DUAL X-RAY ABSORPTIOMETRY MAY NOT BE SENSITIVE ENOUGH TO MEASURE CHANGES IN REGIONAL FAT AFTER ACUTE EXERCISE

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ABSTRACT

Objective: We sought to determine if an acute bout of running accounts for measurable changes in total and regional body fat using Dual X-ray Absorptiometry (DXA). There is limited research about the change in body fat after an acute bout of physical activity. Knowing how body composition changes as a result of exercise is important for populations with symptoms of metabolic syndrome, such as overweight/obesity, increased waist circumference, high triglycerides, low levels of high density lipoprotein (HDL), increased total cholesterol, increased systolic and diastolic blood pressure, increased blood glucose and physical inactivity that can eventually lead to conditions like heart disease and diabetes (1). As the amount of visceral fat a subject has increases, so does their risk for metabolic syndrome. In addition, knowledge of body composition changes is useful in athletic populations for injury prevention and performance in athletes (2).

Methods: Subjects that were between the age of 18 – 40, that reported a history of regular running exercise (approximately 45 minutes, 5 times a week), were otherwise healthy and could commit to running 90 minutes on a treadmill were recruited. All subjects completed a 90-minute run on a treadmill at 60% heart rate reserve (HRR). Body composition was measured before and immediately after the run to determine if DXA was sensitive enough to measure potential changes in body fat following acute exercise. For the present study, total and regional body fat was compared to show if particular regions had a greater change in fat during endurance exercise. The difference in pre-run to post run total and regional body fat was compared using paired t tests. Comparisons between normal weight, overweight/obese, and overall groups were made using nonpaired t tests. Statistical significance was accepted using P-value > 0.05 Changes to total and regional body fat were also compared to the least significant change (LSC) for DXA found in the literature for athletic populations.

Results: A total of 16 lean (female = 7; male = 9; age = 28.1 ± 5.6 yrs; BMI = 22.0 ± 1.6 kg/m^2) and 11 overweight or obese (female = 7; male = 4; age = 32.0 ± 5.2 yrs; BMI = 30.5 ± 4.8 kg/m^2) were recruited and completed both study visits. Weight, VO2max, Body Mass Index (BMI), and baseline percent body fat were significantly different between the normal and overweight/obese groups. Height and age were not significantly different between the groups.

No significant differences were found in the absolute change in normal and overweight/obese group. Absolute fat mass decreased slightly overall (-184.5 ± 443.6 g). There was no significant change in BF from arms or legs within the overall group. A significant loss of fat came from the trunk (-195.1 ± 488.3 g). Android/gynoid ratio change shows significant decrease (-0.02 ± 0.04), in the overall group.

Although significant absolute fat loss from the trunk and the android/gynoid ratio, the difference in fat for the regions was not more than LSC using test-retest technique found in the literature.

Conclusion: Significant differences in fat mass were observed from the trunk and the android/gynoid ratio regions after a 90-minute run at 60% HRR. However, when comparing these changes to the published research on least significant difference of DXA, the change in fat mass observed in this study is less than least significant difference of DXA. This means that the changes in fat measured by DXA in this study may not be accurate.

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LIST OF ABBREVIATIONS

ADP…. Air displacement plethysmography

BIA…. Bioelectrical impedance analysis

BMI…. Body mass index

CT…. Computed tomography

DXA…. Dual X-ray absorptiometry

EKG…. Electrocardiogram

HRR…. Heart rate reserve

LSC…. Least significant change

MF-BIA…. Multifrequency bioelectrical impedance analysis

MRI…. Magnetic resonance imaging

$VO_{2\ max}$…. Maximum uptake of oxygen during peak exercise (mL/kg/min)

CHAPTER 1. INTRODUCTION

Knowing how body composition changes as a result of exercise is important for populations with symptoms of metabolic syndrome, such as overweight/obesity, increased waist circumference, high triglycerides, low levels of high density lipoprotein (HDL), increased total cholesterol, increased systolic and diastolic blood pressure, increased blood glucose and physical inactivity that can eventually lead to conditions like heart disease and diabetes (1). As the amount of visceral fat a subject has increases, so does their risk for metabolic syndrome (1). Body composition is also useful for injury prevention and performance in athletes (2) and for monitoring diseases such as obesity (3) and osteoporosis (4). The purpose of this present study was to determine if an acute bout of running at 60% HRR accounts for measureable changes in total and regional body fat using dual X-ray absorptiometry (DXA), with interest in changes in visceral fat. This is a novel question since there is limited research about change in body composition after an acute bout of physical activity using DXA.

The only other similar study, to our knowledge, analyzes changes in body composition after endurance cycling or resistance activity examines changes in reliability after resistance training and cycling under non fasting condition (5). Some research has shown a decrease in visceral fat after a long-term exercise intervention (6,7). Accumulation of visceral fat is associated with metabolic syndrome risk factors (1). A gap in research remains for effects of acute exercise on body fat (1,6,7). This is the first study, to our knowledge, to examine body composition changes after an acute bout of running in fasted subjects.

Although there are numerous methods for collecting body composition, DXA is considered a gold standard (9). As a person's body begins to adapt to endurance training, muscle glycogen and blood glucose begin to be used less as fat begins to be oxidized more, especially in longer continuous exercise bouts (10). Maximum fat oxidation appears to happen when an athlete is exercising at 65-75% of VO_{2max} (11–13). At about 75% of VO_{2max}, glucose begins to be used more than fat, this is called the "crossover" concept (14). Endurance exercise has positive effects on body composition, such as increased bone density (15) and lean body mass (7,8,15) and decreased fat mass (6–8,15,16). Previous data also noted a decrease in intraabdominal or visceral fat (6–8). Many studies examined the precision of DXA using repositioning between consecutive scans (3,4,17–20) and others examine changes in body composition over many weeks of endurance exercise (6–8,16), and one previous study (5) examines changes in body composition after an acute bout of endurance exercise using DXA. The study recommended having subjects, rested and fasted to minimize biological factors such as food and drink consumption and exercise (5). There are no studies, to our knowledge, that measure changes in body fat after acute bouts of endurance activity with athletes in a rested and fasting condition.

Since few studies exist that examine changes in body fat after an acute bout of physical activity, the purpose of the present work was to test if acute physical activity accounts for measureable changes in fat using DXA. This study recruited trained normal weight and overweight/obese runners. Following a maximal fitness test, trained runners ran at 60% HRR, based off their maximal exercise test, for 90 minutes. Before and after the run total and regional body fat was measured by DXA to determine if specific depots were targeted with continuous aerobic exercise. Changes to body fat were compared to the LSC for DXA found in the literature for athletic populations to determine if the measured change in body fat was beyond the typical error for DXA measurements (18).

Following this introduction are chapters on literature review, methods, results, discussion and conclusion. The literature review includes how DXA can be used, its accuracy, studies that are similar to this study, and why DXA is an important tool. The methods section includes detail about the procedures and equipment used for the two study visits. The results section shows how whole body and regional body fat changes after a 90-minute run. The discussion shows how this study is important to current literature. The last chapter, the conclusion, details why using DXA to measure change in body composition after acute exercise is important and outlines areas for future research.

CHAPTER 2. REVIEW OF LITERATURE

Value of body composition

Knowing one’s body composition is useful in clinical settings because accumulation of fat in specific regions, such as visceral fat, is associated with increased risk of developing hypertension, impaired fasting glucose, diabetes mellitus, and metabolic syndrome (1). In athletic settings, body fat, is critical to injury prevention in athletes and athletic performance (2,4). Various body composition techniques can estimate the fat mass, fat free mass (including muscle mass and bone mass) and total body water volume. Body composition techniques include Densitometry, Ultrasound, Dilution, Skinfold Measurements, Bioelectrical Impedance Analysis (BIA), and Multifrequency Bioelectrical Impedance Analysis (MF-BIA), Magnetic Resonance Imaging (MRI), Computed Tomography (CT) and Dual X-ray absorptiometry (DXA).

Measuring body composition in athletes

Densitometry

Hydrodensitometry and air displacement plethysmography (ADP) are methods for collecting body density measurements, or densitometry (22). Both methods of densitometry are 2 compartment models that divide the body into fat mass and fat free mass (22). By using a constant density for fat mass and fat free mass along with measured body density, percentage of body fat is calculated (22).

Hydrodensitometry

Hydrodensitometry, commonly called underwater weighing, requires subjects to exhale maximally when completely submerged in a tank full of water until three measurements are collected within 100 grams of each other (22,33,34). To determine the body's volume either the volume of water displaced is measured or the subject's weight is measured under water (33,35). The water volume of water displaced is the equivalent of the subject's volume (35). Underwater weight is based on Archimedes Principle, which states a body immersed in a fluid is buoyed by a force equal to the weight of the displaced fluid (35). Since residual volume (air remaining in the lungs after maximal exhalation) effects buoyancy of the subject, it is necessary for residual volume to be calculated (22,35). Residual volume is commonly measured using closed circuit spirometry with helium dilution or oxygen dilution methods and should be done under water since hydrostatic pressure affects lung volume (22,33,35). Body density can be calculated by dividing body mass by the measured volume, which is the difference in body mass and underwater weight divided by water density, minus residual volume (22,35).

$$\mathbf{Db} = \mathbf{BW}/\{[(\mathbf{BW} - \mathbf{UWW})/\mathbf{dw}] - \mathbf{RV}\}$$

Once body density is calculated, it can be used to estimate percent body fat using equations by Brozek et al. (1963) or Siri (1956).

Precision of total body density is excellent and the simplicity of the equation used to determine body composition are benefits of hydrodensitometry (22,35). However, the density of the fat free mass (water and bone mineral content) varies among individuals and different populations and accuracy is limited when a fat free mass constant is used (22,33,35). The assumption that fat free mass is constant is violated in many types of athletes, especially in lean athletes, and because athletes are physical activity regularly (22,33,35). Formulas exist for converting body density to body fat percentage in various populations given the differences in fat free mass (35).Other reasons for individual variations in fat free mass include gender, ethnicity, sexual maturation, age and disease (33,35). The largest variation in body density occurs from the estimation of residual volume most likely due to technical error, subject's understanding of spirometry test and subject fatigue (35).

When compared to DXA, hydrodensitometry reveals higher measurements of fat mass in healthy trained and untrained males (36). Variation in lean body mass contributes to affect results by hydrodensitometry (36). Hydrodensitometry overestimates fat mass in individuals with low body fat and low bone mass and individuals with low body fat and high bone mass such as athletes (36).

Air Displacement plethysmography

Air Displacement plethysmography, or ADP, is a method of measuring body density using sealed air filled chambers, referred to as a BodPod® system

(Life Measurement Inc., Concord, CA, USA), that is an alternative to hydrodensitometry, without the use of water (22,26,33). A BodPod® system is made of fiberglass and is shaped like and egg (35). It uses two chambers linked by an airtight diaphragm that creates small pressure changes between the chambers (22,33,35). One chamber is used for measuring the subject and the other for is for reference and is located under the seat measuring changes in chamber volume (22,33). The BodPod® is calibrated with a known volume of air and the subject sits inside for about 2 minutes so volume of air displaced by the subject can be measured using small pressure changes between the chambers (22,26,33,35). Subjects wear swimwear and swim cap to minimalize entrapment of air (22,35). Since the subject’s body volume is equal to the volume of air in the empty chamber, minus the volume of remaining air in the chamber once the participant is sitting in it (Poisson’s Law), the subject’s volume can be calculated using Boyles Law (22,33,35).

$$\boldsymbol{Boyles\ Law}\colon\ \boldsymbol{P1/P2 = (V2/V1)^{\Upsilon}}$$

P1 and V1 indicate one paired pressure volume and P2 and V2 indicate a second and y is the ratio of the specific heat of the gas at constant pressure to that at constant volume, which is approximately 1.4 for air (35). Because of the behavior of the air in the measuring chamber a breathing maneuver is used to calculate thoracic gas volume and adjustments for gases in the thoracic cavity are added and skin surface area is multiplied to the body volume (22,35). To collect thoracic gas volume, subjects are connected to a breathing tube inside the measuring chamber and asked to breath normally at first and then puffing air gently in and out using their diaphragm (35).

Benefits of using Bod Pod for fat and fat free mass include easy operation, low cost for each test, rapid test results, no exposure to radiation and does not involve water submersion, so it is more comfortable for subjects than underwater weighing (26,35,37). Unlike underwater weighing, BodPod® doesn’t rely on predicting residual lung volume which has been known to elicit large errors in body density and Bod Pod’s predicted thoracic gas volume collection technique is accurate compared to measured thoracic gas volume (35,38). Bod Pod is reliable and valid for determining percent body fat in a group of 68 adults with a variety of body weights, age and ethnicity, when compared to hydrodensitometry (39). Reliability of Bod Pod, determined by two tests in a short time period, was not significantly better than hydrodensitometry (35). Similar to hydrodensitometry ADP , limitations remain in converting body density to body fat percent, since density of fat free mass varies (22,33,35). Moisture on the subject’s body can effects the compressibility of air to the body’s surface and if subjects wear anything but swimwear and a tight fitting silicon cap there is a chance that air may be trapped in clothing or hair both leading to an underestimation of the percentage of body fat (22). Location of Bod Pod needs to be in a room away from windows and doors with monitored temperature and humidity to limit changes in pressure that would necessitate recalibration (22).

Bod Pod estimates of body fat and fat free mass had a smaller correlation with DXA estimates of body fat and fat free mass in subjects with very low BMI and very high BMI (37). Bod Pod overestimated body fat percentage in thinner adults by up to 13.2% and underestimate body fat percentage in heavy adults by up to 8.51%, compared to DXA due to using equations to convert body density into two compartments: fat mass and fat free mass (37). Fat free mass includes many tissues including water, bone, muscle, blood vessels and other tissues and because DXA measures 3 compartments, fat mass, fat free mass and bone density, it has the potential to be more accurate than Bod Pod (37). In a group of college female athletes, competing in a variety of sports, and female non athletes, no significant differences in body fat percentages or fat free mass were found when compared to DXA (40). Since ADP uses constants for calculating fat mass and fat free mass and because it is limited in accuracy at very low and very high BMIs, this method should not be used to compare different groups of athletes, but may be used in specific groups or individuals to track changes in body fat over a short period of time (33).

Ultrasound

Ultra sound imaging uses short ultrasound pulses that travel at the speed of sound through tissue, which is called pulse-echo technique to estimate subcutaneous fat, muscle tissue and skin thickness (22). Most ultrasound machines use a speed of 1540m/s to calculate distance from the probe to the border between two tissues that have different acoustic impedances (22,23). Some of the ultrasound pulse is reflected back to the receiver, the echo, and is converted to electrical energy (23). The echo is shown on the ultra sound machine’s screen as amount of deflection over time (23). Ultrasound machines can show tissue configuration (in B-scan mode) or depth measurements where tissue density changes (in A-scan mode) (23). A-scan mode is used for assessing adipose tissue thickness (23). In B-scan mode, images are created using sequences of ultrasound beams to create images where brightness of the screen is equal to the echo intensity of the scan (22,41). The differences in acoustic impedance in the tissues on either side of subcutaneous adipose tissue are clearly defined (41).

Estimates of total body fat and total subcutaneous fat is more accurate in ultrasound compared to skinfold measurement or BIA (22). A-mode ultrasound has high reliability using test-retest technique for A-mode in male and female collegiate athletes (42). When compared against a three compartment model, A-mode ultrasound significantly underestimated percent body fat in overweight and obese males and females (43). In a population of 93 male and female young athletes in various sports, A-mode ultrasound was in agreement with DXA for body fat and body fat percentage measurements, regardless of gender (44). Although this method has high accuracy and precision, low cost compared to

Magnetic Resonance Imaging (MRI) or Computed Tomography (CT) and results are quickly accessed, ultrasound only measures subcutaneous fat, the fat deposited below the skin (22,23). Ability to decipher tissue types, using proper frequency and measurement error, in the form of varying pressure to the area to be scanned are limitations in estimating subcutaneous fat with ultrasound (22,23). Portable and laboratory ultrasound systems can be used in male and female normal weight athletes to measure subcutaneous fat, muscle and skin thickness accurately, but not visceral fat (22). This method is not recommended for overweight or obese populations (43).

Dilution methods

Dilution methods calculate the total body water using a ratio of the specific amount of tracer that is given to a subject and the concentration in the body after an equilibration period (33). One fluid sample of blood, saliva or urine is collected before intake of the tracer to determine baseline levels (33). The isotopes of hydrogen, deuterium (2H) and tritium (3H) or oxygen-labeled (18 O) water have been used as tracers and are given orally or intravenously (23,26,33,34). A second sample of body fluid is taken after a 3-6 hour equilibration period so the tracer has enough time to distribute throughout the body (33,45). The equilibration time differs base on the fluid collected, 4 hours is preferred for saliva and 6 hours is recommended for blood or urine (45,46). Due to the length of equilibration time, it may be necessary that a cumulative urine sample be collected if subject visits the restroom, so the dose estimate can be adjusted (33). Because body water is associated with fat free mass, total body water can provide an estimate of fat free mass, and therefor fat mass can be calculated within 3%, assuming a constant hydration of 73% or 73.2% (22,23,26,35). However, some research indicates accurate muscle hydration percent can vary in range from 72-73% especially with dehydration and disease (22,26). Since the amount of solution and concentration of the tracer administered is known, the amount of total body water can be calculated based on the concentration of the tracer after equilibration using either mass spectrometry or infrared spectrophotometry (35).

Benefits for using dilution techniques for body composition include minimal subject action, it uses easy to collect samples (saliva, urine or blood), and is easy to administer (22,26).However, since total body water volume is used to calculate fat free mass and fat mass, changes in total body water will alter the results (26). The biggest limiting factor of this method is the variation in hydration levels, which makes this method a poor choice for an active athletic population (22). There is also sizable variation between the amount of water each individual subject has in fat free mass and using a single hydration constant would only produce an approximate mass (35). Another limiting factor for athletes include the 3-6 hour equilibrium time which could interfere with the athlete’s normal nutrition and training practice (22,45,46). Because the purchase of the isotopes used as tracers and mass spectrometer or infrared spectrophotometry is expensive and labor intensive, it is not recommended for large studies (22,23,26).

Dilution methods also have assumptions that, if violated, the ratio of tracer to fluid concentration must be adjusted or the measurement may be inaccurate (33). The assumptions include the tracer must be distributed only in the exchangeable pool, the tracer is equally distributed within this pool, it is not metabolized during the equilibration time, and tracer equilibration is achieved relatively rapidly, the tracer has the same distribution volume as water, it is exchanged by the body in a manner similar to water, and it is nontoxic in the amounts used (23,33).

Skinfold Measurements

Skinfold measurements are made by pinching skin and subcutaneous fat between the thumb and forefinger and pulling away from the body far enough to allow a caliper to grasp the skin (23). The caliper is used to measure the thickness of skin folds twice at specific sites on the body (23). The average thickness at each site is then included in an equation for body composition prediction (23).Equations for many populations are available for assessing body density and body fat, since age, gender, physical training and percent body fat can influence measurement (23,47).

Skinfold measurements and body fat assessment can be collected quickly at low cost (22). Precision of skinfold measurements are affected by subject hydration, compressibility of skin and skill of the staff taking the measurement (22,23). Some limitations to this type of body composition measurement include selecting the correct equation for the subject, such as obese or athletic (22,48). Of the over 100 equations that exist for estimating body fat from skin fold measurements only 3 were found reliable in athletes (22,49). Skinfold measurements can be intrusive for subjects, certain sites may be difficult to collect on some patients and training and consistency is important to accuracy (22).Some skinfold equations agree on average with underwater weighing (48). Taking skinfolds from obese populations may be difficult using calipers which affects the accuracy of the measurement therefore skinfold measurements are not supported in this population (22,48).

Bioelectrical Impedance Analysis

Bioelectrical Impedance Analysis (BIA) is a method that collects total body water, fat mass and fat free mass by sending a single low energy electrical current from one limb, through the body's water pool, to another limb or limbs (24,50). The total volume of a test subject, a conductor, is estimated from its length and resistance to the electrical current using squared length divided by the amount of resistance (22). Fat free mass is calculated using the estimate of total body water and the assumption that 73% of the body's fat free mass is water (26). Since muscle tissue is a good conductor due to water and electrolyte content, it elicits less impedance than other tissue such as fat tissue (50). One four-limb model can estimate visceral fat better than existing 2-limb models because abdominal electrical impedance is determined by subtracting the impedance of both upper limbs from the systemic impedance and the visceral and subcutaneous fat is separated using an equations that takes into account age and sex (50). This four-limb model is the first available to that provides estimates of muscle using the manufacturers algorithm (50).

Advantages of BIA include its portability (26,51), it is simple to use (22,26,51) and shows results quickly (22,24) low cost compared to other methods (26,51) and there are no health risks for subjects (51). Accuracy of DXA is mixed. Some studies have shown BIA has high precision in athletes (22), while others have shown limited accuracy, in obese populations (26,51) and wrestlers (22). Its accuracy is poor (22,24) because the results can be affected by hydration status (22,24,51,52), obesity (26,51) and because assumptions are made that the subject is a uniform cylindrical shape and the electric current is distributed evenly throughout the subject (22). Single frequency BIA has limited ability to differentiate total body water content into intracellular and extracellular compartments (26). Since recent physical activity can affect results, it is recommended that subjects are not active before the test which limits it use in athletes who follow an active training plan (22). Accuracy with BIA can be found in groups of similar athletes, but not across different types of athletes (22,50). BIA is not as precise as DXA (51). Compared to DXA and MRI, BIA provides accurate estimations of muscle mass and fat free mass, but less accurate estimations of total body and visceral fat (50). Although, BIA is not recommended for monitoring body composition in athletes (22), it can be used for epidemiological obesity research and obesity monitoring at home (50).

Multifrequency Bioelectrical Impedance Analysis or Bioimpedance spectroscopy

Multifrequency Bioelectrical Impedance Analysis (MF-BIA) or Bioimpedance spectroscopy (BIS), is similar to BIA, except it uses a range of electric frequency levels to find impedance of body tissues. The multiple frequencies allow total body water to be separated into intracellular water and extra cellular water (22,26). An advantage of MF-BIA over single frequency BIA is it may be used to determine body composition across different populations without the use of separate equations, increasing its use across all populations (51). Another advantage of MF-BIA over single frequency BIA, is that it may provide more accurate estimates of leg muscle (26). MF-BIA also has been shown to provide more accurate fat free mass and fat mass than single frequency BIA in overweight and obese young women(51). Compared to DXA, MF-BIA derived fat free mass was underestimated in normal weight population and overestimated in obese populations (26,51). There is limited data available for athletic and obese populations (22,51), but it can be used to manage conditions where fluid balance and hydration is importing because it shows fluid shifts from intracellular and extracellular compartments (22,26). MF-BIA was more accurate cross sectionally for fat free mass and fat mass than single frequency BIA in overweight and obese young women in weight loss program when compared to DXA as a reference method (51). Single frequency BIA and MF-BIA were both accurate for monitoring changes in body composition throughout weight loss (51). In the same population, MF-BIA estimates were similar with DXA (51).

Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) is used for diagnosis and monitoring of diseases (21). MRI uses a super magnet, a magnetic field and radio signals to generate arrays (pictures) (22). Since neutrons and protons have electric charge, when a magnet is focused on a part of the body, the nuclei in body tissues absorb energy and begin to align with the magnetic field (23). When the magnetic field is removed, the energized nuclei emit the energy they had absorbed and the array of these nuclei is developed using computer software (23). MRI uses nuclei of Hydrogen atoms for estimating soft body tissues, such as adipose tissue, skeletal muscle and organs (23,24). Pixels of specific body tissue in the array are quantified to determine mass (25). MRI and Computed Tomography (CT) are gold standards for assessing intra-abdominal adipose tissue (25). Multi-slice volume MRI and CT are gold standards for assessing total and regional adipose tissue (25).

MRI is accurate for whole body and regional fat and muscle estimation and doesn’t expose patients to radiation, however whole body scanning is not feasible due to the high cost (22,26). Several drawbacks for the use of MRI exist; the equipment is expensive, body composition data is not available immediately and some patients may find enclosed MRI machines uncomfortable (22,26). Single slice MRI is sometimes used to lower cost but with limited accuracy, thus MRI is feasible for accurate assessment of regional fat distribution, not whole body, especially with low cost field methods of body composition available (25).

Computed Tomography

Computed Tomography (CT) uses X-rays to take single slice pictures as a patient is moved through the machine (23). Subjects lay on a platform that moves through the circular opening of the machine and an x-ray beam is rotated around the subject (23). X-ray attenuation is relative to differences in density of body tissue and a 2 dimensional image is formed (23). Using CT software, the single slice pictures are used to estimate the density of the tissues scanned and shade the pictures accordingly (23).

Similar to MRI scans, CT scans are considered gold standard approaches to assessing body composition as far as their accuracy and reproducibility (27). CT and MRI have been used to assess abdominal fat in underweight, normal weight and overweight women and overweight and normal weight men (27,28). However, CT scans expose patients to high levels of ionizing radiation, 2 to 31 millisieverts (μSv) depending on size of the region, and are expensive (22,24,29). The results are also not available immediately like other methods of body composition collection such as DXA and BIA (22).

Dual X-ray Absorptiometry

Dual X-ray absorptiometry (DXA) is a non-invasive method of determining body composition using two X-ray beams. The subject lays on a scanning table while X-ray beams, emitted from below at 2 separate energy levels, are passed through the subject's entire body at the speed of 1 centimeter per second (22,23). An arm passes over the subject at the same speed to collect x-ray energy that was not absorbed by the subject's body (22,23). Changes in attenuation in the x-rays shows where bone and soft tissue, containing fat and lean tissue, are located on the body in pixels. The dose of radiation a participant is exposed to is low, from approximately 0.5 μSv per one whole-body scan, which is much less than what is received from a 7 hour airplane flight or by having an X-ray (5,4,18,26,30,31). A DXA scan takes about 7-20 minutes, depending on the device, the settings and body size (3,31,32).

DXA is commonly identified as a gold standards in body composition analysis, in addition to MRI and CT (9). DXA provides precise regional and whole body fat and lean tissues when compared to CT in cancer patients (25). For precision of total and regional body composition measurements, using Lunar iDXA (technology that allows quantification of visceral fat and allows larger subjects compared to GE Prodigy DXA) with nonobese participants, the coefficient of variation (%CV) between repeated scans was less than 1.0% for total body fat mass and less than 2.5% for all regions except arms (2.8%) (31). In the same study, it was found that iDXA has superior precision for body composition over other GE DXA models, perhaps due to the increased image resolution in iDXA (31). The precision of Lunar iDXA in male and female lean athletes using repositioning, found that Lunar iDXA measured total and regional lean body mass excellently (18). However, greater variability in fat mass and lean mass was noted as total body mass increases (18). The precision of Lunar DXA using duplicate scans with repositioning with normal weight, overweight and obese participants, indicates that as participant BMI and percent body fat increase, so do precision errors for bone mass, lean mass and fat mass (19).

How endurance exercise affects substrate utilization and body composition

As someone begins an endurance training program, such as long distance running or cycling, changes in energy substrate utilization takes place in skeletal muscle as their body adapts to the new stimulus. In trained runners, muscle glycogen and blood glucose are used as fuel for exercise, but at a slower rate than in non-trained runners (10). In individuals with a history of regular endurance training, fat is the main fuel source for mild to moderate endurance exercise (14). Previous work has demonstrated that maximum fat oxidation during exercise happens between 41- 75% of VO_{2max} (11–13,53), with three studies reporting maximum fat oxidation between 65-75% of VO_{2max} (11–13). However, as the exercise intensity increases to 75% of VO_{2max}, a shift toward using more glucose than fat occurs. In the literature, this phenomena of shifting from fat utilization to glucose utilization is called the “crossover” concept (14). One study reported that obese sedentary participants switched from predominately fat to glucose quicker than trained athletes (11).

Schwartz et al. examined change in body composition in healthy younger and older men after a 6 month long walking and jogging endurance training program (6). After the exercise intervention a small reduction in body weight and total fat mass was noted in older men as well as a larger decreases in intraabdominal, subcutaneous chest and subcutaneous abdomen fat depots compared to younger men (6). This suggests that the central fat depots were targeted over other fat depots during training (6). In contrast to older men, young men had a small loss of total body fat and central fat with a greater decrease in peripheral fat (thigh fat) according to CT, this may be due to differences in baseline fat distribution between the young and old men with older men having a greater amount of central fat (6).

Decrease in body fat was shown after training in a study that used under water weighing to collect body composition before and after 20 weeks of aerobic cycling 4-5 days a week for 40 minutes at 80% of maximum heart rate (16). The average baseline body fat percent was 22.0 +/- 8.3 compared to the average body fat after 20 weeks of endurance which was 19.7 +/- 8.1 (16).

After 557 subjects completed 20 weeks of cycling, Wilmore et al. found subject’s total body mass, fat mass, percentage of body fat and total, subcutaneous and visceral abdominal fat decreased while total body density and fat-free mass increased (7).

Body composition changes, similar to what has been described previously in this chapter, were found using DXA and anthropometry, in sedentary individuals before and after 10 weeks of randomized stationary aerobic cycling (8). Subjects in the aerobic group had reduction in body mass, intraabdominal fat mass, total body fat mass, total body fat percent and an increase in total body lean mass (8). For prediction of intraabdominal fat mass, DXA defined regions of interest (5-10 cm above the iliac crest and laterally to the edge of abdominal tissue) were combined with abdominal skinfolds (54).

Although endurance exercise intervention varies in the studies presented, in general, those with endurance training history exhibit greater bone density and lean body mass and decreased fat mass compared to pre-endurance training or to those who are not endurance trained.

Acute changes to regional adipose tissue in response to endurance exercise

Knowing how body fat changes after acute exercise is important because physical activity can decrease visceral fat, which is associated with risk of metabolic risk factors (1). Several studies have measured the precision of DXA using consecutive scans with repositioning in a variety of populations (ex. athletic, overweight and obese) (3,4,17–20,31). Several studies compare changes in body composition over many weeks of endurance exercise intervention (6–8,16). Only one study found in this literature review compares body composition before and after acute endurance exercise to assess reliability using DXA(5). The purpose of that study was to measure body composition changes, in trained participants, following a strength or long distance cycling exercise bout in their training program (5). Subjects were allowed to consume typical before and during exercise food and drink and had three DXA scans in the same day (5). Typically fasting from food, caloric beverages and physical activity is normal DXA preparation, however this shows how typical nutrition and training practices affect DXA reliability (5). The first two DXA scans were consecutive with repositioning in between to determine DXA reliability. The third scan was after consumption of food and drink during the exercise bout (5). Since subjects were allowed to consume food and drink to fuel their workout, changes in body composition more closely resemble true conditions for which athletes exercise compared to fasting (5). The study found the exercise and food and drink consumption greatly affected the reliability of DXA estimates in lean mass and regional body composition (5). The findings from work led to recommendations including having subjects rested and fasted to minimize any biological factors, such as food and drink consumption and exercise, to reveal potentially significant changes in body composition after exercise.

Final argument

Testing for regional fat change after acute exercise is important because physical activity can decrease visceral fat, which is associated with risk of metabolic risk (1). With prevalence of obesity (34.9% of adults 20 years old or older) in the United States (55), body composition has become an important way to monitor weight loss in clinical and research settings, including calculating risk of obesity related diseases. Visceral adipose tissue has been shown to be significantly associated with increased the odds of developing hypertension, impaired fasting glucose, diabetes mellitus, and metabolic syndrome (1). Fox et al. also found that visceral adipose tissue had a stronger correlation with majority of metabolic risk factors than subcutaneous adipose tissue. Physical activity that decreases visceral fat could be used to decrease risk of metabolic risk factors.

CHAPTER 3. METHODOLOGY

Experimental Design

For this study, subjects had two visits to the University of Minnesota campus. At the first visit, subjects completed a graded exercise test to determine their maximal aerobic capacity using indirect calorimetry. Beat by beat heart rate data was collected during the run using a Polar heart rate monitor. HRR was calculated for the second study visit. The second visit was scheduled at least 72 hours after their first visit to give subjects time to recover from maximal exercise. Subjects were instructed to avoid physical activity for 2 days prior to the second visit. Subjects were instructed to avoid consuming anything but water 8 hours prior to having their total and regional body composition measured using dual x-ray absorptiometry (DXA). Following the initial DXA scan, subjects completed 90 minutes of continuous running on a treadmill at 60% of their HRR. Subject heart rate was monitored the duration of the run to ensure 60% HRR was maintained. Following the run subjects completed a second DXA scan. Change in total and regional body composition fat mass were compared to the least significant difference in total and regional fat mass, to see if acute physical activity accounts for measurable changes in fat, using DXA scans. Lean body mass was not compared due to the change in hydration resulting from the run.

Subjects

The University of Minnesota's Institutional Review Board (IRB) approved the study protocol and methods. All participants provided written informed consent prior to study. A total of 16 lean (female = 7; male = 9; age = 28.1 ± 5.6 yrs; BMI = 22.0 ± 1.6 kg/m^2) and 11 overweight or obese (female = 7; male = 4; age = 32.0 ± 5.2 yrs; BMI = 30.5 ± 4.8 kg/m^2) subjects between the age of 18 – 40, that reported a history of regular running exercise (approximately 45 minutes, 5 times a week), were otherwise healthy and could commit to running 90 minutes on a treadmill were recruited and completed the two study visits (Table 1).

Procedures

Study coordinators collected health and exercise information about subjects over the phone to determine if they were generally healthy and regular runners. Subjects were sent information on how to prepare for their visits to the University of Minnesota via email.

Screening Visit

Initial testing occurred at the Human Performance Teaching Laboratory (HPTL) and the rest of the testing occurred at the Masonic Clinical Research Unit (MCRU). Height and weight were collected using a portable stadiometer and digital scale. BMI was calculated using weight in kilograms divided by the square of height in meters. Resting blood pressure and heart rate were obtained using an automatic blood pressure monitor (Colin Press-Mate BP8800C, Colin Medical Instruments Corp., San Antonio, TX, USA), after the subjects were seated in a quiet room for 5 minutes. A 12-lead electrocardiogram (EKG) was given at rest

(GE Marquette MAC 3500 EKG Machine, GE Healthcare, Chicago, IL, USA) to evaluate cardiac health before exercise testing. The EKG was administered by a physician or exercise physiologist and subjects had no signs of ischemia or arrhythmias.

Subjects were instructed to refrain from physical activity 72 hours prior to this visit so a maximal effort could be achieved. Subjects were also recommended to eat a light snack 2-3 hours prior to testing. Maximum oxygen consumption (VO_{2max}) was evaluated by indirect calorimetry using one of two metabolic carts (Ultima Medgraphics CPX-D, Medical Graphics Corporation, St. Paul, MN, or ParvoMedics TrueOne 2400 – OUSW 4.3.4 (20160202), Sandy, UT, USA) depending on testing location using separate treadmill protocols. Overweight subjects were tested using the Bruce protocol (56). Lean subjects were tested using a modified Åstrand protocol (57) that began at subjects' self-selected race pace that was maintained the duration of the test. After lean subjects ran 4 minutes at of 0% elevation, the treadmill increased 2.5% in elevation every two minutes. During the test, subjects wore a mask for measuring inspired and expired air and heart rate was measured using a Polar Heart Rate monitor (Polar Electro Inc., Lake Success, NY, USA). Subjects were instructed to run until volitional fatigue. Subjects were instructed to stop the test when a maximal heart rate (determined by age adjusted estimation) was reached, oxygen uptake plateaued, respiratory exchange ratio (RER) of 1.10 or greater was achieved, and exhaustion was communicated using rating of perceived exertion scale (RPE) (58). Subjects were offered water and a snack before leaving.

Evaluation of body composition changes with acute exercise

Subjects arrived at the Delaware Clinical Research Unit (DCRU) for the second visit after fasting overnight (at least 8 hours). Females of childbearing potential had a negative urine pregnancy test to rule out pregnancy. Height, weight, blood pressure and pulse were collected using a wall stadiometer, digital scale and electric Colin Press-Mate 8800 BP Monitor blood pressure cuff (Colin Medical Instruments Corporation, San Antonio, TX).

Subjects had body composition measured before and after their run using a DXA scanner, the GE Healthcare Lunar iDXA (GE Healthcare Lunar, Madison, WI, USA) with enCORE software version 16.2. Subjects were instructed to lie down on the table of the DXA machine and remain still until the scan was completed (7-15 minutes). The subjects were scanned by one of three DXA Technicians using standard imaging and positioning protocols.

The heart rates at rest and at VO_{2max}, collected at the first visit, were used to calculate the individual running intensity of 60% HRR. The subject’s run pace was initially selected using speed and incline that achieved 60% HRR during the VO_{2max}. All subjects ran for 90 minutes on a treadmill. Heart rate was monitored during the entire run to maintain proper running intensity with Polar heart rate monitor and the Polar Beat Multi-Sport Fitness Tracker smartphone app (Polar

Electro Inc., Lake Success, NY, USA). If heart rate fluctuated >5% HRR, subjects were instructed to decrease or increase speed. After the run, subjects had their second DXA scan and were offered a snack and left.

Data analysis

Data collected were analyzed using SAS 9.3 (SAS Institute, Cary NC). The difference in pre-run to post run total and regional body fat was compared using paired t tests. Comparisons between normal weight, overweight/obese, and overall groups were made using nonpaired t tests. Regions compared were percent body fat, fat mass, arms fat, trunk fat, legs fat, visceral fat, subcutaneous fat, android fat, gynoid fat, and android-gynoid ratio. Data are expressed as means + SD. Statistical significance was accepted using P-value < 0.05.

CHAPTER 4. RESULTS

Subject demographics

Table 1 shows subject characteristics for normal and overweight and obese subjects. The weight, VO_{2max}, BMI, and baseline percent body fat were significantly different between the groups. Height and age were not significantly different between the groups. The data in table one is presented as the mean of each group (normal and overweight/obese) and overall (all subjects) followed by the standard error or number of female participants followed by percent of female participants.

Absolute change in body composition after 90-minute run

Table 2 shows how total and regional body fat changed from before the run to after the run in normal, overweight and combined overall groups. Data in Table 2 is shown as the mean of each subject's pre-run, post-run values in grams and the mean of each subject's change compared to all participants (absolute change) in grams followed by the standard deviation and significance (p-value). Significance (p-value) is also given for normal change compared to overweight and obese change.

No significant differences are found in the absolute change when normal and overweight/obese groups are compared. Absolute fat mass decreased slightly but significantly (-184.5 ± 85.0 g) when normal and overweight/obese groups were combined, in the overall group. There was no significant change in body fat from arms or legs within the overall group. There was a significant loss of fat from the trunk (-195.1 ± 94.0). The android/gynoid ratio change shows significant decrease (-0.02 ± 0.0), in the overall group.

CHAPTER 5. DISCUSSION

In this study subjects completed DXA scans before and after a 90-minute run at 60% HRR so that fat was used as the main energy substrate. The purpose of this study was to test if an acute run accounts for measureable changes in total and regional body fat using DXA. The change in regional body fat was compared to the LSC found in the literature to determine if the change was more than the normal margin of error for DXA. The major observation from this study is that 90 minutes of exercise does not cause large enough changes in total or regional fat mass to be measured accurately by DXA. The changes in body fat observed are less than LSC of DXA found in the literature.

Many studies have focused on accuracy and reliability of DXA in athletes, overweight and obese populations (18,20,31,59). There is limited research on the use of DXA after an acute bout of physical activity. Nana et al., used 27 strength-trained male and 28 male and female cyclists to see how exercise and intra-exercise food and fluid consumption affect reliability in body composition after a strength training or cycling workout of their choice (5). The study observed that exercise and food consumption increased the typical error of measurement by about 10% (5).

To limit error in body composition measurements with DXA, subjects should be fasted and have refrained from physical activity (5). No studies were found in this literature review that examined single bouts of physical activity in subjects who refrained from physical activity and had previously fasted which are both known to decease accuracy of finding true changes in body composition after acute activity (5). This current study is novel because it shows DXA is either not sensitive enough to measure differences in body fat, after an acute bout of physical activity in fasted and rested lean and overweight/obese groups of men and women or that this activity does not cause measureable change in body fat.

Buehring et al., determined that the precision of DXA in 60 Division 1 athletes (30 male and 30 female), using test-retest technique, is excellent (18). The study calculated LSC which is the maximum precision error expected when using DXA for body composition measurements in athletes (18). The total body fat, trunk fat, leg and arm regions of our study are less than the LSC of the Buehring study, when average of right and left arms and right and left legs were compared. This means that the measured change in fat mass by DXA in this study was not more than the typical error expected for athletes and therefore regions did not meaningfully change.

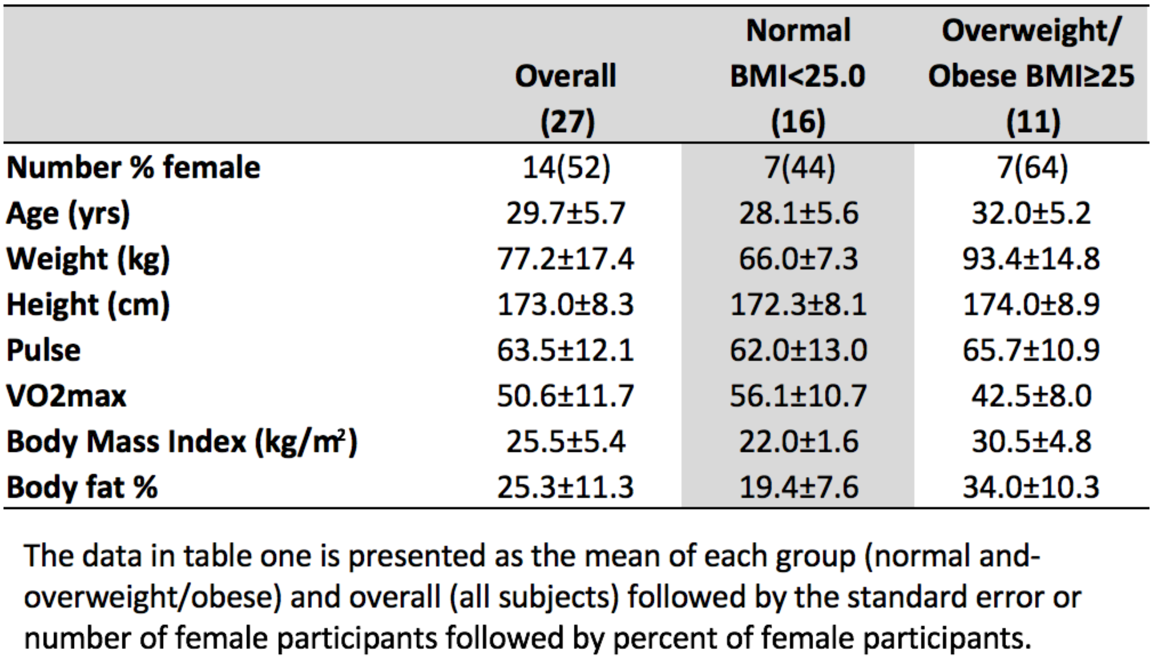
A potential weakness for this study is that the subjects weren't compared by gender. By grouping genders together in this study, we were not able to see if male of female subjects had changes in fat that were greater than the LSC. Since our study has shown DXA may not be sensitive enough to assess change after an acute bout of physical activity with male and female combined, similar studies with analysis by gender are necessary. A strength of this study was including populations of normal weight and overweight/obese. This allows us to assess if BMI affected the change of body fat measured by DXA.

CHAPTER 6. CONCLUSION

DXA was not sensitive enough to determine changes in body fat after the 90-minute run at 60% HRR, when compared to LSC for DXA in athletes since the changes were not more than the typical margin of error. Future studies may calculate the actual fat burned to determine how much activity would be needed to cause a meaningful change.

APPENDIX

Table 1. Participant characteristics



	Overall (27)	Normal BMI<25.0 (16)	Overweight/ Obese BMI≥25 (11)
Number % female	14(52)	7(44)	7(64)
Age (yrs)	29.7±5.7	28.1±5.6	32.0±5.2
Weight (kg)	77.2±17.4	66.0±7.3	93.4±14.8
Height (cm)	173.0±8.3	172.3±8.1	174.0±8.9
Pulse	63.5±12.1	62.0±13.0	65.7±10.9
VO2max	50.6±11.7	56.1±10.7	42.5±8.0
Body Mass Index (kg/m^2)	25.5±5.4	22.0±1.6	30.5±4.8
Body fat %	25.3±11.3	19.4±7.6	34.0±10.3

The data in table one is presented as the mean of each group (normal and-overweight/obese) and overall (all subjects) followed by the standard error or number of female participants followed by percent of female participants.

Table 2. Absolute change in body composition after 90-minute run

	Overall (27)				Normal (16)				Overweight/Obese (11)				Normal vs. Overweight/Obese Absolute Change Comparison
	Mean	±	SD	P-value	Mean	±	SD	P-value	Mean	±	SD	P-value	
Pre percent body fat	25.3	±	11.3		19.4	±	7.6		34.0	±	10.3		
Post percent body fat	25.3	±	11.4		19.3	±	7.4		34.0	±	10.6		
Percent body fat absolute change	0.0	±	0.5	0.93	0.0	±	0.5	0.75	0.1	±	0.5	0.63	0.55
Pre fat mass	19654.7	±	12108.3		12008.7	±	4367.3		30776.1	±	11089.8		
Post fat mass	19470.1	±	12081.3		11880.2	±	4280.0		30510.1	±	11214.7		
Fat mass absolute change	-184.5	±	443.6	0.04	-128.5	±	370.1	0.19	-266.0	±	542.1	0.13	0.44
Pre arms fat	2273.4	±	1526.9		1372.6	±	514.5		3583.6	±	1574.2		
Post arms fat	2271.3	±	1502.3		1388.1	±	515.8		3556.0	±	1550.9		
Arms fat absolute change	-2.1	±	188.2	0.95	15.5	±	68.0	0.38	-27.6	±	289.8	0.76	0.64
Pre trunk fat	9177.8	±	6097.5		5149.9	±	1918.3		15036.5	±	5237.5		
Post trunk fat	8982.7	±	6038.9		4979.1	±	1866.4		14806.2	±	5161.6		
Trunk fat absolute change	-195.1	±	488.3	0.05	-170.8	±	226.2	0.01	-230.4	±	735.5	0.32	0.80
Pre leg field	7345.1	±	4835.9		4657.3	±	2140.4		11254.6	±	5055.6		
Post leg field	7333.2	±	4866.9		4628.2	±	2143.3		11267.8	±	5095.1		
Leg field absolute change	-11.9	±	177.6	0.73	-29.1	±	124.0	0.36	13.2	±	240.4	0.86	0.60
Pre visceral fat	340.1	±	285.0		159.5	±	106.4		602.8	±	257.1		
Post visceral fat	352.4	±	299.1		159.4	±	109.1		633.3	±	261.5		
Visceral fat absolute change	12.3	±	44.4	0.16	-0.1	±	40.2	0.99	30.5	±	45.8	0.05	0.08
Pre subcutaneous fat	1094.4	±	987.4		504.9	±	320.3		1951.8	±	1008.1		
Post subcutaneous fat	1066.6	±	989.0		488.4	±	313.3		1907.7	±	1040.5		
Subcutaneous fat absolute change	-27.8	±	74.3	0.06	-16.6	±	40.8	0.13	-44.1	±	106.6	0.20	0.43
Pre android fat	1434.4	±	1185.1		664.3	±	313.9		2554.6	±	1083.6		
Post android fat	1308.0	±	1127.0		647.8	±	305.1		2268.5	±	1204.3		
Android fat absolute change	-126.4	±	531.9	0.23	-16.6	±	36.2	0.09	-286.2	±	828.4	0.28	0.31
Pre gynoid fat	3572.6	±	2483.9		2124.4	±	1075.4		5679.1	±	2463.8		
Post gynoid fat	3352.6	±	2151.8		2132.6	±	1065.2		5127.1	±	2119.2		
Gynoid fat absolute change	-220.0	±	1288.2	0.38	8.3	±	79.4	0.68	-552.0	±	2025.0	0.39	0.38
Pre android gynoid ratio	0.89	±	0.26		0.79	±	0.22		1.04	±	0.26		
Post android gynoid ratio	0.87	±	0.25		0.76	±	0.20		1.03	±	0.24		
Android gynoid ratio absolute change	-0.02	±	0.04	0.035	-0.02	±	0.03	0.02	-0.01	±	0.05	0.51	0.47

Data in Table 2 is shown as the mean of each subject's pre-run, post-run values in grams and the mean of each subject's change compared to all participants (absolute change) in grams followed by the standard deviation and significance (p-value). Significance (p-value) is also given for normal change compared to overweight and obese change.

REFERENCES

References

1. Fox CS, Massaro JM, Hoffman U, Pou KM, Maurovich-horvat P, Liu C, et al. Abdominal Visceral and Subcutaneous Adipose Tissue Compartments. Circulation. 2007;116(1):39–48.
2. Bosch TA, Burress TP, Weir NL, Fielding KA, Engel BE, Weston TD, et al. Abdominal Body Composition Differences in NFL Football Players. J Strength Cond Res. 2014;28(12):3313–9.
3. Carver TE, Christou N V, Andersen RE. In Vivo Precision of the GE iDXA for the Assessment of Total Body Composition and Fat Distribution in Severely Obese Patients. Obesity. 2013;21(7):2012–4.
4. Knapp KM, Welsman JR, Hopkins SJ, Shallcross A, Fogelman I, Blake GM. Special Section on Obesity Obesity Increases Precision Errors in Total Body Dual-Energy X-Ray Absorptiometry Measurements. J Clin Densitom. 2015;18(2):209–16.
5. Nana A, Slater GJ, Hopkins WG, Burke LM. Effects of Exercise Sessions on DXA Measurements of Body Composition in Active People. Med Sci Sports Exerc. 2012;45(1):178–85.
6. Schwartz RS, Shuman WP, Larson V, Cain KC, Fellingham GW, Beard JC, et al. The Effect of Intensive Endurance Exercise Training Young and Older Men on Body Fat Distribution in Young and Older Men. Metabolism. 1991;40(5):545–51.
7. Wilmore JH, Després J, Stanforth PR, Mandel S, Rice T, Gagnon J, et al. Alterations in body weight and composition consequent to 20 wk of endurance training : the HERITAGE Family Study. Am J Clin Nutr. 1999;70(3):346–52.
8. Donges CE, Duffield ROB, Drinkwater EJ. Effects of Resistance or Aerobic Exercise Training on Interleukin-6, C-Reactive Protein, and Body Composition. Med Sci Sports Exerc. 2010;42(2):304–13.
9. Mourtzakis M, Prado CMM, Lieffers JR, Reiman T, Mccargar LJ, Baracos VE. A practical and precise approach to quantification of body composition in cancer patients using computed tomography images acquired during routine care. Appl Physiol Nutr Metab. 2008;33(5):997–1006.
10. Holloszy JO, Coyle EF. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. Am Physiol Soc. 1984;56(4):831–8.
11. Bircher S, Knechtle B. Relationship Between Fat Oxidation and Lactate Threshold in Athletes and Obese Women and Men. J Sport Sci Med. 2004;3(3):174–81.
12. Romjin JA, Coyle EF, Sidossis LS, Rosenblatt J, Wolfe RR. Substrate Metabolism During Different Exercise Intensities in Endurance-Trained Women. J Appl Physiol. 2000;88(19):1707–14.
13. Romjin JA, Coyle EF, Sidossis LS, Gastaldelli A, Horowitz JF, Endert E, et al. Regulation in relation of endogenous fat and carbohydrate to exercise

intensity and duration metabolism. Am J Physiol. 1993;265(3):380–91.

14. Brooks GA, Mercier J. Balance of carbohydrate and lipid utilization during exercise : the “ crossover ” concept. J Appl Physiol. 1994;76(6):2253–61.
15. Aloia JF, Cohn SH, Babu T, Abesamis C, Kalici N, Ellis K. Skeletal Mass and Body Composition in Marathon Runners. Metabolism. 1978;27(12):1793–6.
16. Despres JP, Bouchard C, Savard R, Tremblay A, Marcotte M, Thlriault G. The Effect of a 20-Week Endurance Training Program on Adipose-Tissue Morphology and Lipolysis in Men and Women. Metabolism. 1984;33(3):235–9.
17. Bilsborough JC, Greenway K, Opar D, Livingstone S, Cordy J, Coutts AJ. The accuracy and precision of DXA for assessing body composition in team sport athletes. J Sports Sci. 2014;32(19):1821–8.
18. Buehring B, Krueger D, Libber J, Heiderscheit B, Sanfilippo J, Johnson B, et al. Dual-Energy X-Ray Absorptiometry Measured Regional Body Composition Least Significant Change : Effect of Region of Interest and Gender in Athletes. J Clin Densitom. 2014;17(1):121–8.
19. Knapp KM, Welsman JR, Hopkins SJ, Shallcross A, Fogelman I, Blake GM. Obesity Increases Precision Errors in Total Body Dual-Energy X-Ray Absorptiometry Measurements. J Clin Densitom [Internet]. Elsevier Ltd; 2014;18(2):209–16. Available from: http://dx.doi.org/10.1016/j.jocd.2014.06.001
20. Knapp KM, Welsman JR, Hopkins SJ, Fogelman I, Blake GM. Obesity Increases Precision Errors in Dual-Energy X-Ray Absorptiometry Measurements. J Clin Densitom. 2012;15(3):315–9.
21. Edelman R, Warach S. Magnetic Resonance Imaging: Second of Two Parts. N Engl J Med. 1993;328(11):785–91.
22. Ackland TR, Lohman TG, Sundgot-borgen J, Maughan RJ, Meyer NL, Stewart AD, et al. Current Status of Body Composition Assessment in Sport. Sport Med. 2012;42(3):227–49.
23. Lukaski C. Methods for the assessment of human body composition: traditional and new. Am J Clin Nutr. 1987;46(4):537–56.
24. Lee SY, Gallagher D. Assessment methods in human body composition. Curr Opin Clin Nutr Metab Care. 2008;11(5):566–72.
25. Shuster A, Patlas M, Pinthus JH, Mourtzakis M. The clinical importance of visceral adiposity : a critical review of methods for visceral adipose tissue analysis. Br J Radiol. 2012;85(1009):1–10.
26. Lee SY, Gallagher D. Assessment methods in human body composition. Curr Opin Clin Nutr Metab Care. 2008;11(5):566–72.
27. Kvist HL, Chowdhury B, Grangard U, Tylen U, Sjostrom L. Total and visceral adipose-tissue volumes derived from measurements with computed tomography in adult men and women: predictive equations. Am J Clin Nutr. 1988;48(6):1351–61.
28. Bredella MA, Ghomi RH, Thomas BJ, Torriani M, Brick DJ, Gerweck A V, et

al. Comparison of DXA and CT in the Assessment of Body Composition in Premenopausal Women With Obesity and Anorexia Nervosa. sObesity. 2010;18(11):2227–33.

29. Smith-bindman R, Lipson J, Marcus R, Kim K, Mahesh M, Gould R, et al. Radiation Dose Associated With Common Computed Tomography Examinations and the Associated Lifetime Attributable Risk of Cancer. JAMA. 2009;169(22):2078–86.
30. Haarbo J, Gotfredsen A, Hassager C, Christiansen C. Validation of body composition by dual energy X-ray absorptiometry (DEXA). Clin Physiol Funct Imaging. 1990;11(4):331–41.
31. Rothney MP, Martin F, Xia Y, Beaumont M, Davis C, Ergun D, et al. Precision of GE Lunar iDXA for the Measurement of Total and Regional Body Composition in Nonobese Adults. J Clin Densitom [Internet]. Elsevier Ltd; 2012;15(4):399–404. Available from: http://dx.doi.org/10.1016/j.jocd.2012.02.009
32. Jensen MD, Kanaley JA, Ph D, Roust LR, Brien PCO, Ph D, et al. Assessment of Body Composition With Use of Dual-Energy X-ray Absorptiometry : Evaluation and Comparison With Other Methods. Mayo Clin Proc [Internet]. Mayo Foundation for Medical Education and Research; 1993;68(9):867–73. Available from: http://dx.doi.org/10.1016/S0025-6196(12)60695-8
33. Ellis KJ. Human Body Composition : In Vivo Methods. Physiol Rev. 2000;80(2):649–80.
34. Demura S, Yamaji S, Kobayashi H, Sato S, Nagasawa Y. Validity and reliability of body composition assessed by the sulphur hexa ¯ uoride dilution method. J Sports Sci. 2002;20:663–70.
35. Wagner DR, Heyward VH. Techniques of Body Composition Assessment : A Review of Laboratory and Field Methods. Res Q Exerc Sport. 1999;70(20):135–49.
36. Johansson G, Forslund A, Sjodin A, Mallmin H, Hambraues L, Ljunghall S. Determination of body composition-a comparison of dual energy x-ray absorptiometry and hydrodensitometry. Am J Clin Nutr. 1993;57(3):323–6.
37. Lowry DW, Tomiyama AJ. Air Displacement Plethysmography versus Dual-Energy X-Ray Absorptiometry in Underweight , Normal-Weight , and Overweight / Obese Individuals. PLoS One. 2015;10(1):1–8.
38. McCrory M, Mole P, Gomez T, Dewey K, Bernauer E. Body composition by air-displacement plethysmography by using predicted and measured thoracic gas volumes. J Appl Physiol. 1998;84(4):1475–9.
39. McCrory M, Gomez T, Bernauer E, Mole P. Evaluation of a new air displacement plethysmo- graph for measuring human body composition_McCrory.pdf. Med Sci Sports Exerc. 1995;27(12):1686–91.
40. Ballard TP, Fafara L, Vukovich MD. Comparison of Bod Pod ® and DXA in Female Collegiate Athletes. Med Sci Sports Exerc. 2004;36(4):731–5.
41. Müller W, Horn M, Fürhapter-rieger A, Kainz P, Kröp JM, Maughan RJ, et

al. Body composition in sport : a comparison of a novel ultrasound imaging technique to measure subcutaneous fat tissue compared with skinfold measurement. Br J Sports Med. 2013;47(16):1028–35.

42. Wagner DR, Cain DL, Clark NW. Validity and Reliability of A-Mode Ultrasound for Body Composition Assessment of NCAA Division I Athletes. PLoS One. 2016;11(4):1–12.
43. Smith-ryan AE, Fultz SN, Melvin MN, Wingfield HL, Woessner MN. Reproducibility and Validity of A-Mode Ultrasound for Body Composition Measurement and Classification in Overweight and Obese Men and Women. 2014;9(3).
44. Pineau J, Filliard JR, Bocquet M. Ultrasound Techniques Applied to Body Fat Measurement in Male and Female Athletes. J Athl Train. 2009;44(2):142–7.
45. Schoeller DA, Dietz W, van Santen E, Klein PD. Validation of saliva sampling for total determination by H2180 dilution. Am J Clin Nutr. 1982;35(3):591–4.
46. Kushner F, Schoeller A. Estimation impedance by bioelectrical. Am J Clin Nutr. 1986;44(3):417–24.
47. Jackson A, Pollock M. Generalized equations for predicting body density of men. Br J Nutr. 1978;40(3):497–504.
48. Fogelholm M, Lichtenbelt WVM. Review Comparison of body composition methods : a literature analysis. Eur J Clin Nutr. 1997;51(8):495–503.
49. Sinning W, Dennis D, Little K, Cunningham L, Racaniello A, Siconofli S, et al. Validity of “generalized” equations for body composition analysis in male athletes. Med Sci Sports Exerc. 1985;17(1):124–30.
50. Wang J-G, Zhang Y, Chen H-E, Li Y, Cheng X-G, Xu L, et al. Comparison of Two Bioelectrical Impedance Analysis Devices with Dual Energy X-Ray Absorptiometry and Magnetic Resonance Imaging in The Estimation of Body Composition. J Strength Cond Res. 2013;27(1):236–43.
51. Thomson R, Brinkworth GD, Buckley JD, Noakes M, Clifton PM. Good agreement between bioelectrical impedance and dual-energy X-ray absorptiometry for estimating changes in body composition during weight loss in overweight young women. Clin Nutr. 2007;26(6):771–7.
52. Bosy-Westphala A, Later W, Hitze B, Sato T, Kossel E, Glüerc C-C, et al. Accuracy of Bioelectrical Impedance Consumer Devices for Measurement of Body Composition in Comparison to Whole Body Magnetic Resonance Imaging and Dual X-Ray Absorptiometry. Eur J Obes. 2008;1(6):319–24.
53. Deriaz O, Dumont M, Bergeron N, Despre J, Brochu M, Prud’homme D. Skeletal muscle low attenuation area and maximal fat oxidation rate during submaximal exercise in male obese individuals. Int J Obes. 2001;25(11):1579–84.
54. Hill AM, Laforgia J, Coates AM, Buckley JD, Howe PRC, Alison M, et al. Estimating Abdominal Adipose Tissue with DXA and Anthropometry. 2007;15(2).

55. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of Childhood and Adult Obesity in the United States, 2011-2012. J Am Med Assoc. 2014;311(8):806–14.
56. Bruce RA, Kusumi F, Hosmer D. Maximal oxygen intake and nomographic assessment of functional aerobic impairment in cardiovascular disease. Am Heart J. 1973;85(4):546–62.
57. Astrand, Ryhming. A nomogram for calculation of aerobic capacity (physical fitness) from pulse rating during sub-maximal work. J Appl Physiol. 1954;7(2):218–21.
58. Borg GAV. Physical performance and perceived exertion. Stud Psychol Paedagog Ser altera, Investig XI. 1962;
59. Santos DA, Silva AM, Matias CN, Fields DA, Heymsfield SB, Sardinha LB. Accuracy of DXA in estimating body composition changes in elite athletes using a four compartment model as the reference method. 2010;