1989). Thus, the relative body fat of children and elderly will be systematically overestimated using the two-compartment model equations.

3. Technical error in the measurement of body density is well documented (Going, 1996). The error from RV measurement represents a major source of body

density and percent body fat. For every 100 ml error in RV or 100 g error in underwater weight, percent fat will be in error by ~0.7 percent body fat units. Typically the error in underwater weight will be less than 100 g, although larger errors may occur when an autopsy scale is used to measure subjects having difficulty remaining motion less underwater. In contrast, discrepancies of 100 to 200 ml (0.7 to 1.4% fat units) can easily occur when RV is measured on land rather than simultaneously with underwater weight , and larger errors (300 to 400 ml) are likely when RV is estimated rather than measured directly. Relatively large errors in water temperature and body weight in air have relatively minor effects on body density and %BF; nevertheless all variables must be measured as accurately as possible to minimize the combined total error (Going, 1996).

4. The UWW equipment is high costly and the procedure are complicate and laboratory test. To perform completely of the UWW experiment, the control is difficult and relies on the subjects ' compliance.

Although there are many limitations in UWW technique, at present, it is commonly considered as the reference method for assessing body composition.

The limitations of BIA

1. The manufacturer did not disclose the exact prediction equations used. Thus, this study did not examine any prediction variables that are unavailable in BIA manufacturer equation. However, the variables of BIA manufacture equation compose of age, sex, height, and weight.

2. Due to the pioneer of BIA occurred by Caucasian scientists in Europe and the United States. The development and production the BIA manufacturer equation were mainly based upon the Caucasian population body composition, such as a theoretically and empirically derived body is whose fat free body is 73.8%, water 6.8% mineral, and 19.4% protein (Brozek et al 1963). The

application of BIA manufacturer equation in other groups, which has different body composition, may not be valid.

As mentioned previously, the limitation of both UWW and BIA methods may contribute in part of high prediction error of BIA in this study. In addition, the equipment used for UWW is applied for estimating body composition. In this study the main limitation is high costly, unavailability of equipment and no practicability of direct RV measurement, especially during performing underwater weighing. Aker and Burkirk (1969) have carefully analyzed the sources of error in body density and shown errors in RV to be major source of variation. The RV estimates in water contribute to decrease the amount of trials in underwater and obtain a reliable and accurate estimates of density (Going, 1996). Nevertheless, good agreement between average measurement of RV on land and in the water is possible (Wilmore, 1969a), and measurements outside the tank are adequate when a group description (average) is the primary goal. Although not recommend for research purposes, in situations where direct measurements of RV volume are not possibly, body density has been calculated using a constant fraction of vital capacity (0.24 in males and 0.28 in females). This was the important reason why this study used a constant fraction of vital capacity to estimate RV values. In addition, Wilmore also showed that the mean difference in body densities calculated using actual and predicted RV is less than 0.001 g/ml. However, significant individual differences were evident with more than 50% of the subjects having deviation in density values ranging from ±0.003 to greater than ±0.0099 g/ml (thus percent body fat deviation ranging from 1 to 4 %). The significance of this finding is evident when one considers that changes in body density with physical training are sometimes less than 0.003 g/ml. Thus, for screening purposes or measuring large groups, acceptable results can be obtained using an estimated RV, but when maximal accuracy is required it is crucial that RV be measured.

Although, relatively large errors in water temperature air have relatively minor effects on body density and %BF, another important limitation of equipment is the absence of the water temperature. The appropriate water temperature close to skin temperature (~32 to 35 °C).

In conclusion

The result of this indicated that BIA manufacturer equation of fat mass and percent body fat war poorly reliable. Although UWW method in this study is the applied equipment for estimating body composition, it acts as the gold standard method. However, the main drawback of this method was no using the direct RV method. This drawback should be corrected if it is possible. And due to no disclosure of BIA manufacturer equation, we do not know the assumption of model in this BIA instrument. The further study should be developed to improve more complete UWW method and the selection of BIA instrument and equation should appropriate to estimate body composition in Thai population.

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APPENDICES

APPENDIX A

Body Composition Analysis : Data Collection Form

Name _____ Date _____

Age _____ Gender _____ Activity level_____

Body Weight _____ (kg) Clothing weight _____(kg) Height _____(cm)

Body and Clothing weight _____ (kg)

1. Vital capacity (average 2 trials within 100 ml)

   Trial 1 _____ Trial 2 _____ Trial 3 _____

2. Residual volume

   Male = 0.24 x _____ ml Female = 0.28 x _____ ml

   = _____ ml = _____ ml

3. Water temperature _____ °C 0

   Water density _____ g/ml

4. Gross Hydrostatic weight (kg)

   Trial 1 _____ Trial 6 _____

   Trial 2 _____ Trial 7 _____

   Trial 3 _____ Trial 8 _____

   Trial 4 _____ Trial 9 _____

   Trial 5 _____ Trial 10 _____

   Average of 3 trials within 100 g

   = _____ kg

5. Tare weight = _____ kg

6. Net UWW = _____ kg

7. Body density = _____ g/ml

8. %body fat (Siri 's equation)

   = _____ %

9. Fat weight = BW x % body fat

   = _____ kg

10. Fat-Free weight = BW – fat weight

    = _____ kg

11. Bioelectrical impedance analysis

    Impedance _____ ohm

    % body fat_____

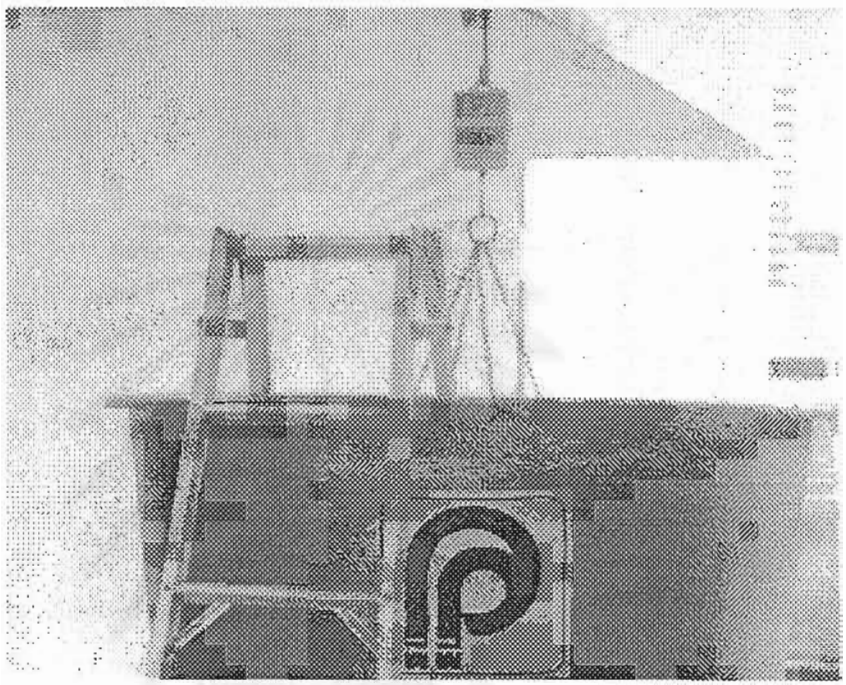
    Fat mass _____ kg

    Fat–free mass _____ kg

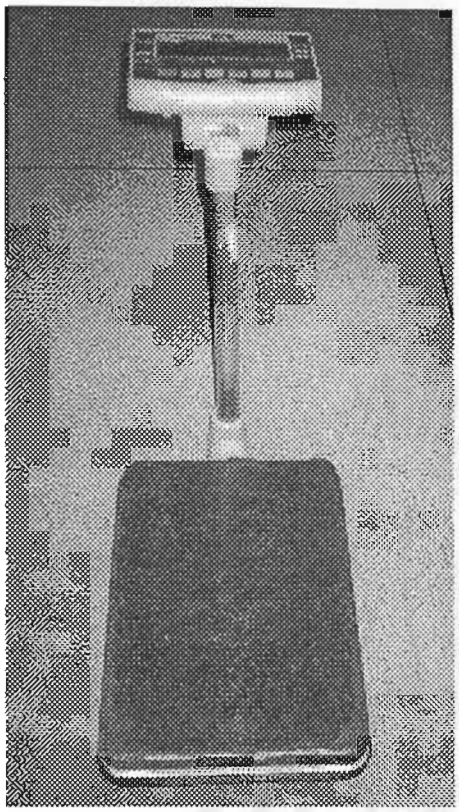
12. Body mass index (BMI) _____

APPENDIX B

Under water weighing method and equipment

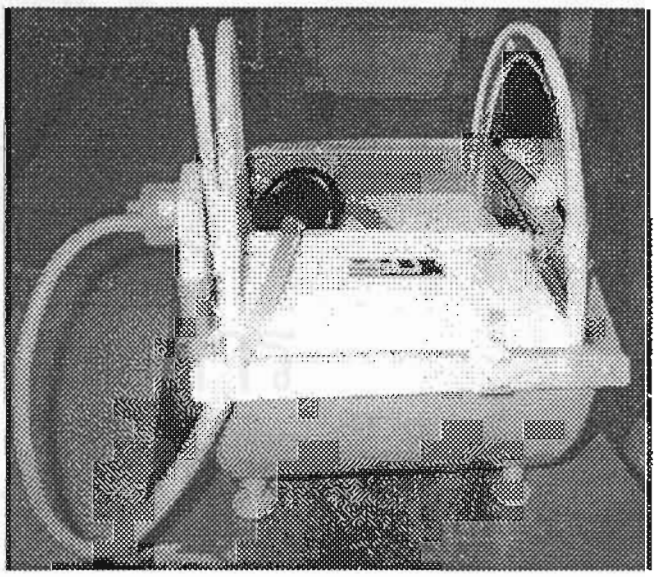


Underwater weighing equipment



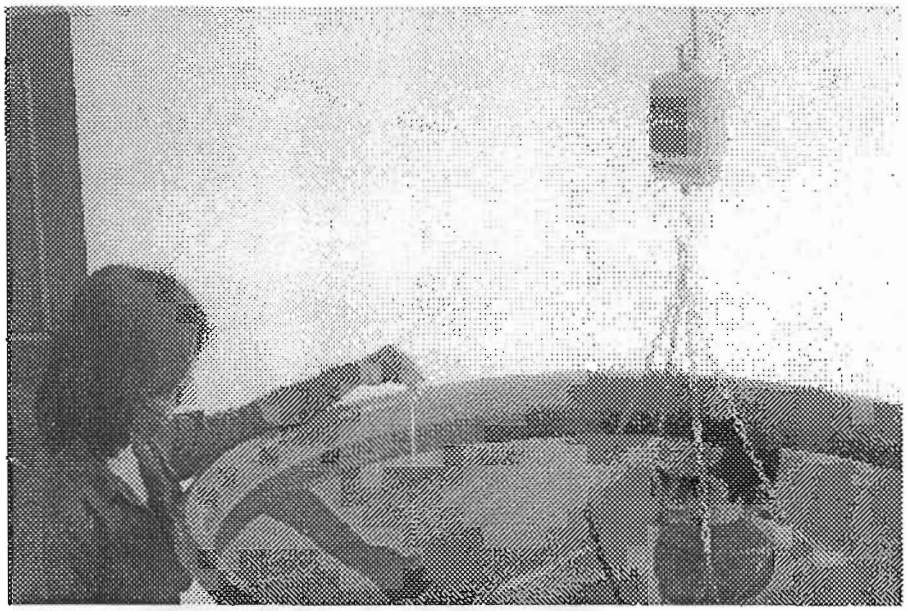
Air weighing machine

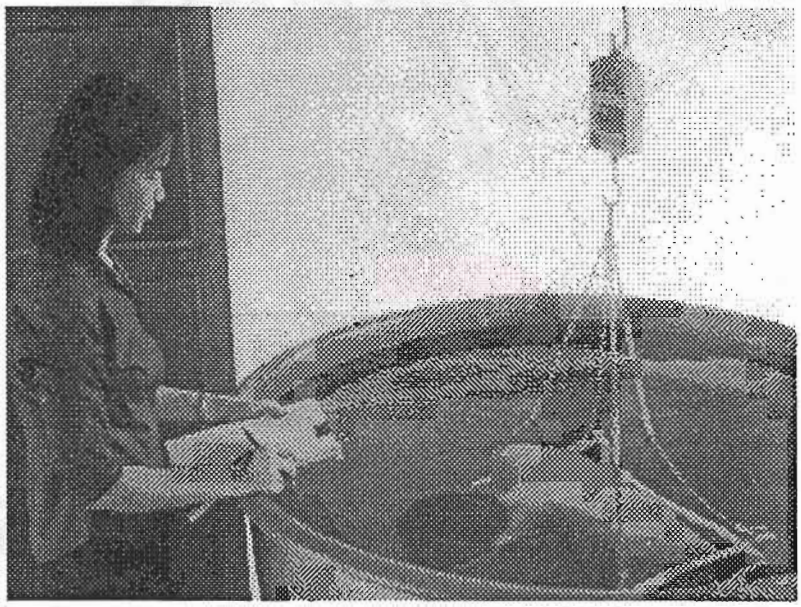
Wet spirometer



Clothing for underwater weighing

RV is measured separated out of the water by percentage of vital capacity.

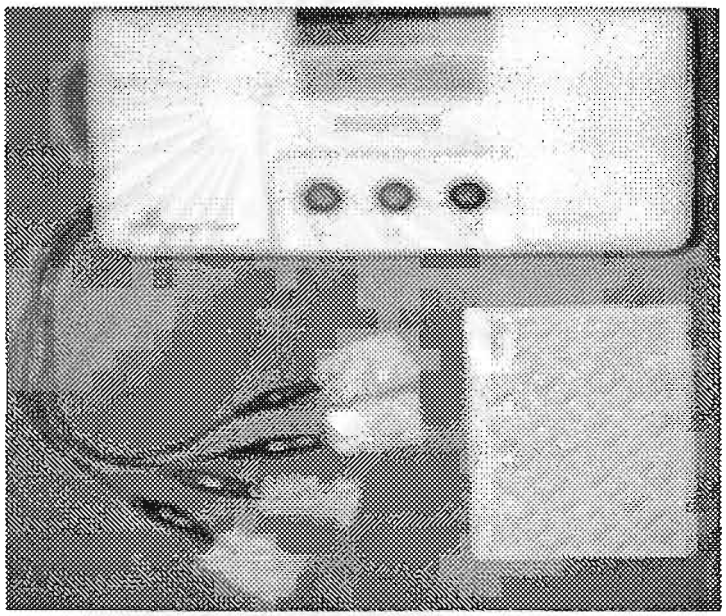




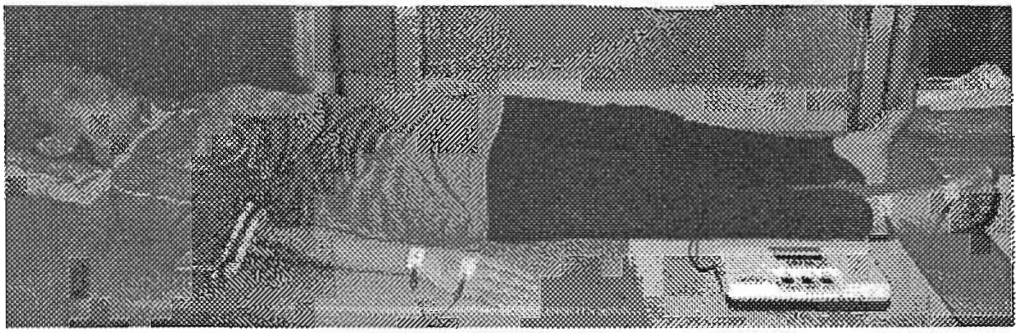
An underwater weighing tank used to determine body density. The subject exhales as fully as possible and bend forward slowly until the head is completely under the water.

APPENDIX C

Bioelectrical impedance analysis equipment and method



Bioelectrical impedance analysis equipment



A subject was prepared for body composition analysis by bioelectrical impedance (BIA). Subjects lied a supine position on a nonconductive bed with arms and legs slightly abducted from the middle line of the body. Shoes and socks were removed. On the right side, two adhesive aluminum foil signal introducing electrodes were placed on the middle of the dorsal surface of the hand and foot at the third metacarpal and matatarsal phalangeal joint, respectively, and two detecting electrodes were placed between styloid processes of radius and ulna and between the medial and lateral malleoli of the ankle.

APPENDIX D

Density of water at different temperatures

Temperature (°C)	Density (g/ml)
23	0.997569
24	0.997327
25	0.997075
26	0.996814
27	0.996544
28	0.996264
29	0.995976
30	0.995678
21	0.995372
32	0.995057
33	0.994734
34	0.994403
35	0.994063
36	0.993716
37	0.993360

BIOGRAPHY

Miss Charintip Khongsakpornchai was born May 23, 1974 in Bangkok, Thailand. She graduated Bachelor of Science in Health Science from Faculty of Science and Technology, Thammasat University in 1995.