Body Composition of athletes assessed by Dual X-ray Absorptiometry and other methods

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Dedication

I would like to dedicate this work to my mother and father, who ensured I had the very best chance to study, who gave me every encouragement to pursue my interests passionately and see things through, and to understand that any contribution to understanding, no matter how small, can make a difference.

Quotation

"Perfection comes about little by little through many numbers"

Polyclitus 460 - 410 BC

sculptor of Greek Gods, heroes and athletes

Declaration

The work contained herein is the result of my own investigations and acknowledgement has been made for all assistance

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ABSTRACT

Introduction. Knowledge of the morphology and composition of the human body has proved useful in clinical, nutritional, physiological and ergonomic settings. Technological developments have led to an increasingly diverse array of available methods, five of which are included in the present study. Of the more recent methods, dual X-ray absorptiometry (DXA) holds the promise of particular utility because it provides separate tissue masses for bone, lean and fat in the whole body and separate sub-regions, and has recently proved more accurate than densitometry alone, when compared with a four compartment model of bone, water, fat and fat-free soft tissue. Despite its widespread popularity for clinical studies, investigation of athletes using DXA is still virtually unknown. This study explores DXA's utility as a new reference method for body composition, and compares its total and regional tissue masses in different athletic groups and controls.

Methods. DXA scans were performed using a Hologic QDR 1000/W (Hologic Inc. Bedford, MA, USA) on 106 male and 30 female athletes. Forty two anthropometric measurements were made on all athletes, and bioelectrical impedance (BIA) was measured on 82 male and 24 female athletes. Six male subjects underwent a total body Potassium scan, and these and a further four subjects undertook a magnetic resonance imaging (MRI) scan of the upper leg. Archived data were used to explore regional fat patterning by skinfolds, and DXA data on 53 controls and 30 anorexic patients were used with permission for comparative purposes.

Findings: Body Composition. Athletes had significantly greater lean tissue and less fat than controls, but bone mineral content (BMC) depended on the type of activity. Anthropometry proved superior to BIA in predicting fat and fat-free mass in male athletes, and the accuracy of predictions of published skinfold equations applied to the athletes appeared to depend upon appropriate skinfold selection. DXA data showed athletes appeared to distribute fat differently from sedentary controls after controlling for age and adiposity, although this was not influenced by exercise type or differences in lean tissue distribution. Female athletes had different distributions of bone, lean and fat tissue to anorexics and controls. A new body composition descriptor - the DXA morphotype - was derived for athletic groups using bone and soft tissue Z scores, which discriminated the groups differently from traditional somatotype measures. Differences in fat content by MRI and DXA of the upper leg were consistent with the variable lipid fraction of adipose tissue with adiposity; while MRI muscle mass was slightly better predicted from anthropometry than DXA lean tissue mass.

Findings: Bone. Eleven out of 26 female athletes had significantly reduced bone mineral density (BMD) at the lumbar spine, which was best predicted from the combined index (CX): the sum of BMI and oestrogen status (OS, defined as 0 for oligo- or amenorrhoea; 1 for eumenorrhoea or supplemented). These data suggest women athletes with a $CX \leq 20.4$ carry a 2.4 fold increased risk of spinal osteopenia. Variation in BMD between male athletic groups exposed differences in physical impact. Rugby showed the highest BMD of all groups, after correction for body size. Compared with controls, running was associated with increased BMD in the legs, while cycling was associated with a decreased BMD in the spine. Cyclists had a mean T score of -1.16, and were seven times as likely as controls to have osteopenia. While unimportant in the legs, muscular torque appeared to contribute to increased BMD in the arms and spine, suggesting different mechanisms for adjusting skeletal architecture may operate at different sites.

Conclusions. Dual X-ray absorptiometry has proved convenient and accurate as a reference method of body composition, making a significant contribution to the understanding of morphological adaptation to specific sports, and their influence on bone, and highlighting the need for further research in specific athletic groups.

Contents

CHAPTER 1.

INTRODUCTION

This chapter seeks to address the context for the measurement of body composition in humans, by providing historical background, by providing a frame of reference for the athletic population in question, and a statement of the aims of this work.

1.1 THE DEVELOPMENT OF HUMAN BODY COMPOSITION STUDIES.

The composition of the human body is subject to both genetic and environmental influences, and the combination of both has perhaps been crucial to our evolutionary ascent from the lower primates. It has been estimated that 98% of the human genotype is shared with the Chimpanzee (Diamond, 1991) and over 99% of the skeletal muscles are common to humans and the Rhesus monkey (Hawes, 1977). Since the relatively rapid spread of *homo sapiens* throughout the globe, evolutionary pressure has forced human size and proportions to vary with climate (Katzmarzyk and Leonard, 1998), amongst other reasons because size has a controlling influence over surface area and temperature regulation. Morphological adaptation to suit different geographical regions has led to significant differences in body composition and physique between Eskimos, Indians, Africans, Mexicans and Manus (New Guinea) natives (Himes, 1988). Skeletal dimensions, muscular development and fat patterning have all been shown to vary between different groups. For instance, a race-specificity in femur : stature ratio has been used extensively as a diagnostic tool in anthropology (Feldesman and Fountain, 1996). American Blacks (unspecified whether African or Caribbean origin) tend to have more muscle and bone than Caucasians (Cohn et al., 1977), and different fat distributions between Caucasians, Blacks, Hispanics, Asians and Indians have led to the development of large numbers of population-specific models to predict fatness (Heyward and Stolarczyk, 1996).

Estimation of the composition of the human body can be traced back to the ancient Greeks, and the application of Archimedes' principle (the volume of an object is equal to the volume of water displaced) has remained in current use ever since. While there would have been clear benefits in battle for individuals possessing strength, speed and endurance, the Greeks are also credited with having pioneered the concept of physical conditioning in their legendary gladiator contests, with a consequent emphasis on muscularity. They discovered that different exercise and training regimens could not only confer

considerable benefits in combat, but also in preparation for athletic competition, and some of their traditional conditioning methods are still in use by athletes today.

Perhaps it is not surprising that the ancient Greeks were the first to attempt to quantify the human physique. Polyclitus (460 - 410BC.) was a bronze sculptor who made gods, heroes and athletes. His fame is largely due to a single work called *Doryphorus* or *spearbearer* which was of a 'virile body', 'suitable for war and athletics' and 'aimed at the mean' (Oxford Classical Dictionary, 1996) which suggests his proportions were not excessive like those of gladiators. Polyclitus also wrote a book called the *Canon* or *rule*, which was based on this work, in which he unequivocally stated that 'perfection comes about little by little through many numbers' and described human proportion in considerable detail, mathematically relating each part of the body to every other part, and to the whole. Many successive sculptors followed this inevitably stereotypical view of human proportions, and the style remained popular throughout the Roman era.

In the early Renaissance, Leon Battista Alberti (1404-1472) produced an innovative treatise on sculpture, *De statua*, which referred to a tool which enabled sculptors to replicate their works, and alter the size without altering proportions. The tool, known variously as the *finatorium* or *definer*, comprised a rotating arm on a protractor base, and a calibrated plumb line. Placed above the sculpture itself, the plumb was lowered at different angular intervals until it contacted the working surface. A numerical 'map' enabled the original to be replicated at a variety of scales. The proportionality of subjects, independent of size was to become a key feature of physique analysis, and remains fundamental to the science of kinanthropometry today.

Today, police use bone measurements to re-construct skeletal remains in much the same way as anthropologists and palaeontologists do. In the 19th century, large numbers of measurements were used in forensic anthropometry. The most famous of these was the total of 243 measurements comprising the *Bertillonage* – the identification system designed by French Police clerk Alphonse Bertillon in 1879,

which remained the most popular method of classifying law-breakers until it was replaced by fingerprinting in 1910 (Olds, 1999). The Italian Cesare Lombroso developed the concept of the "criminal anthropometric profile" based on measurements on the skull, which presumed criminals to be identifiable by facial characteristics. Such methods received sufficient acclaim that it is not altogether surprising that half a century later, Sheldon, in his classification of human physique as variants of three somatotypes, (Sheldon et al., 1940) was seeking a link between human shape and personality.

History recounts that body proportions and physique were originally assessed for purposes of art, sport or combat; today there is a multitude of purposes for measuring body composition. The majority of these are clinical, with the quantification of tissue proportions with a view to optimising health. It is seen from the various perspectives of morbidity and mortality in obesity, proportional and morphological change during growth and development, and as an index of functional capacity as a contributor to fitness and general health. Anthropological, ergonomic, nutritional and physiological research all measure body morphology using the tools of body composition analysis – from defining undernourishment in the third world, to the design and layout of an aircraft cockpit.

While traditional research in body composition has been dominated largely by clinical nutrition and related sciences, more recent interest in the exercise and public health sciences has witnessed a dramatic increase in the use of measurements of body composition, enabling more robust studies to be performed using larger populations. While clinical work has been the driving force behind many new technological advances, many studies are now being performed on healthy groups including athletes, with a consequent growth in recent literature.

Of the broadening array of available methods today, Dual X-ray Absorptiometry is perhaps the most exciting, because by comparison to other imaging techniques it is affordable, while exposing the subject to only a minimal radiation dose. The technology is much less cumbersome than other atomic counting methods, and the subject is merely required to remain supine for 15 minutes (5 minutes with the newer fan

beam models). The data output is more comprehensive and of greater utility than that of other methods, describing bone mass and density, and also fat and lean tissue masses. Its ability to measure regional as well as total composition enables body composition research to proceed along a new direction. Since its development towards the end of the 1980s, DXA is now beyond its infancy, but investigation of athletic populations remain scarce.

1.2 The Context of Exercise in shaping Body Composition

The ability of exercise to influence changes in body morphology is limited by the exercise stimulus and predetermined genetic factors. As a consequence, exercise or conditioning for specific sports must be viewed within the wider context of the genetic effect on body tissues.

Of the transmissible effects, a portion will be truly genetic, while the remainder will be cultural, as identified by biological and adoption family studies. There remains considerable debate over the degree of heritability of the variance in body fat content from approaching zero to as high as 90% using BMI as the criterion (Bouchard and Pérusse, 1988; Stunkard et al., 1986) although twin, adoption and family studies submitted to segregation analysis estimated the genetic component to be about 25% (Bouchard et al., 1988). A figure of 30% was found to be inherited for fat-free mass derived from densitometry (Bouchard et al, 1988), and values exceeding 50% have been reported for skeletal diameters (Bouchard, 1991). Twin studies of bone mass by photon absorptiometry at forearm, lumbar spine and femoral sites also show significant genetic determination (Pocock et al., 1987). Reviews of the heritability of somatotype suggest all three physique components (endomorphy, ectomorphy and mesomorphy) exhibit

genetic effects, though mesomorphy shows the strongest effect in family and twin studies (Carter and Heath, 1990; Song et al, 1993; Song et al, 1994).

Given this genetic template, the body is exposed to the influences of growth and development, which occur in conjunction with what is normally the most physically active phase of life, especially in males. The interdependence of hormonal influence and physical activity has an anabolic effect on body tissues. Following sexual maturation, growth patterns and body morphology of boys and girls become increasingly divergent. Peak height is commonly achieved by the age of 17 and 15 years in boys and girls respectively, following which both sexes continue to gain mass, which, in boys is mostly muscle, and in girls is mostly fat. The quantity and distribution of body fat shows a powerful gender influence, and age – related centralisation of body fat occurs to a greater extent in men than women. Between the age of 20 and 30, bone and muscle mass usually peak, while body fat and total mass continue to increase steadily until middle age. Muscle mass is steadily reduced with advancing age, though the burden of weight bearing per unit of muscle mass is increased, with the implication that physical functional capacity inevitably declines with age.

The effect of exercise at any stage throughout adult life has the potential to affect bone, lean or fat tissue. **Bone:** Before the peak bone mass is reached, weight bearing activities increase bone mass and density, while weight supported activities such as cycling or swimming have little or no effect (American College of Sports Medicine, 1995). The maintenance of optimal skeletal health, particularly in postmenopausal women has also been extensively investigated. Longitudinal studies are scarce, but there is considerable importance attached to achieving a high peak bone mass in early adult life, which will take longer to descend to fracture risk thresholds in later life. Exercise intervention studies have demonstrated that bone mass may actually increase in sedentary adults who begin impact exercise up to 15 years beyond the theoretical peak bone mass at age 30 (Heinonen et al., 1996), but more commonly, exercise maintains existing bone or reduces the rate of its loss in later life.

Lean tissue: Beginning in the late 20s and 30s, the muscle mass and strength in humans shows a gradual decline, which eventually manifests itself in a loss of functional capacity in old age. Conversely, the body never loses the adaptive response to strength training, and such exercise at any stage during life can increase muscle mass, if the body is provided with adequate nutrition. Evidence from body-builders who undergo severe caloric restriction while maintaining high work rates prior to competition, suggests strength is diminished as a result (Bamman et al., 1993). This may be due to a reported shift towards aerobic activity which consumes more calories, but also induces metabolic changes within the muscle cells. Study of unusual feats of human endurance, such as polar exploration or high altitude mountaineering, suggest that the body's energy balance may be upset by a combination of high energy cost of locomotion, suppression of appetite, and cold temperatures increasing metabolic rate. The result is that in addition to fat, muscle may be used in substantial quantities as an energy substrate causing significant reductions in body mass following such excursions, thereby "fuelling the engine by burning the boat" (Stroud, 1993).

Fat: Exercise is associated with enhanced fat distribution involving reduced upper body adiposity (Tremblay et al., 1988; Nindl et al., 1996) and lower total adiposity (Ballor and Keesey, 1991; Björntorp, 1978) although the latter effect is small when no dietary restriction is applied. Evidence from those who suffer eating disorders, including anorexia athletica (disturbed eating patterns in association with a compulsive exercise behaviour), shows not only considerable fat loss, but also significant muscle loss. Essential lipids are considered to occupy 3 – 4% of a healthy individual's mass (Hawes, 1996). Excess lipid can vary from effectively zero in endurance athletes to over 50% of total mass in highly obese individuals. Recently, a third category for lipid has been described in women – the *sex–specific* fat depot, which is non-essential for survival, but desirable for health (Carter, 1999). Amenorrhoeic athletes are thought to have depleted their excess lipid, and have begun utilisation of their sex-specific fat depot.

1.3 BODY COMPOSITION IN ATHLETES

The changes imposed on a sedentary body by sport or exercise – principally of the skeleton, striated muscle and body fat – require a task-specific approach to the assessment of body composition.

Quantification of body composition in athletes has traditionally relied on densitometry, the physical principles of which are discussed by Brodie et al., (1998). Archimedes' principle divided the body into fat, and fat-free compartments of fixed density, and measured density was used to validate skinfold methods in the 1960s and 1970s. However, more recently exercise has been shown to alter not only tissue proportions, but also the density of the fat-free mass (Martin et al., 1986). One study of professional Canadian football players (Adams et al., 1982) illustrated this point by producing negative fat predictions in eight out of 29 subjects, with two as low as –12% fat. The skinfolds of these subjects were substantially greater than those of a population of elite distance runners (Pollock et al., 1977) whose predicted fat was still positive but approached zero. Because these two studies involved subjects of similar age, it is reasonable to conclude that adaptations for strength and endurance activity are associated with different variations from assumed norms for fat quantity and density of the fat-free mass. As a result, studies involving elite athletes negate the use of traditional predictions of body composition derived on a normal (sedentary) population.

The indiscriminate use of generalised equations for predicting fat mass from skinfolds in male athletes was assessed in a comprehensive survey by Sinning et al., (1985) using densitometry as the criterion measure. Of 21 equations, only three by Jackson and Pollock (1978) were deemed to be sufficiently accurate to predict body fat, while those in most common use in the U.K. (Durnin and Womersley, 1974) overestimated fat excessively. This observation was reinvestigated in the present study by using DXA as the criterion method (see chapter 4.2). However, in the same way as body mass or BMI is seen as an

inadequate representation for body composition in sedentary populations, similarly the fat mass and fat free mass are inadequate for describing the physique variables for athletes.

The observation that athletes show a greater variation in muscle mass than fat mass from sedentary individuals has focussed attention on its prediction from corrected girths obtained by subtracting π multiplied by the skinfold thickness from the limb girth (Hawes, 1996). The most commonly used predictor of muscle mass is the equation of Martin et al., (1990) which uses corrected circumferences at the thigh and calf, and an uncorrected circumference at the forearm. Currently, this may offer the best available prediction as it is based upon cadaver evidence, and has exposed differences in muscle mass of various athletic groups (Spenst et al., 1993). However, the methodology will necessarily bias certain sporting disciplines by assuming equal development at the three circumferences, and assume fixed proportional limb lengths to total stature. For instance, muscle mass of weight lifters is likely to be under-predicted because the circumferences include their relatively under-developed calf muscles and take no account of the upper arm and neck muscles which contribute much more significantly to weightlifting performance.

Skeletal dimensions are a good predictor of muscle mass (Behnke, 1959), but are a potential confounder in studies of height – weight relationships unless frame size is adequately assessed. Frame size, traditionally used in the calculation of life insurance premiums from height – weight charts, showed no correlation with fat mass in a study of young adult males (Peters and Eston, 1993). However, in addition to other measures used for establishing minimum recommended weight for athletes (Horswill et al., 1990) knowledge of frame size could inform the decision of ideal competitive weight or category in some sports. Bone dimensions are more heritable than other morphological tissue quantities (Bouchard, 1991) and are a criterion for success in winter sports (Orvanova, 1987), diving (Sovak et al., 1992), gymnastics (Claessens et al., 1991), body-building (Fry et al., 1991), but not cycling (McLean and Parker, 1989).

The study of human physique has been the subject of extensive work spanning several decades. Based upon the original photographic somatotypes of Sheldon (1940), Tanner (1964) characterised Olympic athletes from a morphometric viewpoint, and Heath and Carter (1967) produced a mathematical model on which the entire spectrum of human physique variation could be represented, independently from body size. Of many available variations, Heath – Carter somatotyping is perhaps the most useful in describing the physique of athletes, relying on 10 anthropometric measurements (see methods chapter 3.5).

In many sporting disciplines, world class performers tend to have similar physiques which have been extensively surveyed by somatotype (Tanner, 1964; Orvanova, 1987; Carter and Ackland, 1994; Leake and Carter, 1991). Some of this similarity is undoubtedly the product of self-selection but a substantial proportion is likely to be the consequence of conditioning regimes for specific sports conferring similar adaptations on competitors. There is also evidence for a convergence of somatotypes as the competitive level elevates to world class, which is usefully illustrated on the somatochart (Stepnicka, 1986).
Today the variation in human physique is itself broadening - through more extensive and radical training regimens by athletes on one hand, to increasing sloth and gluttony advancing the prevalence of obesity on the other. As a result, the three poles representing the limits of possible physique variation by Sheldon in 1940, may seem much less extreme today.

A different estimate, also independent of body size, is the "phantom z value"; derived from a unisex phantom and scaled to the subject's height with appropriate dimensional exponents and standard deviations of normative data (Ross and Ward, 1986). This also has been used in comparison of athletes at different competitive levels, and in different sports (Carter and Ackland, 1994; Hawes and Sovak, 1994).

Whichever of the above approaches are used, athletes' morphology departs from non-exercising controls in proportion to the magnitude of the training stimulus. For some, especially in aesthetic sports such as dance, gymnastics and body-building, the altered physique is a performance criterion, while in others it is a consequence of the training process. In either case, the nature of the training places different metabolic

demands on discrete muscle groups, and on circulation, cardio-respiratory function, fuel utilisation, sensory, motor and flexibility systems. While the majority of athletes are concerned with reducing the excess fat compartment to a level approaching zero, and increasing muscle mass to desirable levels, different changes occur in specific sports.

As a consequence of training, athletes' bodies behave differently to sedentary individuals in several important areas. In general terms, exercise would increase muscle mass, reduce fat mass to below that of controls, and enhanced the power : weight ratio and thus the energy cost of locomotion. High muscle mass plus prolonged exposure to physical tasks renders athletes capable of extremely high work rates (up to 30 times resting metabolic rate) and, as a consequence, heat production. Their sweating mechanisms are highly developed and their high capacity for temperature control makes them capable of losses of 5 - 10% of body mass as sweat, although performance decreases significantly at much lower levels of dehydration. However, beyond these general adaptations, specific adaptations are associated with specific body types. X-ray data of muscle : bone thicknesses in limbs of athletes have been shown to discriminate between their specific track and field events, while fat appeared unrelated (Tanner, 1964).
In warm and humid environments, excessive work may predispose strength-trained individuals, whose absolute muscle mass is very high, to hyperthermia (Hayward et al., 1986), while endomorphy and mesomorphy are protective against hypothermia in cross channel swimmers (Ross et al., 1980).

Athletes do not pursue the same training regimen throughout an entire year, but rather a series of cyclical training programmes referred to as periodisation. These cycles are geared to build the foundations of strength, speed, power, endurance and skill in the correct quantities for the required performance, on the required performance occasion. It is widely recognised that the optimal or peak performance represents a temporary state of adaptation which cannot be sustained in the longer term. Furthermore, longitudinal morphological measurements of athletes demonstrate that the processes responsible for peaking increase the muscle : fat ratio which, between competitive phases, reverts to training levels (Hawes and Sovak, 1994). This 'morphological prototype' represents an important future application of knowledge,

combining the identification of sporting potential, variation in body dimensions and tissue masses within observed limits, an awareness of the relationship between physique and performance, and perhaps most importantly, the tracking of physique which can correlate to the health dimension of 'career' athletes.

It is not altogether surprising to discover the illegal use of anabolic agents, which artificially increase muscle mass, has played an increasing role in athletic competitions across a wide spectrum of sports in recent years. The evidence for the effect of pharmacological agents such as anabolic steroids or growth hormone on body morphology and thus performance is all too clear, however the health consequences of such interventions are far reaching, and ensure such agents are on the international list of banned substances.

While sports and exercise scientists will continue to use the simple and effective tools of anthropometry, the increasing medical involvement in elite level sport is likely to bring changes which include more sophisticated body composition techniques being applied to athletes. If not for the detection of illegal substances or the reduced bone density associated with *anorexia athletica*, these will involve tools such as DXA in the pursuit of optimal morphology and recommended lean and fat masses for athletes together with tracking changes over time. Much remains to be discovered regarding how much the human physique can reasonably be expected to vary, and as traditional methods and assumptions are challenged. DXA, with its greatly reduced radiation exposure over conventional X-ray technology, holds the potential, if not to become a gold standard technique, to offer considerable utility as a tool for the measurement of bone and soft tissue composition.

1.4 LEVELS OF ANALYSIS IN BODY COMPOSITION MEASUREMENT

There are several recognised structural levels of approach to investigating body composition: the *atomic*, *molecular*, *cellular*, *tissue* and *whole body* levels. Knowledge of the inter-relationships within a given level or between levels is a prerequisite to a full understanding of individual measurement techniques within the discipline of body composition. This review centres on measurement techniques performed in the present study.

The *atomic level* refers to the sum of the atoms which comprise the body. Approximately 98% of the body is composed of oxygen, carbon, hydrogen, nitrogen, calcium, and phosphorous, with the remaining 2% of body mass representing the other 44 elements present in the body (Hawes, 1996). A frequently cited example of analysis at this level is ^{40}K counting to determine whole body potassium (see methods section). Technological advances have enabled several of these elements to be measured *in vivo*, but many techniques involve exposure to significant doses of ionising radiation.

The *molecular level* refers to over 100, 000 different molecules, the vast bulk of which fall into the categories of water, protein, bone mineral, lipids, and to a much lesser extent, carbohydrate (glycogen). Of these categories, there is most variation and confusion over the lipids. Lipids are generally defined as those chemicals which are insoluble in water, but soluble in organic solvents such as ether (Hawes, 1996). By far the most abundant lipid in the human body is triglyceride. Other lipids include phospho-lipids and cholesterol, which have different molecular structure and higher density. Lipids are frequently characterised into essential and excess categories. Essential lipids comprise those lipids which support life, such as cell membranes and various structures of the nervous system. Excess lipids comprise almost

exclusively triglycerides which, although providing functions of shock absorption and insulation, are not essential for life. DXA is an example of the molecular level of analysis, by virtue of its recognition of the different properties of the body's molecular constituents, which are grouped into categories of fat, lean and bone mineral, and discriminated by differential absorption of a dual energy X-ray beam.

The *Cellular* level includes extracellular fluid, extracellular solids, intracellular fluid, body cell mass and fat mass. Bioelectrical Impedance is an example of a method using this model's approach to measure body composition by assuming the fat component will not conduct an electrical current.

The *Tissue* level comprises adipose tissue, skeletal muscle, non-skeletal muscle, soft tissue and bone. It represents the classical model on which cadaver dissections are based. MRI uses this level of analysis in the present study. Muscle mass predictions from anthropometry are validated against cadaver dissection data, and are a further example of this level of analysis.

Whole body methods involve the subdivision of the body mass into components of measured (or predicted) mass. The two-compartment model involves the fat mass and the fat free mass, derived from densitometry and used to validate the majority of skinfold prediction formulae. The three compartment model is occasionally used where the fat free mass is further subdivided into the water and lean components, but increasingly, the four compartment method, using water, lean and bone compartments of the fat-free mass is used. This method is considered to be the best *in vivo* reference for total body composition, and is used in the validation of other techniques, including DXA.

The levels – of – analysis approach is a useful template for understanding the methods of body composition, especially where comparisons are made between methods which are not on the same level. However, a different approach to whole body analysis is available in modern imaging techniques, where units of the body such as arms, legs and torso can be analysed separately. In the present study, both MRI and DXA are used in this way.

1.5 STATEMENT OF AIMS

This study seeks to exploit DXA's potential in several ways, posing research questions under three broad headings. These are total tissue composition, regional tissue composition and bone density measurement. The pre-text for these questions is that DXA is capable of providing a useful criterion measure against which other methods can be compared, or by which different athletic groups can be discerned. The specific research questions the study asks and answers are listed below.

Total tissue composition.

- Of the portable methods of anthropometry and BIA, which predicts DXA-derived tissue masses more accurately using selected existing formulae?
- Can the present sample of athletes be used to construct prediction formulae which are sufficiently valid for wider use?
- Are these formulae more accurate than existing published formulae?
- Can DXA be adapted to quantify adaptation to specific sporting disciplines in men, and describe the discrete processes of adaptation to conditioning and undernourishment in women?

Regional tissue composition

- Do different athletic groups exhibit regional tissue variation in fat, lean and bone mass?
- Does the muscle development arising from sport-specific adaptation confer any commensurate effect on fat distribution?
- How effectively can anthropometry and DXA predict muscle mass in the upper leg, using MRI as the criterion measure?

- Are differences in fat measurements between DXA and MRI consistent with current understanding of the lipid fraction of adipose tissue?

Bone density

- Do athletes of different sports exhibit differences in bone density, after correction for body size and age?
- Does observation of regional variation in bone density contribute to current understanding of the mechanisms for adjustment to skeletal architecture?
- Can reduced bone density in women athletes be predicted from variables which are easily measured?

CHAPTER 2.

THE UNDERLYING PRINCIPLES OF METHODS

This chapter reviews the physical principles of the methods used in this study. In addition, there is a focus on the use of DXA to date and its validity as a reference method for body composition analysis.

2.1 DUAL X-RAY ABSORPTIOMETRY (DXA)

X rays are produced as a result of energy conversion when a rapidly moving stream of electrons is decelerated by the anode (usually made of Tungsten) of a vacuum diode tube. X-rays comprise a broad spectrum of photon energies. Since their discovery over 100 years ago, X rays have been used for a wide variety of diagnostic purposes in medicine, most notably for discrimination of bone from soft tissues. Quantification proved difficult, partly due to the relative instability of photographic film compared with that available today.

Single photon absorptiometry (SPA) was first performed by using an Iodine 125 radionuclide source, which emits Gamma rays (Cameron and Sorensen, 1963). The attenuation of these rays varies according to the atomic number of the molecules in their path, and as a result an estimate of tissue composition is possible. Single photon absorptiometry measurements required a water bath to provide a 'soft tissue equivalent' which accommodated the variable thickness of soft tissue in subjects.

If photons of two intensities are used, the result is a steeper attenuation at the lower energy, and the ratio of attenuation at the 2 energies - the R value - is a measure of tissue composition. While fat contains practically no atoms with high atomic mass, lean tissue contains several elements - mostly as electrolytes - which have a large effect on the R value - notably Na, P, K, and Cl. This dual photon absorptiometry (DPA) was first done using Gd 153, using energies of 40 and 100keV. However the short half-life of the source meant that it required regular replacement which was expensive. Since the late 1980s, radionuclide sources have been almost totally replaced by DXA, using the same principles but with an X ray source. Thus DXA is essentially a form of DPA.

As far as dual X ray absorptiometry and its development for measuring bone is concerned, the spectrum of photon energies is necessarily compressed into two main energy peaks, which can be done in two ways.

The more common is by means of a "K-edge" filter of a rare earth material (usually Samarium or Cerium) because of electron interaction. The alternative is by pulsing (at mains frequency) the X ray tube between sequential measurement points, while internal calibration at each point is achieved by a rotating filter wheel, through which the beams pass. The latter approach is used in the Hologic QDR 1000 W bone densitometer used in the present study, which has mean energies of 43 and 110keV at 70 and 140kVp respectively.

As photons pass through the subject's tissues, physical interactions occur involving absorption and scattering, generally referred to as attenuation. For homogeneous tissues or other substances, fractional lowering of the beam intensity depends on the substance's linear attenuation coefficient and path length according to the formula

$$I = I_0 e^{-\mu/L}$$

where

I is the resultant intensity of the beam,

I_0 is the incident intensity of the beam

e is the base of natural logarithms

μ is the substance's linear attenuation coefficient

L is the path length

Because linear attenuation coefficient is density (ρ) dependent, a convenient practice when working with tissues which differ in density is to calculate the mass attenuation coefficient μ_m as μ/ρ (Pietrobelli et al., 1996). This removes the physical density dependence of the linear attenuation coefficient.

For heterogeneous absorbers, such as human soft tissues, transmitted photon intensity is related to the substance's fractional mass by the following formula:

$$I = I_0 e^{-fi.\mu m.M}$$

where

M is the mass of the absorber

f_i is the fraction of the i th component of a heterogeneous absorber.

With DXA and many other radiographic methods, "pixel" area is constant and known, so that M represents total mass of the system's volume element or "voxel". For heterogeneous absorbers like human soft tissues, the transmitted photon intensity is related to a substance's fractional mass.

The magnitude of the mass attenuation coefficient is smaller at the higher energy, indicating less photon attenuation. When photons at two different energies are passed through human soft tissue, attenuation at the lower energy can be expressed as a ratio (R) to attenuation at the higher energy. R is thus a function of the mass attenuation of each tissue component and the mass of that component.

Extensive research has produced well documented attenuation coefficients for the abundant elements in the human body (Hubbell, 1969 and 1982; Rao, 1975; White et al., 1980). Because the chemical components of the various molecules which form living soft tissue can be accurately estimated, R values for the molecular constituents of the living body can be calculated independently of direct measurement. Table 2.1 gives an indication of the different R values for the different substances, although the precise R value will depend upon the selected energies.

Table 2.1 R values of different body substances

Fatty acids and Triglycerides	1.20 - 1.22
Protein	1.29
Glycogen	1.30
Water	1.35
Extracellular fluid	1.37
Intracellular fluid	1.39
Bone mineral	2.86

Experiments have shown (Pietrobelli et al., 1996) that predicted and measured R values differ by 1 - 1.5%. Deviations in theoretical and measured values can be expected for several reasons, many of which can be corrected for by machine manufacturers.

Beam Hardening occurs as polyenergetic X-ray photons pass through tissue and low energy components are attenuated more rapidly than higher energy components (Goodsitt, 1992). Because the resulting beam intensity is computed from path length as an exponent of initial beam intensity, the physical size of the subject (or more specifically, thickness) affects the measurements. While it is possible for software to correct for size variation, it is likely that at the extremes of subject size, accuracy is poorer.

Because the output of DXA involves three components - bone mineral, lean tissue and fat, and the underlying methodology allows resolution of only two components, DXA machines resolve the problem as follows. R values are computed for every pixel in the scan of the entire body. Bone-containing pixels will have much higher composite R values than non-bone-containing pixels. A threshold of R is set whereby bone containing pixels are separated from non bone containing pixels. The actual value of this threshold of R will depend upon the measured body composition and bone density (Gotfredsen et al., 1984) and a complicated algorithm which uses histogram analysis of R values, iteration and image processing. The latter involves assumptions that bones are of a minimum size to ensure Calcium deposits elsewhere are not mistaken for bone, and procedures for the delineation of bone boundaries.

In order to calculate the fraction of the fat component f1 and the lean component f2 in soft tissue, it is important to recognise that the R value of the soft tissue will be proportional to the mass fraction and attenuation coefficient of each component according to the formula:

$$R = f1xR1 - (f2xR2)$$

because the sum of f1 and f2 is unity, and thus

$$f1 = (R-R2)/(R1-R2) \text{ and } f2 = (R1-R)/(R1-R2)$$

These equations can be 'validated' by measuring pure fat and pure lean tissue samples in addition to calibration standards.

Estimating the soft tissue composition in bone-containing pixels is more complex and reliant on assumptions, most particularly concerning the distribution of the various tissues in the body. Later revisions of software (e.g. Nord and Payne 1995) include a weighted tissue distribution to justify this methodology. There are problems associated with the method by which DXA predicts soft tissue composition, due to several factors. These include the non-constant composition of adipose and lean tissue (Pawan and Clode, 1960), the variable distribution of fat throughout the body, the variable thickness of subjects and thus path length of X-ray travel, and the variation in all of these with the age of the subject.

Manufacturers have recognised these problems by releasing software which attempts to account for fat distribution, but the models on which they base these are unpublished for commercial reasons. There is little doubt that they are based on animal evidence, phantoms, and perhaps limited measurements of human cadavers. Evidence that fat distribution models 'expect' certain distributions stems from the production of different results following repositioning of the same phantom in different regions of the scanning table. With the Hologic scanner of the present study, the special region of interest facility which examines the tissue in a highlighted region of the body, applies a special correction for which body segment it belongs to. Nord and Payne (1995) acknowledged in their fat distribution model used for Norland machines, the amount of fat nearer the bone was assumed to be less than that farther away from bone, and the algorithm was weighted accordingly. Each of the three major manufacturers of DXA machines (Hologic, Lunar and Norland) applies its own algorithms to calculate bone density and soft tissue composition, which explains why systematic differences are observed between manufacturers (Tothill et al., 1994a and b).

The use of DXA in body composition studies

Dual energy X-ray absorptiometry (DXA) is the gold standard method for the assessment of bone density (Fogelman and Blake, 1998). Since its introduction in the late 1980s, such an endorsement for body composition measurement was deemed premature (Kohrt, 1993; Roubenoff et al., 1993; Laskey, 1996), although improved calibration and precision in more recent years, together with improved technical and software standards, have eliminated much of the scope for its earlier criticism.

Prior to DXA, soft tissue composition had been developed from DPA using Gadolinium-153 (Gotfredsen et al., 1986; Mazess et al., 1984; Wang et al., 1989). As with DXA, calculated R values were computed for every pixel in the scan of the entire body, and software algorithms assigned appropriate quantities of bone, lean and fat tissue. Tissue masses in bone-containing pixels (approximately 40% of the scanned area in most adult humans) were calculated according to models with assumed distributions of fat. These have undergone several revisions since the introduction of DXA, seeking to reflect with increasing accuracy the true pattern throughout the body, for instance assuming that the ratio of lean to fat increases linearly with the proximity to bone in "soft tissue shells" (Nord and Payne, 1995).

DXA, in its standard pencil beam mode, predicts different soft tissue compositions according to different manufacturers and different software versions. The three principal manufacturers - Lunar, Norland and Hologic use different X-ray energies, different calibration procedures and standards, however only those from Lunar, relating R value to % fat has been published (Hansen et al., 1993). While it is established that men and women have different fat distributions, and that age has a centralising effect on fat patterning, these variations have not been incorporated into the fat distribution models to date.

Tothill et al., (1994a and b) investigated soft tissue composition using phantoms and volunteers on machines by all three manufacturers. While the precision was similar, there were significant mean differences in total fat and regional fat, especially in the trunk. Several studies have done cross-

calibration work with different machines, scan mode or software versions (Abrahamsen et al., 1995; Clasey et al., 1997; Economos et al., 1997; Van Loan et al., 1995), or produced correction equations to convert one manufacturer's results into another's (Modlesky et al., 1996). However, to maximise accuracy and precision, DXA is perhaps best viewed as a range of techniques specific to each manufacturer. Particularly for comparison with other techniques, conclusions drawn from DXA studies are peculiar to the scanner type and software version.

Precision, accuracy and validity of DXA measurements

DXA has good precision compared with other techniques. Short-term precision is normally evaluated by repeated scans and calculating the intra-individual standard deviation, or coefficient of variation, CV (SD/mean expressed as a percentage). Mazess et al., (1990) showed precision error SD of 1.2% for total fat for 12 subjects with mean fat content of 21%. A similar value was obtained by Hansen et al., (1993) for 104 subjects, but smaller precision errors are found by investigators with subjects with greater total adiposity (Jensen et al., 1993). CV values are greater for regional fat than total fat, and are greater for fat mass than bone-free lean mass (Mazess et al., 1990; Fuller et al., 1992a).

DXA has been shown to be accurate by comparison with more sophisticated 3 and 4 compartment models, and by comparison with chemical analysis of animal carcasses. Using the Lunar DPX, Mitchell et al., (1996a) showed agreement within 0.4% fat between DXA and the chemical analysis of 48 pigs, with correlation of $r = 0.99$ between the two methods. Svendsen et al., (1993) investigated the in vivo accuracy of DXA in pigs, finding an accuracy (Standard Error of the Estimate (SEE) of DXA estimating chemical composition) of 2.9%, despite homogenisation of carcasses being incomplete.

DXA has been validated against a 4 compartment model based on densitometry where body density is corrected for bone mineral and water content - possibly the most accurate *in vivo* technique available. Using the Lunar DPX, Bergsma-Kadijk et al., (1996) found fat differences (mean $\pm$ 1SD) of $3.1 \pm 1.8\%$ and $5.3 \pm 3.8\%$ in young and old women respectively. By contrast, Friedl et al., (1992) found the

difference to be only 0.4 ± 1.2 % in 10 young soldiers, and found the precision error of underwater weighing to be double that of DXA. Fuller et al., (1992a) found the difference to be 1.4% in a group of 28 healthy adult men and women using a Lunar DPX scanner, and Goran et al., (1998) using a similar machine found DXA to be stable across a broad range of adiposity, with mean differences of 0.3% in elderly men and women, while densitometry was found to be less accurate in older women. In a three compartment comparison (bone mineral, fat and fat-free lean tissue), Fogelholm et al., (1996) found the Norland XR 26 to over-estimate % fat in 34 young women by 7.3 ± 0.8%. In what is the most comprehensive study to date, Prior et al., (1997), using a Hologic QDR 1000W and densitometry assessed body composition in a sample of 172 college men and women, including 111 athletes and mixed ethnicity. The mean DXA - 4 compartment % fat difference was 0.4%.

The study of Prior et al., (1997) provides a justification for DXA's use as a criterion method in preference to densitometry. In their sample DXA predicted body fat % by the 4C method with greater accuracy (Standard error of the estimate and total error 4C-DXA: 2.8% and 2.9% v 4C – densitometry 3.4% and 3.6% respectively). While it is possible to argue that DXA and densitometry each have distinct advantages over the other (Nord and Payne, 1995), in the case of some athletes, the variable density of fat-free mass (Adams et al., 1982) renders densitometry unsuitable as a reference method. The greater bone density which may be anticipated in athletes may be a primary source of the variation of the density of fat-free mass, but DXA's measurement of body composition will be largely unaffected by bone mineral, and less affected than densitometry by fluctuations in body water (Prior et al., 1997). In addition to its advantage in terms of accuracy, DXA may be better than densitometry for subject acceptability because it removes the need for water confidence, which may enable more individuals to participate.
This would offer considerable justification for future studies using DXA as a reference method for body composition, especially those which, like the present work, use the Hologic QDR 1000W scanner and software.

Applications and use of DXA

Since its establishment as the reference method for bone investigations, development of appropriate soft tissue algorithms and software has enabled DXA also to play an increasing role in the field of body composition. In this capacity, much work has compared it to other methods of fat estimation in healthy human subjects (Clark et al., 1993; Forslund et al., 1996; Pierson et al., 1995; Stewart et al., 1993; Wellens et al., 1994). Due to the location of most scanners in major hospitals, it is not surprising that a substantive body of literature has developed regarding different clinical applications, including diabetic patients (Kistorp and Svendsen, 1997; Rosenfalack et al., 1995), anorexics (Hannan et al., 1993) hemodialysis patients (Woodrow et al., 1996; Hart et al., 1993; Formica et al., 1993; Horber et al., 1992) and AIDS patients (Ma, et al., 1996). With its ability to assess regional body composition, DXA has been used for studies of fat patterning (Allemann et al., 1993; Milliken et al., 1997; Nindl et al., 1996; Stewart et al., 1997; Zamboni et al., 1997) and weight change (Fogelholm et al., 1997; Lands et al., 1996; Orphanidou et al., 1997). Increasing attention is being given to using DXA to predict muscle mass (Fuller et al., 1992b; Fuller et al., 1996; Wang et al., 1996; Madsen et al., 1997).

In addition, DXA has been used to assess body composition in adults of differing ethnicity (Cote and Adams, 1993: Mazzetti et al., 1997; Tsunenari et al., 1993), children (Boot et al., 1997; Ellis, et al., 1996; Goran, 1997; Gutin et al., 1996; Manzoni et al., 1996; Ogle et al., 1995; Pintauro et al., 1996), and the elderly (Snead et al., 1993; Treuth et al., 1994).

Snead et al. (1993) measured age-related differences in body composition by measuring 113 women and 72 men both by hydrodensitometry and DXA (Hologic QDR 1000/W). The subjects were grouped into three age cohorts (21 –39; 40 – 59; and > 60 years). While DXA and hydrodensitometry agreed in the young cohort, the differences in % fat from the two methods were significant ($P < 0.05$) at the older age groups. This was more noticeable at the oldest age group, and correction for the diminished bone mineral content measured by DXA failed to explain the difference. In a separate experiment to investigate fat

distribution, nine subjects were scanned with exogenous lard packets of 2 - 3 kg overlying the trunk or thigh. The extra fat was 96% detected on the thigh, but only 55% detected on the trunk, and the authors concluded that DXA underestimates trunk fat, and thus the age-related increase in upper body fat distribution. However, a more recent study using lard placed on the trunk of subjects using updated software was 96% detected by DXA (Kohrt, 1998).

DXA shows good agreement with other methods for predicting body composition. In particular, the Lunar DPX scanner agrees well with underwater weighing in men and women of a wide age range (Wellens et al., 1994). Cote and Adams (1993), also using a Lunar DPX, demonstrated the variability in predicted % fat from densitometry arising from the difference in bone density between black and white women. This highlighted the limitations of the two-compartment methodology, and concluded that for comparison, a four-compartment method was superior which accounted for the race-dependent difference in bone content.

DXA has been used to track the changing body composition during the growth of pigs during weight gain of 62kg (Mitchell et al., 1996b), with validation of final tissue composition by chemical analysis not being significantly different from DXA values ($P > 0.05$). The application of DXA to determine bone mineral and soft tissue composition in domestic animals is becoming more widespread, and is summarised by Grier et al. (1996).

Inter-manufacturer differences have been shown to produce errors, under or over-estimating true fat values in children (Ellis et al., 1994). While there appears to be little variation in the hydration of the fat-free mass (FFM) in normal adults older than 20 years (Scholler, 1989), the FFM of children has more water, less protein and less minerals than that of adults (Lohman et al., 1984; Slaughter et al., 1984; Boileau et al., 1984; Fomon et al., 1982). Pediatric software is available from all manufacturers to enable DXA to assess soft tissue in children, principally by detecting bone at a lower threshold R value. Variation in hydration of the body can affect DXA measures because pure water is detected as roughly

92% lean soft tissue and 8% fat, but fluctuations in hydration induced by hemodialysis produce wide fluctuations in fat-free mass by BIA, while FFM (DXA) reduction was highly correlated with the ultrafiltrate ($r = 0.98$, $P < 0.001$) (Abrahamsen et al., 1996)

DXA has also been compared with more sophisticated medical imaging techniques. Tothill et al., (1996) compared DXA (Norland XR 26), underwater weighing and MRI in healthy women, and found high correlations but poor agreement between the methods. Svendsen et al., (1993b) measured 25 women using DXA and computerised tomography (CT), finding a valid comparison ($r = 0.9$, SEE = 7% fat). Jensen et al., (1995) found the combination of DXA and a single CT slice to be an excellent predictor of CT-derived intra-abdominal fat mass ($r = 0.98$, $P < 0.001$). However, DXA totals all body lipid by mass, whereas MRI and CT record volumes of adipose tissue. Although these more sophisticated methods hold the potential to be reference methods, both the lipid fraction of adipose tissue and measurement artifacts contribute to the variable image density. Analysis involves the setting of thresholds in a subjective manner. For these reasons, and the prohibitive cost of pursuing validation studies with larger numbers of subjects, the reference method for validating DXA is considered to be the 4-compartment method of fat mass, and fat free mass corrected for water and BMC content.

More recently athletes have been investigated (Eckerson et al., 1997; Morris and Payne, 1996; Nichols et al., 1995; Prior et al., 1997; Stubenitsky et al., 1997; Van Marken Lichtenbelt et al., 1995).

Eckerson et al., (1997) measured 18 American football players using the Hologic QDR 2000, comparing the results to BIA, densitometry and near infra-red interactance. DXA proved to be the most accurate in estimating the densitometric result showing a SEE of 2% and total error of 2.1%. However, some doubt must rest on the authors' assumption that the densitometry two compartment model is worthy of being the reference method in this population, in view of the evidence from the alteration of the density of the FFM in Canadian professional football players (Adams et al., 1982).

Morris and Payne (1996) investigated the seasonal fluctuation of body composition in male (n = 12) and female (n = 6) lightweight rowers using DXA and anthropometry throughout the competitive year. Dietary manipulation was surveyed by questionnaire at various regattas throughout the year. FFM was maintained via exercise and the required competitive weight was made by reduction in fat evidenced by DXA and subcutaneous adipose tissue by skinfolds. This study confirmed the ability for skinfolds to detect fat loss in this population.

Nichols et al., (1995) investigated the relationship between regional body composition to bone density in female athletes in collegiate basketball, volleyball, gymnastics and tennis (n = 46) and controls (n = 12). Female athletes of various sports were found to have greater BMD than controls, although no significant differences were found between the various sub-groups. Lean tissue mass was found to be a better predictor of BMD than fat mass, although leg fat mass showed a significant correlation with leg BMD.

Stubenitsky et al., (1997) examined 11 male body builders using DXA (unspecified make), Underwater weighing (UWW) and anthropometry. They found no significant relationship between the % fat results of UWW and DXA, and explained the lower predicted fat content by UWW by the increased BMD. Correcting for the BMD improved the correlation between the UWW 2 compartment and 4 compartment methods. No data were presented as to how body water content was measured.

Van Marken Lichtenbelt et al., (1995) investigated physical activity, body composition and bone density in ballet dancers (n = 24). They used UWW, bromide dilution to estimate extracellular water, and DXA (unspecified model) to produce a 4 compartment model, and found differences between the various fat predictions were related to the total BMD, which was higher than expected due to the weight-bearing nature of dance activity. This finding is at odds with the larger study of Prior et al., (1997) involving 111 athletes of a variety of sports, which found % fat differences between DXA and the 4 compartment model to be unrelated to BMD, race, athletic status or musculo-skeletal development.

DXA is becoming progressively widespread in its use as a reference method of body composition in the pediatric population. This may be due to the increased availability of scanners, together with the ease of subject compliance, fan beam technology reducing scan times and the benefit of deriving bone, lean and fat data in a single measurement session.

Goran et al., (1996) used DXA to assess the effectiveness of skinfold equations and BIA in assessing body composition of 49 girls and 49 boys. They concluded that the existing equations to predict fat mass may be inaccurate, and presented alternative equations using DXA fat as the criterion measure for the predictions. De Lorenzo et al., (1998) used a similar approach to design a prediction for FFM in 35 children of a wide range of adiposity. Height^2 / impedance explained 89% of the variation in FFM derived from DXA, increasing to 96% when body mass was added ($r = 0.96$; SEE = 1.0 kg). Pietrobelli et al., (1998) constructed equations predicting DXA-derived fat mass and % fat from age and BMI in 198 children. R^2 for fat mass and % fat was 0.85 and 0.63 in boys and 0.89 and 0.69 in girls respectively.

To date, few, if any studies in the UK have investigated body composition in athletes using DXA with the exception of the present study and its pilot work (see appendix). Nor have any used DXA as the criterion measurement for soft tissue mass prediction. This work is the summary of data collected between September 1995 and October 1998 on male and female athletes representing a wide variety of sports, and controls.

2.2 ANTHROPOMETRY

Anthropometric measurements of the human body include mass, heights, skeletal breadths, circumferences and skinfolds. These relatively simple measurements can be used in their own right to describe body size, and also be used in various ratios and indices, used in formulae validated by other methods to predict tissue masses, body proportions, desirable weight ranges and somatotype. They

provide an inexpensive and convenient means of obtaining a large amount of information, which is the basis of a considerable body of knowledge regarding body composition. The measures require a minimum of equipment, but a high degree of technical skill. Precision of measurement is assessed by the scatter of replicate measurements made on different subjects on separate testing occasions, or by different recorders. These measures include the reliability coefficient (r), intra and tester error, coefficient of variation (CV%), and technical error of measurement (TEM), expressed as an absolute or a percentage value. Calculations of reliability by these methods are included in the appendix.

Skeletal Breadths

The simplest measures are of mass and of skeletal breadths, which require application of the anthropometer to the appropriate bony landmarks before the measurements are made. An anthropometer is simply a rod of fixed length, which allows sliding blades to move along it, projecting in a perpendicular direction. The Holtain sliding beam anthropometer used in the present study has an accuracy of 0.1cm. The accuracy of breadth measures is likely to depend more on the correct sites being measured, and data on the reliability of the breadth measures used in the present study is scarce, and is not comprehensive across both gender and all age groups. In particular, breathing artifacts affect chest breadth (and depth) measurements, and in athletic subjects, precise siting of the acromial processes can be problematic due to the development of the superior deltoid muscles. A list of measurement sites and reported reliabilities appears in table 2.2, while precise techniques are described in chapter 3.2.

Ratios involving mathematical relationships between measured anthropometric variables include *body mass index* (BMI), *ponderal index, skelic index* and *chest index*. BMI, also known as the Quetlet Index after its pioneer Adolph Quetelet (1796-1874), is the mass in kg divided by the square of the height in m. BMI correlates only moderately well with skinfolds ($r = 0.50$), corrected girths ($r = 0.52$), and bone breadths ($r = 0.51$) in a large population study, explaining fatness only 13% better than pure chance (Ross, 1999). While BMI is used extensively in epidemiological studies and in establishing risk for life assurance, considerable scope exists for failing to discriminate genuinely robust and muscular individuals

from those who are merely overweight. The less common alternative is the *ponderal index*, which is the height divided by the cube root of mass, which, because mass is tri-dimensional and height uni-dimensional, is more logical. The *skelic index* refers to the sitting height: leg length, and the *chest index* refers to the chest depth expressed as a percentage of chest width. Reliabilities of skeletal breadths are illustrated in table 2.2.

Table 2.2 Reported reliabilities of skeletal breadths

Breadth	Reliability	Technical Error	References
Biacromial		0.29cm	Friedlander et al., 1977; Martorell et al., 1975
Chest	CV 1.9%	0.49cm	Cameron, 1978; Carter and Ackland, 1994
Bicristal		0.38cm	Chumlea et al., 1984; Friedlander et al., 1977
Bifemoral	r=0.99		Martin, 1986
Bimalleolar	r=0.97	0.92mm	Himes and Bouchard, 1985
Biepicondylar		0.1cm	Malina, 1968
Bistyloidus	r=0.99		Martin, 1986

CV = coefficient of variation; r = test-retest reliability coefficient; Source of data: Lohman et al., 1988; Carter and Ackland, 1994

Because the size of the adult skeleton does not vary substantially in response to physical training, subsequent physique development is primarily concerned with varying fat and muscle masses. Thus, the skeletal dimensions of athletes can provide useful information regarding self-selection for specific sports.

Skeletal breadth data is used to predict skeletal mass, and used in scaling variables known as "phantom z values", which describe the departure from a theoretical unisex phantom based on a large database (Ross and Ward, 1986) and scaled to the height of the subject.

Limb and Torso Circumferences

Circumference measurements record cross sectional data in the limbs and torso. Together with skinfolds, circumference data can predict relative muscularity, adiposity and fat patterning. Corrected circumference (measured circumference minus the skinfold) have been used to predict the area of muscle plus bone, with the assumption of a cylindrical or conical model for body segments, as summarised by Brodie (1998).

Such models probably simplify the true pattern to an extent, because the cross sections of body segments are not perfectly circular. Furthermore, MRI evidence from this study and others illustrates that the thickness of overlying fat is highly variable, and therefore the location of the skinfold used to correct the circumference has a large effect on the corrected measurement which is used to predict muscle mass.

The accuracy of the measurement of circumferences depends upon several factors, including tape location along the body segment, tape orientation (i.e. at 90 ° to long axis of segment), tape tension, tape stretch, and errors due to parallax affecting recorded readings. Several torso measures are affected by breathing artifacts, and all are influenced by the ability of the subjects to relax (or tense) their muscles according to the precise requirements of the protocol. It is also possible that extremes of hydration as a result of extracellular fluid volume variation affect limb measurements, and more obviously, the contents of the gut may affect torso measurements. These errors have been minimised in the present study by strict attention to measurement procedures, the use of tension-correcting tape (Novell, Rockton, Illinois, USA), and the subjects avoiding exercise on the day of the measurements and arriving for measurements a minimum of two to three hours post prandial, hydrated and voided. Reliability data on the sites measured appear in table 2.3.

Table 2.3 Reliability of circumference measurements

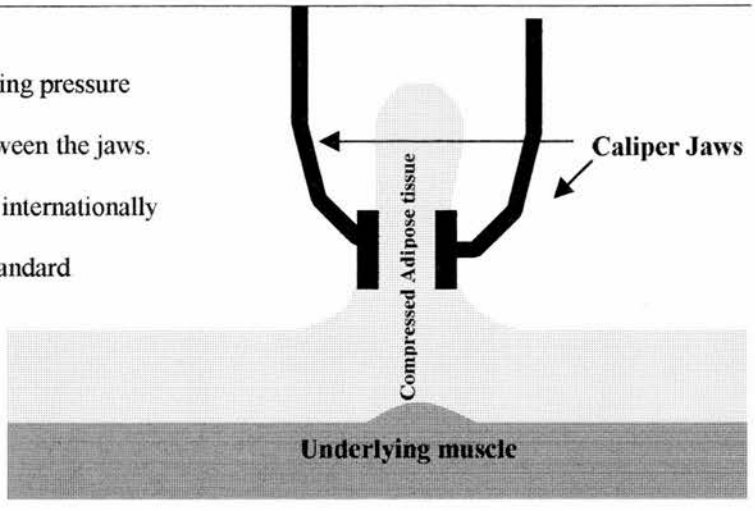
Circumference	Reliability	Technical Error	References
Chest	r=0.94-0.99	0.83cm	Weltman and Katch, 1975; Carter and Ackland, 1994
Waist		0.48-1.56cm 0.59cm	Malina et al., 1973; Chumlea et al., 1984
Abdomen	r=0.99		Wilmore and Behnke, 1969
Hip		1.23-1.38cm	Malina et al., 1973
Thigh		0.33cm	Carter and Ackland, 1994
Calf		0.20cm	Carter and Ackland, 1994
Upper arm		0.35cm	Carter and Ackland, 1994
Upper arm flexed		0.22cm	Carter and Ackland, 1994
Forearm		0.17cm	Carter and Ackland, 1994

Source: Lohman et al., 1988; Carter and Ackland, 1994

Skinfolds

Skinfolds are raised portions of subcutaneous adipose tissue which are used to represent body fatness (see figure 2.1). They are useful measurements in their own right, and can also be converted into % fat (see tissue mass predictions) by applying a series of assumptions.

Figure 2.1 Schematic cross section of a skinfold



The caliper operates with a fixed closing pressure applied across the area of contact between the jaws. Although caliper design has not been internationally standardised, the accepted industry standard adopted by most researchers, and by ISAK (International Society for the Advancement of Kinanthropometry), is the Harpenden Caliper, (British Indicators Ltd., Luton, UK). Two things affect the accuracy of the caliper's ability to measure: the compressibility of the spring, and the calibration of the dial. Both these are checked before release by the manufacturers, with an estimate of approx. 30,000 compressions for the life of the springs. A new instrument was used in and uniquely for the present study, and has been used for approximately 9000 skinfolds throughout the course of data collection.

The skinfold is a raised fold of subcutaneous adipose tissue plus skin, rolled between the thumb and the forefinger of the technician. The fold is drawn out to approximately 2cm from the 'resting surface' level, and with the fold still held by the technician, the jaws of the calipers applied. The spring pressure exerts a force on the skinfold in inverse proportion to the jaw area, and in response, the adipocytes compress, and extracellular fluid and blood is squeezed from the area. The compression is rapid until about two to four seconds, and the reading is normally taken as the reading stabilises, although further compression still

occurs at a much slower rate. The author made all the skinfold measurements throughout the study, and had 20 years' experience of the technique on over 2000 subjects.

Provided the skinfolds are measured with technical competence using a calibrated instrument, according to standard procedures, there remain various assumptions which govern the validity of skinfolds:

1. The compressibility of adipose tissue is constant within individuals and between individuals.
2. Skin thickness and compressibility is constant within individuals and between individuals.
3. Skinfolds record only subcutaneous adipose tissue plus two skin thicknesses, and no muscle tissue.
4. The variation in skinfolds arising from variation in extracellular fluid volume is negligible.

Further assumptions which limit the accuracy of the conversion of skinfolds into % fat are discussed under Tissue Mass Predictions.

Despite these shortcomings, skinfolds have been shown to be sufficiently reproducible to be valid, and the declared technical error of measurement according to ISAK is 5% of the skinfold value. Reliability data for sites used in the present study appear in table 2.4.

Tissue Mass Predictions

Skeletal Mass

The prediction of the skeletal mass uses formulae from Matiegka (1921) and Drinkwater et al., (1986). Such predictions are for wet bone mass, designed to predict the mass of the skeleton from linear breadth data using cadaver dissection as the reference method. While this may be the most accurate reference method available, their subjects were generally elderly and few in numbers and because their resulting predictions take no account of the reduction in bone density with age, younger adults' bone mass is commonly under-predicted as a result. These predictions were compared with total BMC as measured by DXA in chapter 4.3.

Table 2.4 Reliability of skinfold measurements

Skinfold	Reliability coefficient (r)	Intra tester error (mm)	Inter tester error (mm) or r value	Technical Error	References
Chest (pectoral)	0.96; 0.91-0.97		r=0.93		Pollock et al., 1975; 1976; Pollock, 1985
Triceps		0.4-0.8mm	0.8-1.89mm	0.39mm	Johnston et al., 1974; Johnston and Mack, 1985; Malina and Buschang, 1984; Carter and Ackland, 1994
Subscapular		0.88–1.16mm	0.88–1.53mm	0.35mm	Lohman, 1981; Wilmore and Behnke, 1969; Sloan and Shapiro, 1972; Johnston et al., 1972; Carter and Ackland, 1994
Abdominal	0.98	0.89mm		0.67mm	Wilmore and Behnke, 1969; Zavaleta and Malina, 1982; Carter and Ackland, 1994
Mid thigh	r=0.99	r=0.91-0.98	r=0.975	0.64mm	Pollock et al., 1976; Wilmore and Behnke, 1969; Zuti and Golding, 1973; Pollock, 1986; Carter and Ackland, 1994
Biceps				0.2-0.6mm	Meleski, 1980; Zavaleta, 1976
Medial Calf		r=0.94-0.99		0.46mm	Carter and Ackland, 1994
Axilla		1.22mm	0.64mm		Lohman, 1981; Chumlea and Roche, 1979
Suprailiac and Suprailium	r=0.970	0.3-1.0mm		1.7mm	Wilmore and Behnke, 1969; Buschang, 1980; Meleski, 1980; Zavaleta and Malina, 1982; Haas and Flegal, 1981;

Data source: Lohman et al., 1988; and Carter and Ackland, 1994; These data represent the majority of the sites measured in this study. References are in chronological order by column.

Muscle Mass

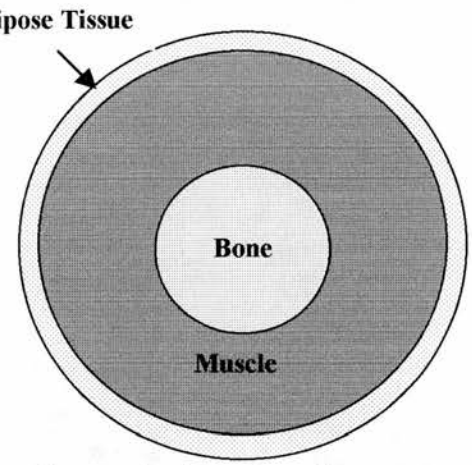
The conversion of corrected circumferences into predicted muscle mass also relies upon cadaver dissection data. Matiegka (1921) pioneered this work, producing a prediction based upon three cadavers, and this was more recently validated by Drinkwater et al., (1986) using 13 female and 12 male cadavers from the Brussels cadaver study. Martin et al., (1990) have produced an alternative prediction which is the most commonly cited, using data from the 12 male cadavers, but a slightly different selection of

circumferences. All three predictions show a high correlation, but a systematic difference between measurements. The theoretical model for the cross section of a limb is shown in figure 2.2. If the cross sectional area is assumed to be circular, then the muscle + bone area is given by the formula:

$$\text{Muscle + bone area} = \pi[(C/2\pi)-(SF/2)]^2$$

where C is the circumference and SF is the skinfold in cm.

Figure 2.2 Schematic cross section of a limb



It is worthy of consideration that these predictions were made on elderly subjects, who were probably sedentary, and may have suffered from a pathological condition which influenced muscle mass before death. The predictions of athletic subjects may be less valid where the training of specific muscle groups may lead to spectacular gains in some circumferences and not in others. For example, in the present study cyclists and runners have large leg muscles, but only modest upper body muscles, while kayakers and climbers have the opposite development. It could be argued, however, that in order to achieve significant upper leg or upper arm muscle development, it is necessary for the muscles to exert force on the torso, and thus be stabilised by synergistic muscles on the trunk which exhibit a commensurate development. This hypothesis was explored further using DXA to weigh limbs and torso separately (chapter 5.2).

Fat Mass

It is logical that the subcutaneous fat depot, the body's largest by some considerable margin under normal circumstances, may be representative of total body fat. Should the proportion of total fat situated on the

surface be constant over a wide range of adiposity, the opportunity for % fat predictions validated against other methods (commonly densitometry) is great, and over 100 such prediction equations have been produced to date, the majority of which are summarised in Brodie (1988). The equations in most common use tend to be large studies based upon validation against a two- compartment model, using subjects whose age and adiposity varies widely. Several population-specific equations have been produced for the young, elderly, obese, anorexic, and for both ethnic and sports-specific groups. For athletes, the adaptation of the body to exercise may render such general equations invalid (Sinning, 1985) however, three published equations were used in the present study for both male and female subjects (Durnin and Womersley, 1974; Jackson and Pollock, 1978; Jackson et al., 1980).

The compliant nature of subcutaneous adipose tissue in young, lean or athletic subjects makes the use of calipers relatively straightforward, although many assumptions influence the validity of converting caliper measurements into % fat.

1. The selected site is representative of subcutaneous fat topography
2. Subcutaneous fat is representative of total body adiposity
3. The lipid fraction of adipose tissue is constant across various levels of adiposity
4. The reference method of assessing total body adiposity is accurate.

Tissue mass ratios can provide a more sensitive index to discriminate between groups or to track change in one individual over time than tissue masses alone. Indices used include muscle : skeletal ratio (Carter and Ackland, 1994) and muscle : fat ratio (Hawes and Sovak, 1994). In this study, DXA allows, in addition to these, the BMC : total mass ratio, and various limb : torso ratios of fat, lean and bone mineral. While these ratios may provide useful information in discriminating between different athletes or between athletes and controls, the error in ratios is the combined error of both single measurements.

Somatotype

Somatotype is a technique for describing human physique, which uses the methods of skeletal breadths, girths, skinfolds and various ratios. It is concerned with body shape and form, and is independent of body size. Its origins relate to work by Kretschmer in 1921 who suggested that any human body was an amalgam of three end forms of variation or 'poles' and to Viola, who, in 1933 expressed the thoracic and abdominal circumference ratios to a 'normatype' (Duquet and Carter, 1996). Sheldon (1940) used both these approaches to assess 'genotypic' morphology in a triaxial scale of endomorphy or relative fatness, mesomorphy or musculo-skeletal robustness, and ectomorphy or relative fragility. Any individual could be represented by three indices which described his relative position on the somatochart between the three poles, for instance 3.5 - 4.0 - 2.5. His work was based on photography and subjective ratings and has subsequently been quantified mathematically by several authors and applied to women subjects as well as men, the most popular and versatile being the Heath-Carter Somatotype method (Heath and Carter, 1967; Carter and Heath, 1990) which uses height, mass, four skinfolds, two circumferences and two skeletal breadths. This 'phenotypic'method enables physiques to be less constrained by artificially imposed qualitative boundaries, which had previously been imagined to be the limits of possible variation.

The relevance of somatotyping is best exhibited by the wide variation of physiques of athletes of different sports, which may share similar indices of BMI or % fat. A bodybuilder may be a 1 - 9 - 1, a gymnast 1-6 - 2 and a distance runner 1 - 3 - 5. All such individuals could have a fat content of 5%, but show a spectacular difference in body shape and form. There is considerable variation in physique exhibited by subjects in the present study from sports such as bodybuilding, marathon running, kayaking, rowing and cycling.

2.3 BIOELECTRICAL IMPEDANCE ANALYSIS (BIA)

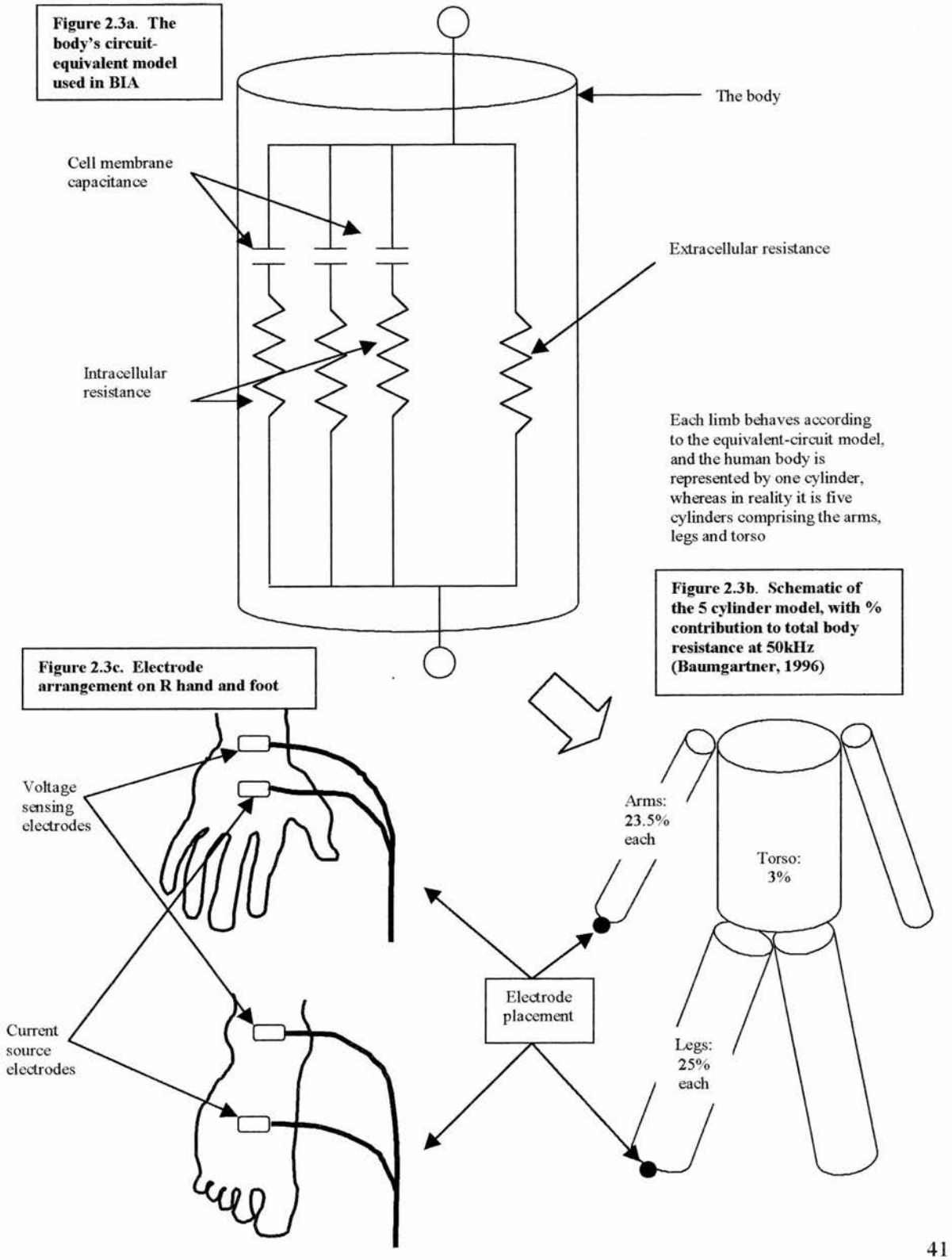
Bioelectrical Impedance is a technique pioneered in the 1960s involving a low level of alternating current passing through the body (Thomasett, 1962; Hoffer et al., 1969). Measured impedance enables the water content of the body to be predicted, and this in turn enables fat and fat-free mass predictions, if hydration coefficients are assumed (Nyboer, 1981).

Impedance (Z) is the frequency-dependent opposition of a conductor to the flow of an alternating current comprising Resistance (R) and Reactance (X). Resistance is the pure opposition to the flow of current in a conductor and is the reciprocal of conductance. Reactance is an additional opposition to electrical flow, and may be inductive (X_l) or capacitive (Xc). In the human body at the frequency normally used for BIA, inductive reactance is negligible but capacitive reactance arises due to momentary charge storage, mostly in cell membranes. Varying the frequency of the alternating current passing through the body affects the path it takes, and the relationship between R and X changes. At low frequencies (<1kHz) current passes primarily through the extracellular fluid, while at higher frequencies (500-800kHz) it penetrates all cell membranes, passing through the intracellular fluid in addition (Lukaski, 1987). The most frequently used current is 500-800μA at 50kHz (as in the present study - see methods chapter 3.3), and several generalised prediction equations have been produced to predict % fat (e.g. Deurenberg et al., 1990; Gray et al., 1989).

In electrical terms, the BIA method assumes the body can be represented by a single cylinder which contains resistive and capacitive elements in series/parallel. Charge storage causes the voltage to lag behind the current and the delay in voltage (expressed as the phase shift) is quantified geometrically as the Arctan(Xc/R). If the circuit is purely resistive, the phase angle will be zero, and if it is purely capacitive, it will be 90 °. BIA analysers measure impedance directly, and phase shift (or phase angle) to divide this into resistance and capacitance. The phase angle in the human body has been estimated to be between 8

and 15 ° at 50 kHz (Baumgartner et al., 1988). Figure 2.3 summarises the schematic view of the body these principles involve.

Figure 2.3 Schematic representation of bioelectrical impedance measurement in the body



At 50kHz, and at lower frequencies, impedance is determined much more from resistance than capacitance, and some predictive equations ignore capacitance altogether. With uniform conductivity, R is proportional to the conductor length, and inversely proportional to the cross-sectional area. In a substance of uniform composition, the resistance (in Ω) can be predicted by the formula:

$$R = \rho L^2 . V^{-1}$$

where ρ is the specific resistivity (in ohm.cm), L is the conductor length (cm) and V is the volume in cm^3. At a constant frequency such as 50 kHz, conductivity in the human body is mainly via ions dissolved in body fluids (Ackmann and Seitz, 1984). The resistivity varies according to fluid volume, and inversely both with ionic concentration (Khaled et al., 1988) and temperature (Geddes and Baker, 1967). Between regions of high conductivity, polar proteins, lipids and tissue interfaces all show poorer conductivity (Baumgartner, 1996).

While the biological tissues of the body are considerably more complex than the underlying theory assumes, athletes may violate the assumptions to a greater extent than sedentary individuals. Assumptions appear in italics below.

The body can be represented by a cylindrical model, in electrical terms, of a measured length and uniform cross sectional area. In a practical sense, the body can be simplified into not one, but five cylinders, comprising the arms, legs and torso (see figure 2.3b). It is estimated that arms and legs are 8% and 34% of total body mass respectively, yet account for 47% and 50% of the total resistance measured (Baumgartner, 1996). Conversely, the trunk may account for 46% of body mass, and have little effect on measured resistance (Baumgartner, et al., 1989).

Impedance (Z) to current flow is directly proportional to its conductor length (i.e. body height) and inversely related to its cross sectional area. Proportionality of limbs is assumed and inter-electrode separation may incompletely represent height in subjects with long arms. Resistivity has been shown to be much greater at 90 ° to muscle fibre orientation, than parallel to it (Rush et al., 1963). While the limbs

contain muscles with longitudinal fibre orientations which can be crudely modelled, the torso contains muscle fibres which run in a variety of directions.

Specific resistivity does not vary among body segments despite differences in tissue type, hydration levels and electrolyte concentration (Kushner, 1992). Muscle fibre direction, together with the lungs, internal organs and visceral fat, means the specific resistivity is 2 to 3 times more variable in the torso than the limbs (Chumlea et al., 1988). Hydration levels are assumed to be constant at 0.732 of FFM for healthy adults (Baumgartner, 1996), although a range of 0.69 – 0.77 has been reported (Streat et al., 1985). Exercise-induced dehydration has been shown to alter capacitance relative to resistance (Kanai et al., 1987), and it is recommended that for subjects whose hydration state varies, prediction equations are selected which include reactance (Baumgartner et al., 1990).

Athletes have different soft tissue distributions to the non-exercising controls used to develop the bioimpedance technique. Athletes have greater total muscle, proportionately greater limb muscle, less total fat and proportionately less torso fat than non-exercising controls. Resistivity of the arms in particular could be dramatically reduced through upper body exercise, while that of the torso may be increased in obesity due to an increased torso fat proportion, after adjusting for differences in size (Chumlea et al., 1993). It is likely that athletes also have a greater water flux than non-athletes of the same mass. The functional adaptation to control temperature during exercise results in greater fluid loss than that of non-exercisers, and a slight dehydration may go unnoticed by subjects, yet may be great enough to influence the results in BIA. A departure of 0.04 from the assumed hydration level would produce a commensurate variation of 5.5 % fat in a typical athletic subject in the present study.

Standardised procedures for measuring bioimpedance include refraining from exhaustive exercise for 8 – 12 hours, avoiding alcohol, coffee or other diuretic substances for a similar period, fasting for at least 2 hours, voiding the bladder before measurement (Kushner, 1992).

The BIA method has been validated against the Total Body Water technique (Hoffer, et al., 1969) and has shown to be a reliable indicator of hydration in clinical settings. The popularity of the technique has been largely due to the convenience of its performance in clinical settings without compromising patient privacy, and the fact that compared with other techniques, it requires minimal skill of the technician. However, at both extremes of fatness, BIA predicts fat content less well than in the mid range (Gray et al., 1989).

2.4 TOTAL BODY POTASSIUM (TBK)

The first chemical analyses of fluids drawn from the living human body in the mid-19th century revealed a chemical homeostasis which was altered under conditions of disease. 100 years or so later, the advent of nuclear chemistry allowed noninvasive chemical assays of the human body. Much detection work centred around nuclear installations and possible contamination of personnel concerned, but in the late 1950s, work measuring the body's own K^{40} radiation established a correlation with fat-free mass (Kulwich et al., 1958) which was later used to predict the body's fat-free mass in humans (Anderson and Langham, 1959; Forbes et al., 1961).

The theoretical principle of K^{40} counting is that naturally occurring potassium in the body exists in three isotopes, whose abundance is known. These are K^{39} (93.1%), K^{41} (6.9%) and the radioactive K^{40} (0.0118%). The latter involves 1.8×10^{18} radioactive atoms per gram of K.

Nuclear physics' fundamental law states the number of disintegrations (dN) per unit time (dt) is given by:

$(dN/dt) = N\lambda$ where N is the number of atoms and λ is the decay constant.

The half life of K^{40} is 1.3×10^9 years, which translates into 1.8×10^3 disintegrations per minute per gram. Of these events, 11% of the decays produce γ-rays, which are of high energy and can be counted when they exit the body. Because of the length of the half-life of K^{40}, corrections for physical decay of the source are unnecessary.

If the average K content of an adult male is in the region of 140g, (Forbes and Lewis, 1956), the K^{40} content is about 15mg. This produces about 30,000 γ-rays per minute. (Female K^{40} contents are proportionately smaller in most cases). These can be monitored by NaI detectors placed close to the subject, although substantial shielding is necessary to eliminate background radiation, which increased substantially as a consequence of global fall-out following atomic bombs dropped during World War II, and, more particularly, after subsequent nuclear weapons testing. Steel production since 1945 has thus been contaminated, and detectors using steel require its manufacture to have pre-dated this time. In the present study, the shield uses 12 tonnes of steel, the source of which was German World War I battleship "*Prinz Wilhelm*" scuttled in Scapa Flow. Amplified detector pulses are recorded via multi channel analyser or computer software.

A background value of K^{40} is normally obtained by scanning a phantom comprising water-filled polythene containers in an anthropomorphic arrangement to replicate the body's own attenuation. Further sensitivity correction can estimate exact size and distribution patterns using orally-administered K^{42} (with a half life of 12.4 hours) although this procedure is somewhat rare due to its limited availability and only modest improvement in accuracy. The difference between the background and subject scans allows the prediction of FFM from established formulae (see methods, chapter 3.7) and subtraction from total mass to derive fat mass.

2.5 MAGNETIC RESONANCE IMAGING (MRI)

MRI represents a low energy window through which it is possible to examine the internal structure of the human body. (Fullarton, 1982). In the early 1950s, the interaction between certain nuclei and radio waves was first discovered, and analysis was produced by early devices referred to as nuclear magnetic resonance spectrometers. Since the development of the Fourier transformation – a mathematical process changing the description of a function into frequency from space or time coordinates (or vice versa), the method has rivaled infra-red spectroscopy in molecular fingerprinting. It was not until the late 1970s that whole body scanners were available for clinical use, and the expense of the equipment has limited its availability to major hospitals and research institutions. Its principal utilities of detecting tissue variation invisible to X-rays and yet being a non-invasive technique have ensured its continued development and increasingly widespread use in clinical medicine today.

Physical bodies possessing charge and spin produce a magnetic moment, which has direction and magnitude. When such a body is placed in a magnetic field, it aligns accordingly. Nuclei with odd numbers of protons and neutrons have such a net spin, and therefore will produce a magnetic moment.

Magnetic force depends on magnetic field strength, and is measured in tesla units (T).

Any nucleus possessing a magnetic moment will attempt to align itself with the ambient magnetic field. This causes the spinning nuclei to precess about the direction of the applied magnetic field with a characteristic angular frequency ω_0 determined by the magnetic field strength and a characteristic magnetogyric constant unique to each nuclear species.

When a nucleus with a magnetic moment is placed in a magnetic field, the energy of the nucleus divides into lower (parallel) and higher (antiparallel) energy levels. Photons of specific energy (frequency) can excite a nucleus from the lower energy state to the higher one. Such energies fall in the radio wave portion of the electromagnetic spectrum. The nucleus will spontaneously revert to the lower energy state in time, releasing radio wave photons according to two decay constants, $1/T_1$ and $1/T_2$. T_1 is the relaxation time characterising the interaction between the nucleus and the lattice environment. At equilibrium, a nucleus experiencing a static magnetic field is preferentially oriented along the magnetic field. Perturbation of the low energy state results in a reversal of that magnetic field involving transfer of energy from the lattice to the nucleus. Returning to its equilibrium via exponential relaxation, energy is transferred from the nucleus back to the lattice, and the nucleus reverts to its original orientation. T_2 is the relaxation time characterising interactions between the nucleus and neighbouring nuclei. Perturbation (re-orientation of the nuclei away from the magnetic field) causes nuclei to precess – initially in phase with one another. Spin-spin interactions cause some nuclei to speed up and others to slow down, resulting in a de-phasing of the nuclei. T_1 is always greater than T_2. In solids T_1 is long, while T_2 is short, while in liquids, T_2 approaches T_1.

In order to measure net magnetisation and relaxation time, a pulsed radio frequency (RF) signal is applied at right angles to the static magnetic field axis, with a frequency equal to the Larmor frequency. The RF signal induces perturbation by rotating the net magnetisation away from the static magnetic field, inducing an electric signal in a receiving coil (free induction decay or FID). The FID oscillates at the Larmor frequency and has a magnitude in direct proportion to the density of nuclei at the measuring site. The FID – a time varying function - is converted via the Fourier transform into its frequency components.

Varying the time parameters of the RF pulse (Time to repeat, TR, and time to echo, TE) can increase the contrast between specific tissue types, such as bone and muscle, or muscle and adipose tissue. In one RF sequence referred to as spin – echo, the TR parameter is adjusted to maximise the difference in T_1 relaxation between muscle and adipose tissue, and is used for the majority of analyses of these tissues.

A complicated procedure involving gradient fields which alter the net magnetic moment and therefore ω_0 with position results in each point having data for density, T_1 and T_2. The gradient fields allow the spatial discrimination necessary for image reconstruction. The MRI image matrix comprises 256 rows x 256 columns to form each pixel. MRI pixel values for a given tissue vary between individuals, and between different locations in the same individual. Heterogeneities in the magnetic field can cause variation in the pixel intensity, and can appear as 'ghosting' in images. Recent advances in hardware and software can minimise this problem, but the requirement for images to be manually corrected or verified, means that the MRI system for measuring tissues is unlikely to be fully automated. Point-counting, using the Cavalieri method remains a convenient method for estimating tissue volumes deduced from separate slices where automated analysis is excessively costly or time consuming (Roberts et al., 1993).

MRI has been used as a reference method for body composition analysis. Ross et al., (1994) reported that whole body lean tissue from MRI can be predicted from anthropometric variables with an accuracy of 3.6% in obese men and 6.5 % in obese women. Predictors for men were mass, together with waist and thigh circumferences, while in women, predictors were mass and hip circumference. Adipose tissue for the whole body was predicted using waist and hip circumferences in women, and these in addition to thigh circumference in men, with an accuracy of about 8%. The observations of Martin et al., (1994), highlighting the wide variation in the density and lipid fraction of adipose tissue, have led to the belief that such error precludes its use as a reference measure of total body fat (Deprés et al., 1996). However, because the greatest health risks are associated with visceral fat (Deprés et al., 1990), much work has focussed on the anthropometric prediction of visceral fat (Deprés et al., 1991) or the relationships between fat distribution and disease. In women, various anthropometric factors explained 74% of the variance in visceral adipose tissue area (Ferland et al., 1989), and age and waist circumference was equally predictive in men (Deprés et al., 1996). Due to the expense of MRI scanning, and clinical pressure for its use, investigations involving athletes are extremely rare.

CHAPTER 3

METHODS

This chapter describes the subjects, permissions and data handling for the study, together with procedures for all the techniques utilised throughout the course of the study.

3.1 SUBJECTS

Subjects were drawn from The University of Edinburgh and the wider athletic community. The principal study using DXA and anthropometry was of 106 male athletes and 30 female athletes recruited by poster advertisement in the University sports centre. A typical appointment session was set at one hour, to interface with the existing computerised booking system for the DXA scanner. In this time, the DXA scanning took approximately 25 minutes, BIA 5 minutes and anthropometry 30 minutes. Control data were gathered synchronously on both males and female volunteers. This pool was extended by including subjects who had undertaken body composition analysis by anthropometry as part of exercise and conditioning advice. A further study of the upper leg using MRI was undertaken using 8 males and 2 females, and TBK was performed on 6 males. Female subjects were asked to complete confidential questionnaires on menstrual history and a seven day dietary recall of a typical training week, the latter forming part of a separate study beyond the scope of this work. Archived data on skinfolds was used from the Fitness Assessment and Sports Injury Centre database, and DXA scans of the whole body and lumbar spine were used from the Medical Physics database of female control and anorexic subjects. A summary of the subjects and studies is contained in table 3.1.

Table 3.1 Subjects and studies

Method	Category	Male Athletes	Male Controls	Female Athletes	Female Controls
DXA	Measured	106	23	30	30
DXA	Archived	0			30 Anorexics
BIA	Measured	82	12	24	
BIA	Archived				30 controls
BIA	Archived				30 Anorexics
Anthropometry	Measured	143			65
TBK	Measured	3	3	0	0
MRI	Measured	5	3	2	0
Exercise history	Measured	143	15		65

3.2 ETHICAL PERMISSION AND CONSENT

Ethical permission was available generically for standard procedures in the Department of Medical Physics for which the risk to subjects is minimal. This included TBK, BIA and DXA. However, specific ethical approval was obtained for each study. Upon appropriate application and clarification of radiation dose (see appendix), such permissions were granted by Lothian Health subcommittee for healthy volunteers in separate applications for female athletes and for male athletes and controls. These permissions were extended by special ruling of the chairman of the ethics committee to enable MRI scans to be performed on 10 individuals. Written, informed consent was obtained by all subjects prior to measurements being made.

3.3 DATA HANDLING AND STATISTICS

Data were collected and entered into Excel 7.0 spreadsheets, before being processed by SPSS software version 7.5 and 8.0, and also Medcalc 4.16. A variety of statistical procedures were used, including descriptive statistics, Pearson correlation, Regression analysis (including stepwise and backwards elimination), paired and independent t-tests using Bonferroni's correction for multiple tests, one way ANOVA using the post-hoc Student-Newman-Keuls test, and MANOVA. Statistical power for certain tests was calculated according to the method outlined in Cohen (1992). The $P < 0.05$, 0.01 and 0.001 levels of significance were selected to describe the relationships between the variables.

3.4 DUAL X-RAY ABSORPTIOMETRY

Athletic subjects were scanned wearing T-shirt and shorts (some controls preferred to wear hospital gowns provided) and all were asked to remove watches and jewelry. All were measured for lumbar spine (L1-L4) bone density and total body bone density and body composition by dual X-ray absorptiometry using a Hologic QDR 1000W scanner (Hologic Inc, MA, USA), and subsequently analysed using software versions 4.47 and enhanced version 5.55 respectively. For the lumbar spine scan, the subject's lower legs were supported by a block of radio-transparent material to ensure the spine was flat during measurement. Because many subjects were taller than average, the maximum allowable scan area was used universally to ensure the L1 – L4 region was captured completely within the scanned region. This scan took about 4 – 5 minutes. Separate calibration using the manufacturer's spine phantom was performed every day by technical staff.

For the whole body scan, the subject lay supine, feet approx. 20cm apart, and arms at the side of the torso. A tissue calibration bar was placed adjacent to the right lower leg of the subject. For very tall subjects (taller than 185 cm), the feet were splayed to the corners of the scanning table, to minimise knee bend, and secured by a board which prevented the feet plantar-flexing which ensured the toes remained within the scanned area. For a very few extremely tall subjects (taller than 190 cm), knee bend was essential to include all body tissue within the scanned area. The subject remained silent until the scanning arm had passed over the head, after which the questionnaire regarding exercise history since childhood, sporting involvement, competitive level and training regime was administered. The scan usually took between 14 and 16 minutes. Repeated scan measurements for investigating precision involved the subject getting up from the table before repositioning.

Scans were analysed in the same systematic way according to the manufacturer's protocol, by dividing the anatomical sub-regions of head, left and right arms, left and right ribs, thoracic and lumbar spine, pelvis,

left and right legs. Sub-regional analysis was performed using different regions, to match the anatomical region of the upper legs used in the MRI study and to investigate which sub regions best reflected overall body composition. Separate investigations of knee bend, leg abduction were performed on the author and a staff member experienced in the use of the DXA scanner.

3.5 ANTHROPOMETRY

This includes mass, skeletal heights and breadths, body segment girths, and skinfolds. All of these measures were made according to standard procedures included in the Anthropometric Standardization Manual (Lohman et al, 1988), or the Anthropometric Definer Manual (Hawes and Soucie, 1993). All measurements were made by the author, an experienced anthropometrist and a full member of ISAK (International Society for the Advancement of Kinanthropometry). In accordance with established procedures measurements of limbs were made on the right side of the body in all cases. In the instance of left handed subjects whose sports were asymmetric, girths were measured on the left side. All subjects wore running shorts and either a vest or a loose fitting T-shirt. Female subjects were measured in pairs, or with another female present, and no measurements required the removal of this clothing.

Body Mass

Mass was measured using a Soehnle Digital S scale (CMS Weighing Equipment Ltd., London, UK) to 0.1kg. Subjects removed footwear and were weighed wearing T-shirt and shorts only. Watches and jewelry were removed.

Skeletal Heights

Stature (total height) was measured using a standard wall-mounted stadiometer (Seca) to 0.5cm. The subject placed the heels together and was instructed to stand as tall as possible, while looking straight

ahead. The moving arm of the stadiometer was lowered until it made contact with the highest point on the subject's skull while in the Frankfort plane (the orbitale and tragion in a horizontal line).

Sitting height was recorded using a Harpenden sliding beam anthropometer (Holtain Ltd., Crymmych, UK) to the nearest 0.5cm, with the subject sitting upright on a hard horizontal surface, with the feet unable to touch the floor. The subject was instructed to 'sit tall' with the head in the Frankfort plane and if necessary, gentle traction was applied to the mandible to ensure the upper body was straight. In some instances, the portable stadiometer was unavailable, and the wall mounted device was used with the subject sitting against it, with knees bent to 90°.

Iliospinale height was measured either with the wall mounted device or Harpenden anthropometer, from the floor vertically up to the most anterior point on the anterior superior iliac spine. The bony projection which marks this point was located via palpation.

Supersternale height was measured by measuring the height of the sternal notch to the superior aspect of the skull with the head in the Frankfort plane using bone calipers to 0.5cm (Young & Sons, Edinburgh, UK), and subtracting this value from stature.

Skeletal Breadths

These were measured using the portable Harpenden anthropometer, and on occasions when this was unavailable, bone calipers (Young & Sons, Edinburgh, UK).

Biacromial breadth was measured to 0.5cm in the horizontal plane with the subject standing relaxed. The left and right acromiales were located by palpation along the spines of the scapulae to their most lateral and superior aspect, with the technician standing behind the subject. Firm pressure was used to ensure overlying soft tissue was compressed to prevent enlarged measurements in muscular subjects.

Bicristal breadth was measured to 0.5cm between the most lateral points on the superior border of the iliac crest. With the technician standing in front of the subject, firm pressure was applied over the iliac sites, with the calipers angled upwards at 45°.

Chest breadth was measured to 0.5cm as the horizontal diameter of the thorax at the level of the sixth rib. The subject stood upright with arms slightly abducted and was instructed to hold the position at the end of a normal expiration. The technician stood behind the subject and inserted the branches of the anthropometer underneath the axillae and a downward angle of about 30°. The technician ensured the anthropometer branches did not slide between the ribs. For muscular subjects, relaxation of the lattissimus dorsi was essential.

Chest depth was measured to 0.5cm using the olive-tip branches of the anthropometer. The subject stood upright, and was instructed to hold the position at the end of a normal expiration. The horizontal distance between the mesosternale (midway between the sternal notch and the xiphoid process) and the spinous process of the corresponding vertebra on the dorsal side or the body was recorded. Large pectoral and trapezius muscles made this measurement problematic in some subjects.

Biepicondylar humerus breadth was measured using a Vernier caliper (Mitutoyo, Japan) to 0.1cm. The subject raised the right hand, flexing the elbow to 90°, with the dorsum of the hand facing the technician. The caliper was applied at a slight angle to the epicondyles, because the medial epicondyle is distal to the lateral one. Slight pressure was applied to compress overlying tissue.

Bistyloidus breadth was measured using the Vernier caliper to 0.1cm as the distance between the most medial aspect of the ulnar styloid to the most lateral aspect of the radial styloid, with the dorsum of the hand facing the observer.

Biepicondylar femur breadth was measured to 0.1cm as the horizontal distance between the most medial and most lateral aspects of the femoral condyles. The subject stood with feet shoulder width apart and knees slightly flexed. The branches of the caliper were angled slightly downwards.

Bimalleolar breadth was measured to 0.1cm as the most medial extension of the medial malleolus to the most lateral extension of the lateral malleolus in a horizontal oblique plane. The subject stood with feet shoulder width apart, with weight evenly distributed on both.

Girths

Girths are circumferences of a limb or torso segment. In all cases, these were measured using a tension-correcting anthropometric tape (Rockton, Illinois. USA).

Maximum upper arm girth was measured to 0.1cm with the subject abducting the arm to the horizontal level. The circumference was measured at the insertion of the superior deltoid, perpendicular to the long axis of the arm.

Maximum forearm girth was measured to 0.1cm as the maximum circumference obtained perpendicular to the long axis of the arm with the arm abducted to the horizontal level. The tape was applied loosely and slid along until a maximum value was obtained.

Mid thigh girth was measured to 0.1cm as a horizontal circumference midway between trochanterion and the tibiale, with the subject standing in a relaxed manner, feet about 10cm apart. In a few subjects, additional thigh circumferences were made at superior (highest measurable horizontal circumference) and inferior (1 cm above the patellar border) locations, and at measured distances midway between these points and the mid-thigh location.

Maximum calf girth was measured to 0.1cm with the subject standing in a relaxed manner, with the feet about 10cm apart. The tape was slid vertically up and down the lower leg until the point of greatest horizontal circumference was located.

Chest girth was measured to 0.5cm at the level of the mesosternale, at the end of a normal expiration. Subjects stood in a relaxed manner with the arms abducted slightly, and were encouraged to relax, as measurements were made.

Flexed biceps girth was measured to 0.1cm with the arm flexed to 45° at the elbow, with the biceps brachii tensed. The measurement was made in a vertical plane as the maximum circumference obtained. It was noted that in order to tense the biceps, the brachialis muscle acts as an antagonist and is also under tension, also contributing to the magnitude of the reading.

Waist girth, defined as the smallest horizontal circumference between the thorax and the iliac crest was measured to 0.5cm. With the subject standing in a relaxed manner, the measurement was made at the end of a normal expiration, with the anthropometrist standing behind.

Abdominal girth, defined as the horizontal circumference taken at the maximum anterior extension of the abdomen, was measured to 0.5cm at the end of a normal expiration without compression of the subcutaneous tissues. Many subjects had flat abdomens, rendering this measurement hard to distinguish from the waist. Most commonly, this location was immediately inferior to the umbilicus.

Hip girth, defined as the horizontal circumference measured at the maximum extension of the buttocks, was measured to 0.5cm over the clothing worn by subjects, with the anthropometrist standing to the side of the subject, who stood in a relaxed manner (without tensing the gluteal muscles).

Skinfolds

Skinfolds were measured on all subjects using the same pair of calibrated calipers (Harpenden Ltd., British Indicators, UK) in a total of 19 sites. Measurements were made according to standard procedures, with the left hand of the technician raising a fold by placing the thumb and forefinger/middlefinger approximately 8cm apart and palpating as necessary to raise a fold. This fold was pulled approximately 2cm away from the main tissue, and the jaws of the calipers applied adjacent to the fingers and thumb, ensuring the sides of the fold were parallel. All readings were made 2-3 seconds after applying the calipers, to 0.1mm. No measurements were made if the skin was broken. On some occasions, oily skin required drying with paper toweling before measurements could be made to prevent the calipers sliding over the skin surface. In order to comply with patient acceptability, skinfolds were measured at all sites only twice at maximum (unless the two measurements were more than one millimetre different) with the mean entered. On those occasions when available time did not permit replicate measurements, only a single measurement was made on all 19 sites.

The reliability of the author's replicate measurements has previously been rated at $r = 0.98$, which equates to that of published reliabilities (in chapter 2). Applying the mathematical theory illustrated in Himes, (1989), little is gained by adding replicate measurements (as is the case for height, mass, skeletal breadths and girths) if the reliability is greater than 0.94. However, because the reliability is different at different sites, technical error of measurement was calculated at all sites by 36 paired measurements on male and female athletes on consecutive days. These data are summarised in chapter 4.1.

The cheek skinfold was measured at a slight angle under the lateral corner of the eye on the line connecting the tragus and the nostrils.

The chin skinfold was measured as a vertical fold below the hyoid bone, with the head lifted slightly, though not sufficient to stretch the neck.

Pectoral skinfolds were measured in different locations for male and female subjects. For males this was the mid-point between the anterior axillary fold and the nipple. For females, the site was immediately medial to the anterior axillary fold. This site was obtained with the subject pushing up the sleeve of her T-shirt, and using the left hand to pull the garment obliquely across the body.

The triceps skinfold was measured as a vertical fold midway between the acromion and the olecranon. Palpation of the skin was necessary in some individuals in order to be confident of including only subcutaneous adipose tissue and not underlying muscle.

The subscapular skinfold was measured in a 45° orientation along the natural cleavage lines of the skin, approximately 1cm below the inferior angle of the scapula.

The chest (thorax) skinfold was measured above the 10^{th} rib at the point where it intersects the anterior axillary line. The direction of the skinfold is close to the horizontal, parallel to the rib.

The supersternale skinfold (also referred to as the iliocristale skinfold) is taken 7cm proximal and medial to the spinale on the anterior axillary line, and inclined 45° to the horizontal, following the natural cleavage lines of the skin.

The abdominal skinfold was taken in the horizontal plane 3cm lateral to and 1cm inferior to the omphalion, with the subject standing.

The patella skinfold was measured vertically in the midsagittal plane 2cm above the superior border of the patella, with the subject standing in a relaxed manner.

The thigh skinfold was measured vertically at the midpoint between the trochanterion and the tibiale. In practice, this was most easily achieved by the subjects flexing the hip to define the inguinal crease, and

placing the edge of the hand in this fold, to determine the precise location of this skinfold. It was necessary to encourage some subjects to relax the quadriceps muscles for this measurement to be possible.

The proximal calf skinfold was obtained on the posterior aspect of the lower leg with the subject standing, but with the leg flexed very slightly. The vertical fold was raised approximately 5cm inferior to the fossa poplitea.

The mid-calf skinfold was taken posteriorly in the vertical plane at the level of maximal calf girth.

The biceps skinfold was taken on the anterior aspect of the upper arm in the vertical plane overlying the mid-point of the biceps muscle, midway between the anterior border of the acromion and the antecubital fossa. This measurement was made with the subject standing in a relaxed manner, with the hand directed anteriorly.

The forearm (mid) skinfold was measured in the vertical plane with the hand supinated. It was located at the mid-point of the anterior aspect of the arm, at the level of maximum forearm girth.

The forearm (radiale) skinfold was taken on the lateral side at the point of its greatest circumference.

The medial calf skinfold was measured with the subject placing the right foot flat on a chair, with the knee flexed to 90°. The vertical skinfold measurement was made on the medial aspect of the calf at the point of its greatest circumference.

The suprailium skinfold was measured as an oblique fold immediately superior to the crest of the ilium where it intersects the anterior axillary line.

The suprailiac skinfold was measured as a horizontal fold taken in the midaxillary line immediately superior to the iliac crest.

The axilla skinfold was taken as a vertical fold on the midaxillary line, at the level of the xiphoid process of the sternum.

Skinfolds and corrected girths were used with existing predictions of fat (Durnin and Womersley, 1974; Jackson and Pollock, 1978) and muscle (Martin et al., 1990).

Somatotype

Somatotypes were calculated according to the Heath – Carter method (Carter, 1980) used in *Anthropometric Definer* software version 1.5 (Hawes and Soucie, 1993). This involved some 10 of the raw anthropometric measures, subjected to the various mathematical transformations. Somatotype scores are calculated as follows:

$$\text{Endomorphy} = 0.1451X - 0.00068X^2 + 0.0000014X^3 - 0.7172$$

where X = Σ[triceps + subscapular + supraspinale] skinfolds in mm.

$$\text{Mesomorphy} = 0.858\ HB + 0.601\ FB + 0.188\ CUAG + 0.161\ CCG - 0.131H + 4.5$$

where H = height in cm

HB = humerus breadth in cm.

FB = femur breadth in cm.

CUAG = corrected upper arm girth (circumference – triceps skinfold in cm)

CCG = corrected calf girth (calf circumference – medial calf skinfold in cm)

Ectomorphy is defined on the basis of the reciprocal of the Ponderal index (PI),

$$\text{where } PI = H.M^{-0.333}$$

where H = height in cm

M = mass in kg

If PI > 40.75, ectomorphy = 0.732(PI) – 28.58; if PI < 40.75, ectomorphy = 0.463 (PI) – 17.63

3.6 BIOELECTRICAL IMPEDANCE ANALYSIS

Impedance measurements were made on the scanning table immediately following the DXA scan using a self calibrating RJL BIA 101 analyser operating at 50 kHz and 800 μA (RJL Systems, Detroit, USA) which provided separate measurements of resistance and reactance. The legs and arms of subjects were abducted to the maximum width of the surface. A tetrapolar electrode arrangement was used, with the electrodes placed on right limbs as follows: voltage-sensing electrodes were placed on the wrist midway between the styloid processes, and on the anterior surface of the ankle midway between the malleoli; source electrodes were located approximately 5cm distally from these sites, on the third metacarpo-phalangeal and third metatarso-phalangeal joints respectively. Sites were prepared by cleaning with an alcohol swab prior to application of self-adhesive electrodes (Bodystat Ltd. Douglas, Isle of Man, UK). The measured impedance was used to predict fat and fat-free masses measured by DXA, in addition to using equations derived on a six-centre combined sample (Lohman, 1992) and one recommended for use with male athletes (Lukaski and Bolonchuk, 1987).

$FFM = 0.485\ (ht^2/R) + 0.338\ (M) + 5.32$ (Lohman, 1992)

$FFM = 0.734\ (ht^2/R) + 0.116\ (M) + 0.096\ (Xc) - 3.152$ (Lukaski and Bolonchuk, 1987)

where FFM is the fat-free mass in kg; M is total body mass in kg; ht is height in cm; R is resistance in Ω; Xc is reactance in Ω.

Precision of impedance measurements is summarised in chapter 4.1.

3.7 TOTAL BODY POTASSIUM

The whole body potassium counter (Nuclear Enterprises type NE 8108) comprised four NaI (Tl) detectors 15cm across and 10cm thick, two above and two below a moveable couch. To screen out background radiation, low emission steel from a World War I battleship was used in blocks 15cm thick, with a total mass of 12 tonnes. In this shield, adjustable lead collimators defined the field of view of the NaI (Tl) detectors. Amplified detector pulses were digitised in a multi-channel pulse height analyser (Canberra series 35, Canberra Industries Inc. Meriden, CT, USA). The γ ray spectrum was transferred to a BBC computer for subsequent analysis.

Subjects were either fasted or 2 – 3 hours post prandial, fully hydrated and voided before being scanned. The protocol involved subjects wearing a hospital gown lying supine on the scanning table, which slowly passed between the detectors twice during the 40 minute scan. A background value had previously been obtained by scanning a phantom comprising water-filled polythene containers in an anthropomorphic arrangement to replicate the body's attenuation. The background-corrected count-rate in the subject spectrum was compared with that from an anthropomorphic phantom containing a known mass (1kg) of potassium. Further sensitivity correction to estimate exact size and distribution patterns using orally-administered K^{42} (half life of 12.4 hours) (see chapter 2.4) was not performed but a separate study established individual variation in potassium distribution increased uncertainty in a typical estimate by

only 0.5% (Hannan, 1990). A series of 10 measurements made on one control subject produced 142.0 ± 4.76 g potassium. The relationship of FFM to K used in the computer programme was

$$FFM\ (kg) = TBK\ (g) / 2.519$$

which is very close to the mean value of a review of literature (Boddy et al., 1973).

3.8 MAGNETIC RESONANCE IMAGING

The MRI scan was performed on a Siemens 42 SPE imager (Erlangen, Germany) operating at 1 tesla. A T_1 weighted sequence was used with repetition time, TR 570 ms, spin echo time TE 15 ms. The field of view was 500 x 500mm and the matrix was 128 x 256. The slices were 10mm thick, with a gap of 2mm between the slices. Each subject presented fasted or a minimum of 2 – 3 hours post prandial, and wore either a hospital gown or a T-shirt and shorts for the scan. The scan was performed axially from the neck to the feet, one region of the body at a time. The centre of the region was moved to the iso-centre of the magnet for scanning in order to obtain the most homogeneous images possible. The number of slices which could be scanned simultaneously varied with the mass of the subject because of radio frequency-induced heating (between 15 in the lightest subject and 19 in the heaviest subject). After each block of slices was complete, the subject was moved by a distance equal to number of slices + slice gap in order to provide contiguous images without overlap. The images were transferred to a Sun workstation and converted into a file format compatible with Analyze software (Mayo Foundation, Rochester USA). The images were matched with the region of the upper leg, and analysed using volume rendering for total tissue volume. The images were sampled for pixel density of muscle in several locations in several slices in order to determine the appropriate thresholds for discriminating different tissue types, which had the effect of screening out fat, bone and connective tissue where appropriate using a semi-automated process. Volumes were calculated automatically by system software for muscle tissue (including tendons), adipose tissue (excluding marrow), and for total upper leg volume, once appropriate thresholds had been set.

CHAPTER 4.

FINDINGS: VALIDITY OF DXA AND PREDICTION OF TOTAL TISSUE COMPOSITION USING DXA AS THE REFERENCE METHOD

The existing predictions of body composition in athletes are largely and justifiably based upon portable methods - anthropometry and bioimpedance. This chapter is concerned with placing existing predictions against DXA as the reference method, examining the predictor variables themselves, and then finding an optimum prediction of fat and of fat-free mass for this population. DXA reference data are also used to construct a 'morphotype' for body composition to describe variation between different athletic groups.

4.1 OBSERVATIONS ON VALIDITY OF DXA BASED ON EXPERIMENTATION

Intra-subject reproducibility

Because many reproducibility studies are performed on consecutive days or weeks, variation in the results represents the sum of the measurement error and the biological variation in the subject. This experiment involved one subject being scanned 8 times consecutively in a single testing session lasting approximately three hours, with repositioning before each scan. The subject consumed neither foods nor fluids during the testing session. %CV (the standard deviation divided by the mean, expressed as a percentage) is a measure of the precision error. Results for bone, lean, fat and regional totals appear in tables 4.1 – 4.4.

Table 4.1 Reproducibility measures for bone

	Mean	SD	%CV
Left arm BMC	206.1	3.7	1.8
Right arm BMC	205.6	5.5	2.7
Left leg BMC	637.3	6.0	0.9
R leg BMC	634.3	3.7	0.6
Torso BMC	850.4	9.3	1.1
Head BMC	426.1	6.0	1.4
Total BMC	2959.3	12.6	0.4
Left arm BMD	0.949	0.009	1.0
Right arm BMD	0.939	0.017	1.8
Left leg BMD	1.430	0.014	1.0
Right leg BMD	1.434	0.008	0.6
Lumbar spine BMD	1.093	0.018	1.7
Total BMD	1.262	0.005	0.4

BMC values are in g; BMD values are in $g.cm^{-2}$

Table 4.2 Reproducibility measures for lean tissue

	Mean	SD	%CV
Left arm lean	2983.6	26.1	0.9
Right arm lean	3256.9	27.2	0.9
Left leg lean	9402.3	120.5	1.3
Right leg lean	9583.8	77.9	0.8
Torso lean	29855.3	351.5	1.2
Head lean	3359.8	52.0	1.6
Total lean	58441.1	467.5	0.8

Tissue masses are in g.

Table 4.3 Reproducibility measures for fat tissue

	Mean	SD	%CV
Left arm fat	477.4	33.5	7.0
Right arm fat	448.3	34.2	7.6
Left leg fat	1743.8	116.7	6.7
Right leg fat	1675.4	53.3	3.2
Torso fat	3492.4	112.4	3.2
Head fat	715.5	10.5	1.5
Total fat	8552.0	286.5	3.4

Tissue masses are in g.

Table 4.4 Reproducibility measures for regional tissue

	Mean	SD	%CV
Left arm	3667.1	31.6	0.9
Right arm	3910.8	35.2	0.9
Left leg	11783.3	106.6	0.9
Right leg	11893.4	61.5	0.5
Torso	34198.0	272.1	0.8
Head	4501.4	64.4	1.4
Total	69952.5	215.0	0.3

Tissue masses are in g.

Inter-subject reproducibility

17 male athletes agreed to be scanned twice, with repositioning between the scans. Neither foods nor fluids were consumed during the testing session.

Table 4.5 Reproducibility of BMC in 17 male athletes

	Scan One		Scan Two	
	Mean	SD	Mean	SD
Left arm BMC	214.0	28.8	214.7	26.2
Right arm BMC	217.5	26.4	219.4	26.2
Left leg BMC	658.4	72.3	661.1	75.1
Right leg BMC	661.7	71.1	659.8	72.7
Torso BMC	863.1	102.2	864.2	99.6
Total BMC	3141.1	312.3	3141.1	312.2

BMC values are in g.

Table 4.6 Reproducibility of lean tissue mass in 17 male athletes

	Scan One		Scan Two	
	Mean	SD	Mean	SD
Left arm lean	3322	473	3315	474
Right arm lean	3507	466	3521	471
Left leg lean	10282	1447	10189	1470
Right leg lean	10322	1387	10165	1363
Torso lean	31616	2812	31821	2840
Total lean	62650	6028	62750	6218

Tissue masses in g.

Table 4.7 Reproducibility of fat mass in 17 male athletes

	Scan One		Scan Two	
	Mean	SD	Mean	SD
Left arm fat	572	263	584	282
Right arm fat	551	267	544	266
Left leg fat	2076	983	2036	954
Right leg fat	2124	1002	2130	1097
Torso fat	3429	2553	3485	2527
Total fat	9538	4820	9573	4892

Tissue masses in g.

Differences between successive scans were calculated for each tissue in arms, legs and torso, and compared with data from Fuller et al. (1992b) using the Lunar DPX scanner. These data appear in table 4.8. The reproducibility is broadly similar in both the present study and the study of Fuller et al. (1992b), which pooled data for 12 females and 16 males. The athletes of the present study were more muscular and less fat, which accounts for the differences in %CV where the SD is similar.

Table 4.8 Differences between duplicate measures in the present study and Fuller et al. (1992b)

	Present study n = 17		Fuller et al., 1992 n = 28	
	SD	%CV	SD	%CV
Arm fat	64	5.7	110	9.0
Leg fat	246	6.4	200	3.4
Torso fat	219	6.4	250	4.4
Total fat	390	3.0	420	3.0
Arm lean	156	2.3	150	2.8
Leg lean	425	2.1	230	1.2
Torso lean	382	1.2	240	1.0
Total lean	459	0.7	420	0.8
Arm BMC	13	3.0	10	2.0
Leg BMC	22	1.7	20	1.4
Torso BMC	16	1.9	20	2.0
Total BMC	28	0.9	30	0.9

All masses are in g.

Sub-regional analysis reproducibility

DXA software permits, in addition to the default analysis of the whole body as arms, legs, head, torso and pelvis, any designated area which is a multiple of its pixel dimensions (point size 1.3cm; resolution 0.2cm). The same 17 pairs of whole body scans were analysed for sub-regions covering the left upper leg using a fixed pixel area for each scan. The SD of the differences and %CV appear similar to regional reproducibility for whole body analysis, and appear in table 4.9. Paired T tests compared the results from each set of scans. No parameter showed any significant difference ($P > 0.05$). While the bone parameters remained remarkably constant, more variance was observed with the soft tissue masses. The mean difference in total mass was smaller than the mean difference for either fat or lean tissue separately. This suggests a relative sensitivity in the threshold for detecting lean from fat tissue which, to an extent, is compensated for when total tissue masses are compared between successive scans. However, because the body was repositioned between scans, locating the region of interest in precisely the same location varies in difficulty according to the extent of the difference between successive locations and the point size (see appendix for data on pixel size and point resolution).

Table 4.9 Differences between duplicate measures of a sub region

	Actual difference	SD of difference	%CV
BMC (g)	0.6	4.8	2.9
BMD ($g.cm^{-2}$)	0.0037	0.030	1.8
Bone area (cm^2)	0.18	3.2	3.1
Fat (g)	13.3	38.2	6.4
Lean (g)	20.6	139.5	3.0
Total (g)	10.1	164.4	3.0

The sub-regional analysis software permits the use of up to seven regions simultaneously, and an experiment was carried out to investigate the regional variation in fat by using slices (one pixel longitudinally) across the upper arm, upper leg, lower leg and torso in 33 male athletes representing the full range of adiposity of the athletes in the study. Percentage fat values for each slice are listed in table 4.10.

Table 4.10 % fat for DXA slices

Region	Mean	SD
Whole body	12.3	6.1
Abdomen (umbilicus)	15.1	9.8
Left Upper arm	20.6	11.0
Right Upper arm	18.8	9.3
Left upper leg	7.4	6.1
Right Upper leg	7.6	5.9
Left lower leg	10.8	5.6
Right lower leg	10.7	6.5
Weighted average for 7 regions	12.2	7.5

While there would be little reason to attempt to predict DXA fat from measurements which require a full body scan in any case, the examination of regional fat distribution allows an insight as to how the fat distribution model might have been constructed. In virtually all areas, regional fat SD was greater than the variation in total fat SD, and thus the error from a departure from the assumed variation in one place will be compensated for, at least to a degree, by a commensurate departure in another area.

The tissue distribution, in percentage terms, showed a significant difference in arm fat ($P < 0.01$), although this was mostly due to the increased lean tissue mass in the right arm (all subjects in this experiment were right handed). Left and right limb regions were summed, and total fat % correlated with upper arm, upper leg and lower leg slices. The results appear in table 4.11.

Table 4.11 Correlation of % fat by regional slices

	Total fat	Abdominal	Upper arm	Upper leg	Lower leg
Total fat	1.0	0.95	0.91	0.95	0.76
Abdominal		1.0	0.88	0.85	0.65
Upper arm			1.0	0.84	0.70
Upper leg				1.0	0.77

These slices were used to predict total fat % using stepwise regression analysis. The regression produced was as follows:

$$\text{DXA fat \%} = 0.322(\text{Abdominal fat \%}) + 0.518(\text{Upper leg fat \%}) + 3.592$$

$$(r^2 = 0.97;\ \text{SEE} = 1.07;\ P < 0.001)$$

These sites corresponded to skinfold sites which best predict total adiposity (chapter 4.2).

The reason that the full range of adiposity in athletes was selected for sub-regional analysis is because DXA fat distribution models constructed by the manufacturers will have been made to 'expect' subjects whose fat quantity and distribution lies within the normal range. Athletes are generally less fat than non-athletes, and at the extreme lean end of the spectrum of fatness, DXA may fail to detect any fat at all in some sub-regions. Of the 33 subjects, zero fat was detected in three individuals, all in the upper leg site. Based on the R value measured at the site in question, the software predicts a negative fat value which is compensated for by 'borrowing' from other regions in the final averaging calculation. The head is used for this averaging in the whole body software, and total adiposity outside the head was only 170g in one anorexic female. Her regions of predicted 'negative fat' were on one arm and on the torso. Even more bizarre is the scan of an anorexic male (subject one in chapter 5.1) whose entire body excluding the head was predicted to have –1.1% fat. Ironically, his bones were extremely dense, and because the bone-containing pixels are likely to represent a higher proportion in anorexic individuals, this will increase the error accordingly.

What is clear, is that at the extremes of body composition, DXA will lose accuracy, and that quantification of soft tissue masses in extreme cases is likely to be affected by bone. The fat distribution model for the whole body can achieve encouraging results by compensating 'errors' in individual distribution, but such a methodology breaks down as fat in specific regions approaches zero.

Normal procedures for scanning involve lying supine on the table with legs outstretched. This enabled individuals as tall as about 190 cm to be scanned with legs straight, but those taller than this were scanned with knees bent by as much as was necessary to maintain all body tissues within the area of scanning. In

such individuals, this will have had the effect of reducing the number of pixels of scanned tissue, and increasing the tissue represented by each. While this will have clear implications for BMD, the effect was quantified by investigating knee bend, with the popliteal surface of the knee 40 cm off the scanning table. This represents a significant exaggeration of the knee bent exhibited even by very tall subjects. Three trials were performed by two subjects with knees straight and with knees bent. Paired T tests were used to establish whether differences were significant. The results are summarised for the two subjects in table 4.12.

Experiments on a similar scanner to the one used in the present study showed tissue thickness or height to have a marked influence on X-ray attenuation at thicknesses exceeding 25cm which is interpreted as increased fat (Jebb et al., 1993). Such tissue thicknesses only occur in obese subjects, except if knee bend is significant. The portion of the subjects' knee and leg represents mostly lean tissue, and the greatest observed differences were for leg lean tissue. However, the trend for total detected mass to be reduced with knee bend suggests that in individuals who do not fit onto the scanning table, the approach of Prior et al., (1997) whereby two overlapping scans are made of the subject and subsequently merged, may be a more accurate solution.

Table 4.12 The effect of bending the knee on tissue mass (percentage change from supine)

	Subject 1 (40 yr. 17% fat)	Subject 2 (76 yr. 10% fat)
Total BMC	-1.7 *	-4.0**
Leg BMC	-7.3*	-10.4*
Torso BMC	+4.2	+3.3
Total lean	-2.0*	-2.8**
Leg lean	-9.2**	-8.9**
Torso lean	+0.3	no change
Total fat	+5.7	+13.3*
Leg fat	+12.9*	+26.5*
Torso fat	+0.3	+12.5
Total tissue mass	-0.5$^{\phi}$	-0.8$^{\theta}$

* $P < 0.05$; ** $P < 0.01$; $^{\phi}$ $P = 0.059$ $^{\theta}$ $P = 0.071$

Caution is necessary in interpreting the error attributable to knee bend because of the precision errors (%CV) listed in able 4.8. The fact that one elderly subject appeared to show greater changes may be due to his lesser stature and a greater knee bend necessary to achieve the same height. While these data suggest a substantial knee bend causes anomalies in BMC, lean and fat tissues, it is also possible that rotation of the pelvis affects the regional boundary between the leg and the pelvis which describes a different angular plane with bent knees.

The final validity check investigated by experimentation in the present study was the effect of varying foot angle on scanned tissue. The foot plate was secured in position and scans were performed with the medial surfaces of the feet parallel and at 90° divergent. Three trials were performed on one subject in each position. While there was a tendency for the abducted foot to be associated with greater lean and less fat tissue in the leg, the only parameter to show a significant difference was bone area ($P < 0.05$). The mean difference in area was only $5cm^2$ in each leg and the reason this figure appears so small is perhaps due to the effect of exposing more foot bone in abducted position which is, to a degree, compensated for by a transposition of the tibia and fibula. This indicates the consequences for bone or soft tissue masses of varying foot abduction are insignificant.

Precision of Bioimpedance and Anthropometric Measurements

Precision of the portable methods is commonly estimated by the technical error of measurement (TEM) which is defined according to the formula: $TEM = [\Sigma(x_2 - x_1)^2 / 2n]^{0.5}$

This is expressed as a percentage of the mean value of replicate pairs as $\%TEM = 100 \times (TEM / M_1)$ where x_1 and x_2 are replicated measurements, n is the number of replicated pairs and M_1 is the mean of the first measurements.

Precision of the RJL BIA 101 analyser was previously determined by replicate measurements on 16 healthy volunteers (archived data). TEM was 1.8Ω for resistance, and 1.3Ω for reactance, which represents a %TEM of 0.3% and 1.8% respectively.

Anthropometric measurements were assessed for precision by replicate measurements on 36 male and female athletes (representing the full range of adiposity of the sample) on separate days. Circumferences had %TEM of 0.9%, and skinfolds averaged 3.8%, ranging from 2.1% at the subscapular site to 6.5% at the suprailium site. These values are generally within the acceptable precision (1% for circumferences and 5% for skinfolds). Table 4.13 provides %TEM values for each skinfold site.

Table 4.13 Percentage Technical Error of Measurement in 36 male and female athletes

skinfold	%TEM
cheek	6.7
chin	4.8
pectoral	3.9
triceps	3.1
subscapular	2.1
chest (thorax)	5.4
supraspinale	2.8
abdominal	3.1
patella	2.9
thigh	3.1
proximal calf	4.2
mid-calf	5.2
biceps	5.5
forearm	3.6
forearm (radiale)	2.8
medial calf	2.5
suprailium	6.5
axilla	3.4
suprailiac	5.1

4.2 PREDICTION OF TISSUE MASS IN ATHLETES

Fat mass in male athletes

Of the 106 athletes who were measured using DXA and anthropometry, a total of 82 were measured for bioimpedance. Thus, BIA and anthropometry were compared in their ability to predict DXA fat by using these 82 subjects. Subsequent validation of anthropometric predictions used the remaining 24 athletes, and further predictions were made from the total sample of 106 athletes. Comparisons were also made using the 15 controls for whom complete anthropometric data were obtained.

Table 4.14. Physical Characteristics of Subjects

	Sample one (n = 82)		Sample two (n = 24)		Total sample (n = 106)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Age (yr)	28.1	7.5	29.0	7.0	28.3	7.4
Height (cm)	180.4	7.1	180.6	7.8	180.4	7.2
Mass (kg)	76.4	9.6	81.0*	9.2	77.4	9.6
BMI ($kg.m^{-2}$)	23.4	2.2	24.8**	2.5	23.7	2.3
% fat (DXA)	10.2	4.5	13.2**	5.4	10.9	4.9
Endomorphy	2.7	0.8	3.1*	1.0	2.8	0.8
Mesomorphy	4.9	1.2	5.3	1.1	5.0	1.2
Ectomorphy	2.7	1.0	2.2*	1.0	2.6	1.0
Training ($Hrs.wk^{-1}$)	9.3	4.8	8.8	4.6	9.2	4.7

* Different from sample one $P < 0.05$; ** $P < 0.01$

Initially, a comparison of predictions using published formulae was made. With respect to anthropometry, these were made by calculating body density (BD) as follows:

BD = 1.1631 – 0.0632log (biceps + triceps + subscapular + suprailiac) for ages 20 – 29 yr. (Durnin and Womersley, 1974)

BD = 1.1422 – 0.0544log (biceps + triceps + subscapular + suprailiac) for ages 30 – 39 yr. (Durnin and Womersley, 1974)

BD = 1.10938 – 0.0008267(pectoral + abdominal + thigh) +0.0000016 (pectoral + abdominal + thigh)^2 – 0.0002574(age) (Jackson and Pollock, 1978)

where skinfolds are in mm; and BD = body density, which was converted into % fat by the formula of Siri, (1956) : % fat = [(4.95/BD) – 4.5] *100

Bioimpedance equations were derived on a six-centre combined sample (Lohman, 1992) and male athletes (Lukaski and Bolonchuk, 1987). Fat mass was calculated by subtracting FFM from total mass and expressing the result as a percentage of total mass. The formulae were as follows:

FFM = 0.485 (ht^2/R) + 0.338 (M) + 5.32 (Lohman, 1992)

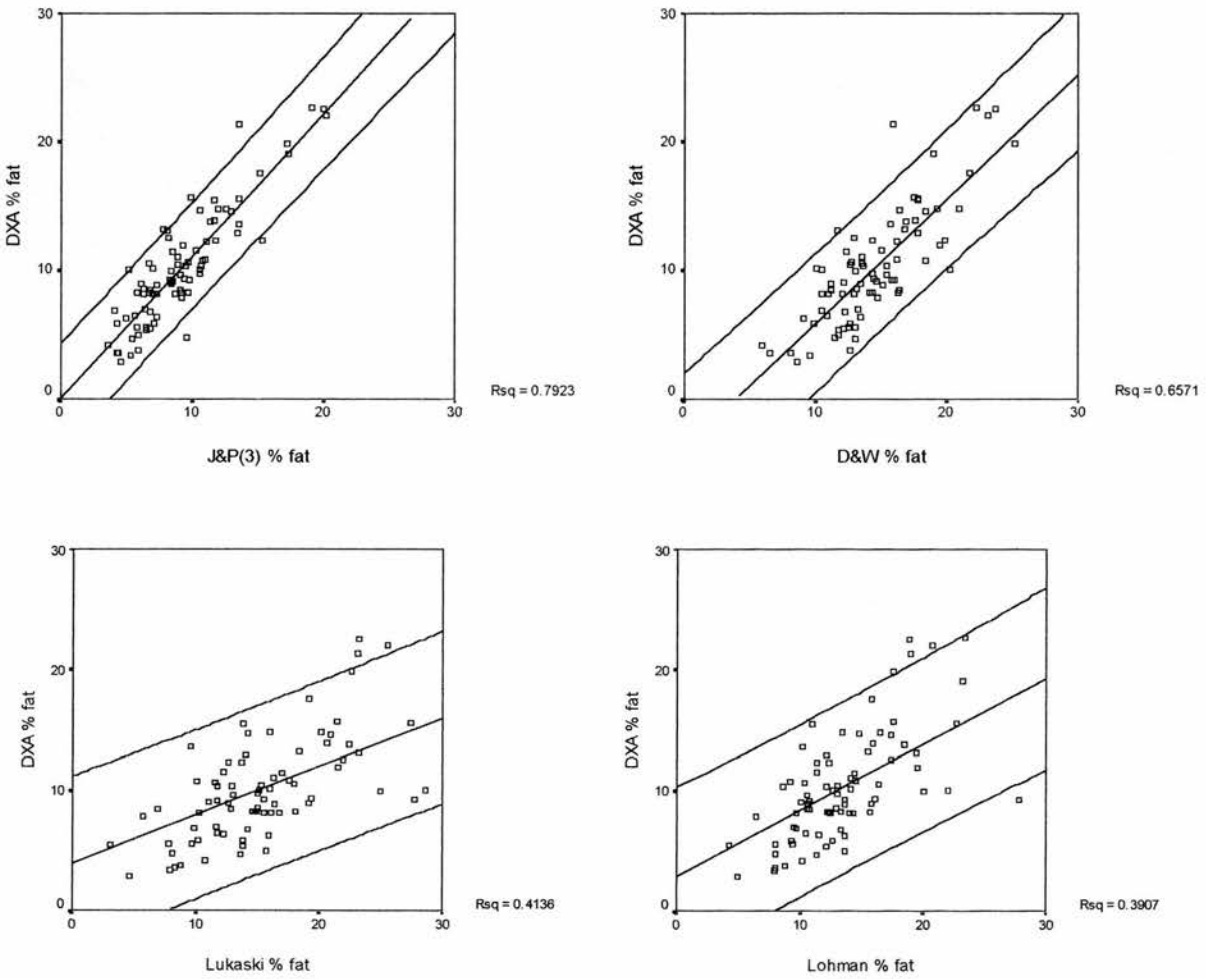
FFM = 0.734 (ht^2/R) + 0.116 (M) + 0.096 (Xc) – 3.152 (Lukaski and Bolonchuk, 1987)

where FFM is the fat-free mass in kg; M is total body mass in kg; ht is height in cm; R is resistance in Ω; Xc is reactance in Ω.

From examination of the slopes, intercepts and 95% CI of the best fit regressions against DXA % fat, these data suggest that % fat is better predicted from anthropometry than BIA, which only explain about 40% of the variation in DXA % fat. Of the anthropometric predictions, the equation which employs three-sites of Jackson and Pollock (1978) appears to have a closer agreement to the DXA value and a smaller prediction error value than that of Durnin and Womersley (1974). The issue of whether the reason for the difference related to the skinfold selection or the model (i.e. logarithmic or quadratic) was investigated by

regressing various skinfold sums against DXA % fat. There was little difference between results using 82 or all 106 athletes. The results for 106 athletes appear in table 4.15

Figure 4.1 Comparison of BIA and skinfold predicted fat against DXA % fat in 82 athletes



Predictions indicate linear regression and 95% CI.
J&P3 refers to the 3-site caliper prediction of Jackson and Pollock (1978).
D&W refers to the 4 site caliper prediction of Durnin and Womersley (1974)
Lukaski refers to the BIA prediction of Lukaski and Bolonchuck (1987)
Lohman refers to the BIA prediction of Lohman (1992)

From the data illustrated in table 4.15, it can be seen that simple summation of skinfolds at sites in question has a considerable consequence for the accuracy of the prediction. The sum of the three sites in the Jackson and Pollock (1978) equation provide the optimal sum, while those in the Durnin and

Womersley (1974) equation represent the poorest accuracy. With more sites selected – as in the 7 and 19 site predictions, there is a degree of compensation for different fat distribution between individuals, but the prediction is less accurate than the simple three-site sum. Neither log nor quadratic transformations improved the accuracy of the predictions, although notably, the logarithmic model of Durnin and Womersley performed better than the quadratic one for the sum of their 4 sites, while the reverse was true for the quadratic models of Jackson and Pollock.

Table 4.15 Prediction of DXA % fat from sums of skinfolds in 106 athletes

Prediction	r^2	SEE
% fat = 0.377 (Σ pec + ab + thigh) – 1.28	0.80	2.21
% fat = 27.99 log (Σ pec + ab + thigh) – 30.62	0.75	2.45
% fat = 0.004844 (Σ pec + ab + thigh)^2 + 5.21	0.77	2.32
% fat = 0.367 (Σ bi + triceps + subscap + suprailiac) – 1.51	0.66	2.84
% fat = 30.77 log (Σ bi + triceps + subscap + suprailiac) – 35.53	0.66	2.87
% fat = 0.004225 (Σ bi + triceps + subscap + suprailiac)^2 + 5.59	0.61	3.05
% fat = 0.211 (Σ pec + ax + subscap + suprailium + tri + thigh + ab) – 2.579	0.77	2.35
% fat = 32.08 log (Σ pec + ax + subscap + suprailium + tri + thigh + ab) – 46.40	0.75	2.47
% fat = 0.00136 (Σ pec + ax + subscap + suprailium + tri + thigh + ab)^2 + 4.78	0.74	2.51
% fat = 0.09661 (Σ all 19 skinfolds *) – 4.407	0.77	2.35
% fat = 36.62 log (Σ all 19 skinfolds *) – 69.09	0.75	2.46
% fat = 0.0002557 (Σ all 19 skinfolds *)^2 + 3.99	0.74	2.51

* see methods 4.4; ab : abdominal; ax : axilla; bi: biceps; pec ; pectoral; subscap : subscapular; tri : triceps;
All predictions and predictors significant at $P < 0.001$.

On the 82 subjects, regression analysis was employed using fat mass by DXA as the dependent variable, with predictors of mass, height, resistance and reactance entered. BIA predicted % fat (DXA) according to the formula:

$$\text{fat mass (g)} = 429.4\ (M) - 283.6\ (Ht^2.R^{-1}) - 73.1\ (Xc) - 134.1$$

($r^2 = 0.50$; SEE = 2805g; $P < 0.001$)

where M is the total body mass in kg; Ht is the height in metres; R is the resistance and Xc is the reactance in ohms.

Using the same 82 subjects, a selection of skinfolds was entered into a stepwise regression. The following formula was produced predicting fat mass:

$$\text{fat mass (g)} = 331.5(\text{abdominal}) + 356.2\ (\text{thigh}) + 111.9\ (\text{M}) - 9108$$

$$(r^2 = 0.81;\ \text{SEE} = 1732\text{g};\ P < 0.001)$$

where skinfolds are in mm. and M is the total body mass in kg.

Both methods were employed to predict fat - free mass. Bioelectrical impedance analysis best predicted FFM according to the equation:

$$\text{Fat} - \text{free mass (g)} = 294.3\ (Ht^2.R^{-1}) + 662.7\ (\text{M}) + 71.8(\text{Xc}) + 662.7$$

$$(r^2 = 0.90;\ \text{SEE} = 2680\text{g};\ P < 0.001)$$

where Ht is the height in m, R is the resistance in Ω, M is the total body mass in kg and Xc is the reactance in Ω.

The equivalent prediction from anthropometric variables was investigated using corrected circumferences generated by the circumference minus the average of two skinfolds taken along it, similar to the method of Martin et al. (1990). Stepwise regression analysis was used to select the variables which best predicted fat-free mass. The following equation was produced:

$$\text{Fat-free mass (g)} = 689\ (\text{M}) + 285\ (\text{SH}) + 1025\ (\text{CFG}) + 534\ (\text{CCG}) - 473\ (\text{CWG}) - 20895$$

$$(r^2 = 0.90;\ \text{SEE} = 2653\text{g};\ P < 0.001)$$

where M is the total body mass in kg, SH is the sitting height in cm, CFG is the corrected forearm girth (forearm girth minus π x the mean of forearm (anterior) and radial skinfolds), CCG is the corrected calf girth (calf girth minus π x medial calf skinfold), and CWG is the corrected waist girth (waist girth minus π x the mean of abdominal and suprailiac skinfolds). All girths and skinfolds are in cm.

Corrected girth methodology was designed for estimating dissected skeletal muscle mass, and DXA lean tissue mass represents considerable non-muscle mass in addition. The hypotheses that DXA lean tissue mass could be better predicted by subtracting fat mass from total mass than with the corrected girths model was tested by entering a variety of skinfolds into a stepwise regression analysis with DXA fat-free mass as the dependent variable. The following equation was produced:

$$\text{Fat-free mass (g)} = 888\,(M) - 252\,(\text{abdominal}) - 382\,(\text{suprailium}^*) - 335\,(\text{thigh}) + 9120$$

$$(r^2 = 0.96;\ \text{SEE} = 1738\text{g};\ P < 0.001)$$

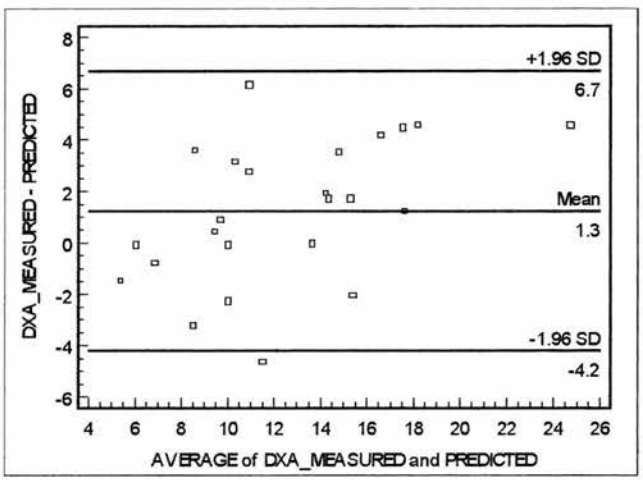
* The suprailium site is an oblique fold on the anterior axillary line, as used in the Jackson and Pollock (1978) equations and differs from the suprailiac site, which is a horizontal fold taken in the midaxillary line, used in the Durnin and Womersley (1974) equations.

Where M is the mass in kg, and skinfolds are in mm. These SEE and r values of this prediction approach the accuracy of those of the prediction of muscle mass in Martin et al. (1990).

Once the optimal prediction for determining fat and fat-free mass had been established, these were applied to a further sample of 24 athletes of equivalent competitive standard (including 7 international athletes) age and training, but who were heavier (mean 81.0 ± 9.2kg; $P < 0.05$) and had higher BMI (24.8 ± 2.5kg.m^{-2}; $P < 0.01$) and greater % fat (13.2 ± 5.4%; $P < 0.01$). The derivation, validation and total samples are described in table 4.14.

Total error (TE), calculated according to the formula: $TE = [\Sigma(DXA\ mass - predicted\ mass)^2 / n]$ where n is the number of subjects, was 2310g (2.9%) and 2159g (2.7%) for fat and fat free mass respectively. Predicted and measured fat and fat-free mass showed a correlation of 0.86 and 0.96 respectively ($P < 0.01$). The limits of agreement, calculated according to the method of Bland and Altman (1986) showed a mean bias (± 2SD) of 1.3 ± 5.4% for % fat and -0.8 ± 5.1% for % fat-free mass, as illustrated in figure 4.2.

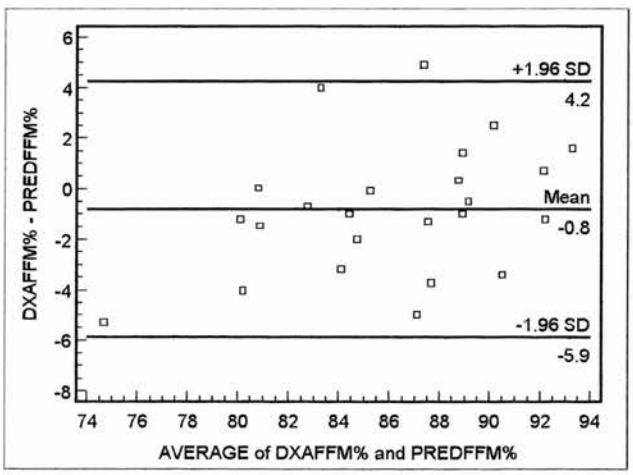
Figure 4.2a. Bland and Altman Plot of predicted and measured % fat in 24 athletes



The Bland and Altman plot is a means of assessing the agreement between two methods of measurement, or, in this case, a prediction and a measurement. The ±1.96 SD lines represent the 95% confidence limits for agreement, assuming normal data distribution. The adequacy of the mean difference (bias) in this comparison is a judgement based upon levels which are scientifically relevant. In this instance, mean bias is well within ±3% of the measured value and the prediction can be judged valid.

% fat predicted from the formula: fat mass (g) = 331.5(A) + 356.2(T) + 111.9(M) – 9108
where A = abdominal, T = thigh skinfolds in mm; M = total mass in kg; and converted into a % of total mass

Figure 4.2b. Bland and Altman Plot of predicted and measured % fat-free mass in 24 athletes



As figure 4.2a, the plot shows predicted % fat-free mass to be well within an acceptable range of ±3%, and thus the prediction is judged valid.

% fat free mass predicted from the formula: fat free mass (g) = 888(M) -252(A) – 382(S) -335(T) + 9120
where M = total mass in kg; A = abdominal, S = suprailium, T = thigh skinfolds in mm; and converted into a % of total mass

Subsequent predictions of fat and fat-free mass using anthropometry were made using data from all 106 athletes. When fat mass was predicted using the thigh and abdominal sites only, the r^2 was 0.82 and SEE 1843g, equivalent to 2.4% for a typical athlete in the sample. When all the skinfolds were entered into a stepwise regression using 106 subjects, fat and fat free mass were best predicted from the following formulae:

Fat mass (g) = 105.2 (M) + 189.5 (A) +345.2 (P) –521.1 (FR) + 215.9 (MC) +258.3(T) +293 (S) – 8334.4 (r^2 = 0.85; SEE = 1679g; TE = 1614g; $P < 0.001$)

Fat-free mass (g) = 890 (M) - 261 (A) - 244 (T) - 342 (S) -237 (TP) + 9966 (r^2 = 0.96; SEE = 1694g; TE = 1645g; $P < 0.001$)

where M = mass (kg); A = abdominal; P = pectoral; FR = forearm radial; MC = medial calf; T = thigh; S = suprailium; TP = thigh-patellar skinfolds, all in mm. For both predictions, SEE and TE values are 2.1 - 2.2% for a typical athlete in the sample. Model summary and change statistics appear in table 4.15.

Data from the 82 athletes for which both anthropometry and BIA were performed indicated that fat and fat-free masses in male athletes were best predicted from skinfolds, especially at the abdominal, thigh and suprailium sites. Application of the prediction equations to fatter athletes resulted in a small mean difference between predicted and measured values, and the SD of the predicted values was slightly greater than measured values (4.1% v 5.4% fat) in the 24 subjects of the validation group. This difference may have resulted from differences in regional fat distribution between athletes of different adiposity, or the possibility that the smaller validation group of athletes was more heterogeneous. Combining the initial and validation samples introduced greater heterogeneity into the total sample, and the optimal prediction of fat mass used six skinfolds in producing a similar SEE (1.7kg), though this explained a further 4% of

the variation in DXA-derived fat. Combining samples produced no change in SEE or r^2 in the fat-free mass prediction.

Table 4.16a. Stepwise regression analysis predicting fat mass from skinfolds in 106 athletes

Model	r^2	SEE (g)	r^2 change	F change	df	Sig. F change
1	.667	2460	.667	208.4	104	.000
2	.756	2117	.089	37.4	103	.000
3	.798	1934	.042	21.4	102	.000
4	.823	1821	.025	14.0	101	.000
5	.834	1770	.011	6.9	100	.010
6	.841	1744	.007	4.1	99	.045
7	.837	1754	-.004	2.2	101	.138
8	.845	1718	.008	5.3	99	.024
9	.854	1679	.009	5.7	98	.019

Stepwise criteria: Probability of F-to-enter <=0.05; probability of F-to-remove >=0.1;
Predictors (constant in all predictions):
Model 1: abdominal
Model 2: abdominal, medial calf
Model 3: abdominal, medial calf, mass
Model 4: abdominal, medial calf, mass, thigh
Model 5: abdominal, medial calf, mass, thigh, suprailium
Model 6: abdominal, medial calf, mass, thigh, suprailium, pectoral
Model 7: abdominal, mass, thigh, suprailium, pectoral
Model 8: abdominal, mass, thigh, suprailium, pectoral, forearm (radial)
Model 9: abdominal, mass, thigh, suprailium, pectoral, forearm (radial), medial calf

Table 4.16b. Stepwise regression analysis predicting fat-free mass from skinfolds in 106 athletes

Model	r^2	SEE (g)	r^2 change	F change	df	Sig. F change
1	.809	3628	.809	441.5	104	.000
2	.929	2220	.120	174.6	103	.000
3	.951	1852	.022	46.1	102	.000
4	.957	1738	.006	14.8	101	.000
5	.960	1694	.003	6.4	100	.013

Stepwise criteria: Probability of F-to-enter <=0.05; probability of F-to-remove >=0.1;
Predictors (constant in all predictions):
Model 1: mass
Model 2: mass, abdominal
Model 3: mass, abdominal, thigh
Model 4: mass, abdominal, thigh, suprailium
Model 5: mass, abdominal, thigh, suprailium, thigh-patella

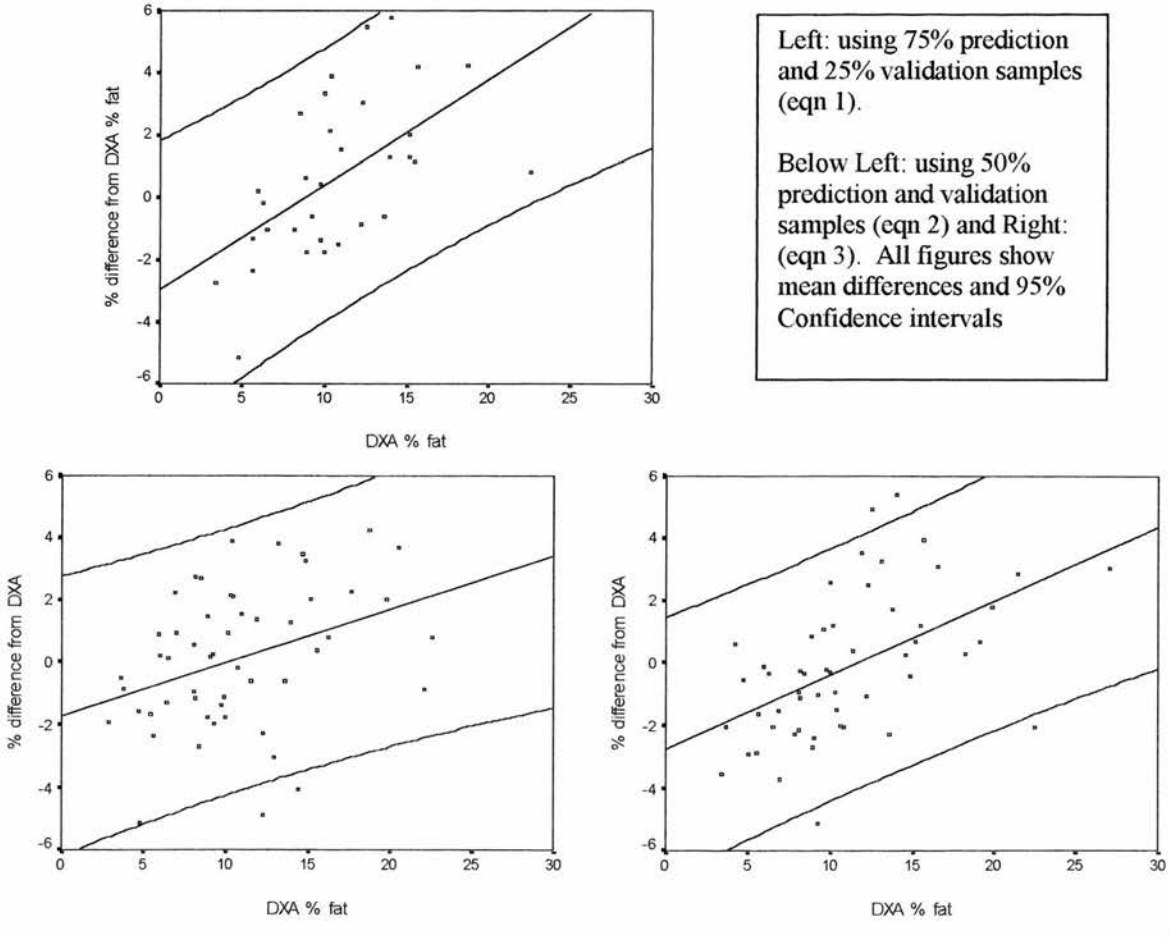
The effect of sampling bias was investigated by randomly subdividing the 106 athletic subjects into two groups, deriving a % fat prediction on one and validating it on the other. This process was first done using the roughly $\frac{3}{4} : \frac{1}{4}$ distribution for the derivation and validation samples as used by Jackson and Pollock, and then with a 50: 50 split. The latter was repeated in a crossover design, reversing the prediction and validation groups. The regressions for the plots appear in table 4.17 and plots of differences between predicted and measured fat % are illustrated in figure 4.3.

Table 4.17 Prediction equations used in validation

Sample	Eqn	Prediction	r^2	SEE
75%	1	% fat = 0.492 (abdominal) + 0.434 (thigh) – 1.038	0.84	2.1
50%	2	% fat = 0.525 (abdominal) + 0.435 (thigh) – 1.198	0.80	2.3
50%	3	% fat = 0.408 (abdominal) + 0.517 (thigh) – 0.613	0.79	2.2

All predictions and skinfold predictors significant at $P < 0.001$

Figure 4.3 Validation plots of subgroups of the 106 athletes



Left: using 75% prediction and 25% validation samples (eqn 1).

Below Left: using 50% prediction and validation samples (eqn 2) and Right: (eqn 3). All figures show mean differences and 95% Confidence intervals

The mean bias of all predictions is close to zero, but the trend for less accuracy with increasing adiposity is common to all, with the equations under-predicting fat. While the sampling was the result of random selection, the 75% sample is likely to have contained a disproportionate number of the fatter athletes.

The present study may be among the largest using a sample of athletes to date, whose numbers may exceed the physically active individuals of generalised prediction equations. Because the sample is more homogeneous than that of generalised studies, it could be a reasonable expectation for its predictions to be more accurate. The use of a large selection of skinfolds in the analysis enabled an optimal prediction of fat mass which was able to accommodate the differing fat 'topography' associated with physical fitness.

The agreement of predicted and measured values in the validation sample (n = 24) was well within 95% confidence limits, but showed a mean bias of +1.3% for % fat and -0.8% for % FFM. This suggests that there may be a tendency for the fatter athletes of the validation group to display more heterogeneity in fat distribution, and that increasing error will be found if the prediction equation is applied to increasingly fat athletes. Bioimpedance data were not collected on the validation sample, and therefore it is not known if a similar anomaly would occur with fatter athletes using BIA.

The agreement of skinfold prediction equations by Jackson and Pollock (1978) and Durnin and Womersley (1974) with % fat derived by DXA, varied with adiposity. Compared with the DXA value, mean differences were within 3% over ranges 0 – 18% fat for Jackson and Pollock's equation, and > 14% fat for Durnin and Womersley's equation. These ranges represent 91% and 23% of the athlete cohort respectively. Close inspection of the data of both studies reveals that subjects of Jackson and Pollock were taller and leaner, and were closer in body composition to the athletes of the present study than those

of Durnin and Womersley. It is also possible that the study of Jackson and Pollock (1978) included a greater proportion of athletic or physically active individuals than the study of Durnin and Womersley (1974).

A difference in fat distribution between athletes and controls would affect the optimal skinfold selection to predict total fatness in each category. In the present study of 82 athletes, the sum of four sites (biceps, triceps, subscapular and suprailiac - as used by Durnin and Womersley) explained 63% of the variation in DXA % fat (SEE = 2.8%, $P < 0.001$) while the sum of three (abdominal + chest + thigh – as used by Jackson and Pollock) explained 79% (SEE = 2.1%, $P < 0.001$). Including all 106 athletes increased the explained variance in DXA % fat to 80%, and the SEE to 2.2%. This would suggest that appropriate skinfold selection is a key element in the accuracy of a prediction equation, and that those sites chosen by Jackson and Pollock (1978) have included the two, which, according to the present study, best describe total fatness in athletes. This supports previous observations on preferred skinfold selection using existing formulae (Stewart and Eston, 1997). The much closer correlation between DXA and the existing predictions when applied to controls ($r = 0.90$ for Durnin & Womersley and $r = 0.94$ and 0.93 for Jackson & Pollock's 7 and 3 site equations respectively) suggests skinfold selection may be less critical in sedentary subjects of greater overall adiposity.

Despite its greater precision, BIA offers a less accurate prediction of % fat than skinfolds. The difference in accuracy may be more closely linked to lean tissue rather than fat tissue distribution. Resistance in the human body is essentially determined by body water, whose electrolytes are excellent conductors. Total body water is therefore estimated directly from the impedance measurement, FFM is predicted by assuming a fixed hydration and fat mass is predicted by subtraction of FFM from total mass. Errors in fat prediction are likely to be higher than in lean tissue prediction because the FFM represents a much greater mass and similar variance. Using densitometry as the reference method, Lohman (1992) postulated that existing equations using BIA with fat mass as the dependent variable, should have standard errors of the estimate which fall within the range of 2.1 – 2.9 kg. Those from the present data fall centrally within this

range, while those from anthropometry are substantially lower. While anthropometric predictions could be affected by differences in fat patterning between athletes and controls, in addition to this, bioimpedance could be influenced by differences in hydration, to which DXA is relatively impervious (Pietrobelli et al., 1998), as well as differences in lean tissue distribution.

The hydration level for the fat – free mass is commonly assumed to be 0.732 (Baumgartner, 1996), although this is an average figure, and a range between 0.69 - 0.77 has been reported (Streat et al., 1985). Close inspection of the data reveals the range of 0.68 – 0.77 in the predominantly active sample of Prior et al., (1997), measured by deuterium dilution. Departure from the assumed average will reduce the accuracy of the body composition prediction by BIA, and dehydration following sweat loss is one of several factors which could introduce errors (Brodie et al., 1991; Heyward and Stolarczyk, 1996). In the athletes of the present study whose weekly training averaged 9.3 hours, daily fluid balance and thus the hydration of FFM may have fluctuated widely, and there is at least the possibility that some individuals may not have been fully re-hydrated since exercising the previous day.

Athletes also have greater lean tissue than non-athletes, and such muscular development - particularly in the arms, may dramatically enhance the conductivity. Site-specific muscular development could introduce greater variance of conductivity in athletes than in non-exercising controls, rendering BIA less accurate. In this respect, it is perhaps surprising that the equation of Lukaski and Bolonchuk (1987) which was derived on athletes, did not predict fat better. The difference in % fat prediction between DXA and BIA from either formula was significantly correlated with mesomorphy ($r = 0.26$; $P < 0.05$) which suggests that BIA predictions may be population-specific to the extent that strength athletes may be poorly represented by a predictive formula based on endurance athletes. No equivalent correlation was observed in anthropometric predictions.

While it may be logical to expect subcutaneous fat - the largest compartment - to be representative of total body fat, the concept becomes less tenable as greater understanding emerges of the proportional distribution of fat between compartments (Hawes, 1996). In an investigation of regional fat placement in

165 soldiers, Nindl et al., (1996) recognised a hierarchy of regional fat mobilisation by DXA as abdomen > arms > legs as a consequence of weight loss, but this was not supported by skinfold evidence in the arms and legs. Such an observation could be explained by a change in the partitioning between subcutaneous and internal fat with weight loss. Evidence on fat partitioning from medical imaging techniques is conflicting. Ross et al., (1994) using magnetic resonance imaging, found no significant relationship between visceral adipose tissue and total adipose tissue in 17 obese men (over 30% fat), while Després et al., (1991) using computed tomography, found deep abdominal fat to correlate with total fat and age ($r = 0.76$ and 0.63 respectively, $P < 0.0001$) in 110 men whose fatness ranged from 2.2 - 39.8% (mean 22.9, *s* 8.9%). The proportional distribution of fat in athletes is unknown, although high proportion of total fat situated in the subcutaneous compartment would explain why skinfolds are relatively accurate in predicting total fat.

Dual X-ray absorptiometry is calibrated for a normal expected range of tissue thickness, beyond which the accuracy may be reduced (Wahner and Fogelman, 1994). Separate corrections are therefore necessary to accommodate growth studies (Mitchell et al., 1996a). Ryde et al., (1998) found DXA (Lunar DPX model) to under and overestimate small and large changes respectively in fat loss in 10 obese women, compared with a 4C model. Experimental work on the same machine as that of the present study indicated that fat measurements on subjects were stable up to a total tissue depth of 25centimetres (Jebb et al., 1993), which exceeds the anterior – posterior thickness of any of the athletic subjects. Inter-manufacturer differences (Tothill et al., 1994a and b) are greater for sub-regions than the whole body, but of the three manufacturers, Hologic (as used in the present study) is the scanner type best validated for accuracy in an athletic sample. The study of Prior et al., (1997) found differences between % fat by 4C and DXA to be weakly related to BMI but not mesomorphy, suggesting that increasing fat mass, rather than lean tissue mass contributes to the discrepancy.

While concern has been expressed over DXA's accuracy in obese individuals, extremely lean subjects represent a greater threat to accuracy in the current sample. There was a total of 9 athletes whose fat was

measured by DXA to be less than 5% (a suggested minimal weight criterion used by Lohman, 1992), the lowest being only 2.9%. While this might appear plausible by comparison to two subjects in the densitometric study of Adams et al., (1982) whose fat was predicted to be –12%, +2.9% is still a remarkably low value. This figure could theoretically represent only essential lipids in the leanest athletes who had reduced their "excess fat" to zero. DXA failed to record any fat in the torso region of the leanest three individuals. The regression of torso fat against total % fat predicted zero torso fat to occur at 3.3% total fat (95%CI 2.2 - 4.4). However, even for these leanest subjects, abdominal skinfold averaged 5.8 ± 0.3 mm. In addition, essential lipids in the liver and other organs means that zero fat in the torso is, in practical terms, impossible. A more plausible explanation is that accuracy is diminished at the lean extreme of this sample for several reasons. Poorer reproducibility may be expected in very lean individuals because the variance between replicate measures will represent a greater proportion of the measurement value. In addition, the bone-containing pixels, which DXA must interpolate across in order to derive a fat content are most prevalent in the torso. The leanest individuals have proportionately fewer soft tissue pixels to provide the information for this interpolation and accuracy is reduced as a consequence. DXA is unable to measure soft tissue inside the skull, and the software used by the present study assumes a fixed value of 17% fat. Lastly, the algorithms which predict fat content, which are clearly different between manufacturers and software versions, are designed to be robust for subjects of a normal range of adiposity, and extremes at both ends of this scale will compromise their stability.

The findings of Sinning et al., (1985) indicate that using the 2C method (fat and fat-free mass) as the criterion, the majority of generalised equations were not valid for predicting the fat content of athletes, with the exception of three equations from Jackson and Pollock (1978). While the three-site equation of Jackson and Pollock is clearly the best of the four predictions compared in the present study, the conclusion of Adams et al., (1982) was that this methodology was inappropriate for some athletes because of their increased density of the fat-free mass. The finding of the present study is not altogether surprising because the athletes in the present study (sample n = 82) and of the study of Sinning et al., (1985) had mean BMI values within 0.3kg.m^{-2} and mean % fat within 1%. This contrasts with the equivalent figures

of Prior et al., (1997) whose male sample (67 athletes and 24 non-athletes) had mean BMI values of 27.0 ± 4.6 and % fat of 13.1 ± 5.7% (by DXA). The greater mesomorphy of the Prior sample than the athletes of the present study (6.2 ± 1.7 v 5.0 ± 1.2) suggests considerable musculo-skeletal development and potentially greater variation in the density of FFM. While good agreement has been reported between DXA and densitometry in 128 men and women (Wellens et al., 1994), in the case of the study of Prior et al., (1997), variability in the density of the FFM beyond that expected in a sedentary population could explain the poorer association of % fat by densitometry and the 4C model than by DXA and the 4C model. The relative accuracy of both methods relative to the 4C model was compared in 78 young and older men and women by Clasey et al., (1999). Overall, DXA was slightly more accurate (TE for DXA - 4C and densitometry - 4C was 5.3% and 5.7% respectively), but the difference was greatest for young men and older women. Although physical activity was not reported, higher bone density in young men and lower bone density in older women may have violated the assumption of the constant density for FFM more than with the other groups.

The criteria of Lohman (1996) suggest the evaluation of a new method of predicting % fat accurately should include having a SEE within 3% of body mass. Using the data from Prior et al., (1997), DXA fulfils the criteria (SEE = 2.8%), while densitometry does not (SEE = 3.4%). The implications of this are that DXA may be superior to densitometry as a reference method. Thus, until a 4C criterion or alternative prediction is established for athletes, DXA may be the criterion method of choice. Compared with the best of the existing equations used (Jackson and Pollock, 1978), the SEE of the prediction of % fat in the present study is smaller (2.2% v 3.3%), and r^2 values comparable (0.82 for the equation of Jackson and Pollock; 0.81 for the sample of 82 athletes, and 0.85 for all 106 athletes of the present study). This adds weight to the case for promoting their use in predicting the fat content of athletes. Fat-free mass predictions using the anthropometric corrected girth model were of equivalent accuracy to bioimpedance, although both were less accurate than using skinfolds. While FFM was predicted with greater accuracy than fat, it was predicted slightly less accurately than the muscle mass prediction of Martin et al., (1990)

which may be of greater utility with athletic groups. Predicting lean tissue (i.e. FFM – total BMC) was achieved with a similar r^2, and a negligible improvement in accuracy (SEE = 2563g v 2680g).

While the major thrust of this investigation was prediction of DXA tissue mass based on anthropometry and bioimpedance, a group of six male volunteers (3 athletes and 3 medical staff) provided scans of total body potassium on the same occasion as DXA scans and anthropometry, enabling a limited comparison to be made. The time necessary for all three measurements exceeded two hours and precluded obtaining a large sample. In addition, this comparison is further limited by the non-uniform distribution of age, adiposity and muscularity between the subjects. A summary of the characteristics of each subject is detailed in table 4.18.

Table 4.18. Physical characteristics of subjects

Subject	Age (yr)	Mass (kg)	BMI ($kg.m^{-2}$)	fat mass (kg) by DXA	muscle mass (kg)*
1[a]	38	71.2	22.7	9.0	40.4
2[a]	32	73.2	23.8	12.2	39.8
3[a]	23	66.2	26.5	9.3	38.6
4	74	64.4	22.3	7.7	33.6
5	49	73.2	25.0	15.7	35.3
6	34	78.2	23.7	19.2	36.3

[a] athlete; * Predicted from Martin et al., (1990)

The correlation between TBK and DXA for FFM was 0.57 ($P > 0.05$), as illustrated in figure 4.4. Wide individual variation in the predictions was observed, although the mean differences were close to zero for this group, as illustrated in figure 4.5. Large individual variation may not be surprising in such a diverse group. The data show considerable discrepancy between the methods in two subjects. One was the elderly subject who was exceedingly lean, in whom DXA FFM was underestimated by TBK by 4.8kg, while in the youngest subject, who was additionally the smallest of the 106 athletes and a competitive power lifter, had DXA FFM overestimated by 5.9kg by TBK. This would suggest the relationship

between potassium and lean tissue is likely to be affected by the metabolic activity of muscle, by age, or by both factors.

Figure 4.4 Plot of Fat Free Mass by TBK v DXA in 6 male subjects

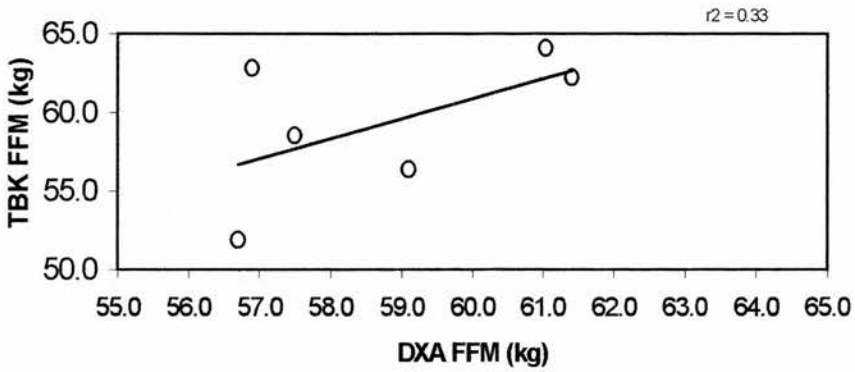
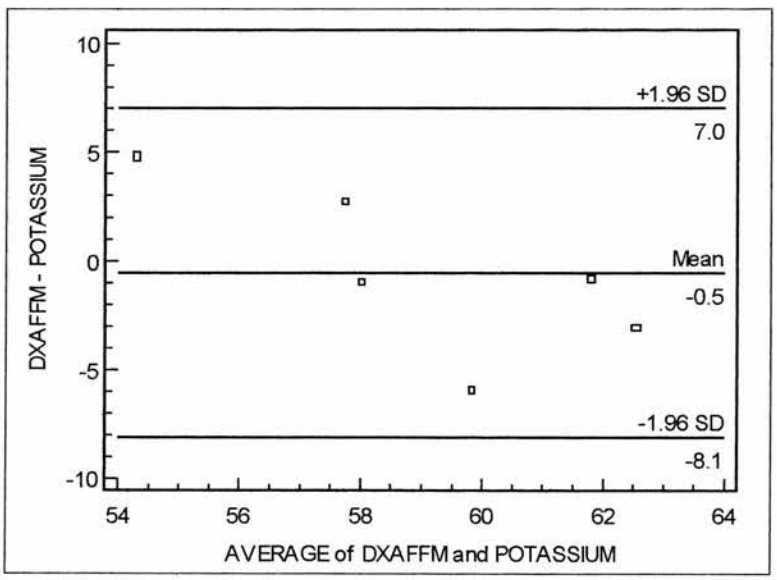


Figure 4.5 Bland and Altman plot of DXA FFM and TBK FFM in 6 male subjects



Prediction of fat tissue in female subjects

Bioimpedance data were available on anorexic and controls (previously collected by technical staff), but clinical use or machine maintenance prevented some athletes from being measured. The 24 female athletes measured were not different in physical characteristics to the cohort of 30 athletes ($P > 0.05$). Physical characteristics of the groups appear in table 4.19. Predictions of fat mass from bioimpedance measurements appear in table 4.20.

Table 4.19 Physical Characteristics of subjects compared for bioimpedance

Group (n)	Age (yr)	Height (cm)	Mass (kg)	BMI ($kg.m^{-2}$)	% fat (DXA)
Anorexics (30)	28.0 ± 6.4	163.1 ± 5.4	39.6 ± 4.5	14.8 ± 1.2	10.7 ± 5.0
Athletes (24)	25.1 ± 4.5	168.0 ± 6.7	57.1 ± 7.4	20.2 ± 2.2	17.2 ± 6.5
Controls (30)	28.1 ± 4.6	165.0 ± 6.9	63.7 ± 9.1	23.4 ± 2.8	29.2 ± 5.0

Table 4.20 Prediction of DXA fat mass from bioimpedance in women subjects

Group	**Prediction**	**r^2; SEE**
Anorexics	Fat mass (g) = 344.9(M) – 262.6 ($Ht^2.R^{-1}$) + 1028	0.67; 1188g (2.9%)
Athletes	Fat mass (g) = 763.9(M) – 626.4 ($Ht^2.R^{-1}$) - 3753	0.77; 2345g (4.2%)
Controls	Fat mass (g) = 806.0(M) – 551.6 ($Ht^2.R^{-1}$) - 6297	0.89; 1800g (2.9%)
Total Group	Fat mass (g) = 734.7(M) – 518.7 ($Ht^2.R^{-1}$) - 4775	0.88; 2616g (4.9%)

M = mass in kg; Ht = height in cm; R = resistance in Ω; % refers to SEE for mean subject of sample

While numbers preclude constructing predictions which are likely to be capable of generalisation to other samples, the difference in the accuracy of the predictions for each of the groups indicates their population – specific nature. These regressions explained least variation of fat mass in anorexics, however in percentage body mass terms, athletes had a larger SEE. As in male subjects, different proportionality, specific muscle development or incomplete re-hydration after exercise the previous day, could explain the lower prediction accuracy. For the anorexic group, altered homeostasis could have affected body fluids, electrolyte concentrations and thus BIA measurements.

For the 30 female athletes for whom DXA and skinfold data were obtained, correlations with DXA % fat of the predictions of Jackson and Pollock (1978) were 0.96 for both the 3 site and 7 site formula, while Durnin and Womersley (1974) 4 site formula was 0.95. However these high correlations are due in part to the large range of % fat values obtained which show a close relationship rather than close agreement. As a consequence, the difference between the skinfold - predicted and DXA – measured % fat was calculated, and the acceptability judged by the % fat range of mean differences falling within 3% of the DXA value. This value was chosen because an 'acceptable' method of body composition is required to be within 3.5% of the reference method value, but a portion of the error must be attributed to the reference method itself (Lohman, 1992). The sums of the skinfolds used for these predictions were separately regressed against the DXA value, and finally individual skinfolds were inserted into stepwise regression analysis to establish the optimum prediction using the present data.

Stability of the prediction equations of Jackson and Pollock (1978) was 1 – 20% fat for the 3 site prediction and 3 – 20% for the 7 site method. For both there was a trend for differences to increase with % fat. By contrast the Durnin and Womersley prediction fell outside the acceptable range for values below 24% fat, but was satisfactory for values above this. Regressing the sums of skinfolds against DXA % fat produced the expressions in table 4.21.

Table 4.21 Regression of sums of skinfolds against DXA % fat in 30 athletes

Prediction	r^2; SEE
% fat = 0.474 (Σ triceps + suprailium + thigh) + 0.206	0.89; 2.2
% fat = 0.409 (Σ biceps + triceps + subscapular + suprailiac) + 3.60	0.86; 2.5
% fat = 0.235 (Σ pectoral + axilla + triceps + abdominal + subscapular + suprailium + thigh) + 0.206	0.90; 2.1

All predictions significant at $P < 0.001$

Regressing a selection of individual skinfolds against % fat using stepwise regression resulted in the following expression:

$$\% \text{ fat} = 0.603 \text{ (thigh)} + 0.448 \text{ (abdominal)} + 0.782 \qquad r^2 = 0.93;\ SEE = 1.8;\ P < 0.001$$

No difference in accuracy was obtained using fat mass as the dependent variable and including total body mass in the regression.

The similarity between the predictions for % fat in male and female athletes is striking, stepwise regressions selecting the abdominal and thigh sites. While there is a rationale for densitometry producing gender specific algorithms for fat prediction due to variation in bone density, no such rationale exists in DXA because the model already accounts for this. As a result, a male algorithm was applied to the female athletes. Using the male prediction based on 75% of the male athlete sample, a correlation of 0.96 was obtained between predicted and DXA – measured fat in the female athletes. The difference in agreement became greater with greater adiposity, and was within 3% of the measured value only from 0 – 10% fat, representing only four of the 30 subjects.

This similarity may be a consequence of a unisex fat distribution model for DXA (i.e. DXA 'expects' fat to conform to a certain pattern) de-emphasising the gender specific fat patterns such as breast tissue in females and the typical wedge-shaped lobe of fat around the iliac crest in males. It may represent a process of convergence where the morphology of males and females become increasingly similar with certain types of, or greater amounts of athletic training. The more highly trained female athletes were better represented by the male prediction, and include three distance runners and one race walker who averaged 11.8 hours per week of athletic training (more than four hours more than the rest of the group). There could be an endocrine rationale to explain this process, drawing on the lipolytic activity in adipocytes in the leg and torso but such investigations were not part of this study.

Prediction of lean mass in female subjects

As in the prediction of fat, availability of archived data allowed a useful comparison for the prediction of FFM. Results for the three groups appear in table 4.22.

All these predictions are of acceptable accuracy, judged by recent published recommendations (Heyward and Stolarczyk, 1996), although it is evident that the poorest prediction group is the athletes, who also have the highest FFM.

Table 4.22 Prediction of FFM (DXA) in female subjects

Group	N	Prediction	r^2; SEE
Athletes	24	FFM (kg) = 0.225(M) + 0.655($Ht^2.R^{-1}$) + 2.42	0.82; 2.35kg (5.1%)
Anorexics	30	FFM (kg) = 0.618(M) + 0.282($Ht^2.R^{-1}$) –0.469	0.93; 1.17kg (3.4%)
Controls	30	FFM (kg) = 0.189(M) + 0.547($Ht^2.R^{-1}$) + 6.713	0.94; 1.35kg (3.0%)
Total Group	84	FFM (kg) = 0.268(M) + 0.517($Ht^2.R^{-1}$) + 4.392	0.89; 2.39kg (5.7%)

M = total mass in kg; Ht = height in cm; R = resistance in Ω; brackets contain SEE as % total mass for a typical subject of each group

Prediction of DXA lean tissue mass (FFM – BMC) using the same methodology produced virtually identical results which were not significantly different from FFM predictions in accuracy ($P > 0.05$). Because comprehensive anthropometric data were not collected on anorexics or controls, no comparison between these groups was possible on the basis of predicting lean mass based on skinfolds or girths.

Prediction of BMC in male athletes

Predicting bone mass is rare from the perspective of exercise science, although predictions for skeletal mass based on cadaver evidence do exist (Mateigka, 1921; Drinkwater et al., 1986). Skeletal mass includes the non-mineralised portion of bone, plus marrow, together with other structures such as

ligaments and cartilage. The present study is using BMC to represent the skeleton in such predictions, and uses skeletal breadths as predictors. Within the 106 male athletes, the correlation of total BMC with total BMD was 0.92 ($P < 0.01$) which suggests that individuals with more bone in total also have denser bone. Regression of total BMC against all the skeletal breadths and heights revealed that only total mass and sitting height were significant predictors, explaining 72% of the variation in total BMC. This suggests other factors such as exercise type and duration could account for some of the remainder. Including the 15 controls for whom complete exercise history was known, total BMC was regressed against mass, height, hours of exercise and impact which was 0 for controls and weight – supported sports (cycling and rowing) and 1 for weight-bearing sports (running, triathlon, strength, fitness, racket, rugby and upper body. The resulting regression explained 73% of the variance in total BMC, with all the variables highly significant ($P < 0.001$), with hours of exercise less so ($P < 0.05$). Such a process includes different individuals who train in different disciplines to differing extents, but at least points to the underlying relationships which merit further investigation. Skeletal dimensions are only modest predictors of BMC, and that impact may be one of a number of factors which share an influence. It is clear that isolating the variables which are responsible for adjustments in skeletal architecture is fraught with difficulty with the subject groups as they are. However, impact and non-impact sports (as exemplified by running and cycling) are more fully evaluated and described for their influence on bone in chapter 6.3.

In female subjects, the number of influences on bone is probably greater than in men. Total BMC and total BMD showed a correlation of 0.88 ($P < 0.01$), and regressing skeletal dimensions against total BMC as the dependent variable using stepwise regression analysis selected only the malleolar breadth and total mass as predictors, explaining 64% of the variation in total BMC. The predictability of reduced bone density in women athletes is addressed in chapter 6.2.

While DXA is able to be used to test the predictive strengths of other methods, DXA data for bone, lean and fat components can be useful in describing tissue mass and proportions. These have been constructed in a new way, summarising body composition, referred to as the 'DXA morphotype'.

4.3 THE DXA MORPHOTYPE

While traditional somatotype scores give a size-independent index of shape, varying between the three polar axes of endomorphy, mesomorphy and ectomorphy, they are somewhat limited in their precision at describing composition. The concept presumes musculo-skeletal development and adiposity conform to fixed patterns. While larger bone breadths have been associated with greater muscular development in athletes (Tanner, 1960), the relationships are complex. Traditional mesomorphy rating based on only two bone breadths and two corrected girths may fail to recognise some specific morphological adaptations to training in some athletic groups. Similarly, the endomorphy component is limited by its selection of skinfolds, none of which have proved the strongest predictors of adiposity in the athletic subjects of this study.

The *'morphological prototype'* (Hawes and Sovak, 1994) represents an evolutionary and adaptive concept whereby conditioning status and body morphology are interdependent. While the rationale for this concept is sound, the means of calculating physique change introduces errors. These result from somatotype (Heath and Carter 1967) which is essentially a static measure which assumes symmetry, proportionality, and fails to account for alterations in fat patterning with changing adiposity, or for specific muscle development. The morphological prototype, which assesses change in morphology over time, is by definition a dynamic concept which can use somatotype, fat (or skinfolds), muscle or bone quantities to assess change. It is logical for the *'DXA Morphotype'* to be included in this list.

The *'DXA Morphotype'* is a combination of DXA output data, arranged in a way to describe tissue proportions and tissue masses. It requires reference data to describe average values, and departure from these averages by any one subject or subject group. It utilises tissue mass or percentages of fat, lean and BMC – the normal output for a whole body scan. The database mean and standard deviation are calculated – in this instance using data from 15 controls. Departure from the mean is assigned a Z score according to the formula for standardised deviate scores (also known as standard scores):

Z = (subject value - mean) / population SD.

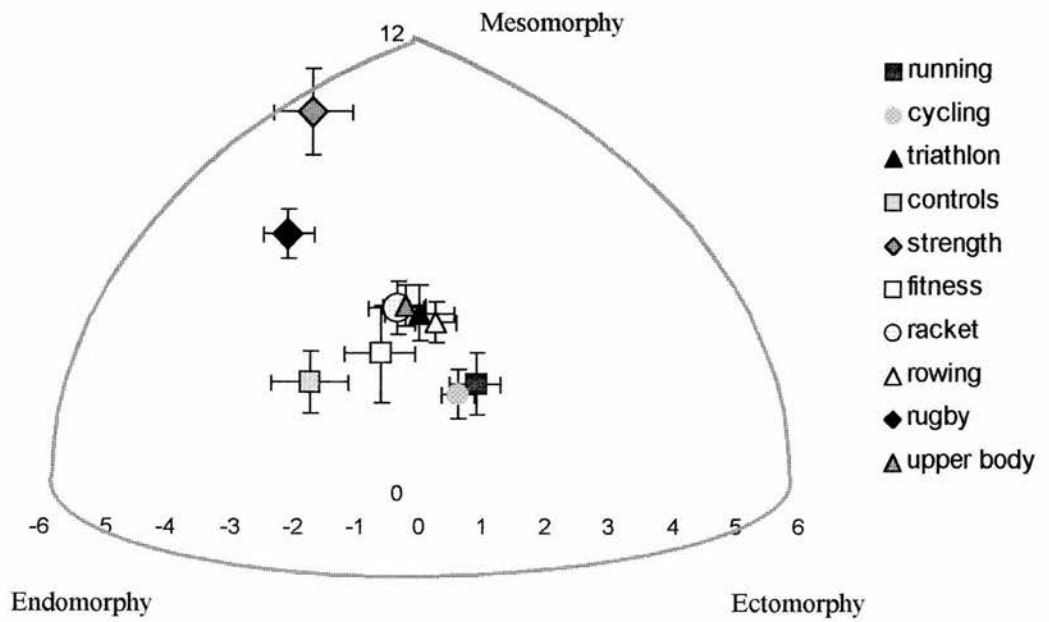
Z scores may then be plotted on a bone axis and a soft tissue axis in a similar manner to recording somatotypes on a chart:

Bone co-ordinate = 2* BMC Z score – (fat Z + lean Z scores)

Soft tissue co-ordinate = lean Z score – fat Z score

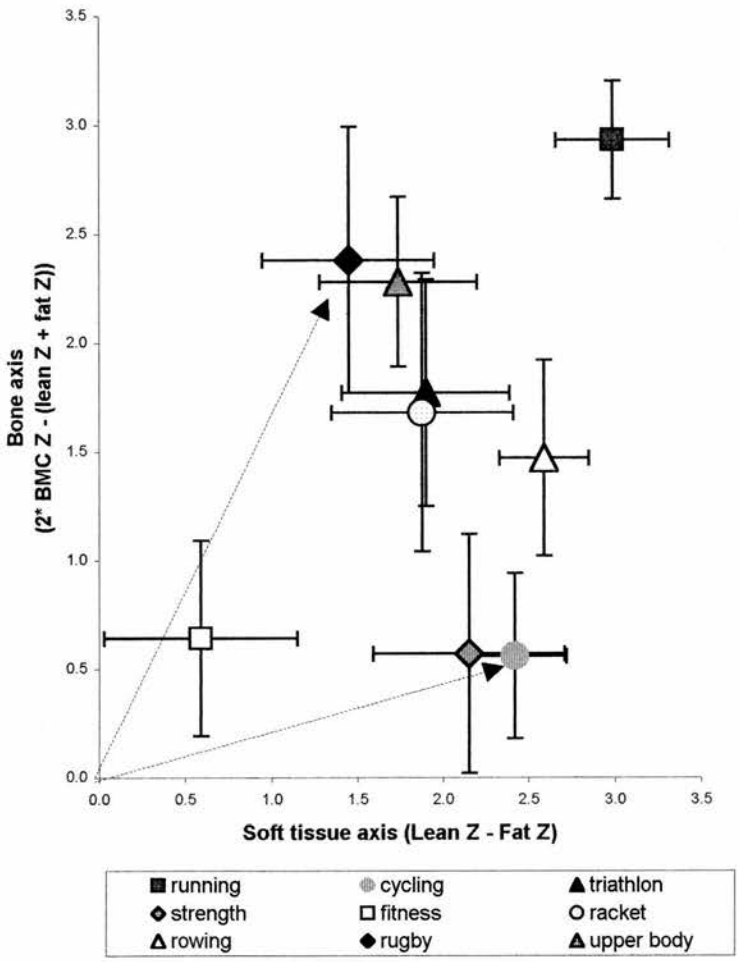
While this represents size independent composition, tissue masses themselves can also be used to calculate Z scores from DXA data in a similar way. These would discriminate between groups of differing size more effectively, and may be more appropriate for examining weight loss or weight gain. Traditional somatotypes are illustrated in figures 4.6 and DXA morphotypes for male subjects and 4.7 and 4.8 for %mass and tissue mass respectively.

Figure 4.6 Somatotypes of 106 male athletes and 15 controls by athletic group



Somatotypes calculated according to Heath & Carter (1967);
Error bars for specific groups refer to ±1SE

Figure 4.7 DXA Morphotypes in 106 male athletes and 15 controls based on % tissue mass



Arrows indicate the range of relative adaptation of specific sporting groups, assuming controls represent baseline values.

Rugby represents the greatest adaptation in bone, relative to soft tissue, while cycling represents the greatest adaptation in soft tissue, relative to bone.

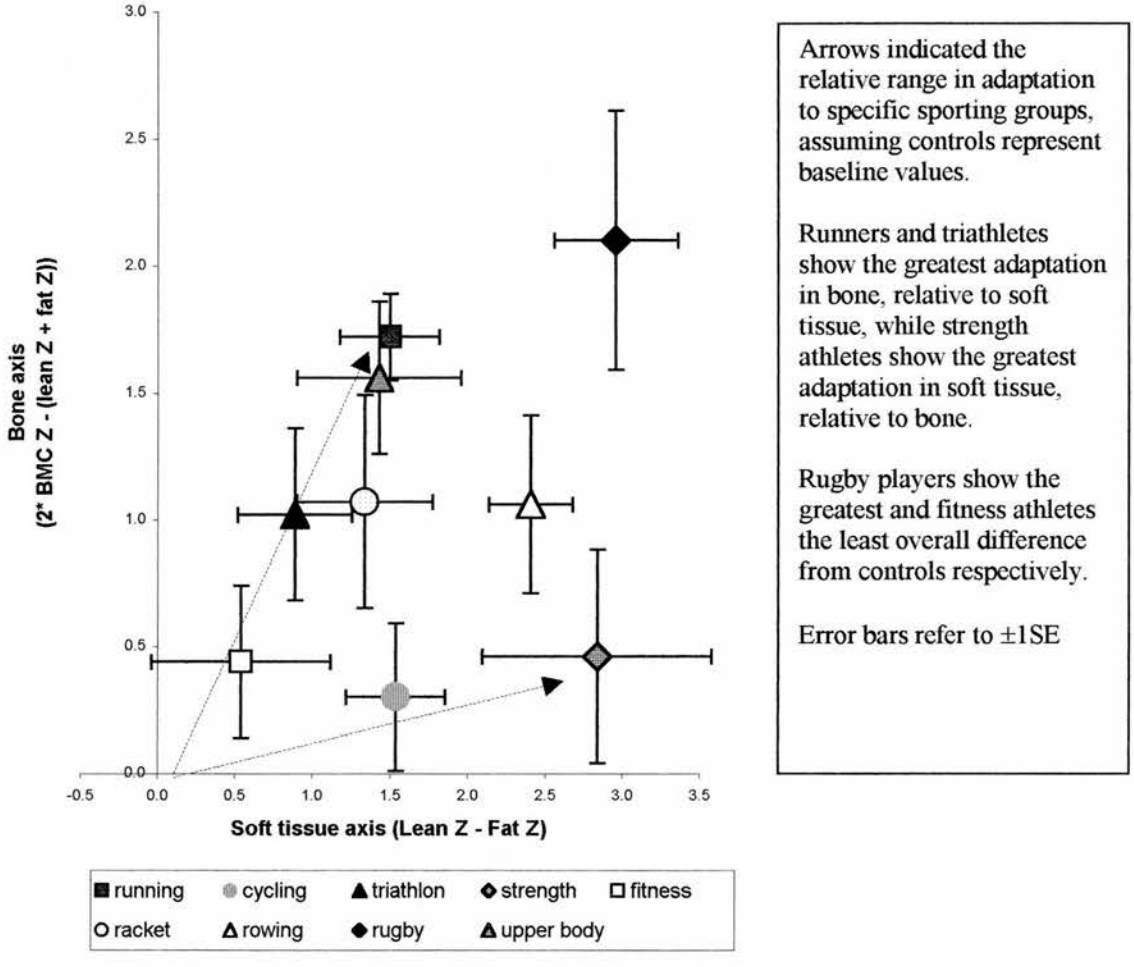
Runners show the greatest and fitness athletes the least total adaptation (distance from the origin of the plot) respectively.

Error bars refer to ±1SE

The utility of this method may highlight differences between groups of athletes whose physiques are outwardly identical, but whose bones vary. Runners and cyclists are an example of this category, with runners placing positive and cyclists negative on the bone axis. In physique terms, the cyclists and strength athletes are very different, but the DXA morphotype shows their composition to be similar.

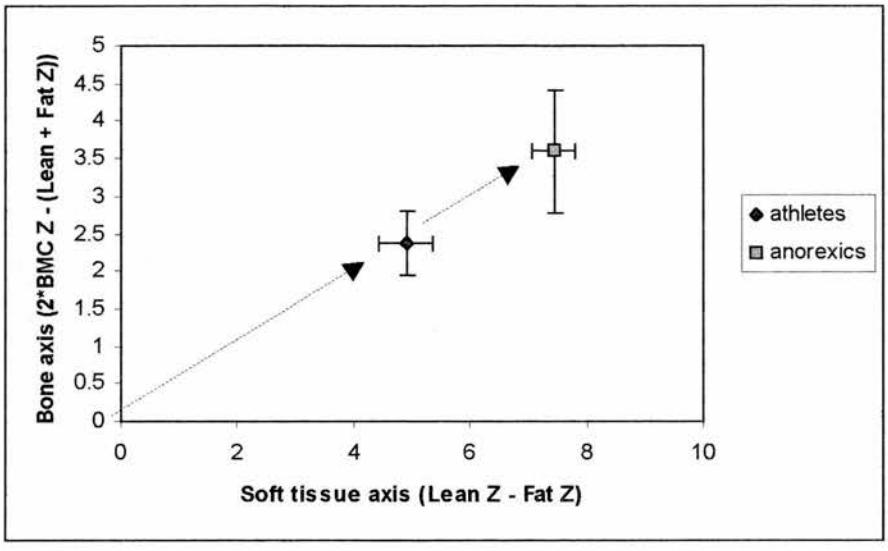
Using the same methodology but deriving Z scores based on total mass, rather than percentage mass, gives a size-dependent image, which complements the former composition-dependent image.

Figure 4.8 DXA Morphotypes in 106 male athletes and 15 controls by tissue mass



In female subjects, limited numbers precluded identifying sporting sub-groups of the athletes. However a comparison with anorexics in both DXA morphotype plots is illustrated in figures 4.9 and 4.10.

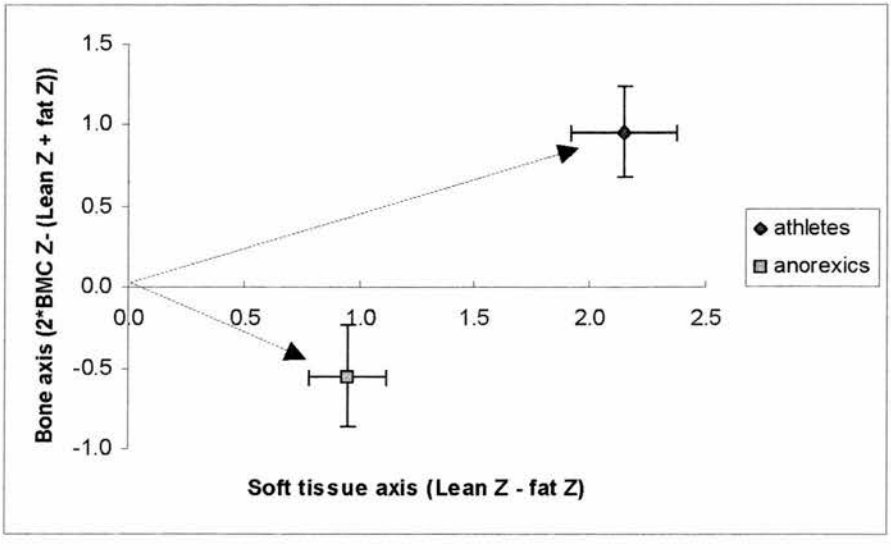
Figure 4.9 DXA morphotype plot of 30 female athletes and 30 anorexics by % tissue mass



Arrows show that athletes and anorexics have the same vector: i.e. the same relative change in bone to soft tissue from controls, in % terms.

Error bars refer to ±1SE

Figure 4.10 DXA morphotype plot of 30 female athletes and 30 anorexics by tissue mass



Arrows show in tissue mass terms, athletes and anorexics show different adaptations, relative to controls.

Error bars refer to ± 1SE

Both male and female athletes have reduced fat substantially, and increased muscle, relative to controls. In female subjects, the two morphotype plots appear to be describing different phenomena, because the vectors are parallel in % tissue terms, and yet divergent in tissue mass terms. In reality, this apparent

anomaly is an artefact of the means of calculating the co-ordinates for the plots, coupled with the samples being of different body mass. In comparing such groups, the tissue mass plot may be more useful, as it suggests that in response the physiological adaptations to starvation and exercise appear to have different anatomical consequences. In relative terms, anorexics appear to have diminished lean mass to a reduced extent, compared with fat and bone.

As with male athletes, there is a danger in assuming that female athletes were equivalent to controls prior to training. This assumption may not be justified because specific anatomical and morphological advantages are discriminants for success in different sports may have selected the athletes in a non-random manner. Furthermore, the size and range of athletes' represented in this limited sample may not span the full range which exists in the sport as a whole. Control data had insufficient numbers (n = 15 males and n = 30 females) for absolute quantification of Z scores to be made with confidence. However, despite these limitations, the DXA morphotype can be useful in describing differences between groups in a cross sectional analysis, and is also capable of tracking change in individuals or groups as a consequence of not only exercise conditioning, but growth, development and ageing. It may hold promise for future developmental and collaborative work.

CHAPTER 5.

FINDINGS: REGIONAL VARIATION IN TISSUE DISTRIBUTION

This chapter explores relationships of regional tissue distribution, with a view to evaluating the influence of exercise on body morphology. Anthropometry and DXA are used, and archived data from both techniques allow comparisons using greater numbers. A study of the upper leg utilises sub-regional analysis of what is a structurally simple region, yet crucially important for exercise.

Introduction

Estimation of soft tissue composition, particularly fat mass indicates little of its distribution, i.e. the relative amount of each tissue in different anatomical regions. Several factors can influence fat distribution including age, sex, maturation, ethnicity and exercise (Malina, 1996; Nindl et al., 1996). There appears no standard method for assessing adipose tissue distribution. Approaches include summing trunk and extremity tissue thickness on radiographs (Reynolds, 1950), measuring a series of trunk and extremity skinfold sites (Hammond, 1955; Hawes and Soucie, 1993), or analysis of ratios of skinfolds and circumferences (Baumgartner et al, 1986). Broad findings suggest an increase in subcutaneous adipose tissue with age in both males and females, and a demonstrable increase in ratios of central : peripheral sites in males, but not females (Malina and Bouchard, 1988). Significant differences for age-adjusted upper and lower extremity skinfolds are also noted between different ethnic groups (Malina, 1996), however all subjects in the present study are Caucasian and therefore no ethnic variation can account for regional differences observed.

From as early as age 9, males and females exhibit different patterns of fat storage, and these become accentuated during maturation (Durnin and Womersley, 1974; Malina and Bouchard, 1991). Typically, females deposit more fat on the thigh and the hip regions (the gynoid distribution) while men deposit more fat on the torso, especially at the abdomen (the android distribution). Much work has centred on the waist to hip ratio as being an index of fat distribution, because it is easily measured and has been shown to be a useful indicator of health disorders (Hartz et al., 1984). The epidemiological literature suggests not only an increase in total fat, but an increasing centralisation of body fat with age (Schwartz et al., 1990; Borkan et al., 1983). This involves an increase in subcutaneous fat on the torso, together with an increased visceral fat deposition, both of which appear more marked in men than women. Physical activity (or inactivity) is also known to have an influence of on fat quantity (Klesges et al., 1991) and distribution (Kohrt et al., 1992), but the strength of these combined influences is poorly investigated.

Analysis of the distribution of fat is fraught with the principal difficulty of the combined influences of age, exercise and total adiposity, which are difficult to isolate in any given population. In addition, epidemiological studies involve groups which are largely sedentary and physical activity or sports are seldom considered.

5.1 ANTHROPOMETRY

Anthropometric analysis of regional variation in body composition was possible on four discrete samples of subjects. The largest of these was a group of 222 males and 163 females representing a large age range, who were pursuing exercise programmes and monitored by fitness testing (University of Edinburgh, archived data). Comprehensive anthropometry involving 42 measurements and a complete exercise history was obtained on a different sample of 138 male and 65 female adults, including those 106 male athletes, 15 male controls and 30 female athletes who had undergone DXA scans. Eight male individuals (all former athletes, with current physical activity variable) were previously observed for body composition 10 years before (Stewart, 1987; unpublished M.Phil thesis). The same observations were repeated in order to investigate longitudinally the variations in skinfold patterns. Lastly, two extreme examples of morphological adaptation to training – one strength athlete and one endurance athlete, were measured and compared to the 106 athletes for whom complete data were available.

Archived data on skinfolds

Data of 222 male and 163 female physically active subjects from the Fitness Assessment and Sports Injury Centre database (The University of Edinburgh) allowed a comparison of subscapular skinfold, triceps skinfold and ST ratio (the ratio of subscapular : triceps) which included greater numbers of subjects in the higher age ranges. All measurements were made according to the same methods, using the same instrument (see methods). While five different individuals recorded these data, each was highly trained and experienced. Inter-recorder error is largely negated when using skinfold ratios rather than the

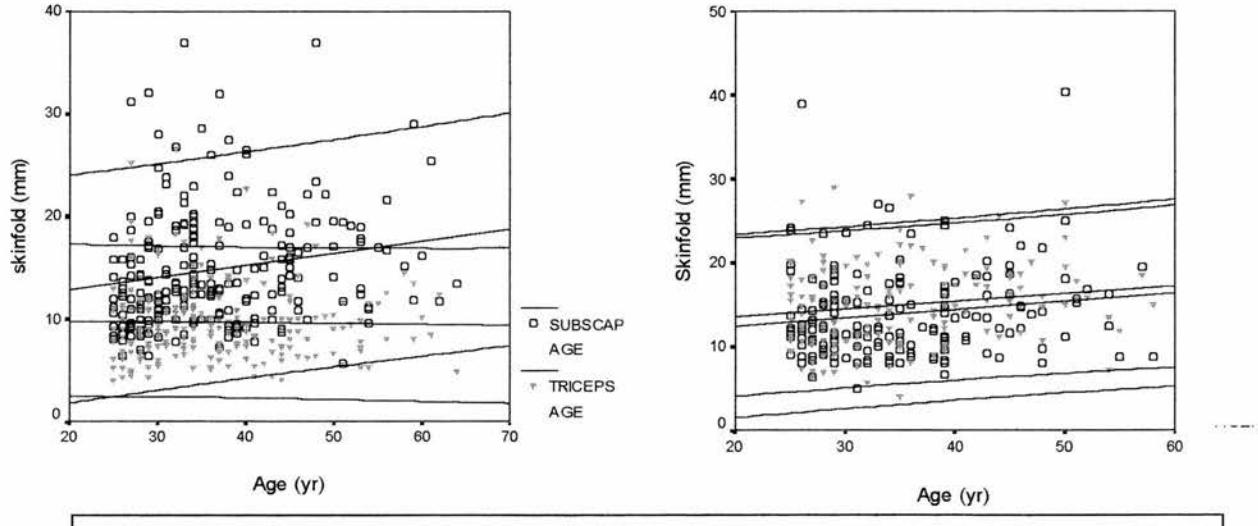
raw values, and ANOVA of intra and inter-recorder error has been shown to be equivalent to 0.27% fat in a similar recording group (Stewart, 1987). Correlations of skinfolds and age appear in table 5.1. Plots of subscapular and triceps skinfolds against age appear in figures 5.1a and 5.1b; and ST ratio for 222 males and 163 females across age ranges and quintiles appear in figures 5.2a and 5.2b.

Table 5.1 Pearson Correlation coefficients of Age and skinfolds

n = 222 males	**Subscapular**	**Triceps**	**ST ratio**
Age	0.19**	-0.026	0.24**
Subscapular		0.56**	0.47**
Triceps			-0.43**
n = 163 females			
Age	0.15	0.16*	0.01
Subscapular		0.55**	0.51**
Triceps			-0.38**

* P < 0.05; ** P < 0.01

Figure 5.1a Plot of skinfolds against age in 222 males and 5.1b, 163 females



Left: In males, the relationship between each skinfold and age is different: triceps stays constant and only subscapular changes, thus affecting the ST ratio; Right: In women each skinfold shows a similar trend, thus ST ratio remains constant; Lines show means and 95% CI

Figure 5.1 indicates the difference in trend of skinfolds with age. In males there is a significant increase in the subscapular skinfold with age ($P < 0.01$), while the triceps remains constant, suggesting a redistribution of fat towards the torso. In women, the trend of both skinfolds with age is virtually identical, and the ST ratio was only age-dependent in males ($P < 0.01$). The results are grouped by age bands and quintiles to illustrate the effect in Figures 5.2a and 5.2b.

Figure 5.2a ST ratio by age and quintiles in 222 male exercisers

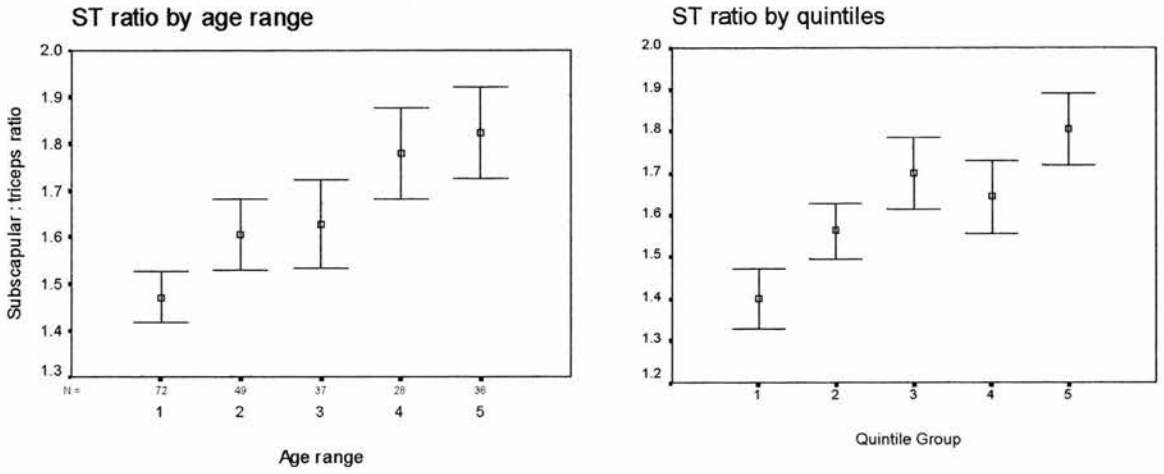
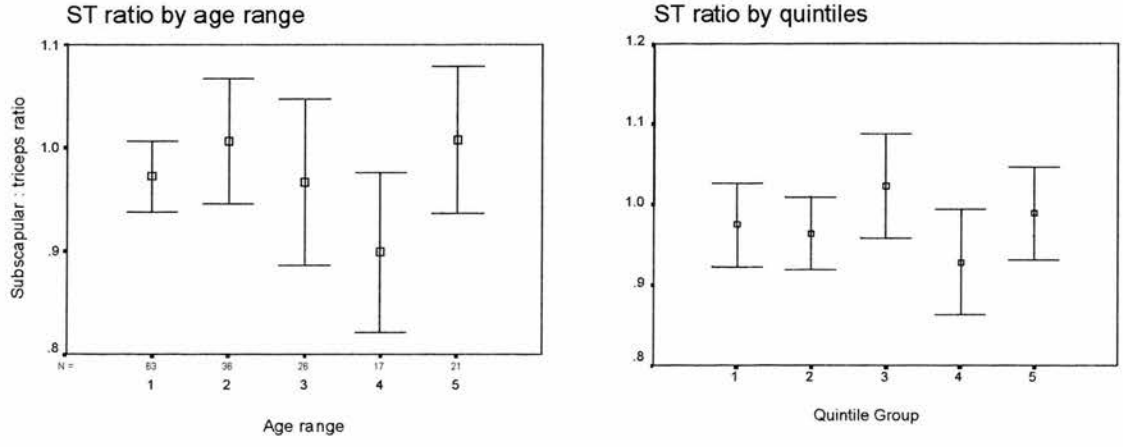


Figure 5.2b ST ratio by age and quintiles in 163 female exercisers



Age ranges: 1: under 30; 2 : 31 – 35; 3 : 36 – 39; 4 : 40 – 44; 5 : 45 and over

The mean ST ratios are similar to those reported in a study of Caucasians which formed part of an ethnic comparison (Malina, 1996). Results from both studies are illustrated in table 5.2.

Table 5.2 ST ratios from 222 men and 163 women compared with data from Malina (1996)

	US Caucasians (Malina, 1996)			Physically active UK Caucasians			
Males							
Age	**n**	**Mean**	**SD**	**Age**	**n**	**Mean**	**SD**
25 - 34	90	1.52	0.58	25 - 29	72	1.47	0.46
35 - 44	115	1.63	0.52	30 – 34	49	1.60	0.53
45 – 54	88	1.77	0.68	35 - 39	37	1.63	0.58
55 – 69	79	1.74	0.58	40 – 44	28	1.78	0.52
				≥ 45	36	1.82	0.59
Females							
Age	**n**	**Mean**	**SD**	**Age**	**n**	**Mean**	**SD**
25 - 34	115	0.96	0.31	25 - 29	63	0.97	0.27
35 - 44	158	0.94	0.30	30 – 34	36	1.01	0.36
45 – 54	89	0.95	0.27	35 - 39	26	0.96	0.41
55 – 69	86	0.96	0.31	40 – 44	17	0.90	0.32
				≥ 45	21	0.98	0.33

While the specific details of physical activity are largely unknown in either group, their influence cannot be quantified. However, these data suggest that increasing age is associated with a progressive increase in ST ratio in males, but not females. Variation in values in the female 40 –44 yr. group of the present study may reflect an increasingly selected sample of active individuals.

Detailed anthropometric analysis of 138 males and 65 female exercisers

This sample was distinct from the larger previous one, and all measurements were made by the author. Because ST ratio may not adequately reflect alterations in fat patterning, a larger number of measurements were made on fewer subjects. The large number of skinfold sites sampled for each subject enabled a

useful comparison of adipose tissue thickness within and between individuals, and made possible an investigation of the influences of exercise and age on adipose tissue distribution. All 19 skinfolds were totalled, and two measurement ratios calculated to reflect the degree of centralisation of adipose tissue in addition to the ST ratio. These were the abdominal : medial calf (AMC) ratio, and trunk : extremity (TE) ratio (Malina and Bouchard, 1988), calculated from the sum of three skinfolds in the trunk (abdominal, subscapular and suprailiac) and three in the extremities (biceps, triceps and medial calf). Correlation between these factors is presented in table 5.3.

Table 5.3 Correlation Coefficients of age, exercise and skinfold ratios in 138 males and 65 females

Males	**Exercise ($hrs.wk^{-1}$)**	**ST ratio**	**AMC ratio**	**TE ratio**	**Total of 19 skinfolds**
Age (yr.)	-0.18*	0.26**	0.32**	0.31**	0.18*
Exercise ($hrs.wk^{-1}$)		-0.07	-0.14	-0.25**	-0.29**
ST ratio			0.33**	0.46**	0.04
AMC ratio				0.87**	0.17*
TE ratio					0.33**
Females					
Age (yr.)	-0.27*	0.21	0.40**	0.33**	0.29*
Exercise ($hrs.wk^{-1}$)		0.17	-0.22	-0.18	-0.59**
ST ratio			0.35**	0.65**	0.09
AMC ratio				0.82**	0.34**
TE ratio					0.42**

* $P < 0.05$; ** $P < 0.01$ (2 tailed)

Regression analysis was used to estimate the extent to which ST ratio, AMC ratio and TE ratio were explained by age and exercise hours. Significant correlations were obtained with all ratios and age with the exception of ST ratio in females. This can be explained by the commensurate increases in the triceps and subscapular skinfolds with age. In both men and women, the AMC ratio has the highest correlation with age, exceeding that of TE ratio. While no correlation with exercise hours was found with any of the

ratios in women, in men the TE ratio had a significant negative correlation with exercise. This distinction may be a gender difference, or a threshold of exercise load, the men exercising for longer than the women. However these data suggest that the TE ratio appears sensitive to exercise, while not to age, with the reverse being the case for ST ratio. Caution in interpretation of these data is necessary because while weekly exercise duration has a more normal distribution, the age of both male and female subjects is skewed towards younger subjects. It is also possible, if not probable, that the subjective reporting of the number of hours of exercise per week is a poor measure of its metabolic cost to each individual, which is governed both by the duration and the intensity of exercise.

Longitudinal observations in 8 male subjects over a 10 year period.

While the vast majority of fat distribution data are cross sectional, a limited amount of longitudinal data was gathered on a cohort of former athletes who were part of a previous study (Stewart, 1987), and all of whom were currently physically active, though to a variable extent. None of these subjects was included in any of the other samples. The study involved physical activity profiles, mass, and skinfolds at biceps, triceps, subscapular and suprailiac sites. The repeat measurements were carried out by the same recorder, using a similar calibrated instrument as used in the previous work (see methods, chapter 3.5).

Table 5.4 Repeated skinfold measurements over 10 years in 8 subjects

	Initial measurement		10 yr. measurement	
	Mean	SD	Mean	SD
Age (yr.)	28.8	4.1	38.8*	4.1
Mass (kg)	68.5	7.8	72.8*	8.0
Biceps (mm)	2.9	0.4	4.5	2.6
Triceps (mm)	5.3	1.4	7.3*	2.4
Subscapular (mm)	8.0	1.3	11.4	5.3
Suprailiac (mm)	4.7	1.5	8.7	6.8

* Different from initial measurement at $P < 0.05$, using a paired t-test

Mean differences were 1.7, 2.0, 3.4 and 4.0 at the biceps, triceps, subscapular and suprailiac sites respectively, the only significant difference in skinfolds occurred at the triceps site, where, ironically, a relatively small increase was observed. This is due in part to the lowest standard deviation of all skinfolds occurring at this site. However, the relative physical activity after 10 years was highly variable, and the range of skinfold total which was initially 16.8 – 27.9 mm (66 % spread of data) expanded to 19.2 – 60.3 (314% spread of data). None of the proportional contributions of individual to the total of all four measurements showed a significant change over the study period, though the differences in proportions were greatest at subscapular and suprailiac sites.

Table 5.5 Correlation of Mass, activity and skinfold differences over 10 years

	Mass	Biceps	Triceps	Subscapular	Suprailiac
Activity	-0.81*	-0.72*	-0.43	-0.81	-0.66
Mass	1.0	0.89**	0.65	0.98**	0.92**
Biceps		1.0	0.57	0.80*	0.84**
Triceps			1.0	0.70	0.87**
Subscapular				1.0	0.93**

* Correlation is significant at $P < 0.05$; ** $P < 0.01$

While the differences in individual sites (initial measurement – 10 yr. measurement) showed significant correlations with weight change at biceps, subscapular and suprailiac sites ($P < 0.01$), no significant correlation was found at the triceps site.

In this very limited group, no firm conclusions can be drawn as regards the detailed response of the body, because the activity has been subjectively reported. However it illustrates the wide variation in response of skinfolds with weight change. In three individuals, two of whom reported no reduction in physical activity, the ST ratio actually decreased over the 10 year period, suggesting that in some individuals, the trend for it to increase due to age, may be offset by physical activity. (The average value for all eight individuals also showed a slight decrease, though this was not significant; $P > 0.05$). However, the

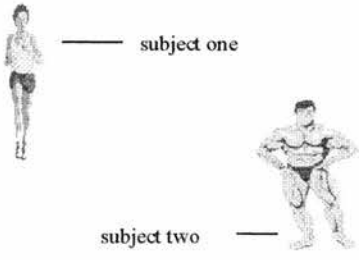
difference in ST ratio over the study period showed a correlation of 0.83 with weight gain ($P < 0.05$), which suggests that change in skinfold ratios may be more influenced by adiposity than age.

In summary, this longitudinal study highlights the considerable individual variation which occurs in skinfold thickness response to age and activity, with weight gain being associated with a reduction in activity, and manifested by increased skinfolds, especially on torso sites.

Extreme morphological adaptation in two male athletes

While it could be argued that the presence or absence of exercise might confer the greatest variation in physique which an adult can experience, within physically active individuals there is immense diversity in shape and form, independent of body size. While power athletes are mainly concerned with gaining muscle bulk, bodybuilders are also concerned with the definition of muscle groups which become apparent only when the subcutaneous adipose layer is thin. Endurance athletes on the other hand, tend not to strength train muscle groups to any significant extent, but to pursue whole-body exercise. One male example from each group is presented here, and compared with the male athlete cohort (n = 106).

Subject one was an endurance exerciser, with a medical condition involving compulsive exercise behaviour and inadequate nutrition. *Subject two* was a semi–professional bodybuilder whose training regime regularly includes fasting before competitions.



While it is possible to calculate Z scores using standard kinanthropometric procedures based on standard deviations from a stature-corrected mean (Ross et al., 1980), it would be surprising if subjects of the present study were not more muscular and less fat than a theoretical unisex phantom. As a more useful alternative, Z scores have been calculated according to standard deviations taken from the 106 male athletes for whom complete data exist.

A summary of body morphology is presented in table 5.6. Detailed skeletal dimensions and phantom z-scores are presented in table 5.7.

Table 5.6 Summary of body morphology in two male athletes

Factor	Subject one	Subject two	Average of 106 athletes
Age (yr.)	21	31	28.3 ± 7.4
Height (cm.)	182.5	175.5	180.5 ± 7.2
Mass (kg.)	55.0	102.2	77.4 ± 9.7
BMI ($kg.m^{-2}$)	16.51	33.17	23.7 ± 2.3
Exercise ($hrs.wk^{-1}$)	12.0	14.0	9.2 ± 4.7
% skeletal mass[a]	17.5	9.9	12.4 ± 1.0
% muscle mass[b]	50.7	64.7	57.9 ± 4.4
% fat[c]	2.5	10.9	9.1 ± 3.4
endomorphy	1.21	3.16	2.76 ± 0.85
mesomorphy	2.38	9.33	4.98 ± 1.18
ectomorphy	6.59	0.10	2.56 ± 1.02

[a] Drinkwater et al., (1986) [b] Martin et al., (1990) [c] Jackson & Pollock (1978);

Table 5.7 Detailed kinanthropometric analysis of skeletal dimensions in two male athletes

Factor	Subject one	Z score	Subject two	Z score	Average of 106 athletes
Height	182.5	0.31	175.5	-0.67	180.3 ± 7.2
Sitting height	94.9	0.38	94.6	0.30	93.4 ± 4.0
Iliospinale Height	102.9	0.15	94.4*	-1.43	102.1 ± 5.4
Biacromial breadth	39.3	-0.55	42.6*	1.28	40.3 ± 1.8
Bicristal breadth	31.0	0.89	30.5	0.42	29.3 ± 1.9
Chest breadth	27.2*	-1.57	34.6*	1.95	30.5 ± 2.1
Chest depth	17.3*	-1.50	22.5*	1.75	19.7 ± 1.6
Humerus breadth	7.2	0.40	7.4	0.80	7.0 ± 0.5
Wrist breadth	5.5	-0.50	6.0	0.75	5.7 ± 0.4
Femoral breadth	10.1	-0.20	11.1*	1.8	10.2 ± 0.5
Ankle breadth	7.5	0.50	7.2	-0.25	7.3 ± 0.4

Average is mean ± SD. All measurements in cm. * More than ± 1 SD from average of 106 male athletes

Table 5. 8 Comparison of Unisex (U-Z) and athlete-derived Z scores (A-Z) in two male athletes

	Subject one		Subject two	
Factor	**U-Z score**	**A-Z score**	**U-Z score**	**A -Z score**
Height		0.31		-0.67
Sitting height		0.38		0.30
Iliospinale Height		0.15		-1.43
Biacromial breadth	-0.73	-0.55	1.70	1.28
Bicristal breadth	0.04	0.89	0.42	0.42
Chest breadth	-1.47	-1.57	3.24	1.95
Chest depth	-0.99	-1.50	3.13	1.75
Humerus breadth	0.67	0.40	1.99	0.80
Wrist breadth	-0.29	-0.50	2.17	0.75
Femoral breadth	-0.21	-0.20	2.59	1.80
Ankle breadth	0.87	0.50	0.84	-0.25

Table 5.9 Detailed kinanthropometric analysis of circumferences in two male athletes

Factor	**Subject one**	**Z score**	**Subject two**	**Z score**	**Average of 106 athletes**
Upper arm relaxed	23.5	-2.18	39.7	3.61	29.6 ± 2.8
Upper arm flexed	26.5	-2.20	43.6	3.50	33.1 ± 3.0
Forearm	24.5	-1.72	32.5	2.72	27.6 ± 1.8
Thigh	41.5	-3.63	65.1	2.58	55.3 ± 3.8
Calf	29.0	-2.65	42.0	1.18	38.0 ± 3.4
Chest	86.5	-1.61	119.0	3.90	96.0 ± 5.9
Waist	63.0	-2.98	88.3	1.88	78.5 ± 5.2
Abdomen	67.0	-2.56	92.0	1.54	82.6 ± 6.1
Hip	85.0	-2.02	104.5	1.88	95.1 ± 5.0

Average is mean ± SD. All measurements in cm. Z scores calculated according to Ross et al., (1980).

Table 5.10 Detailed kinanthropometric analysis of skinfolds in two male athletes

Factor	Subject one	Z score	Subject two	Z score	Average of 106 athletes
Cheek	6.5	-2.64	15.8	1.59	12.3 ± 2.2
Chin	3.2	-1.00	6.7	1.33	4.7 ±1.5
Chest (pectoral)	3.3	-1.41	9.3	0.81	7.1 ± 2.7
Triceps	3.2	-1.70	4.6	-1.19	7.8 ± 2.7
Subscapular	6.1	-1.44	16.4	2.37	10.0 ± 2.7
Chest	3.6	-1.12	11.0	1.34	7.1 ± 2.9
Supraspinale	4.9	-1.20	9.9	0.02	9.8 ± 4.1
Abdominal	5.5	-1.36	15.3	0.31	13.5 ± 5.9
Thigh patella	5.0	-0.97	11.2	0.72	9.4 ± 2.5
Thigh	3.8	-1.78	7.4	-0.98	11.8 ± 4.5
Proximal calf	2.7	-1.78	5.5	-0.74	7.5 ± 2.7
Mid-calf	3.1	-2.06	8.0	-0.69	10.5 ± 3.6
Biceps	2.3	-1.38	4.4	0.23	4.1 ± 1.3
Forearm	2.5	-1.82	3.9	-0.54	4.5 ± 1.1
Forearm – radial	2.7	-2.00	5.4	-0.07	5.5 ± 1.4
Medial calf	3.0	-1.30	4.2	-0.94	7.3 ± 3.3
Suprailium	3.4	-1.20	5.2	-0.32	6.4 ± 2.5
Axilla	3.5	-1.29	9.9	0.77	7.5 ± 3.1
Suprailiac	4.6	-1.28	10.4	-0.26	11.9 ± 5.7
Total of 19	72.9	-1.94	164.5	0.14	158.5 ± 44.2

Average is ± SD. All measurements in mm. Z scores calculated according to Ross et al., (1980).

Z scores are available for all these variables based on a unisex phantom, scaled dimensional exponents and a large heterogeneous database (Ross et al., 1980). Under such scrutiny subjects appear very lean although subject one's slenderness is somewhat hidden, while subject two appears excessively stocky. However such Z scores may mask the pattern exhibited by subjects one and two, because they are more muscular and less fat than average male subjects. If the departure from the mean of the athletic group is taken as a measure of uniqueness, with the assumption that the underlying distribution is normal, then

comparing 'athletic Z scores' from various measurements allows an understanding of how extreme these subjects' morphology is within an athletic context. Distribution of a selection of skeletal variables is illustrated in table 5.8. A comparison of proportional tissue masses is presented in figure 5.3.

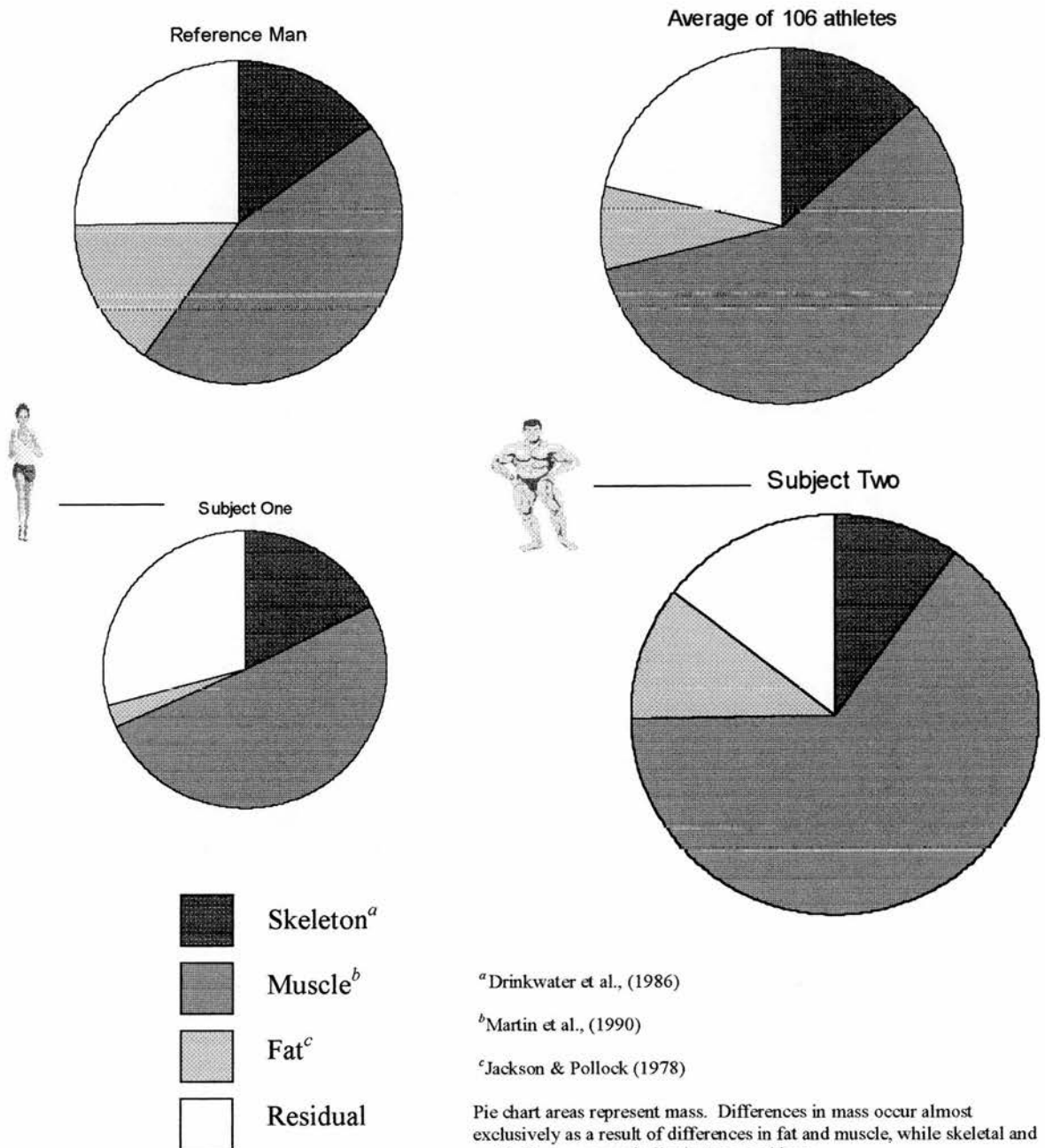
An overview would be that there is little unusual in either the skeletal dimensions, or skinfolds, but that circumferences of both subjects exhibit the widest departures from average. Subject one exhibits very low athletic z-scores for mass (-2.31) and BMI (-3.13), largely arising as a result of a slender frame. The average of 10 skeletal measurements' athletic z-scores is –0.22, suggesting his frame is far from extreme, and supports a low muscle mass and low fat mass. Because the total mass is low, the contribution of each tissue is somewhat distorted, with, for instance a higher % muscle than sedentary individuals, but lower than average for athletes. The fat is very low, even for athletes, but the low muscle mass is the more extreme. Equivalent athletic z-scores for the average of 9 circumferences and 19 skinfolds are -2.39 and -1.52 respectively. The high % skeletal mass is ironic in view of his extreme ectomorphic physique characterised by 'skeletal fragility' (Heath and Carter, 1967).

Subject two is the athletic antithesis of *subject one*. He has very high athletic z-scores for mass (+ 2.56) and BMI (+ 4.12). His extreme mesomorph physique is largely the result of a very large muscle mass, average fat mass (for an athlete) and a large frame. The average of 10 skeletal measurements' athletic z scores is + 0.70, and corresponding values are + 2.53 for 9 circumferences and + 0.21 for 19 skinfolds. By comparison, subject one appeared to have most diminished girth values at the waist, abdomen and calf, while subject two appears to have most enlarged at the chest and upper arm sites (table 5.9). By comparison, skinfold topography appears more evenly distributed (table 5.10).

The residual component for both athletes (largely comprising the nervous system and all internal organs, occupies 29.3% of subject one and 14.5% of subject two. However, this translates into a roughly equivalent 16.1kg and 14.8 kg respectively. Seen together, it appears that excessive muscle mass appears associated with modest additional fat accumulation, while excessive loss of fat appears associated with

loss of muscle in addition. Such speculation is borne out by the successful heavyweight power-lifter athletes' physiques, and, by contrast, observation of the body's response to starvation.

Figure 5.3 Relative proportions of tissue mass



[a] Drinkwater et al., (1986)

[b] Martin et al., (1990)

[c] Jackson & Pollock (1978)

Pie chart areas represent mass. Differences in mass occur almost exclusively as a result of differences in fat and muscle, while skeletal and residual masses are similar between subjects.
Reference Man data is from Behnke & Wilmore (1974)
Images of subjects one and two are purely schematic.

5.2. DXA SUB-REGIONAL TISSUE MORPHOMETRY

DXA offers a relatively unique facility by allowing each anatomical region to be considered separately in terms of bone mineral, lean tissue and fat. Analysis of whole body scans therefore permits comparisons of absolute tissue masses for each tissue type, proportional distribution of each tissue type, and comparisons between gender, age and physical activity. However, studies using DXA for regional composition are rare. In one study (Nindl et al., 1996) DXA and anthropometric assessment of fat loss following military training yielded conflicting results, a finding which the authors link to regional differences in lipoprotein lipase activity, regional blood flow and responsiveness to systemic hormones (Smith et al, 1979). Given that DXA measures total lipid, while anthropometry measures subcutaneous adipose tissue, differences arising from internal : surface adipose tissue distribution, together with the variable lipid fraction of adipose tissue with total adiposity (Martin et al., 1994) could also explain the discrepancy.

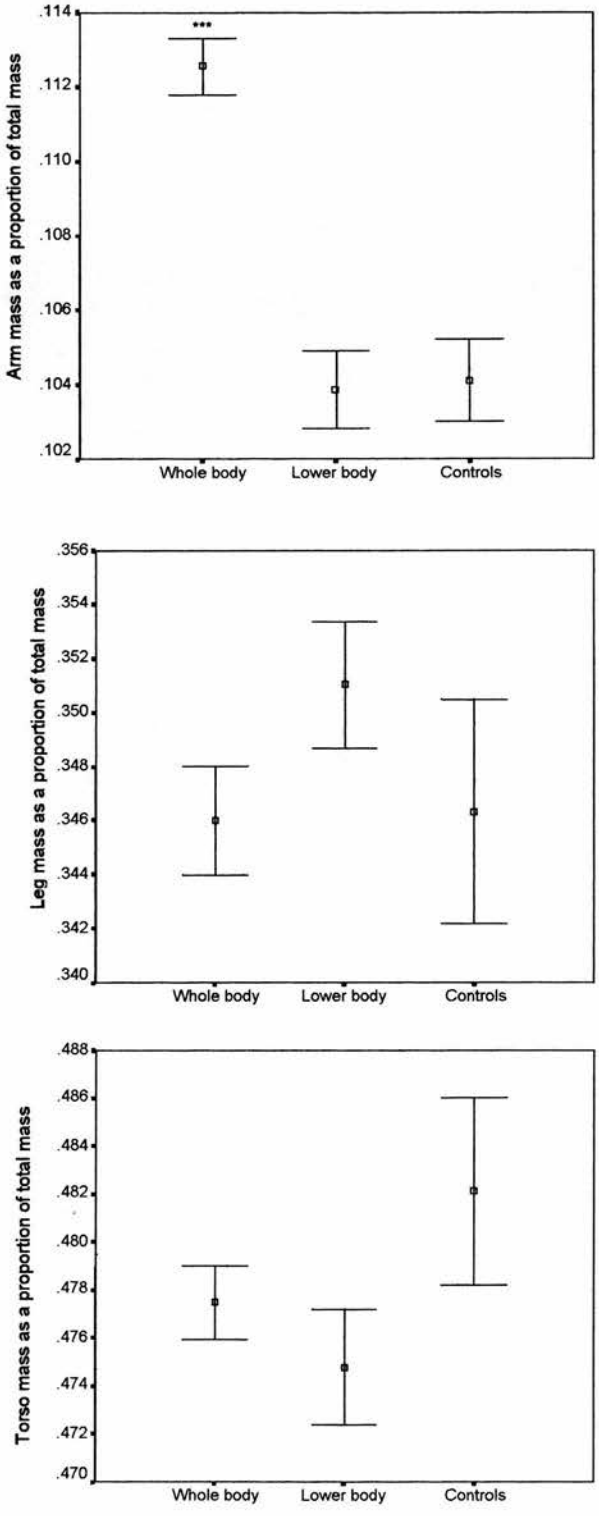
This aspect of the study focuses on DXA and aims to investigate whether BMC, lean and fat proportions and their distribution varies between different athletic groups in male subjects.

Whole body and lower body comparison.

The effect of total, bone, lean and fat tissue distribution was compared initially by subdividing the 106 athletes of the present study into groups whose sport stressed the lower body alone (cyclists and runners; n =28), or the whole body (all other athletic groups; n =78). These were compared with controls known to take no deliberate exercise (n = 15).

Regional proportion of total mass, BMC, lean mass and fat are illustrated in figures 5.4, 5.5, 5.6 and 5.7 respectively. Arm mass as a proportion of total mass was the only region to show a difference between whole body exercisers and both other groups ($P < 0.001$).

Figure 5.4 Proportional distribution of total mass in 78 whole body athletes, 28 lower body athletes and 15 controls



*** Difference from controls $P < 0.001$; Error bars refer to mean ± 1SE

Figure 5.5 Proportional distribution of total BMC in 78 whole body athletes, 28 lower body athletes and 15 controls

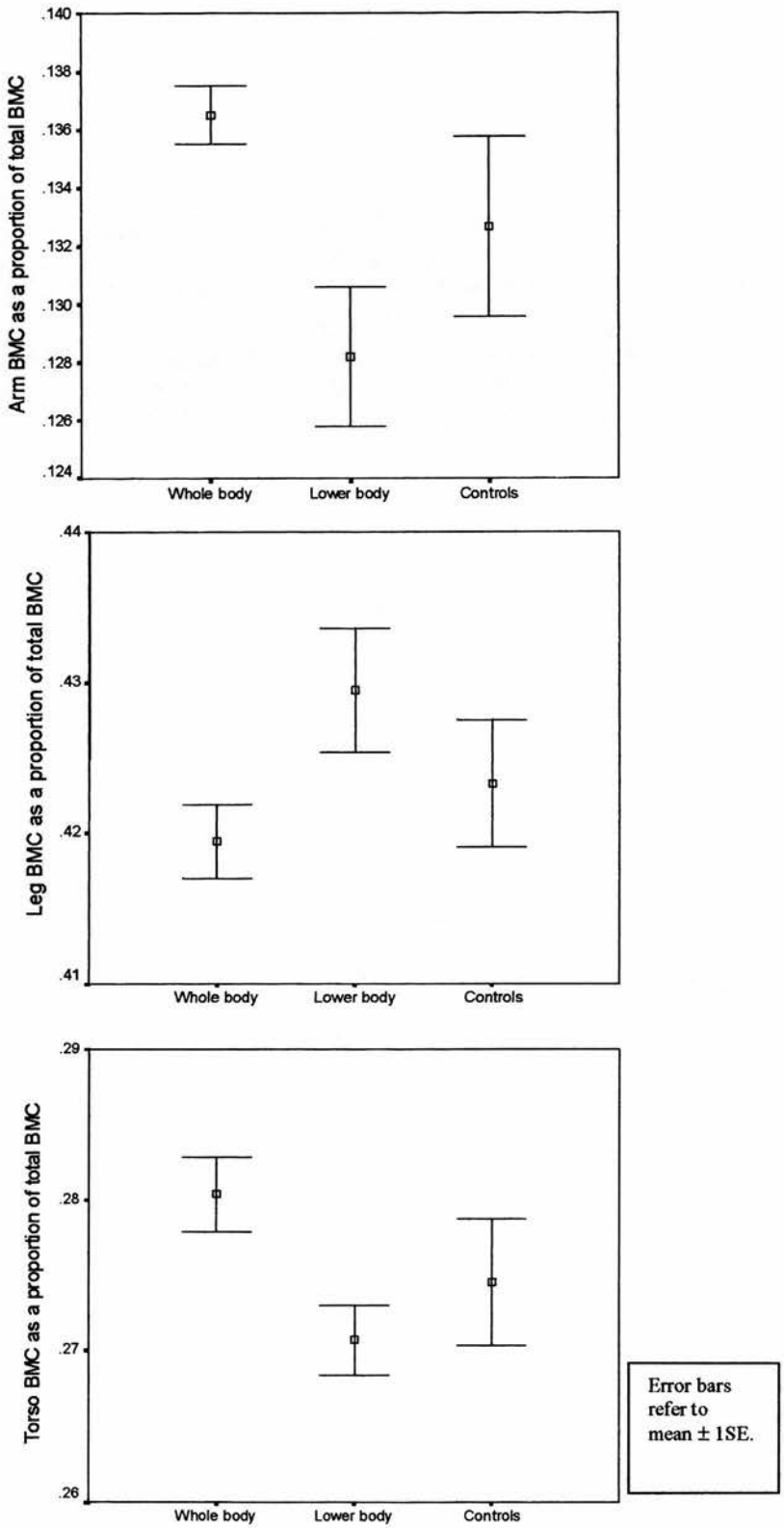


Figure 5.6 Proportional distribution of lean mass in 78 whole body athletes, 28 lower body athletes and 15 controls

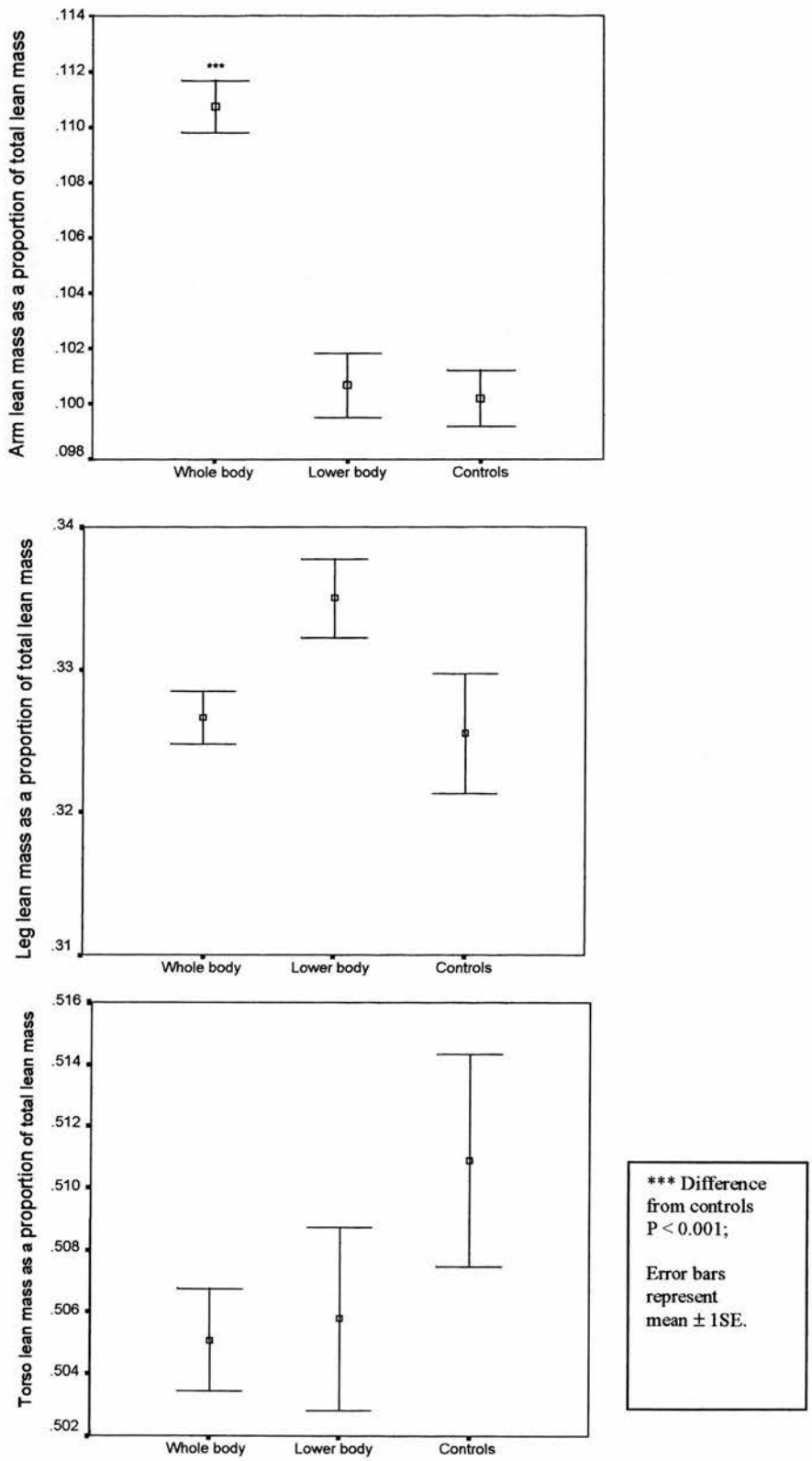
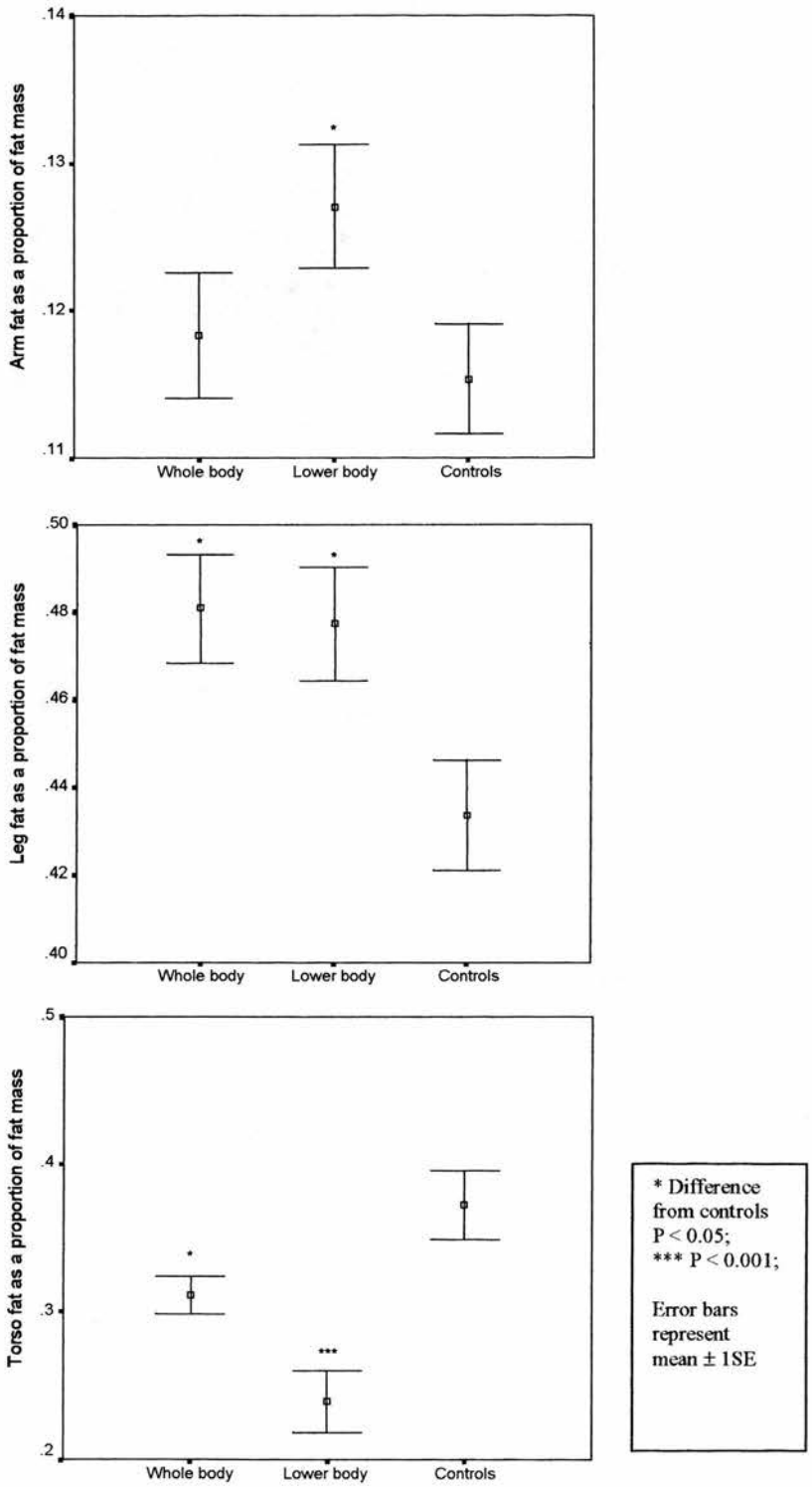


Figure 5.7 Proportional distribution in of fat in 78 whole body athletes, 28 lower body athletes and 15 controls



There were no significant differences in regional bone proportions between the controls and either of the athletic groups ($P > 0.05$). There were, however, BMC differences between the two athletic groups. Whole body exercisers had greater arm BMC : total BMC and torso BMC : total BMC ($P < 0.01$), but less leg BMC : total BMC ($P < 0.05$) than lower body exercisers, as illustrated in figure 5.5.

Whole body athletes had greater arm proportions of total lean mass than lower body athletes or controls ($P < 0.001$), as illustrated in figure 5.6.

Whole body athletes had greater leg proportions and lesser torso proportions of total fat than controls ($P < 0.05$). Lower body athletes had greater arm proportions ($P < 0.05$), greater leg proportions ($P < 0.05$) but lesser torso proportions ($P < 0.001$) than controls, as illustrated in figure 5.7. However, differences in fat distributions were not significant after controlling for % fat in multivariate analysis ($P > 0.05$).

Lean tissue distribution was investigated by calculating the ratio of lean tissue in the arms : legs (LAL) and in the trunk : legs (LTL). Whole body exercisers had greater LAL than lower body exercisers and controls ($P < 0.001$), but there were no differences in LTL ratio. Arm lean mass, as a proportion of total lean mass was the only region to show a difference between whole body exercisers and both other groups ($P <0.001$)

Fat distribution was examined by the ratio of fat on the arms : legs (FAL) and on the torso : legs (FTL). There were no differences in FAL ratio between the groups. Whole body exercisers had greater FTL ratio than lower body exercisers ($P < 0.05$), and lower body exercisers had lower FTL than controls ($P < 0.001$).

Lower body exercisers had a greater proportion of fat situated on the arms than both other groups ($P < 0.05$), while controls had a lesser proportion on the legs than both athletic groups ($P < 0.05$). Controls had greater fat proportion on the torso than whole body exercisers ($P < 0.05$) or lower body exercisers ($P <$

0.001), but differences were no longer significant after controlling for % fat using multivariate analysis ($P > 0.05$).

Comparison of individual sporting groups by tissue ratios.

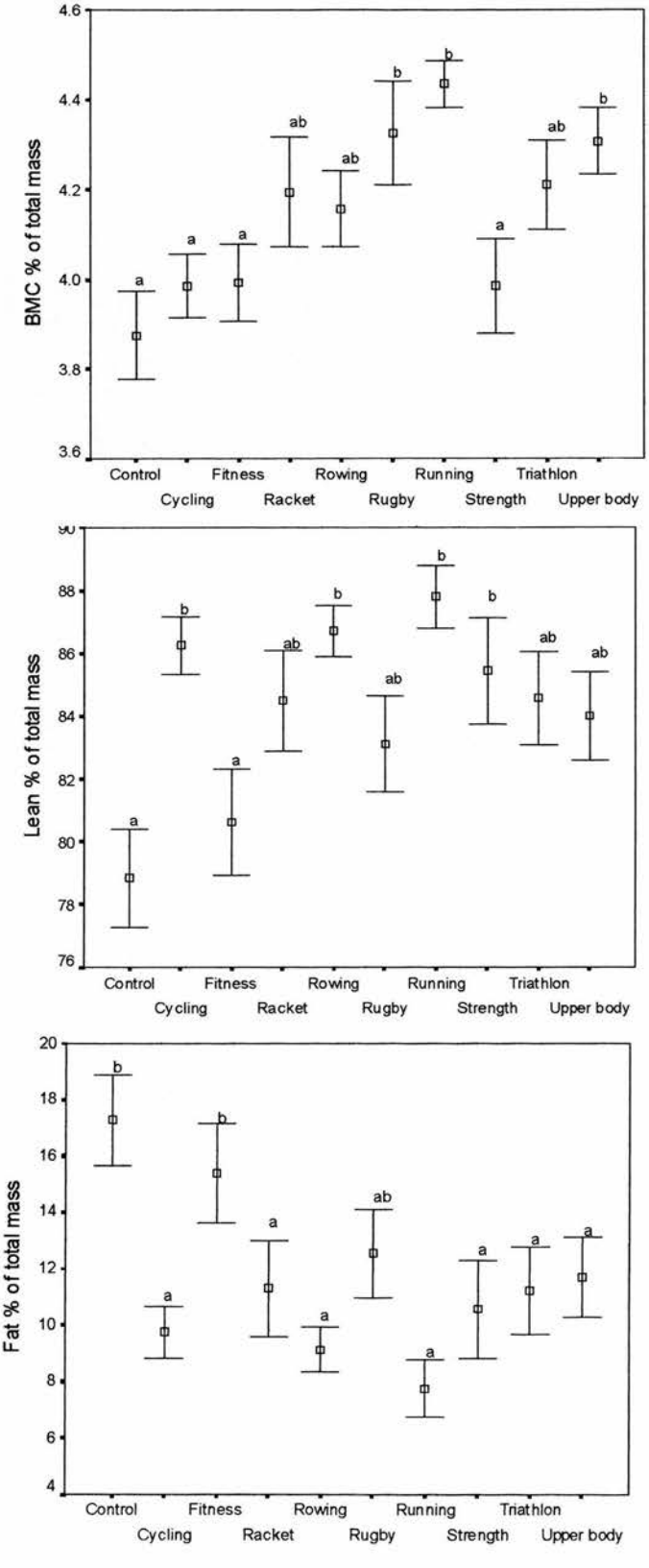
Athletes were then divided into their individual sports to investigate the distribution of BMC, lean tissue and fat. These were controls (n = 15), runners (n = 12), cyclists (n = 16), triathletes (n = 15), strength athletes (n = 6) comprising weightlifters and bodybuilders, racket sports players (n = 12), rowers (n = 15), rugby players (n = 11) non-competitive fitness participants (n = 9), and a group whose sports stressed the upper body (n = 10) comprising kayakers, rock climbers, combative athletes and swimmers.

Tissue distribution was assessed by calculating ratios of tissues in the arm : leg and torso : leg. Because DXA is limited in its ability to measure inside the skull, head data were omitted from these calculations. The ratios included arm BMC: leg BMC (BAL) ratio; torso BMC : leg BMC (BTL) ratio; arm lean : leg lean (LAL) ratio; torso lean : leg lean (LTL) ratio; arm fat : leg fat (FAL) ratio and the torso fat : leg fat (FTL) ratio. These ratios enabled regional comparison to be made between groups without requiring a correction for total mass. ANOVA showed significant differences between the groups for all tissue types ($P < 0.001$), and Student Newman Keuls post hoc tests for homogeneous subsets grouped various sports together, as illustrated in figure 5.8, which is an alternative means of expressing the DXA morphotype, as defined in chapter 4.3.

BMC

ANOVA showed significant differences in BAL ratio ($P < 0.001$), with runners exhibiting lower values than all other categories. SNK post – hoc tests put runners in a separate group with low values ($P < 0.05$), and fitness, rugby players, racket players, upper body athletes and strength athletes, having higher values than other athletes were all grouped together ($P < 0.05$).

Figure 5.8. Tissue percentages of total mass in male athletic groups

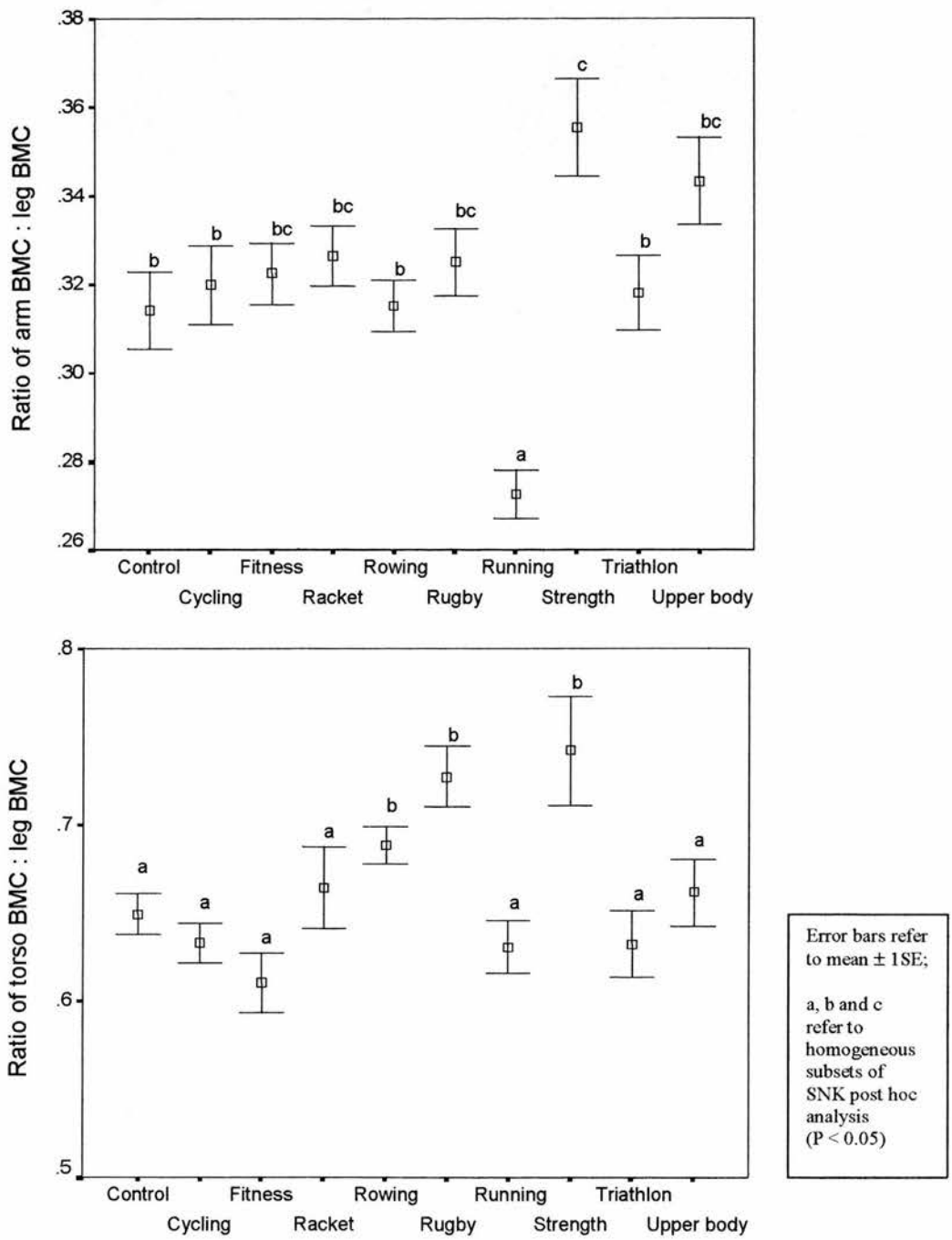


Error bars refer to mean ± 1SE;

a and b refer to homogeneous subsets of SNK post-hoc analysis ($P < 0.05$)

BTL ratios were also significantly different between the groups by ANOVA ($P < 0.001$). SNK post-hoc tests included strength athletes, rugby players and rowers in a separate group with higher values ($P < 0.05$). BAL and BTL ratios are illustrated in figure 5.9.

Figure 5.9. BAL and BTL ratios in male athletic groups



Lean tissue

One way ANOVA showed significant differences between the groups for LAL ratio ($P < 0.001$). Strength athletes, Racket sports players and rugby players showed higher ratios than controls ($P < 0.001$), as did rowers ($P < 0.01$). Student-Newman-Keuls (SNK) post-hoc tests put strength athletes separate from other groups but included racket players, rowers, rugby players upper body athletes and triathletes into one homogeneous group ($P < 0.05$), while controls, runners, cyclists and fitness categories were also grouped together ($P < 0.05$).

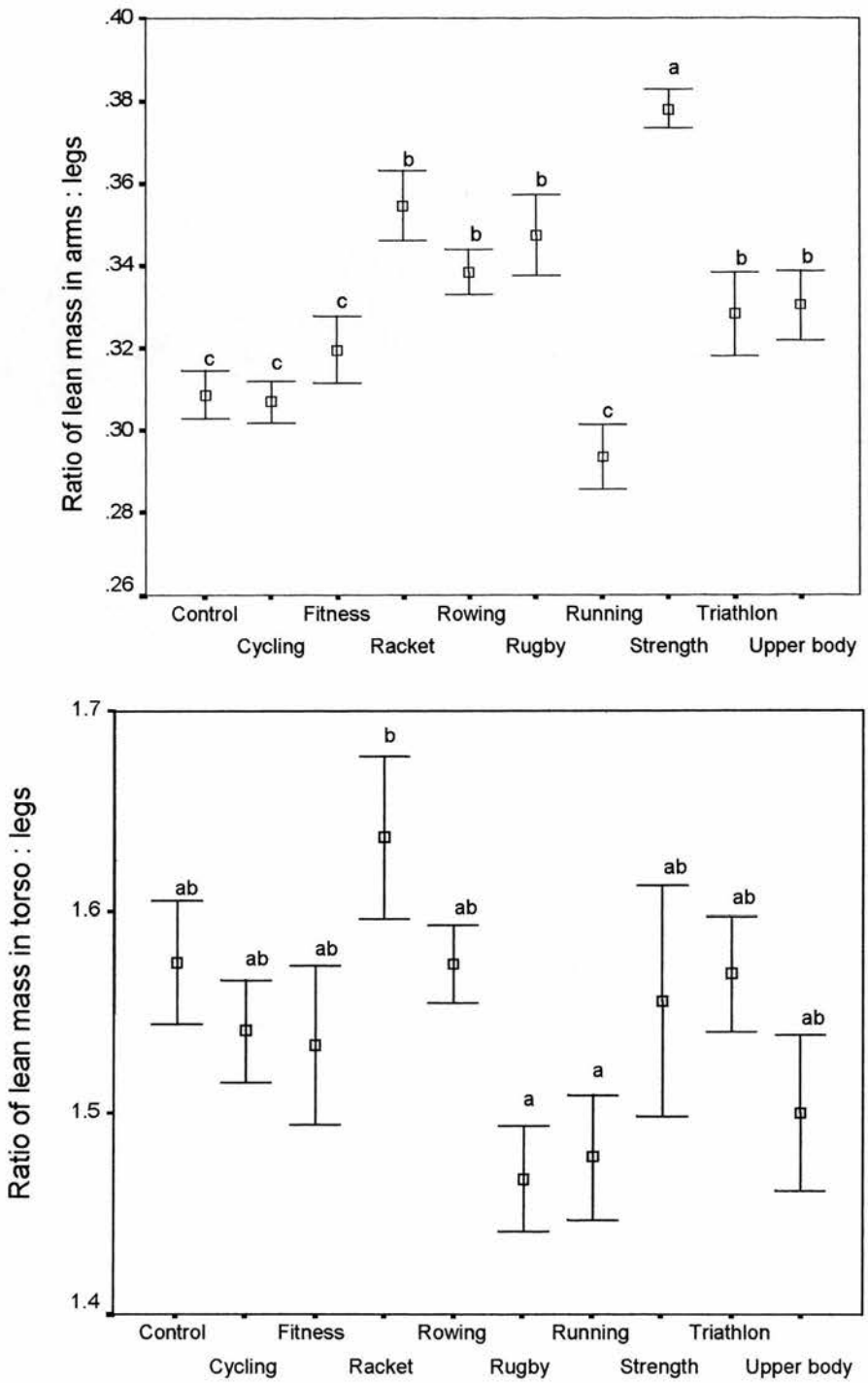
LTL ratio showed less variation between the groups. ANOVA found a significant difference ($P < 0.05$), but only runners and rugby players were different from controls ($P < 0.05$). SNK post-hoc tests put all groups in one homogenous category. Lean tissue ratios are illustrated in figure 5.10.

Fat

Fat distribution was less variable between the groups, and ANOVA failed to find a significant inter-group difference in FAL ratio ($P > 0.05$). However the apparently low value in rowers was investigated by multiple T tests with controls and other endurance athletes (runners, cyclists and triathletes) using the Bonferroni correction. Rowers had lower ratios than controls, runners and triathletes ($P < 0.01$), but the difference was of borderline significance compared with cyclists ($P = 0.018$). Rowers were significantly younger than controls, runners ($P < 0.05$) and triathletes ($P < 0.001$), and controlling for age in multivariate analysis, the difference in FAL was no longer significant ($P > 0.05$).

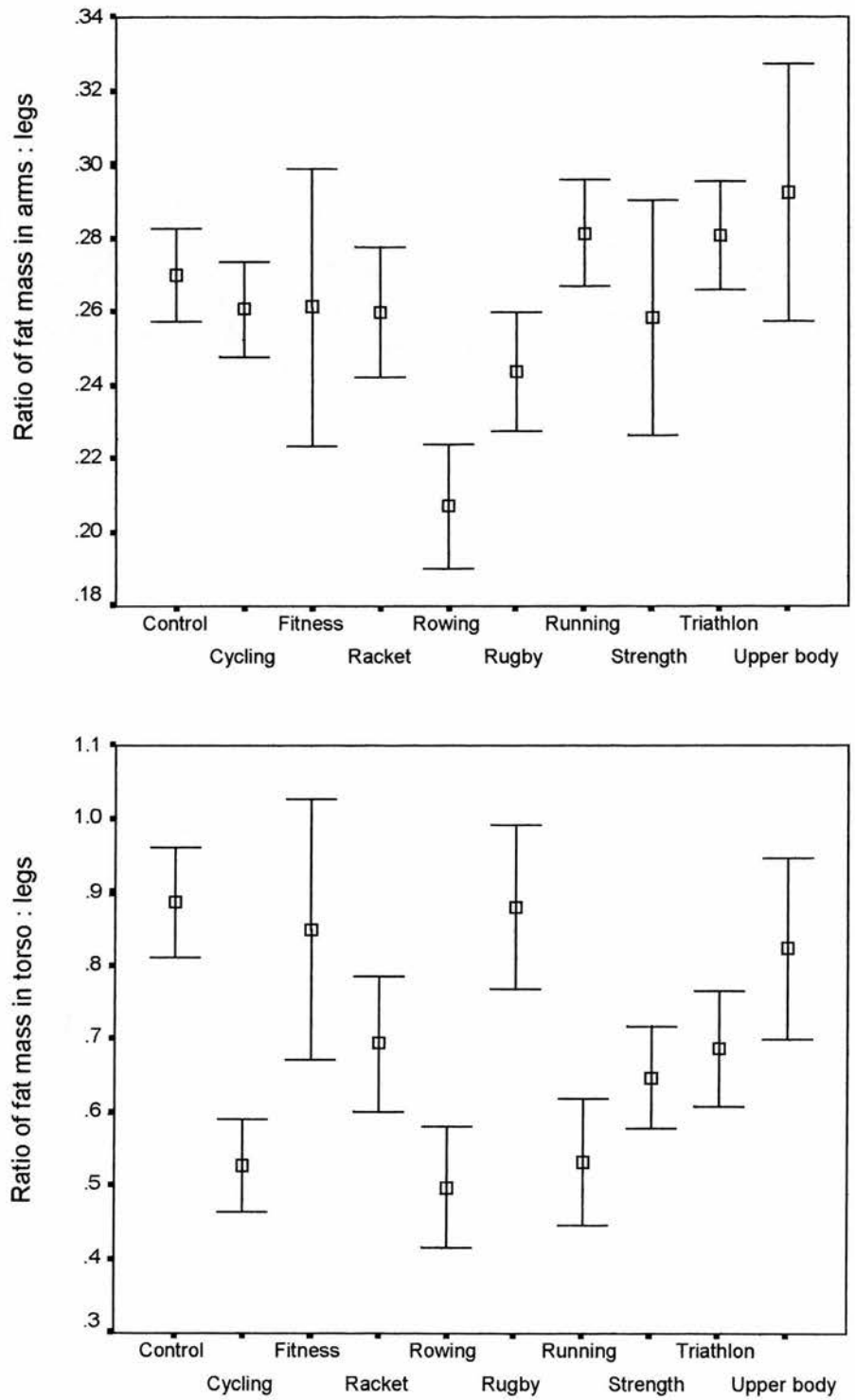
FTL ratio ANOVA showed significant inter-group differences ($P < 0.01$), although SNK post hoc tests placed all athletic groups together. Multiple T tests with the Bonferroni correction showed runners, cyclists and rowers to have lower ratios than controls ($P < 0.01$). Fat tissue ratios are illustrated in figure 5.11.

Figure 5.10. LAL and LTL ratios in male athletic groups



Error bars refer to mean ± 1SE; a and b refer to homogeneous subsets of SNK post hoc analysis ($P < 0.05$)

Figure 5.11. FAL and FTL ratios in male athletic groups



Error bars refer to mean ± 1SE.

The initial analysis involving whole body athletes, lower body and controls determined differences in tissue mass which were largely a result of lean tissue distribution. Differences in fat distribution between the athletic groups were not significant after controlling for % fat, but the data suggest that of the regions investigated, torso fat appears to fluctuate the most with alterations in total % fat, which agrees with the finding that the abdominal adipose depot appears to be more readily mobilised than peripheral depots with endurance exercise (Bouchard et al., 1993). When athletes from all sports were considered, no pattern of fat distribution was significantly different amongst the sporting groups ($P > 0.05$), with the exception of the rowers, whose low torso fat proportion was not significant after correction for age.

Every athletic group showed significant differences from controls for FTL ratio after correction for age and total fat % ($P < 0.01$), although in fitness athletes this difference was less significant ($P < 0.05$). Fitness athletes spent fewer hours per week exercising than triathletes, strength athletes, racket players ($P < 0.05$); runners, cyclists, rowers ($P < 0.01$) and rugby players ($P < 0.001$). Furthermore, the extra intensity of exercise offered by competitive sports may have contributed an additional effect on fat distribution. This suggests that exercise is important in altering fat distribution, but the type of exercise is unimportant. These data suggest an absence of exercise is associated with larger total fat and increased FTL ratio. While the metabolic activity of the muscle was not measured, differences in lean mass distribution, which imply metabolic activity, were not associated with any local effect on fat distribution.

Based on 17 paired measurements, the %CV for total fat, lean and BMC were 3.0%, 0.7% and 0.9% respectively, very similar to previously reported precision (Fuller et al., 1992b), and better than an earlier study which concluded that DXA provides precise total and regional body composition (Mazess et al., 1990). Regional precision is poorer than total body precision, with the present study showing %CV of 5 – 6% for fat, 1 – 2% for lean and 1 – 3% for BMC. In all cases, precision was poorest in the arms, probably because this represents the smallest regional mass, and delineation of the arm : torso boundary may be more difficult than other boundaries to establish in some subjects due to arms being 'forced' parallel to the torso in order for larger subjects to remain within the scanned area.

The use of tissue ratios may exacerbate the precision error in analysis, but because methodological and systematic errors are avoided by the same scanner and operator being used throughout the present study, these errors are minimised. Nevertheless, comparison of regional data from DXA scanners of different manufacturers must be done with caution, as pixel size, X-ray voltages and bone shape and edge-detection algorithms differ. Thus DXA is best viewed as a range of techniques specific to each manufacturer as far as regional tissue distribution is concerned, despite cross-calibration equations developed to convert between results from different manufacturers for total tissue mass (Modlesky et al., 1996).

Gender differences in regional tissue distribution can be easily identified. Examples of male and female results from the present study are given in table 5.11.

Table 5.11 Regional body composition in 106 male and 30 female athletes and 23 male and 30 female controls using DXA

	controls				athletes			
Males	**BMC**	**Lean**	**Fat**	**Total**	**BMC**	**Lean**	**Fat**	**Total**
Arms	402	6152	1699	8253	430	7054	986	8470
Legs	1257	19392	5678	26327	1349	21366	3885	26600
Torso	804	30621	5339	36764	894	32773	2805	36472
Total *	2961	59954	13559	76474	3199	64912	8409	76520
Females								
Arms	301	3720	3000	7021	253	3801	1274	5328
Legs	905	13673	9424	24002	900	13994	5194	20088
Torso	512	23600	6960	31072	604	23853	2627	27084
Total*	2311	42649	20108	65068	2225	44750	9765	56740

* Includes head data

The sub-regional analysis study of Mazess et al., (1990) performed on a Lunar DPX, (Lunar Corporation, Madison, WI. USA), is one of few studies which render comparison possible. However, the sub-regional analysis may have been performed according to a different protocol involving different anatomical landmarks delineating the boundaries between the regions. Nevertheless, a comparison of male and female subjects is comparable within and between the studies.

Of the non-athletic subjects, males appear to have about half the total fat of females, and while males have fractionally more fat on the torso, females have noticeably more on the legs. In athletes, the difference between total fat in males and females is much less, and for both groups the torso fat is much less than leg fat. Thus it is likely that that the preferential mobilisation of torso fat as a response to chronic exercise applies equally well to both sexes.

The ratio of lean tissue mass between arms and legs is close to 1 : 3 in males and 1 : 4 in females and appears similar for athletes and non-athletes. However, there is a small difference in the lean tissue on the torso as a percentage of the total lean mass. In the present study male and female athletes had approximately 50% and 52% respectively, while the non-athletes had 51% and 53% respectively, suggesting exercise adds more limb muscle than torso muscle. The equivalent figure from Mazess et al., (1990) is 45% for six young male and 43% for six young female adults. Age and ethnicity were not given, so a precise comparison is not possible, but it is likely that the differences arise more from a systematic difference in analysis protocol arising from different manufacturers' procedures, than any true difference in regional composition. This highlights an important point – while comparisons of total body composition between DXA machines from different manufacturers is difficult, sub-regional analysis comparisons are even more tenuous. In addition, gender comparisons within the present study may not be able to evaluate the effect of exercise, because female athletes have a reduced strength component to their training compared with men, which de-emphasises the upper body compared with the legs. The consequence – the fact that athletic participation causes different morphometric adaptations in men and women in terms of lean and BMC regional distribution – could be explained at least partly by differences in training type in addition to gender. The fact that female athletes have less arm BMC than controls despite having more lean tissue (whereas male athletes have greater quantities of both) may lead to speculation that female bone adaptation and architecture may be the result of a greater number of influences in this group.

Comparison of female athletes, anorexics and controls.

Because there were insufficient numbers of subjects to compare different sporting disciplines in female athletes, athletes in general (n = 30) were compared with archived data from 30 controls and 30 individuals being treated for anorexia nervosa, whose total body composition was described in chapter 4.3. Results of regional body composition appear in table 5.12.

Table 5.12 Regional body composition in 30 female athletes, 30 anorexics and 30 controls

Variable	Athletes	Anorexics	Controls
Age (yr.)	25.9 ± 5.5	28.0 ± 6.4	28.1 ± 4.6
Height (cm)	167.9 ± 6.3	163.1 ± 5.4	165.0 ± 6.9
Mass (kg)	56.9 ± 7.2**	39.6 ± 4.5***	63.7 ± 9.1
BMI ($kg.m^{-2}$)	20.02 ± 2.2***	14.8 ± 1.2***	23.4 ± 2.8
Bone			
Arm BMC	253 ± 50***	169 ± 40***	296 ± 41
Leg BMC	900 ± 140	602 ± 162***	893 ± 128
Torso BMC	606 ± 100	399 ± 82***	629 ± 101
Total BMC	2225 ± 312	1593 ± 292***	2274 ± 265
Lean			
Arm lean	3801 ± 604**	2814 ± 463***	3368 ± 554
Leg lean	13994 ± 1977**	9954 ± 1470***	12641 ± 1777
Torso lean	23854 ± 2547	17931 ± 2265***	23304 ± 2998
Total lean	44612 ± 5073	33548 ± 4092***	42278 ± 5075
Fat			
Arm fat	1274 ± 613***	597 ± 262***	2783 ± 853
Leg fat	5193 ± 1934***	2117 ± 1165***	9090 ± 2149
Torso fat	2627 ± 1999***	940 ± 735***	6491 ± 2727
Total fat	9765 ± 4411***	4282 ± 1990***	19029 ± 5310

Tissue masses are in g. * Difference from controls $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$;

These data suggest that anorexics are significantly different from controls in BMC, lean and fat content in all regions. Compared with controls, athletes had less arm bone ($P < 0.05$), more arm lean and leg lean ($P < 0.01$) and less fat in all regions ($P < 0.001$). Compared with anorexics, athletes had more BMC, lean and fat tissue in all regions ($P < 0.001$). However the tissue quantities may not discriminate between all regional differences which could help determine if athletes are, for instance, intermediate between controls and anorexics, as far as fat distribution is concerned. Such patterns of tissue quantity were examined as proportions of the tissue totals and of the regional totals. The results appear in tables 5.13 – 5.15.

Table 5.13 Fat proportions in 30 female athletes, 30 anorexics and 30 controls.

	Athletes	Anorexics	Controls
Arm fat / fat mass	0.131 ± 0.02**	0.146 ± 0.03	0.146 ± 0.01
Arm fat / arm mass	0.234 ± 0.10***	0.166 ± 0.06***	0.426 ± 0.07
Leg fat / fat mass	0.548 ± 0.06***	0.481 ± 0.1	0.483 ± 0.06
Leg fat / leg mass	0.253 ± 0.07***	0.164 ± 0.08***	0.397 ± 0.05
Torso fat / fat mass	0.234 ± 0.1***	0.188 ± 0.1***	0.330 ± 0.07
Torso fat / torso mass	0.096 ± 0.06***	0.050 ± 0.04***	0.212 ± 0.06

* Difference from controls $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$;

While it is clear that both athletic and anorexic groups had less fat in all regions than controls, examination of the proportions of the differences in distribution indicates anorexics and athletes had different distributions from each other. Arm fat as a proportion of arm mass is significantly lower for athletes and anorexics than controls ($P < 0.001$), but while arm fat as a proportion of fat mass was lower in athletes ($P < 0.01$), in anorexics it was no different from controls ($P > 0.05$). Athletes had a lower proportion of total fat on arms ($P < 0.05$) and higher proportion on legs ($P < 0.001$) than anorexics, although the proportion on the torso was no different ($P > 0.05$).

Leg fat, as a proportion of leg mass was lower in both athletes and anorexics than controls ($P < 0.001$) but as a proportion of fat mass it was higher in athletes than anorexics ($P < 0.001$). Torso fat showed lower

proportions of torso mass in athletes and anorexics than controls ($P < 0.001$), and was also higher in athletes than anorexics ($P < 0.01$). Only in athletes was the contribution of arm fat to arm mass less than the contribution of leg fat to leg mass. In anorexics, the arm and leg fat proportions of limb masses are virtually identical. Male and female athletes had similar arm : leg fat ratios (approx. 0.25) while controls had higher ratios. In females, the ratio was similar in anorexics and controls ($P > 0.05$). Torso : leg fat ratios were much higher in male than female athletes (0.66 v 0.45), and correspondingly higher in controls of both sexes.

Table 5.14 Lean proportions in 30 female athletes, 30 anorexics and 30 controls.

	Athletes	Anorexics	Controls
Arm lean / lean mass	0.085 ± 0.007**	0.084 ± 0.008*	0.079 ± 0.006
Arm lean / arm mass	0.719 ± 0.09***	0.787 ± 0.07***	0.528 ± 0.07
Leg lean / lean mass	0.313 ± 0.015**	0.296 ± 0.015	0.299 ± 0.019
Leg lean / leg mass	0.702 ± 0.07***	0.789 ± 0.08***	0.563 ± 0.05
Torso lean / lean mass	0.521 ± 0.013**	0.523 ± 0.017**	0.551 ± 0.018
Torso lean / torso mass	0.881 ± 0.06***	0.929 ± 0.04***	0.767 ± 0.06

* Difference from controls $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$;

Compared with controls, athletes and anorexics had higher proportions of lean tissue in all regions by mass ($P < 0.001$), with anorexics having higher proportions than athletes in arms and torso ($P < 0.01$) and legs ($P < 0.001$). When regional lean mass as a proportion of total lean mass was calculated, athletes had higher values than controls in arms and legs and lower values in the torso ($P < 0.01$). Anorexics had higher values than controls in arms ($P < 0.05$), and lower values in the torso ($P < 0.01$), while leg proportion was similar ($P > 0.05$). The anabolic effect of exercise would seem to focus on increasing lean tissue in the limbs, while the catabolic effect of under-nourishment would decrease lean tissue primarily on the torso. However, such an interpretation is based on the assumption that limb : torso length, remained constant between the groups, and such anthropometric measurements were not recorded on archived data.

Table 5.15 BMC proportions in 30 female athletes, 30 anorexics and 30 controls

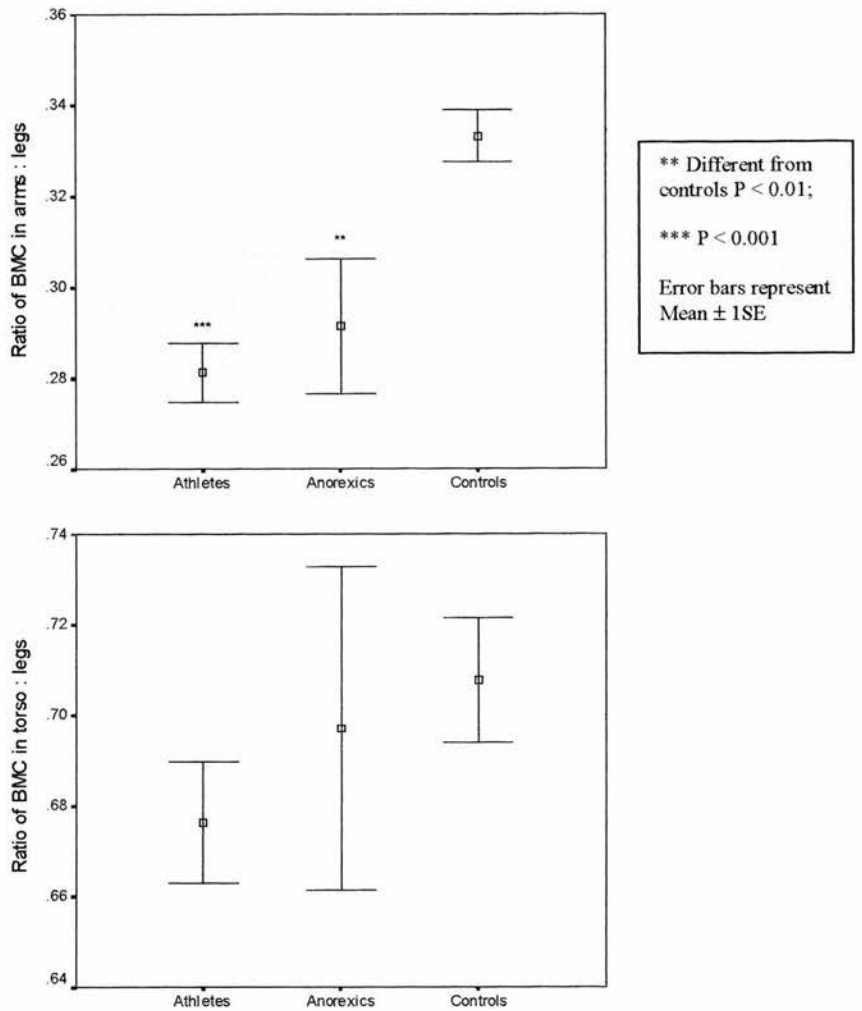
	Athletes	Anorexics	Controls
Arm BMC / total BMC	0.113 ± 0.01***	0.102 ± 0.02***	0.130 ± 0.01
Arm BMC / arm mass	0.047 ± 0.004	0.047 ± 0.01	0.0464± 0.004
Leg BMC / total BMC	0.404 ± 0.02*	0.373 ± 0.05	0.393 ± 0.02
Leg BMC / leg mass	0.045 ± 0.004***	0.047 ± 0.01**	0.040 ± 0.004
Torso BMC / total BMC	0.272 ± 0.02	0.251 ± 0.03***	0.276 ± 0.02
Torso BMC / torso mass	0.023 ± 0.003**	0.021 ± 0.003	0.021 ± 0.002
% BMC / total mass	3.91 ± 0.3***	4.02 ± 0.5***	3.57 ± 0.3

* Difference from controls $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$;

Compared with controls, athletes had less of their total BMC in the arms ($P < 0.001$), more in the legs ($P < 0.05$), and had similar values in the trunk. Compared with controls, anorexics had less of their total BMC in the arms and torso ($P < 0.001$), and had similar values in the legs. Compared with anorexics, athletes had proportionately greater BMC in the legs ($P < 0.05$) and torso ($P < 0.001$). As a proportion of leg mass, athletes had more leg BMC than did controls ($P < 0.001$), and as a proportion of torso mass, they had greater torso BMC ($P < 0.01$). Anorexics had greater leg BMC as a proportion of leg mass than controls ($P < 0.01$) and athletes had more torso BMC as a proportion of torso mass than anorexics.

Using the same analysis as with the male athletes, arm : leg and torso : leg ratios were calculated for BMC, lean tissue, and fat, and illustrated in figures 5.12, 5.13 and 5.14 respectively. When subjected to ANOVA, there were significant inter-group differences for FAL, FTL, LTL and BAL ratios ($P < 0.01$). Homogeneous subset analysis using SNK post-hoc test showed athletes were different from anorexics and controls for FAL and LTL ratios ($P < 0.05$) and both athletic and anorexic groups differed from controls for FTL and BAL ($P < 0.05$).

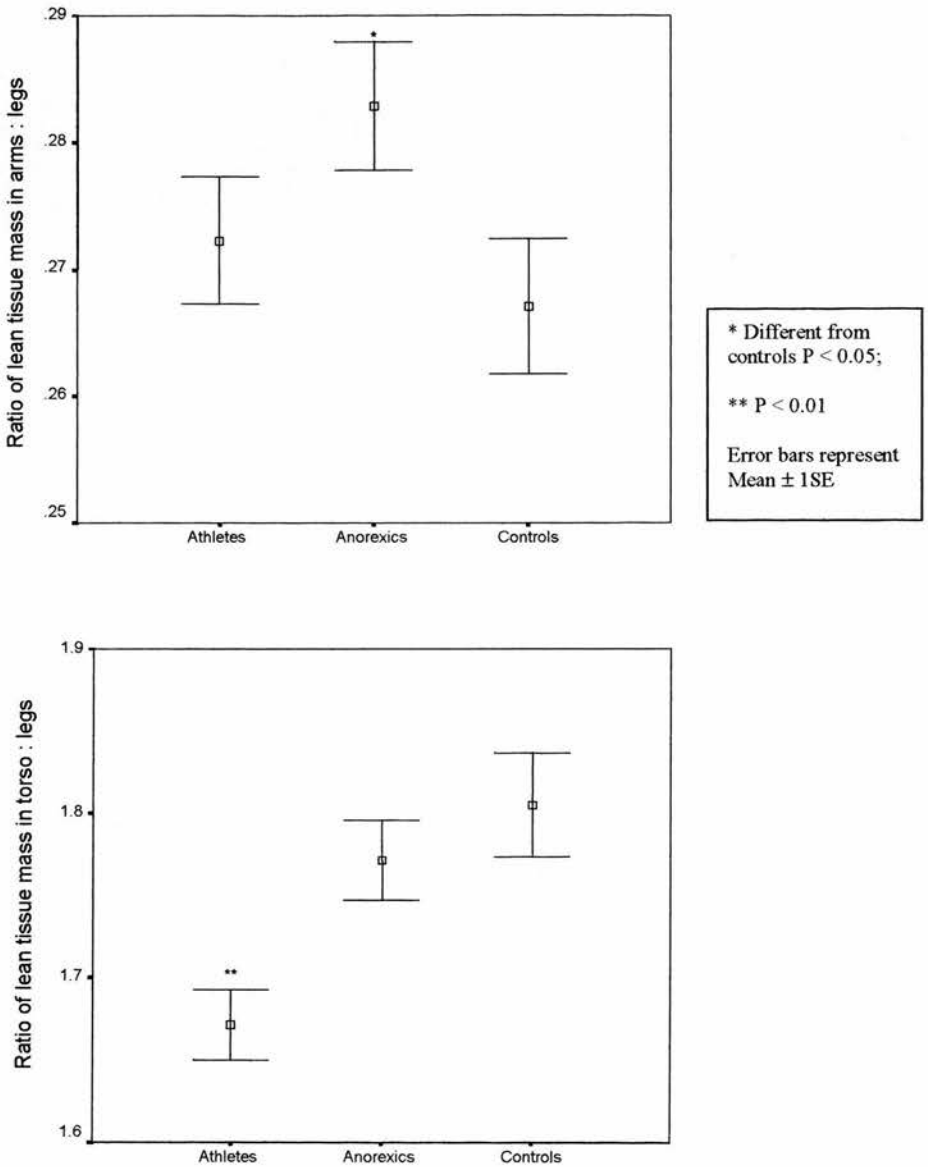
Figure 5.12 BAL and BTL ratios in 30 female athletes, 30 anorexics and 30 controls



An explanation of these findings usefully draws on observation both of absolute and proportional differences in tissue types. On one hand, anorexics had, for their body mass, more BMC (and by implication skeletal mass) than either controls or athletes, and yet by difference, had on average, over 600g of BMC less than the other groups. While full exercise data were not collected for the anorexics, their mean mass of 39.6kg would preclude exhaustive exercise. The observed low absolute but high relative BMC could be the hormonally-regulated consequence of the body's altered homeostasis in the absence of sufficient dietary calcium. In the female athletes, lower than expected arm BMC values are harder to explain. Because there were relatively few in the group whose sports involved significant arm

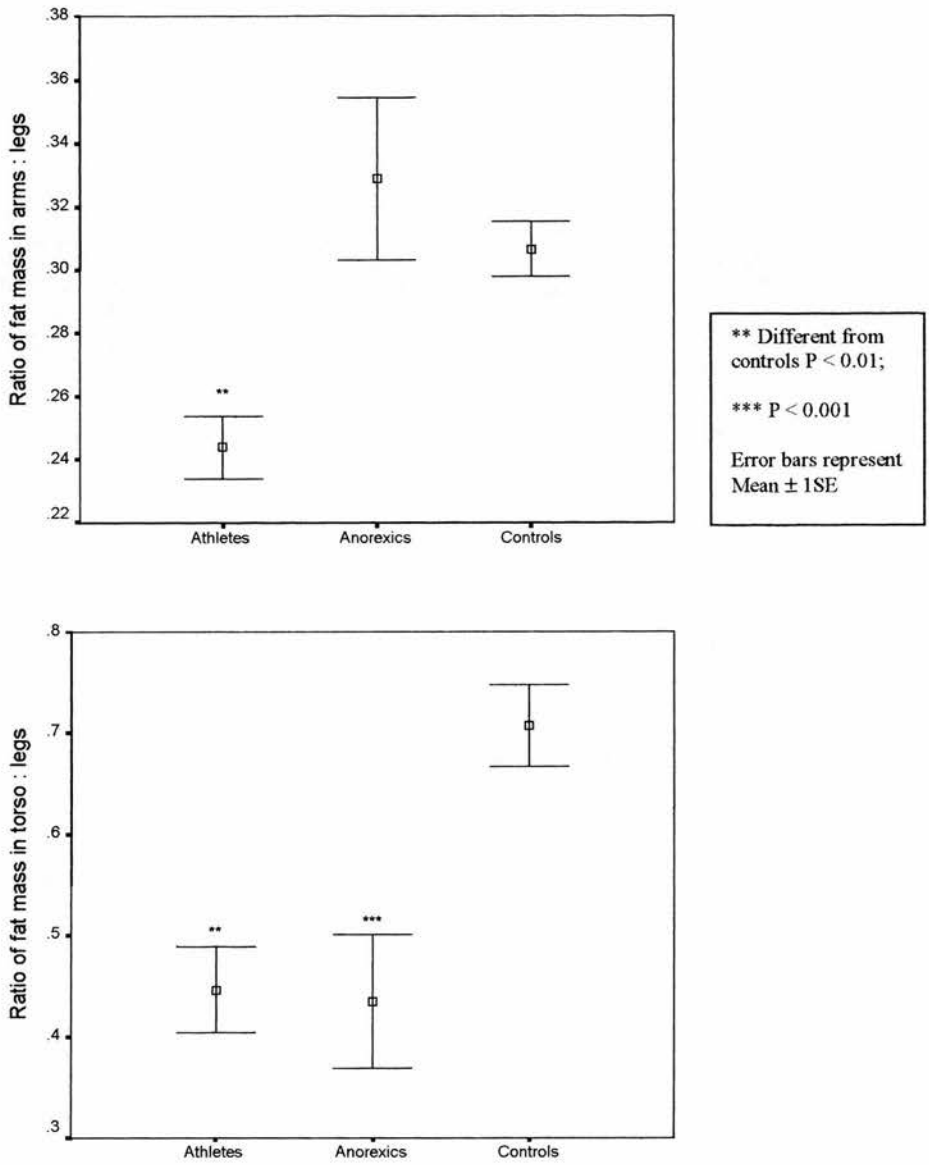
impact, reduced arm bone could be explained by the response of the skeleton contributing calcium as required for body processes from skeletal sites experiencing minimal strain.

Table 5.13 LAL and LTL ratios in 30 female athletes, 30 anorexics and 30 controls



The variable LAL ratio seen in anorexics may be the reflection of the body's usage of the considerable lean tissue present in the upper leg as a substrate for energy production. The effect of the exercise on the athletes is a preferential increase of lean tissue in the legs relative to the torso compared with controls.

Figure 5. 14 FAL and FTL ratios in 30 female athletes, 30 anorexics and 30 controls



The morphometric analysis of tissue relationships only permits a limited explanation in the absence of other data. Fat distribution appears different in each group, and the cross-sectional study design relies on the assumption that such differences are a consequence of either exercise or undernourishment. If this assumption is made, then athletes and anorexics share the preferential mobilisation of torso fat relative to controls. However, the athletes appear to have reduced arm fat and increased leg fat as proportions of

total fat, which renders the FAL ratio more sensitive than absolute values. Analysis of data from male subjects failed to reveal any influence over local fat as a consequence of local muscle action, and such a conclusion would be unlikely. But it did determine that exercise *per se* influenced fat distribution by reducing the relative amount on the torso. While this would explain the FTL ratio difference, the FAL ratio remains less clear. Measurement precision is one explanation, but tissue thickness and absolute body size could be expected to have most influence on the comparison between the anorexics and controls, which showed similar results.

The response of the body to anorexia, as measured by differences from controls, appears to vary between tissue types and body regions. Bone, lean tissue and fat are all substantially reduced, with the implication that lean tissue has been used as an energy substrate. Most bone is lost from the arm, while most fat and lean tissue is lost from the torso. There appears relatively less reduction in arm lean tissue, possibly reflecting a functional baseline level of arm muscle bulk having been reached. While it would be difficult to argue that there appears a preferential 'sparing' of lean tissue in certain regions from the present findings, such a mechanism could have existed as a consequence of evolutionary pressure to maintain capacity for locomotion during severe undernourishment.

DXA algorithms are designed to be stable for subjects in normal ranges, and become less reliable at the extremes of measurement. It is possible that a combination of effects occur commensurately in anorexics, which account for such a loss of accuracy. These include the preferential use of torso fat during chronic undernourishment, anomalies in normal lean tissue distribution arising from muscle wasting, and the less than normal tissue depth. The implication for both anorexics and athletes is that body composition predictions based on other methods such as anthropometry or bioimpedance will produce errors if the prediction equations used were not derived on the group in question.

5.3 UPPER LEG MORPHOMETRY

A COMPARISON OF MAGNETIC RESONANCE IMAGING, DUAL X-RAY ABSORPTIOMETRY AND ANTHROPOMETRIC METHODS IN HEALTHY ADULTS.

The soft tissue composition of the upper leg is a determinant of performance of muscular activity. While the quadriceps and hamstrings combine to power the most forceful lever in the human body, the upper leg is also associated with considerable adipose tissue accumulation. Knowledge of the tissue composition of the upper leg is therefore a useful indicator of functional capacity.

The leg can be modelled using truncated cone geometry using girths and skinfolds to discriminate between total volume and 'lean' volume (Jones & Pearson, 1969). This model, or a variation quantifying the thigh alone is frequently used in correcting power measurements in exercise tests for peak power production (Winter et al, 1991), although similar anthropometric models have been reported to exaggerate the active muscle volume (Heymsfield et al, 1982).

More sophisticated models of upper leg morphometry have been developed using computed tomography (CT) scanning to compare different age groups (Overend et al, 1993) or adaptations to different exercise regimens (Sipila & Suominen, 1995). However, CT scanning involves significant radiation doses to the subject. Dual X-ray absorptiometry (DXA) is recognised as a precise and accurate technique for measuring body composition (Prior, 1997; Kohrt, 1998) involving minimal radiation exposure, and the special region-of-interest facility can be used to examine tissue masses in any body region. Several studies have used Dual Photon Absorptiometry (DPA) and, more recently DXA to estimate muscle mass

by comparison with other methods (Heymsfield et al, 1990; Fuller et al., 1992b; Madsen et al, 1997; Wang et al, 1996).

Magnetic Resonance Imaging (MRI) is noninvasive and is widely viewed as the gold standard for in vivo tissue morphometry (Baumgartner et al, 1992; Fowler et al, 1991; Ross et al, 1994), although it is reported that the error associated with converting adipose tissue volumes into lipid mass precludes the use of either MRI or CT as a reference method for body fat (Després et al, 1996). Using MRI, the slices which provide a 'photographic' image of the region of interest are derived by the tissue-specific relaxation time of protons in fat and tissue water. By adjusting the timing of the radio pulses which are absorbed by the nuclei, and also the timing of the read-out of the absorption signal, the contrast can be adjusted to expose maximum differentiation between tissue types, in this instance adipose tissue and skeletal muscle. Thus, rather than include all lean soft tissue in the same compartment, muscle mass can be measured directly from MRI images. This study compared a simple anthropometric model of the upper leg and tissue mass measurements from DXA, with MRI images, with a view to predicting lipid and muscle masses from anthropometry.

10 healthy volunteers (aged 21 – 51), 3 male controls, 5 male athletes and 2 female athletes representing a range of physical activity and adiposity volunteered for the study. Each subject underwent a series of measurements on the same day comprising a MRI whole body scan, a DXA whole body scan, and an anthropometric assessment of the upper legs using girths and skinfolds.

MRI volumes were calculated separately for muscle tissue (including tendons), adipose tissue (excluding marrow), and for total upper leg volume, defined as the portion of the upper leg between the highest horizontal circumference obtainable with the subject standing, and 1cm above the superior border of the patella.

Each subject presented fasted or a minimum of 2 – 3 hours post prandial, and wore either a hospital gown or a T shirt and shorts for the MRI scan. This was performed on a Siemens 42 SPE imager (Erlangen, Germany) according to the method outlined in chapter 3.8. Once tissues had been sampled for the setting of thresholds to discriminate tissue types, an automatic volume rendering by system software produced results for adipose tissue, muscle and total upper leg volume.

The whole body DXA scan was performed on a Hologic QDR 1000W scanner (Hologic Inc, Bedford, MA, USA) and analysed using enhanced version 5.55 software for special region of interest to coincide with the same region selected for MRI analysis. The resulting tissue masses of bone mineral content, lean tissue and fat were summed for each leg segment.

Horizontal circumferences were measured in five locations of both upper legs with the subject standing using a tension-correcting anthropometric tape (Rockton, Illinois. USA). The sites were the gluteal (the highest possible horizontal circumference), patellar (1cm above the superior border of the patella) and three others at measured distances equally spaced between these two. The upper leg distance was defined as the distance between the gluteal and the patellar circumferences along the anterior aspect of the skin. Skinfolds were measured using Harpenden spring calipers (British Indicators, Luton, UK) on both legs on the anterior aspect of these circumferences, using a vertical raised fold. These measures corresponded with the locations of the regions in both the scanning procedures. Measurements were made on both legs and averaged. Volumes of total and lean upper leg volume were calculated by geometric principles assuming a circular cross section as outlined in Hawes (1996). Because of the small differences in regions measured (mostly arising due to pixel size between DXA and MRI), correction factors were calculated to match the length of the regions to the nearest millimetre.

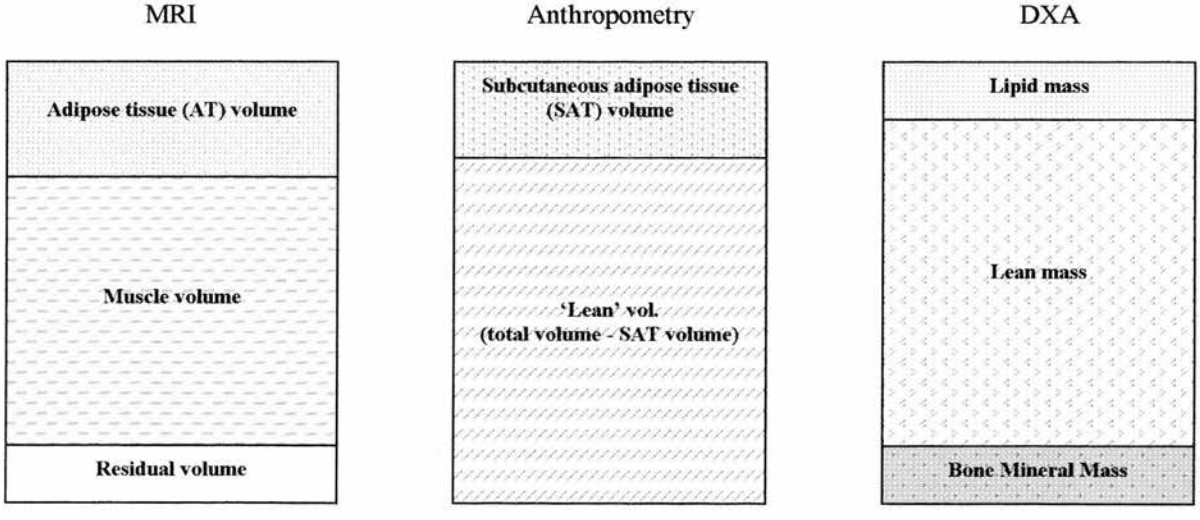
Physical characteristics of subjects appear in table 5.16

Table 5.16 Physical Characteristics of Subjects (8 male, 2 female)

Parameter	Mean ± SD	Range
Age (yr.)	33.2 ± 7.5	23 - 49
Height (cm.)	173.7 ± 6.4	157.5 – 180.0
Mass (kg.)	70.5 ± 6.1	59.8 – 80.6
Upper leg distance (cm)	29.3 ± 1.5	26.5 – 31.0
Exercise per week (hrs)	6.0 ± 3.9	0 - 12

The models of the upper leg by anthropometry, DXA and MRI depend on different measurements and different assumptions. The measurement components of each model are summarised in figure 5.15.

Figure 5.15. Measurement components of MRI, Anthropometric and DXA models



The conversion of tissue volumes into mass was made by constants of reported density. These were 1.0414 $g.cm^{-3}$ for the density of skeletal muscle (Snyder, 1974), and 0.948 $g.cm^{-3}$ for adipose tissue, the median of a recent study using cadaver evidence (Martin et al., 1994). Lipid mass was calculated from a regression which predicted lipid fraction from total adiposity (Martin et al., 1994). DXA lean mass was converted into an estimated muscle mass by subtracting skin mass calculated from the surface area of

skin using the anthropometric model, and applying the reported thicknesses of 1.56 and 1.20mm for men and women respectively, together with the estimated skin density of 1.145 $g.cm^{-3}$ (Snyder, 1974).

MRI was used as the reference method for muscle mass, while DXA was used as the reference method for lipid mass. The comparisons of DXA-predicted muscle mass and anthropometric 'lean' mass with MRI muscle mass appear in figure 5.16. Comparisons of lipid mass predicted from MRI and skinfolds with DXA lipid appear in figure 5.17. The total upper leg volumes showed good agreement between MRI and anthropometry (r = 0.94).

Figure 5.16 Muscle mass of the upper leg

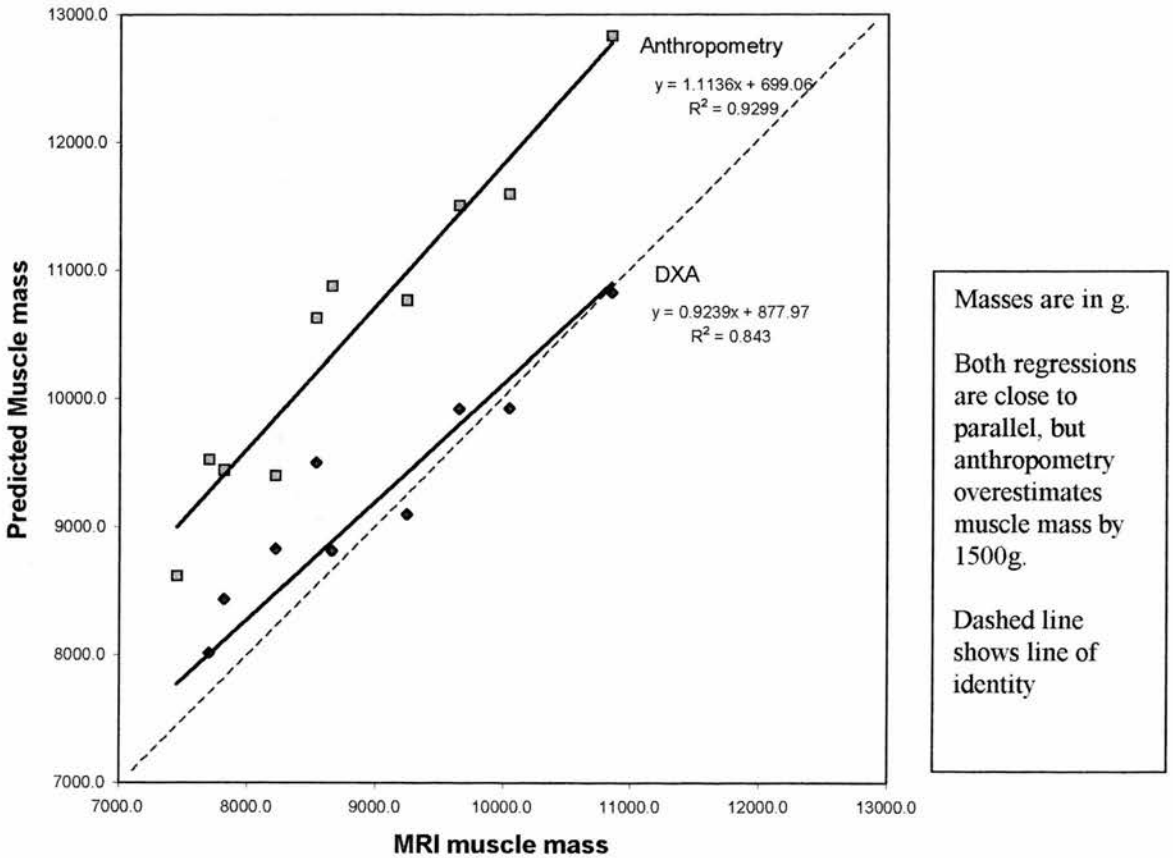
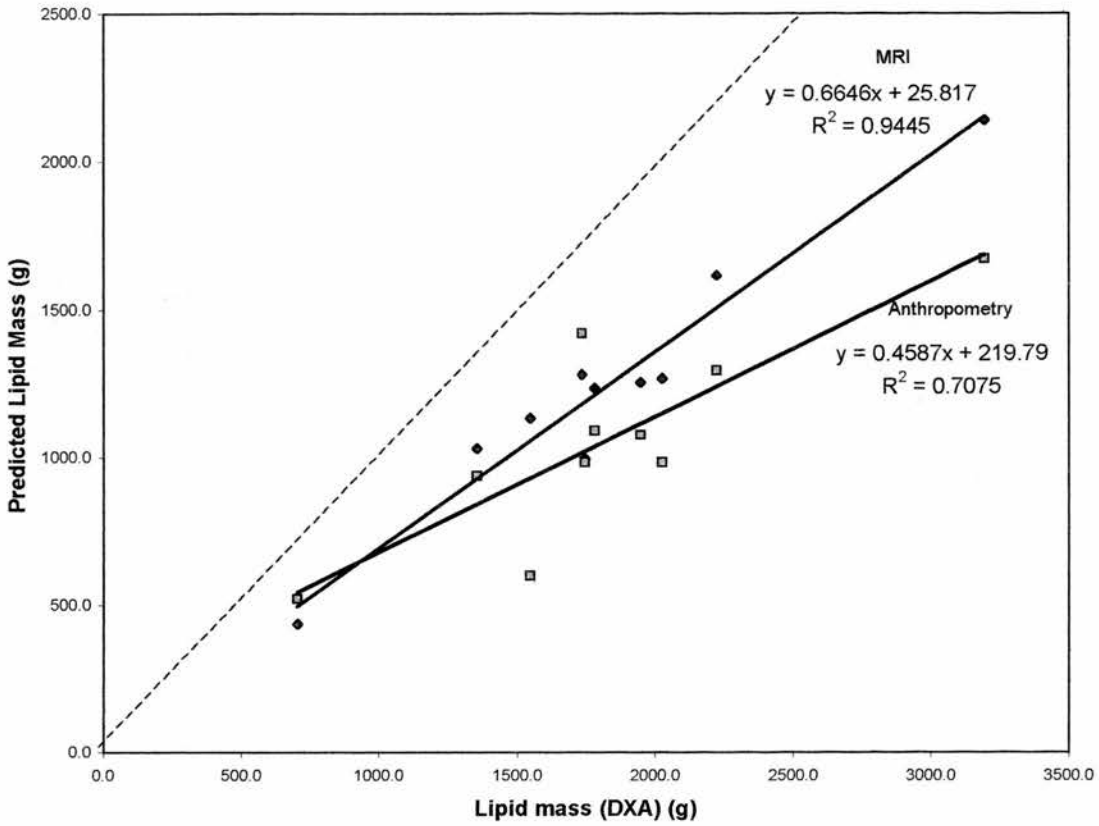


Figure 5.17 Lipid mass of the upper leg



dashed line shows line of identity

While anthropometry may be viewed as the least precise technique, measurement error was comparable to other published data on athletes. The technical error of measurement (TEM) recorded by the same operator on 46 male and female subjects (mostly athletes) on consecutive days was 0.9% for the thigh girth, and 3.5% for the thigh skinfold. Comparable measures from an international study of elite athletes (Carter and Ackland, 1994), were 0.6 - 0.8% and 4.0% respectively.

The reproducibility of the DXA region of interest had been previously tested using repeated sub-regions representing the same area of upper leg on two consecutive scans of 17 male athletes (see chapter 4.1). Paired T tests showed no significant differences in bone area, mineral, density, fat or lean components

between the tests and produced SEM of 9.3g for fat, 33.8g of lean tissue and 1.2g for BMC, with CV of 6.3%, 3.0% and 2.9% respectively.

MRI images vary in intensity according to the tissue under scrutiny, because the radio frequency coil does not have uniform reception across all locations. In order to be certain that muscle and adipose tissue were both accurately assigned, a sampling facility was employed on a slice - by - slice basis, matched with anatomical landmarks to confirm individual muscle location. The ranges of intensity observed for each subject were then used as the criteria for selecting thresholds for counting the total muscle volume, including tendons. This method is more accurate than interpolation between slices where only a portion of the actual muscle tissue is sampled. For muscle, this process involved totalling inter-connected pixels between the established thresholds using a 'volume-rendering' function. For adipose tissue, the fat deposits are not inter-connected and were calculated by summing the volume of isolated deposits.

The automated method for MRI analysis based on a single set of scans precludes reproducibility measures, as it will always yield the same result if programmed with the same thresholds. As a result, soft tissue reproducibility was estimated by two operators manually fitting the nadir separating adipose tissue from muscle in a histogram displaying the range of intensity. This was carried out on 6 of the 10 subjects, and then these different thresholds were applied to the volume rendering process. Paired T tests showed no significant differences for fat or muscle volumes for the different operators and produced SEM of 89.4g for adipose tissue and 63.1g for muscle, with CV values of 12.1% and 2.2% respectively. A 10% shift in intensity of the threshold altered results by less than 3% for muscle, but by 11 – 16% for fat. The reason for the poorer reproducibility in adipose tissue is most likely to be due to the considerably greater range in real density of adipose tissue than muscle, together with the 'partial volume' effect of pixels sampling small isolated fat deposits, which has the consequence of altering the apparent density.

Corrected girths for each region (gluteal – suprapatellar) multiplied by upper leg length, and DXA lean mass were regressed against muscle mass from MRI. The results obtained are illustrated in table 5.17.

Table 5.17. Prediction of upper leg muscle mass

Predictor	r^2	SEE (g)	P <
Gluteal	0.73	637	0.01
Upper thigh	0.90	382	0.001
Mid thigh	0.94	305	0.001
Lower thigh	0.83	506	0.001
Suprapatellar	0.42	941	0.05
Mean corrected girth	0.85	480	0.001
DXA lean mass	0.84	465	0.001

Anthropometric predictors were multiplied by MRI distance before entry into regression

Muscle mass (MRI) was best predicted by the following regressions:

$$\text{Muscle mass (g)} = 0.981(\text{mid-thigh corrected girth} * \text{MRI thigh length}) - 4519$$

$$r^2 = 0.94;\ \text{SEE} = 305;\ P < 0.001$$

Where corrected girth is in cm, and MRI thigh length is in mm. The mid thigh corrected girth exceeded the average of all 5 girths in accuracy of prediction.

$$\text{Muscle mass (g)} = 0.892(\text{DXA lean mass}) + 390$$

$$r^2 = 0.84;\ \text{SEE} = 465;\ P < 0.001$$

Correcting for skin mass produced a nearly identical result, but offered no improvement in the prediction.

Lipid mass (DXA) was best predicted by the following regressions:

$$\text{Lipid mass (g)} = 1.421(\text{MRI lipid}) + 65 \quad r^2 = 0.95;\ \text{SEE} = 159;\ P < 0.001$$

where MRI lipid is calculated using adipose tissue density and lipid fraction data from Martin et al., (1994).

$$\text{Lipid mass (g)} = 1.542(\text{anthropometric lipid}) + 195 \quad r^2 = 0.71;\ \text{SEE} = 366;\ P < 0.01$$

where anthropometric lipid is calculated using adipose tissue volumes from anthropometry and adipose tissue density and lipid fraction data from Martin et al. (1994).

The systematic difference between anthropometric 'lean ' mass and MRI muscle mass is likely to be due to the inter and intra muscular fat, together with the bone component being included in the anthropometric prediction. DXA measures lipid mass, but this can only equate to a volume with assumed lipid fraction and density of adipose tissue. The fat content of marrow is not detected by DXA, and marrow fat was excluded from the MRI analysis. DXA's total body composition software is validated on anthropomorphic phantoms, and using Hologic software, the region of interest facility has in-built corrections for tissue thickness and 'anticipated' fat distribution corresponding to the region in question.

DXA muscle mass showed much closer agreement, but slightly poorer correlation with MRI muscle mass, compared with that between anthropometry lean tissue mass and MRI muscle mass ($r = 0.92$ v 0.96). By contrast, MRI lipid showed much closer agreement with DXA lipid than did anthropometrically-derived lipid ($r = 0.97$ v 0.84).

This study presents a comparison of three techniques, each with its limitations and assumptions, but each of which contributes to the understanding of 'true' tissue composition. Due to the sheer volume of data

which both MRI and to a lesser extent DXA provide, such comparisons are unlikely using the entire body, as data analysis is time consuming. Examining one specific body region renders such comparisons more practical to execute, but as a consequence the results cannot be generalised to the whole body. This study uses the entire data available for the thigh region, rather than an interpolation between slices measured for composition, which may be more practical for use in the entire body (Ross et al., 1994).

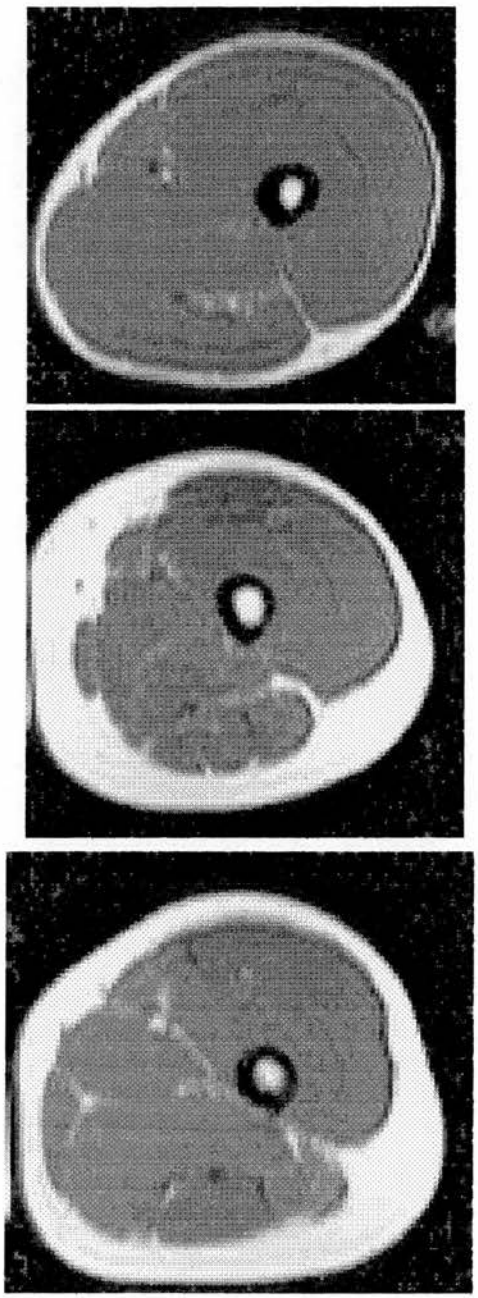
Little or no published work has included region-of-interest comparisons of DXA. However precise each measurement modality is, the delineation of the boundaries of the region of interest is likely to be a source of error when the comparison between methods is made. Anthropometry was measured with the subject standing, which could possibly be expected to produce larger volumes than the recumbent position due to the flattening effect of lying compressing tissue during the scan. The difference (mean value nearly $300cm^3$ with MRI volumes being greater) was not significant ($P > 0.05$). Errors could also have arisen from the pixel size being different in MRI and DXA scans, and perhaps more crucially, the landmarks which identify the region of interest in one method being difficult to observe in the other (Baumgartner et al., 1996).

The sexual dimorphism in fat patterning may threaten the validity of combining male and female data in a single subject pool. Female subjects have more fat situated laterally on the thigh, while in males, the fat is situated more medially. The variation in individual fat patterning in the mid-section of the thigh is readily observed in MRI images, as illustrated in figure 5.18. The extent to which the anterior thigh may be a reasonable estimate of subcutaneous adipose tissue thickness is debatable. Skinfolds are useful because of their convenience, which depends upon sites being readily identified and easily measured. Subjectively, the anterior thigh appears to be more representative in both the male and female athletes than the male control. There were no data gathered on female controls, so it is thus impossible to separate the gender, total adiposity and exercise influences on this distribution. However, the female

athlete with 12% total fat (by DXA) appears to have a similar adipose tissue area to that of the male control with 25% total fat, suggesting a very clear difference in distribution (see below).

Figure 5.18 Cross section of upper thigh in male and female subjects

Medial Lateral



A Male athlete, age 32, 7% fat

B Male control, age 34, 25% fat

C Female athlete, age 28, 12% fat

Heymsfield et al (1982) found a 20 – 25% over-estimation in arm muscle area by anthropometry when compared with CT, attributed to the inclusion of bone, and an exaggeration arising from the assumed circular cross-section, which is, in reality, a flattened ellipse. While the present study uses the density to predict masses from volumes in MRI and anthropometry, applying the density factors to DXA tissue masses produces a volume 13% less than MRI. Less difference in data from the present study may be due to subjects having greater than average muscle development, and having less inter-muscular fat. The density values which enable such conversions (Snyder et al., 1974), are based on a small number of subjects who, in anatomical terms, may fail to represent the subjects in the present study.

Many recent studies have sought to use DXA to predict muscle mass, but rely on an assumed ratio of muscle in the limbs to the total of 0.75 (Snyder et al., 1974) which may not be valid for athletes, whose limb muscle may be greater. Wang et al., (1996) found the ratio to be 0.79 by comparing DXA with CT in 17 healthy men and 8 patients with auto-immune deficiency syndrome (AIDS) whose mean ages were within 2 years of the mean of subjects in the present study. Hansen et al., (1999) assume skeletal muscle mass in the arms and legs represents 0.75 of total muscle. However, they make no correction for skin tissue, which from its elemental composition is likely to be registered as lean mass by DXA, which would have the effect of increasing predicted muscle mass by DXA because the surface area of the limbs exceeds that of the torso. Chowdhury et al., (1994) derived volumes which accounted for the skin, and found that arm plus leg muscle only accounted for 55% of total skeletal muscle.

Limb lean tissue by DPA has been validated against total body potassium and nitrogen methods (Heymsfield et al, 1990) by assuming muscle occupies a fixed proportion of the lean tissue. Thigh muscle + bone area measured by anthropometry had a correlation of 0.88 with muscle mass from DPA in a sample of 34 healthy adults, compared with 0.92 in the present study.

Fuller et al., (1992b) measured 12 female and 16 male subjects by DXA and calculated anthropometric volumes based on one circumference (corrected for skinfolds) for upper and lower arms and legs. Additional measures of total body Potassium and body density by underwater weighing were also made. Detailed calculations of muscle mass in the limbs was based on the mass of skin, bone, and fat, with assumed fat content of muscle and hydration of adipose tissue. DXA tissue masses were used to calculate a predicted volume using assumed densities of $0.9 g.ml^{-1}$ for fat and $1.1 g.ml^{-1}$ for fat-free mass. However there was a discrepancy of 18.8% of the calculated volume between DXA and anthropometry despite DXA including the feet and anthropometry omitting them. Such discrepancy was not observed in the arms. Such a discrepancy could arise as a consequence of variation in the lipid fraction of adipose tissue, and thus the volume of adipose tissue required to contain a given mass of lipid (Martin et al., 1994) or else variation in the density of the fat-free mass (Martin et al., 1986). Lipid fraction variation with adiposity is supported by earlier evidence that fatter individuals had greater concentrations of long-chain fatty acids than leaner individuals (Remenchik and Bernsohn, 1963). Using DXA BMC and lean tissue masses, it is theoretically possible to calculate bone volume and muscle volumes separately using a cylindrical model of in vivo bone density of the femur of $1.95 g.cm^{-3}$, and skeletal muscle, about $1.0414 g.cm^{-3}$ (Snyder et al, 1974). The range in this calculated density of fat-free mass was small in the subjects of the present study (1.095 – 1.109), departing relatively little from the assumed figure, and may suggest that variation in lipid fraction may best explain the volume discrepancies in the data of Fuller et al., (1992b).

The issue of whether lean upper leg volume or lean upper leg mass is calculated will depend on available methods. In predicting muscle mass, anthropometry proved slightly superior to DXA, and these data suggest the current practice of measuring mid-thigh corrected girth and thigh length was only slightly poorer than the calculation based upon all five corrected girths. Anthropometric lean volumes produced in the present study are within 4% of those of the male cohort of young physically active subjects in the study of Winter et al (1991) and approximately 12% greater than those of Overend et al (1993).

The study of Jones & Pearson (1969) is frequently cited as the model for anthropometric corrections. Their study included many more subjects than the present study, but omitted SEE values for the anthropometric prediction, and a quantification of the relationship between X-ray derived fat and caliper -derived adipose tissue. Their volume results appear very large compared with those of the present study, and those of Winter et al., (1991) and Overend et al (1993).

Winter et al., (1991) highlighted functional differences between maximal exercise performance between men and women which are independent of lean leg volume. Attempts to compare young and old men highlighted an increase of inter-muscular fat in the thigh in older subjects which increased errors in the prediction of muscle cross sectional area (Overend et al., 1993). As these authors imply, prediction of thigh muscle cross sectional area can have clinical significance for normalising strength measures per unit of muscle size. There is considerable scope by using MRI to render a total muscle volume in improving such a prediction, and establishing a muscle mass, which can be an index of functional capacity in the elderly or sporting performance in athletes, or of diseases of muscle wasting.

Errors resulting from threshold selection affecting tissue classification by MRI require more investigation. A sophisticated use of anthropometry involved predicting thigh muscle area in 18 young men and women (Knapik et al., 1996) calculated bone area and quantified the overestimate of muscle area due to the assumed circular model. However, the study failed to address the issue of quantifying errors in soft tissue recognition by MRI, which varies within and between individuals. The establishment of a standard procedure for threshold identification could assist this process. The failure to correct adipose tissue for lipid fraction may lead to errors, which could be easily corrected, and further investigation of lipid fraction in young and athletic populations would enable such a transformation to be made with greater confidence.

CHAPTER 6

FINDINGS: BONE MINERAL DENSITY

This chapter provides a review of current understanding of bone processes and seeks to examine the skeletal integrity of athletic subjects, best described by bone mineral density. Other factors are used to predict bone density, and in the case of female athletes, develop a method which can assign risk of reduced bone density. Different sporting groups are compared in male athletes, with the influences of cycling and running evaluated in greater detail.

6.1 EXERCISE AND THE SKELETON:

A REVIEW OF BONE PROCESSES

The human skeleton has many functions. On a physical level it provides form and shape for the body, protection for internal organs and a means for muscles to exert leverage to produce movement. On a cellular level it has roles in blood formation, immune function and as a dynamic store of calcium and phosphorus.

In volumetric terms, bone comprises roughly equal proportions of water, protein (collagen) and Calcium Hydroxyapatite or bone mineral. These constituents are organised into two distinct architectures - cortical and trabecular. The cortical architecture is crystalline, and relatively dense, while the trabecular architecture is a meshwork of bony plates like 'scaffolding' inside a cortical shell. Skeletal sites with mostly trabecular bone are associated with fracture risk later in life.

Bone adjusts to mechanical stress by changing shape, and it remains elastic up to about three quarters of its breaking stress. Bone continually adjusts its architecture according to prevailing circumstances by two mechanisms - modelling and remodelling. Modelling is most active during growth and is the change in shape or size of a bone by formation on one surface and resorption on another. Remodelling is the turnover of bone in small cellular packets called basic multicellular units, which repairs microdamage arising from wear and tear. In the present study, where subjects' growth has ceased, remodelling is the predominant means of adjustment in bone, and can replace between 10 and 30 % of the skeleton in a year.

In both men and women, BMD and BMC increase from early childhood and throughout adolescence, and ascend to a peak, usually in the 3rd decade. This is followed by a slow decrease in BMC and BMD in men, and in women, the effect is magnified after the menopause. Approximately 90% of bone mass is

present at the end of skeletal maturation (Slemenda, 1994). The peak bone mass achieved between 20 and 30 years is an important determinant of bone mass in later life and BMD can explain roughly 80% of the variation in bone strength (Aloia, 1993). However for the age group of subjects under scrutiny in this study, BMC and BMD are at their most constant level throughout life.

While genetic influence on BMD has been claimed to be as high as 75 – 85% (Gueguen et al., 1995) a number of lifestyle factors such as alcohol, smoking, BMI and diet all contribute to its variation. However, the variation imposed by physical activity can be considerable, especially in subjects who exercise as extensively as the athletes of the present work.

In addition to its numerous other health benefits, weight-bearing physical activity is essential for the normal development and maintenance of a healthy skeleton, and even in sedentary individuals exercise may increase bone mass slightly, as well as prevent bone loss as a result of inactivity (ACSM, 1995).

Different types of exercise cause different effects on bone remodelling, and apart from accidental trauma, the largest strains on bone appear to be the result of large muscle groups working against several times body weight using inefficient levers to cause movement under the earth's gravity (Frost, 1997). Frequency of strain appears relatively unimportant in stimulating bone adaptation. Animal studies suggest strain rate to be important in stimulating new bone formation (Skerry, 1997) and increasing strain magnitude has been shown to produce a dose-dependent increase in bone mass (Rubin & Lanyon, 1987). In humans the case remains unproven, although a variety of related evidence now exists. Removing gravity virtually eliminates strain on bone and astronauts may lose up to 19% of their weight-bearing bone on extended missions (Hughes-Fulford & Lewis, 1996). Exercise countermeasures are employed in an attempt to minimise the loss of bone and muscle, and resistance exercise appears to be more important than endurance exercise in this respect (Baldwin et al, 1996). Impact activity such as running can induce strain as a result of the momentary loading of several times body mass. By contrast, activities such as road cycling and swimming are weight-supported, involve minimal strain, and involve a prone orientation for considerable periods of time. While self-selection of preferred genotypes could explain why athletes

of specific sports have unique adaptations (Skerry, 1997), it is likely that specific training for certain sports plays a pivotal role in skeletal adaptation. Understanding such effects could inform exercise advice given to young athletes who may be unaware of the risks of stress fractures arising through training to excess, or reduced bone density later in life.

The interdependence of exercise and endocrine function has been described in the form of a balance (Bailey and McCulloch, 1990) where Parathyroid hormone and Calcitriol push the balance towards resorption, while oestrogen levels and the osteogenic effect of mechanical strain oppose this by stimulating bone formation. According to the model, the only thing to be capable of stimulating a sustained increase in bone mass which is not subsequently negated by resorption is high, or 'inappropriate' strains. More recent evidence has shown Selective Estrogen Receptor Modulator drugs (SERMS) such as *Tamoxifen* to inhibit strain-related responses in bone cells. This implies that strain uses the machinery of the oestrogen receptor to produce its effects on bone (Lanyon, 1998).

This part of the study seeks to establish, within the limits of its cross-sectional design, the consequences of exercise type on bone, and to determine whether such individuals are assisting or hindering their skeletal health.

6.2 The Incidence and Prediction of Reduced Bone Density in Women Athletes

Conditioning programmes followed by most athletes normally target the cardiovascular and muscular systems with the aim of maximising performance. Frequently this may result in modest gains of muscle and significant loss of fat, but in women the pressure to achieve or maintain an unrealistically low body mass may have severe consequences. Energy intake levels may be reduced to the point where disordered eating patterns are developed (Yeager et al., 1993), and underlie the *female athlete triad* of disordered eating, amenorrhoea and osteoporosis (Otis et al., 1997). Although current understanding remains incomplete, the association and inter-dependence of these disorders means that the presence of one is a likely risk factor for the presence or development of others. Therefore identification of any of these factors will potentially aid recognition of the triad, and enable appropriate intervention.

The loss of regular menstrual function has been considered to be attributable to a specific % fat threshold which is required for oestrogen production. This has been identified as 17% (Frisch and McArthur, 1974) and more recently; 12% (Cantu and Micheli, 1991), although other evidence points to a lack of association between menstrual function and body fat (Sanborn et al., 1987; Drinkwater et al., 1984). A lack of oestrogen, in addition to affecting long-term reproductive function, has consequences for the skeletal system by demineralising bone. While impact exercise in the presence of oestrogen produces a net gain of bone, the training-associated loss of menstrual function has been shown to lead to bone loss (Drinkwater et al., 1984). Evidence shows intense exercise may offset the impact of amenorrhoea, but that runners in particular may be at high risk of exercise-related fractures (Marcus et al., 1985).

Comparatively little is known of the prevalence of reduced bone density in women athletes. Because dual X-ray absorptiometry (DXA) scans are not widely available, only those athletes with severe orthopaedic, gynaecological or psychiatric symptoms are likely to have been investigated clinically. While there is a large number of influences on bone density, athletes may be at an increased risk of stress fracture because demineralised bone is structurally weaker than normal bone, and rigorous conditioning regimes expose the skeleton to greater stresses. Predicting individuals at risk using easily measured variables may enable earlier identification of risk and assist in the development of recommended mass ranges for individual athletes. This part of the study aims to explore the incidence of reduced bone density in female athletes and to investigate the feasibility of predicting individuals at risk from easily measured variables.

Of the female athlete cohort, a total of 26 completed all aspects of the protocol, and those who did not were excluded from the analysis. All were required to have been in their selected sports for three years or more, and to train for a minimum of four hours per week. Subjects with a history of bone disease, eating disorder, or those who trained for power sports were excluded from the analysis. All were involved in sports associated with a risk of the female athlete triad (Otis et al., 1997) and included 9 runners, 4 dancers and 4 lightweight rowers, with the remainder drawn from a variety of other sports. The competitive standard was club or University level, but not international standard. Athletes who competed in weight supported exercise (rowing or cycling) also performed running based circuit or weight training as a part of their conditioning regime.

Bone mineral density was measured by dual X-ray absorptiometry using a Hologic QDR 1000W (see methods chapter 3.4) in whole body mode (enhanced software version 5.55) with a separate bone density scan performed for the lumbar spine L1 – L4 (software version 4.47). Whole body software produced % fat which was used in subsequent predictions. A total of 42 anthropometric measurements of skinfolds,

girths and skeletal breadths were measured on each subject (see methods chapter 3.5) enabling the prediction of skeletal muscle mass (Drinkwater et al., 1986), and somatotype (Heath and Carter, 1967).

A comprehensive training background was obtained by administered questionnaire, and training hours were calculated as the weekly average hours spent conditioning or competing in the previous year. An additional questionnaire on detailed menstrual history, hormone replacement and oral contraceptive use was completed by all subjects. This was evaluated by a specialist doctor in sports medicine and scored for oestrogen status (1 for eumenorrhoea or supplemented oestrogen; 0 for oligo or amenorrhoea defined as fewer than six normal periods in the previous 12 months in the absence of pregnancy).

Physical characteristics of subjects appear in table 6.1.

Table 6.1. Physical characteristics of 26 female athletes

Variable	mean ± SD
Age (yr)	26.0 ± 5.2
Height (cm)	168.2 ± 6.2
Mass (kg)	55.6 ± 6.0
BMI ($kg.m^{-2}$)	20.0 ± 1.9
endomorphy	2.8 ± 1.2
mesomorphy	3.3 ± 1.1
ectomorphy	3.7 ± 1.1
Training ($hrs.wk^{-1}$)	9.2 ± 3.0
% fat (DXA)	16.1 ± 6.2
% muscle[a]	46.2 ± 3.8

[a] Drinkwater et al., (1986)

These data suggest the athletes were, in general, slender, lean, and of low body mass for their height. While % muscle was high for women, mesomorphy was not in most cases, and the predominant somatotypes were balanced or mesomorphic ectomorphs.

Bone data from the DXA scans appear in table 6.2.

Table 6.2. Bone parameters of 26 female athletes

Bone parameter	mean ± SD	range
Total BMC (g)	2223 ± 291	1689 - 2790
Total BMD ($g.cm^{-2}$)	1.13 ± 0.08	0.99 – 1.28
Spine BMD ($g.cm^{-2}$)	0.98 ± 0.14	0.72 – 1.25
Spine T-score*	-0.713 ± 1.15	-3.01 - +1.8

*Defined as measured bone density expressed as the standard deviation above or below predicted peak bone mass

Ten of the 26 athletes were osteopenic and one was osteoporotic, as defined by the World Health Organisation as a T-score of between -1 and –2.5, and <2.5 respectively (Kanis, 1994). These were six runners (including the one osteoporotic subject), two dancers, one cyclist, one aerobics instructor/competitor and one race - walker. The correlation of T-score with various physical measurements appears in table 6.3.

Table 6.3. Correlation of spine T-score with selected variables in 26 female athletes

Variable	r
Height	0.02
Sitting Height	0.13
Mass	0.61**
BMI	0.72**
SBMI$^{\phi}$	0.70**
Endomorphy	0.41*
Mesomorphy	0.59**
Ectomorphy	-0.60**

*P < 0.05; ** P < 0.01 $^{\phi}$BMI based on sitting height, rather than total height

Regression analysis was used to predict spine T-score from selected physique variables and oestrogen status (OS). The best single predictor was BMI, which explained 52% of T-score variation. Ectomorphy was best of the somatotype predictors, explaining 36% of T-score variation. BMI alone explained 52%

of the variation in T-score, with OS adding a further 6%. The optimum prediction of these two factors separately was no better than the sum of BMI + OS. The regressions produced were as follows:

$$\text{T-score} = 0.434(\text{BMI}) + 0.543(\text{OS}) - 9.566 \qquad r^2 = 0.58;\ \text{SEE} = 0.778;\ P < 0.001.$$

$$\text{T-score} = 0.446(\text{BMI} + \text{OS}) - 9.743 \qquad r^2 = 0.58;\ \text{SEE} = 0.763;\ P < 0.001.$$

Somatotype is perhaps the most thorough of physique descriptors, offering a size – independent picture of body shape. In examining the relationship between physique and T-score, the data show higher scores of both endomorphy (relative fatness) and mesomorphy (musculo-skeletal robustness) to be protective against, and higher scores of ectomorphy (linearity or relative fragility) to be an indicator of spinal osteopenia. Because ectomorphy, along with low body fat, has been shown to be a discriminant for success in distance running (Bale et al., 1985), and dancers and rowers are required to keep more strict patterns of weight control, it is clear that adaptations for enhancing performance in sport may be in conflict with that for optimal bone health. While a regression of mesomorphy and SBMI (mass. sitting height^{-2}) explained nearly as much T-score variation as the combined index, the practicalities of measuring mesomorphy from corrected girths and skeletal breadths (Heath and Carter, 1967) may be less straightforward in a clinical setting.

In a comprehensive study, severity of menstrual irregularity was assessed for its affect on bone in 97 young athletes (Drinkwater et al., 1990). The combination of menstrual pattern and body mass explained 43% of the variation of the BMD at the lumbar spine, and suggests that oligo or amenorrhoea appears to have a residual effect on bone density at this site. It may be not only more practical and more accurate, but also more prudent to assess menstrual status and endocrine function to assist the athlete establish sensible practices for defining healthy minimum mass (Drinkwater et al., 1990).

Using the optimal predictors of Spine T-score: % fat, oestrogen status and BMI, there remains considerable potential for error in predicting osteopenia in any individual athlete. Because of genetic differences, two athletes with the same physique variables and training may have radically differing bone densities, beyond detection by variables used in the present study. A further difficulty is that the tools of measurement may not be as precise as widely believed. Precision errors in establishing % fat by underwater weighing can be at least 3%, and with skinfold caliper predictions this error is higher, particularly if generalised equations are used which have not been validated on athletes (Sinning et al., 1985). By comparison with % fat, BMI and OS can be established with greater ease, precision and accuracy.

Because regression analysis showed the prediction of spine T-score by BMI could be improved by 6% by adding oestrogen status, this sum, and the various other thresholds of % fat and BMI were subjected to receiver operating characteristic (ROC) analysis (Bernstein, 1997), to establish the best predictive test and threshold for predicting the presence of spinal osteopenia in the group. ROC analysis proceeds by establishing the *sensitivity* (the proportion of subjects who have osteopenia and test positive for it) and *specificity* (the proportion of subjects who do not have osteopenia and test negative for it).

ROC analysis curves which plot sensitivity against (1 – specificity) can be constructed for the group to establish the maximum area (the maximum probability that a randomly selected normal subject has a higher test variable result (BMI or % fat) than a randomly selected osteopenic subject), and to establish the optimum threshold for such a test being most accurate. Figures 6.1 and 6.2 show ROC curves for % fat (from DXA) and the sum of BMI and OS (BMIOS) respectively.

Comparison of the ROC curves reveals no differences in area between the BMIOS curve and the DXA % fat curve (0.82; 95%CI: 0.62 – 0.94; v 0.80; 95%CI: 0.60 – 0.93). Optimal accuracy was obtained using a DXA fat level of 13.3%, or a BMI + OS sum of 20.4. These data suggest that for women whose BMI +

OS is less than 20.4, there is an increased relative risk of osteopenia of 2.4 (95%CI 1.2 – 4.7; $P < 0.05$).

Figure 6.1 ROC analysis of DXA % fat for predicting osteopenia in 26 female athletes

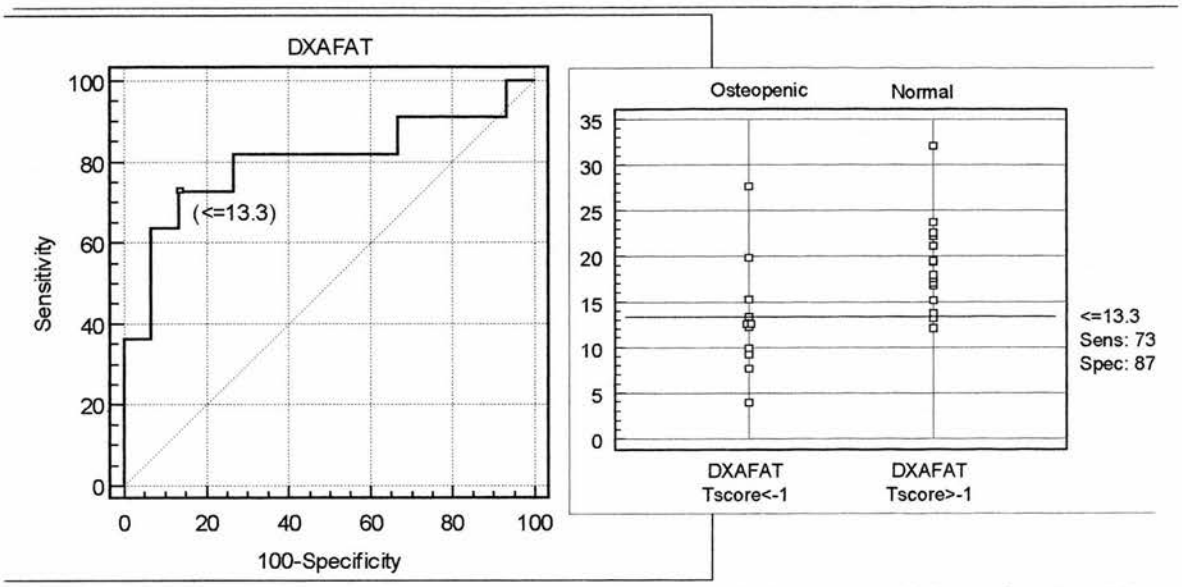
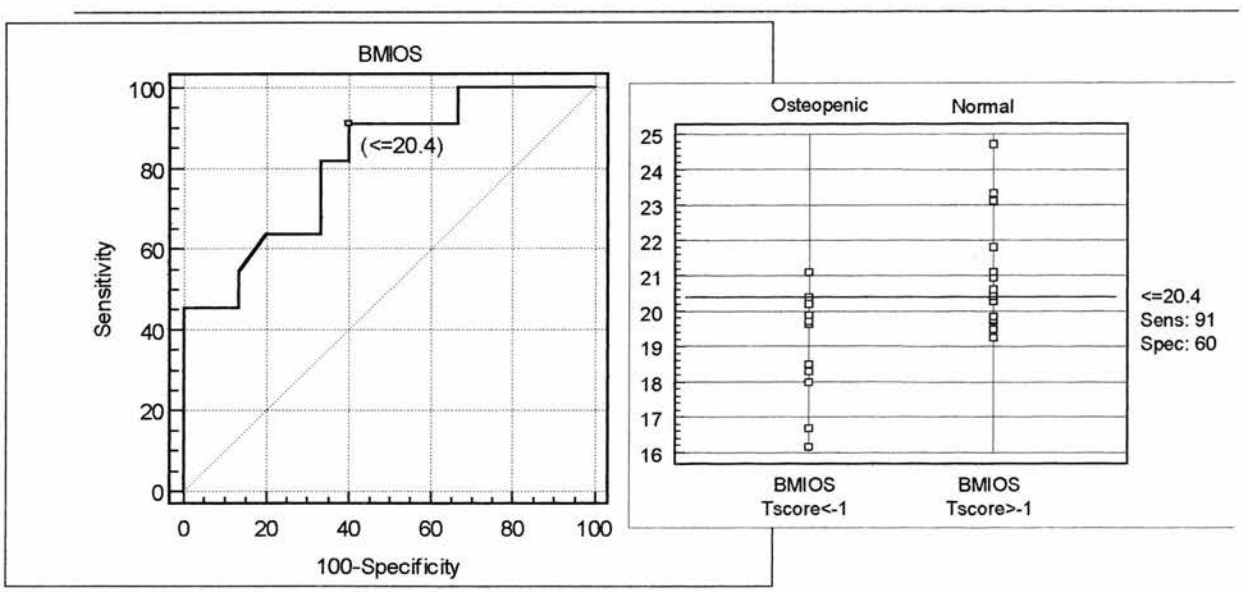


Figure 6.2 ROC analysis of BMI + Oestrogen Status for predicting osteopenia in 26 female athletes



It is important to appreciate the dynamic nature of human physique, which is the physical expression of a complex training periodicity, which alters with proximity to competitive performance (Hawes and Sovak, 1994). Applying this concept to bone density, this means that temporary phases of lower body mass than

the appropriate threshold can be survived with minimal ill effect, provided the mass is regained within a matter of months. More permanent reductions in mass below the minimum mass carry increased risk. Resumption of menses following decreased training and gain of mass (Drinkwater et al., 1986b) may provide a protective mechanism safeguarding longer term bone health. Wide fluctuations in mass and thus BMI will induce alterations in bone architecture which are likely to lag behind soft tissue mass changes. While the period of the remodelling cycle has been reported to be approximately 3 – 6 months (Eriksen, 1986), only 10 – 30% of the adult skeleton is remodelled in any one year (Bailey and McCulloch, 1990). Because this time frame for adjustment in bone architecture is substantially longer than the time necessary for alterations in fat and muscle, predictions based on any body composition or morphology variable must be viewed with caution if the athlete's mass has fluctuated or is likely to fluctuate widely.

It is recognised that reduced bone density is not a truly dichotomous variable, and that athletes may be at risk with T-scores above –1. Such individuals can be identified via regression analysis with the appropriate standard error of the estimate. For clinical use, where a simple test is required, this analysis provides a suggested template for applying such data as this to groups for whom DXA measurements are unavailable.

It appears that there exists a window of opportunity which childhood and adolescence presents to the young athlete in order to lay the foundations for a strong skeleton, and health professionals are determined that advice given to athletes must reflect a more life-long health, rather than performance based approach (Skolnick, 1996). While this sample may not necessarily reflect the true occurrence of osteopenia in the wider population of female athletes, data from this study suggests young adult women athletes need an equivalent awareness, emphasising adequate mass and normal hormone function, in order to allow the peak bone mass to reach its potential, thereby safeguarding against reduced bone density later in life.

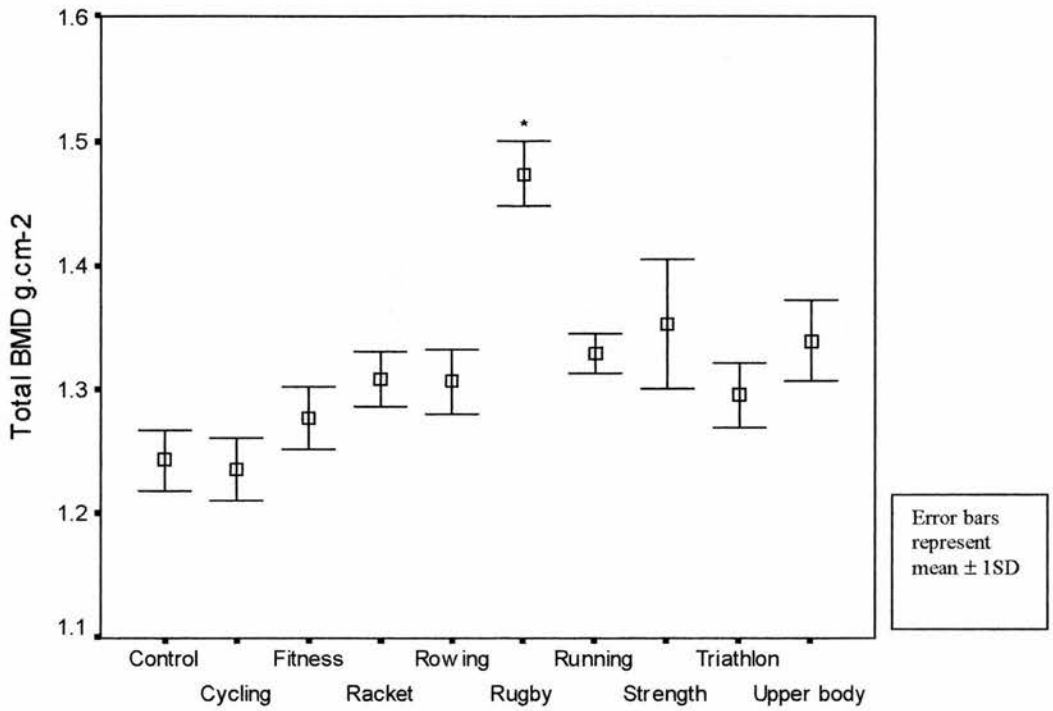
6.3 BONE MINERAL DENSITY IN MALE ATHLETES

While there is a significant and justifiable focus of studies on bone density of female athletes – largely arising from related factors such as menstrual disturbance, disordered eating and social pressure to be slim, there is a paucity of data on male athletes. Osteopenia and osteoporosis, while present in young male adults, has not been associated with athletes, and investigations are therefore very scarce.

Skeletal integrity is best measured by BMD, which is an areal measure of bone projected onto a two dimensional surface, arising as the measured BMC divided by the projected bone area outlined by edge-detection algorithms which recognise a steep rate of change in R value. So-called '*areal* bone density' is not a true *volumetric* density, because a true density is a function of volume and not area. However this is not a significant problem in cross sectional studies where groups of individuals of similar size are being compared. It can present problems where larger individuals are being compared with smaller ones, or perhaps a generalised study comparing males with females. The reason for this is straightforward: If two cylindrical bones of differing size but the same volumetric density are compared, then the difference will be proportional to the square of the radius. If bone A is 5cm in diameter, and bone B is 4cm in diameter, and both bones are the same length, then there will be a 20% difference in bone area in the DXA scan, but a 56% difference in BMC. This translates into a 25% difference in areal BMD which is purely artefactual. Therefore in correcting for the anomaly, an adjustment for bone size should be performed, most practically by linear skeletal dimensions or mass. In reality, bones are not perfect geometric shapes, and it is very difficult to calculate a true volumetric density without assumptions which simplify the true shape or structure of bone *in vivo*.

Figure 6.3 shows the BMD amongst the various athletic groups, and illustrates this point. The greater density of rugby players compared with all other groups was still significant after controlling for height and mass in multivariate analysis ($P < 0.05$).

Figure 6. 3 Total BMD in 106 male athletes and 15 controls by athletic group



* Significantly different from other groups P > 0.05 using SNK post-hoc ANOVA, and after controlling for height and mass

Figure 6.4 Spine BMD in 106 male athletes and 15 controls by athletic group

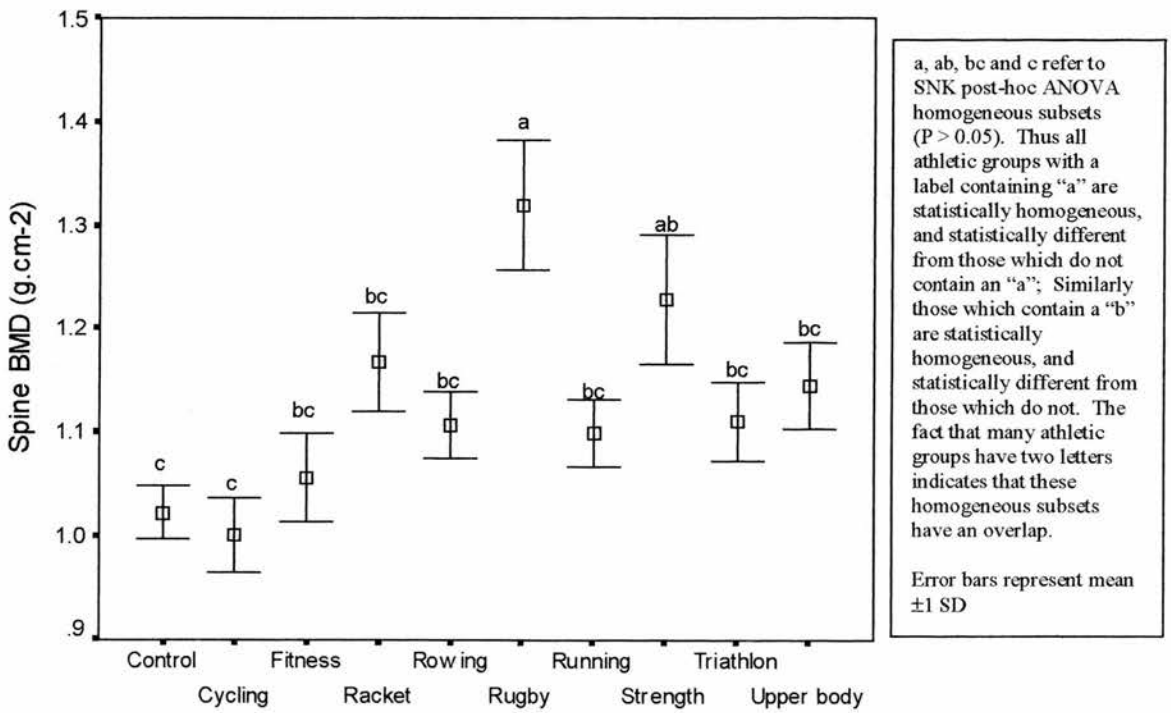


Figure 6.4 illustrates the Spine BMD amongst the athletic groups, which show more inter-group variation than total BMD. Rugby players had greater BMD in total and at the spine, after correcting for size, which can be readily explained by theirs being the only athletic group habitually exposed to 'collision' type forces in addition to the impact of body weight. Total BMD and spine BMD showed a correlation of 0.84 ($P < 0.01$) amongst the entire group.

The skeletal dimensions recorded (see methods chapter 3.5) were used to predict Total and spine BMD and entered into a stepwise regression. For both, only chest breadth and malleolar breadth were selected and predictions were as follows:

$$\text{Total BMD} = 0.184(\text{chest breadth}) + 0.0825(\text{malleolar breadth}) + 0.152$$

$$r^2 = 0.28;\ \text{SEE} = 0.093;\ P < 0.01$$

$$\text{Spine BMD} = 0.0258(\text{chest breadth}) + 0.0883(\text{malleolar breadth}) - 0.31$$

$$r^2 = 0.20;\ \text{SEE} = 0.151;\ P < 0.01$$

Height was not a significant predictor of either total or spine BMD ($P > 0.05$) although mass was, explaining 38% and 26% of BMD variation respectively ($P < 0.01$). Of the physique variables, only ectomorphy was a significant predictor of total BMD ($r^2 = 0.11$; SEE = 0.105; $P < 0.01$) while endomorphy and ectomorphy were both significant predictors of spine BMD ($r^2 = 0.14$; SEE = 0.154; $P < 0.01$). Total BMD and total BMC showed a correlation of 0.92 ($P < 0.01$).

These results suggested the relationships between bone density and physique were not particularly strong, and as a consequence of the model for adjusting bone architecture being strain dependent, and thus enhanced under conditions of physical impact, a 'dummy variable' was created which scored 1 for load bearing (i.e. impact) and 0 for weight supported sports. Initially, this was regressed, along with mass and

weekly hours of exercise against total and spine BMD for all athletes and 15 controls. The following regressions were produced:

$$\text{Total BMD} = 0.0699(\text{impact}) + 0.00669(\text{mass}) + 0.00284(\text{hours}) + 0.724$$

$$r^2 = 0.50;\ \text{SEE} = 0.078;\ P < 0.001$$

$$\text{Spine BMD} = 0.00807(\text{mass}) + 0.00436(\text{hours}) + 0.0105(\text{impact}) + 0.386$$

$$r^2 = 0.38;\ \text{SEE} = 0.13;\ P < 0.001$$

Thus, it appears that impact is an important determinant of BMD, while skeletal dimensions are relatively poor predictors by comparison, particularly in view of the projection anomaly, which accounts for some of the association. However some athletes undertook a mixture of impact and non-impact as a consequence of their training, and the total was reduced to 63 whose regimes involved all impact or no impact. Results of the comparison with controls appear in table 6.4.

Table 6.4 BMD in Impact and Weight-supported groups of male athletes and controls

	Impact (n = 28)	Weight-supported (n=35)	Controls (n=23)
Age (yr)	26.9 ± 6.6	26.2 ± 7.4	26.6 ± 6.0
Mass (kg)	80.7 ± 12.2	76.8 ± 7.7	77.6 ± 9.1
BMI ($kg.m^{-2}$)	24.6 ± 2.9	22.9 ± 1.5*	24.1 ± 2.5
% fat (DXA)	10.1 ± 4.7***	9.8 ± 3.3***	17.2 ± 6.5
Weekly training (hr)	10.4 ± 5.6	10.2 ± 4.4	none
Total BMD ($g.cm^{-2}$)	1.39 ± 0.11***	1.27 ± 0.10	1.24 ± 0.11
Spine BMD ($g.cm^{-2}$)	1.23 ± 0.19***	1.06 ± 0.14	1.04 ± 0.12

Mean ± SD; * difference from controls $P < 0.05$; *** $P < 0.001$

When mass and BMI were factored out in multivariate analysis, BMD differences from controls were still highly significant ($P < 0.001$). These data support the American College of Sports Medicine position

statement on osteoporosis and exercise (ACSM, 1995) by suggesting weight–supported exercise does little to influence bone density, while impact exercise has a powerful effect.

This fits, in broad terms, with current understanding of bone remodelling with high strains or strain rates significantly increasing bone. However, to suggest that impact is equivalent amongst all the sports included in this analysis is an over-simplification. Rugby and combative sports involve significant trauma as a natural consequence of participation, but this is very difficult to translate into strain within living human bone. In addition to this, the requirements for skeletal adjustments depend on whether the site is weight bearing or not. One single study has, to date, measured bone strain in a living human, where miniature strain gauges were surgically implanted in the skull and tibia (Hillam et al., 1995). The highest recorded strains were 0.21% in the tibia when jumping down from 1.5 metres, but the same event caused a strain of just 0.02% in the skull. The authors infer differences in strain sensing, either by different thresholds or different mechanisms to prevail, and conclude that a whole raft of work is needed to investigate the sensing of strain and the response under various pathological bone disease states.

A more exact study of the effect of two exercise influences – running and cycling – was possible by including the athletes of each discipline, together with triathletes and controls. None of the runners undertook any cycling training, and none of the cyclists did exercise other than cycling (two cyclists were excluded because of impact arising from manual work or racket sports). Triathletes were participants in multi-sport events of running and cycling only (N = 2) or in addition, swimming (N = 9) or kayaking (N = 3). All were scanned for whole body and lumbar spine (see methods chapter 3.4).

With these numbers, the study had 80% power to detect an effect size of 1.0 assuming a one-tailed α of 0.05. An effect size of 1.0 was selected due to the World Health Organization's designation of osteopenia as 1SD below peak bone mass. Unpaired *t*-tests were used to determine whether differences between groups were significant. Statistical significance was accepted at the $P < 0.05$ level. Regression analysis was performed to assess the contribution of various factors to BMD variance.

When the athletic groups were compared with one another, there were no significant differences in age, height, mass, BMI, % fat or hours of training ($P > 0.05$).

Table 6.5 Bone parameters of runners, cyclists, triathletes and controls

Variable	Controls n = 23	Runners n = 12	Cyclists n = 14	Triathletes n = 13
Total BMD ($g.cm^{-2}$)	1.24 ± 0.11	1.32 ± 0.07*	1.21 ± 0.07	1.31 ± 0.09*
Spine BMD ($g.cm^{-2}$)	1.04 ± 0.12	1.09 ± 0.10	0.96 ± 0.11§	1.14 ± 0.12
Arm BMD ($g.cm^{-2}$)	0.87 ± 0.09	0.86 ± 0.04	0.84 ± 0.04	0.94 ± 0.07**
Leg BMD ($g.cm^{-2}$)	1.46 ± 0.14	1.60 ± 0.13*	1.42 ± 0.12	1.51 ± 0.13
Leg BMC/wt ($g.kg^{-1}$)	16.2 ± 1.84	19.3 ± 1.20***	16.4 ± 1.29	18.0 ± 1.17*

* Difference from controls at $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; § $P = 0.05$ assuming equal variance

Bone data are summarized in table 6.5 and illustrated in figures 6.5 – 6.8. Compared with controls, runners had greater total BMD ($P < 0.01$), greater leg BMD ($P < 0.01$) and greater leg BMC corrected for total body mass ($P < 0.001$). Compared with controls, cyclists had less spine BMD ($P = 0.05$). Compared with controls, triathletes had greater total BMD ($P < 0.05$), arm BMD ($P < 0.05$) and leg BMC corrected for mass ($P < 0.01$).

Compared with runners, cyclists had less total BMD ($P < 0.001$), less spine BMD ($P < 0.01$), less leg BMD ($P < 0.01$) and less leg BMC corrected for mass ($P < 0.001$). Compared with runners, triathletes had greater arm BMD ($P < 0.01$), but less leg BMC corrected for mass ($P < 0.01$). Compared with cyclists, triathletes had greater total BMD ($P < 0.01$), spine BMD ($P < 0.001$), arm BMD ($P < 0.001$), and leg BMC corrected for mass ($P < 0.01$).

Figure 6.5 Total BMD in male runners, cyclists, triathletes and controls

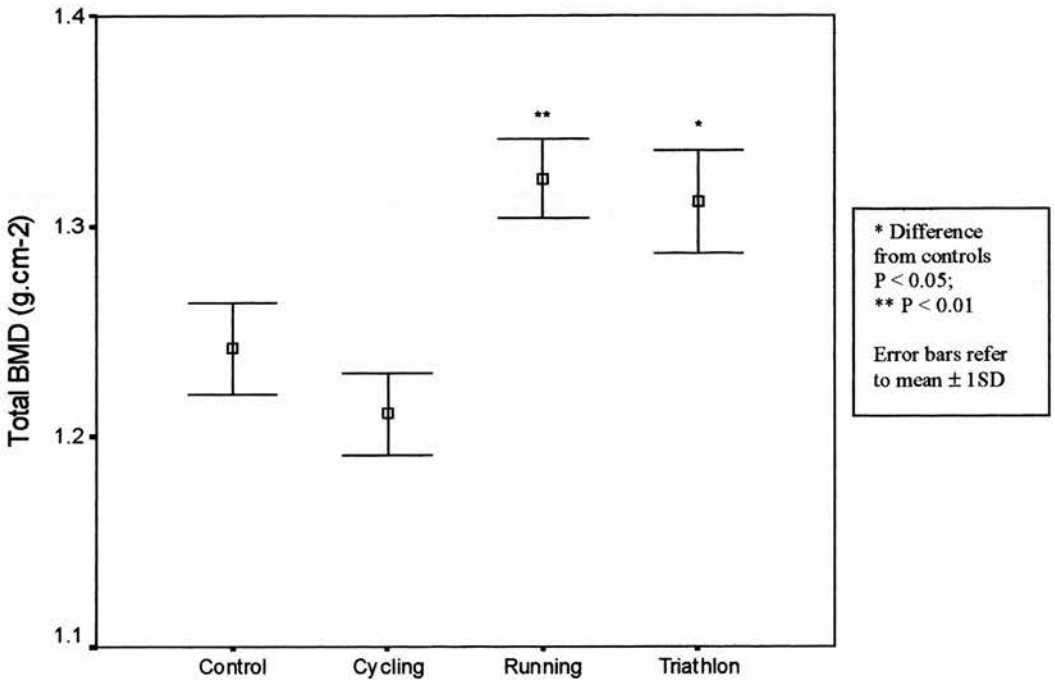


Figure 6.6 Spine BMD in male runners, cyclists, triathletes and controls

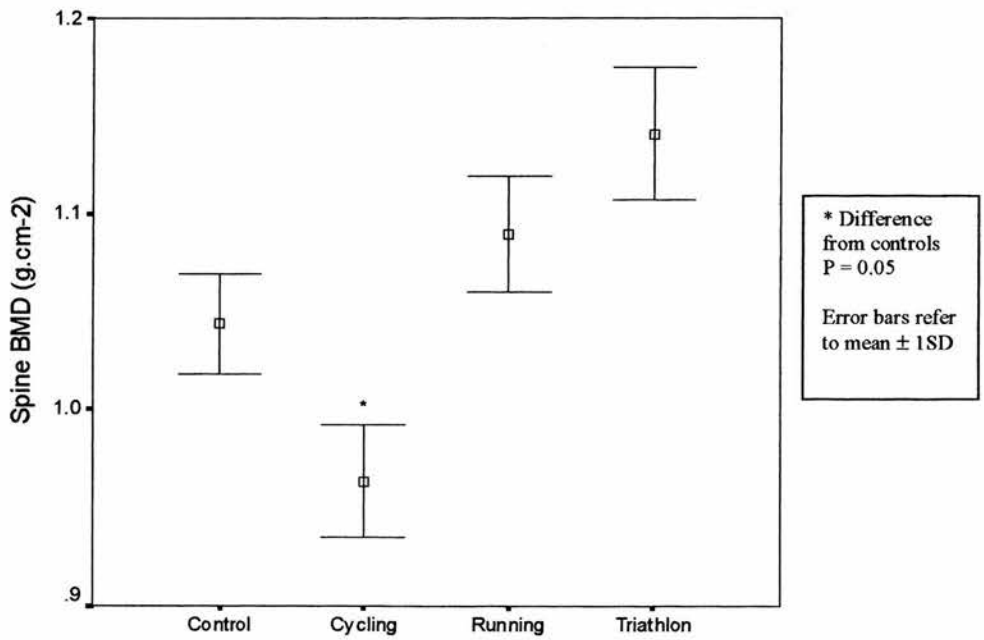


Figure 6.7 Leg BMD in male runners, cyclists, triathletes and controls

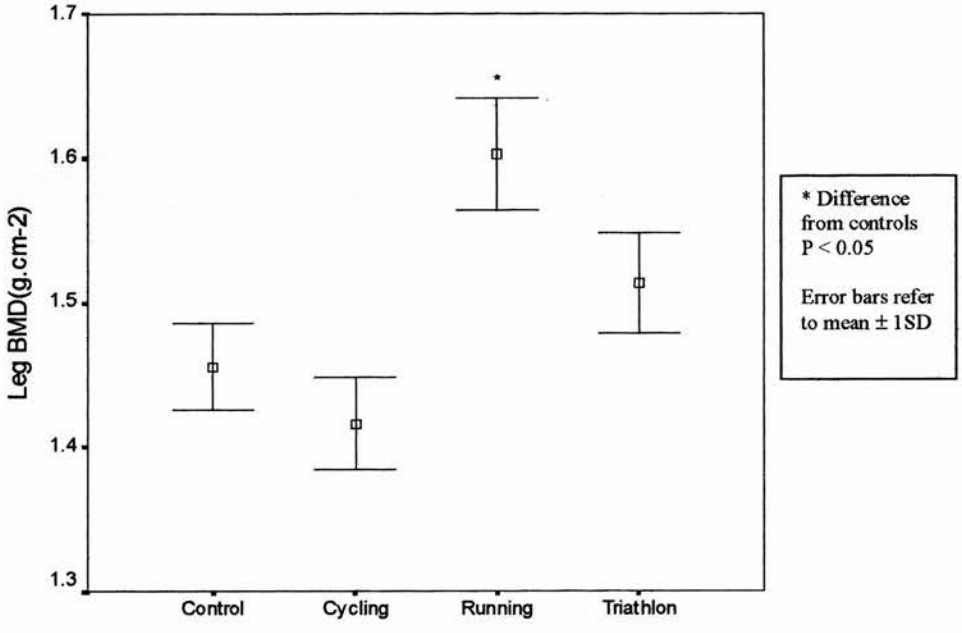
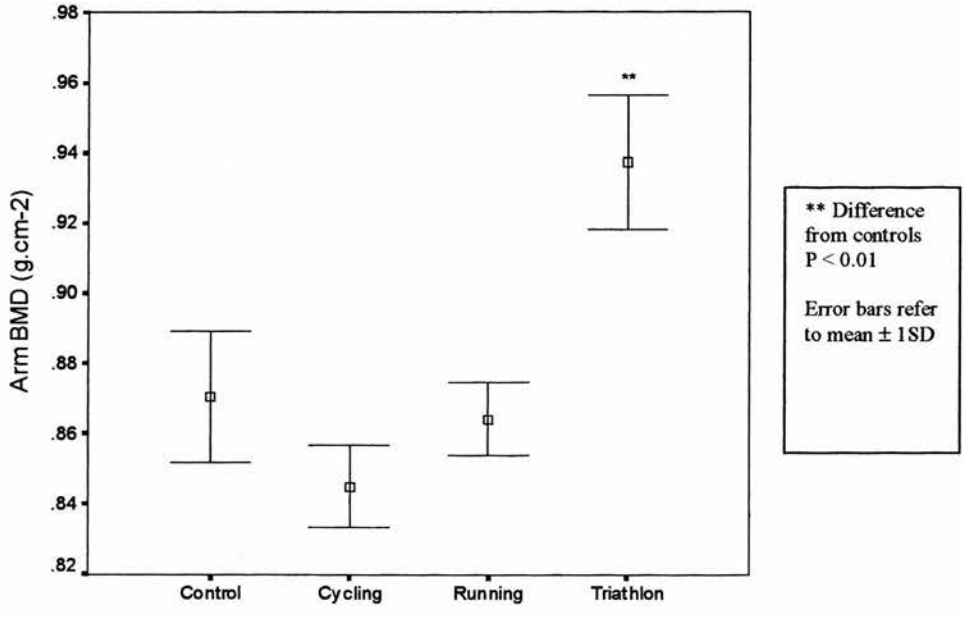


Figure 6.8 Arm BMD in male runners, cyclists, triathletes and controls



Regression analysis was performed to evaluate the extent to which the variance in total and spine BMD was explained by mass alone, or mass together with running status (RS) or cycling status (CS), each of which was assigned a "1" for presence and "0" for absence. When athletes and controls were combined (N = 62), mass alone explained 18% of total BMD, while adding the factors explained 52%. Mass alone explained 12% of the variation in spine BMD, increasing to 38% when the other factors were included ($P < 0.001$). When athletes were treated as one separate group (N = 39), mass alone explained 21% of the variation in total BMD, rising to 60% with the factors included. Mass explained 14% of the variation in spine BMD, rising to 50% with the other factors included ($P < 0.001$).

This study shows that each of the athletic groups displayed a significant difference from each other and from controls with respect to BMD or mass corrected BMC. As there were no age or anthropometric differences between any of the athletic groups ($P > 0.05$), it is likely that these differences can be attributed to differences in exercise and training which has placed different loading on the skeleton.

The mechanism of bone remodeling in adult life responds to commonly-experienced strains and has been referred to as the "mechanostat", similar to the principle of a thermostat (Frost, 1987). The sequence of remodelling occurs in a period of time between 100 days and one year via negative feedback loops involving systemic hormones. Thus, although the general configuration of the skeleton is genetically determined, the internal structure is in the process of dynamic change according to external stimuli. In this respect, running and cycling appear to cause different adjustments to the "mechanostat". Experimental data show dynamic weight-bearing activity using high strains appears to be most effective in promoting skeletal development in animals (O'Conner et al., 1982; Rubin and Lanyon, 1984) and that in humans, power athletes have superior bone density to endurance athletes, although the bone response to mechanical loading is site-specific (Bennell et al., 1997). Heinonen et al., (1996) reported gains in BMD of 1.4 - 3.7% in a period of 18 months of "jump training" which induces impacts approaching six times body weight. Their study was succeeded by a follow up which divided the original training group into

training and control sub-groups. Despite the mean age of 40yrs, BMD continued to rise over the subsequent 26 months in the training sub-group. Marcus (1996) suggests jogging can produce forces of 3 - 4 times body weight, and suggests that the extra intensity of activities which stress the skeleton, may also be sufficiently rigorous to compromise safety.

Studies investigating the bone density of competitive cyclists are rare. One study of female athletes of different endurance sports, weight lifters and controls (Heinonen et al., 1993) showed cyclists had no difference in BMD from controls at any site, whereas orienteers had higher BMD at distal femur and proximal tibia, suggesting a site-specific osteogenic influence of running.

Rico et al., (1993) investigated 22 young male cyclists who trained 10 hours per week for two years. Using a Norland XR 26 bone densitometer (Norland Medical Systems Inc. Fort Atkinson, WI) they found no difference in total body bone mineral (TBBM) between the cyclists and age-matched controls, but found the cyclists had less leg bone mineral than controls. This difference was eliminated when results were normalized for body mass. No measurements at the spine were presented. While it has been suggested that more than 90% of adult bone mineral is present at the end of skeletal maturation (Slemenda et al., 1994), the mean height of the cyclists in Rico's study was only 171cm, 4cm less than the controls, suggesting further growth of the cyclists was probable. This could also explain why their leg BMC data were lower in cyclists than controls. In the present study, the height of the cyclists was not different from that of controls ($P > 0.05$), and both cyclists and controls had virtually identical proportions of total mass as leg mass, and leg BMC divided by total mass or leg mass ($P > 0.05$).

Warner and Dalsky (1997) investigated 30 elite competitive male cyclists with similar age and height to the cyclists of the present study. When compared with recreationally active male controls, there was no difference in BMD or mass-adjusted BMD at any regional site. The ethnicity and physical activity of the recreational athletes was not presented, nor was it stated that the cyclists undertook no impact activity. The significant difference in total mass and % fat would suggest that their cyclists trained harder than their

recreational athletes. Despite their study being performed on a Lunar DPX-L (Lunar Corporation, Madison, WI), and the spine scan being L2-L4, rather than L1-L4 as in the present study, the difference in findings between their work and the present study cannot be accounted for by reported inter-scanner differences (Tothill et al., 1994a). The results of the present study, which suggest that cycling is associated with reduced bone mineral, are at odds with their findings.

Non weight-bearing activities such as swimming and cycling have not been promoted for increasing BMD (ACSM, 1995), and the lack of weight bearing exercise is considered harmful to the skeleton (Bailey and McCulloch, 1990). While this could explain why cyclists have no different bone density from controls, why cycling should exert a negative influence on BMD independent of mass, is something of a paradox. Possible explanations for cyclists' apparent low bone content include the failure of cycling to produce strains above the remodeling threshold, and the fact that body weight is distributed horizontally in the axial skeleton. Given that the average weekly training time in this position is 11 hours, this represents a substantial proportion of total waking hours during which the skeleton is exposed to minimal strain, while the spine is exposed to the equivalent of bed rest. Such a flat cycling position is likely to cause the arms to assume a proportion of upper body weight. However, because this loading is largely static in nature, the increase in strain on arm bones is probably minimal and no commensurate increase in arm BMD relative to runners or controls was observed.

Individuals who engaged in manual work or other impact sports such as tennis, were excluded from the study in an attempt to focus the investigation on cycling or running. However, the lack of other strain-producing activity in the cyclists is equally crucial to this argument. While the regional and total BMD disparity between cyclists and controls disappeared when corrected for mass ($P > 0.05$), the co-efficient of cycling status remained negative in the regression. Thus, the fact that cyclists had lower BMI than controls could explain most, but not all of their lower BMD. However, given that all subjects in the study were at or close to their theoretical peak bone mass, a low value may predispose increased fracture risk later in life, especially if other risk factors for osteoporosis are present. The mean spine T score (number

of standard deviations from a peak BMD reference range supplied by the manufacturer) of the cyclists was –1.16, which would be diagnosed as osteopenic (significantly reduced BMD). Using manufacturer's data, this represents a seven – fold increased relative risk of a cyclist having osteopenia than a control. One cyclist aged 36, with no family history of osteoporosis, had a T score of – 2.72 (classified by the World Health Organization as osteoporotic). While endocrine data were not collected as part of this study, this subject, following further clinical assessment, was shown to have normal endocrine function. Without further evidence it would be misleading to suggest that cycling *per se* causes the observed low bone density, but the occurrence of reduced bone density appears greater than expected amongst those who cycle to the exclusion of physical activity involving impact.

For the triathletes, excluding all other exercise except cycling and running would have enabled a more complete understanding of the interactive effect of both activities. The fact that athletes who both cycled and ran largely engaged in significant additional exercise (11 out of 13 individuals), means the contribution of upper body exercise could be viewed as a confounding variable. Nevertheless, the greater arm bone density of triathletes is highly suggestive of the influence of upper body exercise on overall skeletal adaptation. The heavier build of these athletes, coupled with the diversity of their training can also explain that their mass-corrected leg BMC was less than that of runners. However, the fact that such upper body exercise is non-impact suggests a different mechanism for adjusting bone architecture may operate which can be implemented at lower bone strains. Evidence of human bone strain *in vivo* is extremely difficult to collect, and has not as yet included the sporting disciplines under investigation here.

Seen comparatively, the data on all three athletic groups suggest cycling in the absence of other sports is associated with a reduction in bone, whereas running is associated with an increase in bone at load bearing sites. The effect of running would appear to counteract the effect of cycling in those athletes who do both, and upper body exercise appears to increase arm and spine bone significantly.

What remains to be investigated is the extent to which cyclists can improve their skeletal mass by impact bearing activity. Recovery of lost bone has been shown to be possible, but is probably site-specific (Hughes-Fulford and Lewis, 1996). Interventions which have shown increases in bone mass in middle age (Heinonen et al., 1996) have not been undertaken by athletes, but logically, there is a case supporting its potential effectiveness which remains to be investigated.

Clearly cycling is a sport where the inducements of success may predispose a performance-based view of training, where future health concerns may be little considered. The extensive training on roads which is required, appears largely to the exclusion of other exercise in the majority of subjects. However, data from the present study suggests there may be considerable health benefit for cyclists to pursue some impact exercise involving skeletal loading in addition to bike riding itself. Future work could usefully concentrate on the dose-response of exercise regimens, together with more specific *in vivo* measurements. Nonetheless, it would appear that caution is advised on cycling to the exclusion of other physical activity, particularly in young competitive athletes who are ascending towards their peak bone mass.

CHAPTER 7.

SUMMARY AND CONCLUSIONS

This chapter is concerned with providing an overview of the work, and an assessment of its true meaning and implications. In appraising the utility of DXA, it is reasonable to assume that it makes a very significant contribution to the field of body composition, although several unanswered questions remain as subjects for further research.

7.1 SUMMARY OF FINDINGS

In applying a new technique to populations already extensively investigated by other methods, DXA has been able to add a considerable amount to current knowledge. Its principal utility, its ability to provide bone, lean and fat tissue in regional as well as total masses, offers a vast array of information, and more than most specific research questions require.

While DXA has moved beyond its infancy and exercise studies in the elderly are becoming more common, studies with athletes are rare, the majority involving females only. In addition to its developmental purpose of measuring bone, DXA has become an integral part of the gold standard body composition measure in humans: the 4 compartment model. Not least through its chemical validation by use of pig carcasses (Mitchell et al., 1996a), total body composition has become widely accepted using DXA alone as the reference method. While there remain inter-manufacturer differences, conclusions drawn from DXA scan data are, for the time being at least, specific to each manufacturer. Hologic is arguably the best validated of these, and the study by Prior et al., (1997) using the same model of scanner as that of the present study suggests DXA is more accurate than underwater weighing for assessing the body composition of athletes.

Fat

Athletes exhibit a different fat pattern to sedentary controls, and DXA and skinfold thickness have provided conflicting results in other studies. While the subcutaneous : internal fat distribution in athletes remains unknown, the reliable prediction of total fat afforded by skinfolds suggests this ratio is probably high.

In both men and women athletes, total fat is better predicted from skinfolds than BIA, and in both, the thigh and abdominal sites are significant predictors. These sites are not included in several traditional

prediction equations which are still in use today with athletic populations. Data from the present study suggest such predictions may be highly inaccurate, although one prediction validated against densitometry which contained these sites was accurate (SEE < 3%) over a range from 0 -18% fat, representing 93% of the male athlete cohort. The prediction equations derived from the present study have smaller total error and SEE, while explaining a similar amount of variation in the total fat content. As long as it remains impractical to develop a 4-compartment validation for athletes, the equation predicting male fat in the present study may be the most accurate, and it is likely that future studies will seek to use DXA as the reference method.

There is a sexual dimorphism in regional fat distribution which becomes less distinct as total fat decreases. For both male and female athletes there was a lesser proportion of total fat on the torso than for controls, DXA evidence supporting a 'fit-fat' distribution theory (Nindl et al., 1996). Weekly exercise duration correlated with trunk : extremity skinfold (TE) ratios in men, but not women, which could be explained by either their greater potential for altering fat topography, or their more extensive exercise regimes. Age had a centralising influence independently of exercise, which best correlated with the abdominal : medial calf (AMC) skinfold ratio. The apparent low torso fat in rowers was not significant after controlling for age in multivariate analysis. Lean tissue development, with its implied metabolic activity of muscle, appeared to have no effect on fat distribution.

In females, anorexic fat distribution in arms : legs was different from that of athletes ($P < 0.01$), suggesting starvation and exercise cause different adaptations, although it is recognised that measurement accuracy is less at the extremes of body composition.

Lean tissue

The specific development exhibited by athletes of certain sports indicates the extent to which the physique responds to training. While there are anthropometric predictions of muscle mass, DXA lean mass includes muscle plus organs, body fluids and glycogen. The inability of DXA to distinguish between

these various components of lean tissue may render DXA of lesser utility in predicting performance than a technique which estimates muscle mass, but regional analysis of DXA lean tissue is a useful means of recognising the significant variation in development between different sporting groups. The ratio of assumed muscle development in previous DXA studies (75% on limbs; 25% on the torso) may not be the case for athletes, and the placement of field boundaries arising from different operators or manufacturers limits the validity of comparisons.

The present study indicates a sexual dimorphism in lean tissue, with males having greater amounts in absolute and proportional terms. The optimal prediction of lean tissue was from subtracting adipose tissue mass from total mass, and anthropometry appeared superior to BIA in predictive accuracy. This was probably due to variation in specific muscle mass, although it may have been possible for incomplete re-hydration after prior exercise to have contributed to the poorer accuracy of BIA in some individuals.

Regional differences in lean tissue distribution show significant differences between the various male athletic groups, which were most marked in the ratio of lean tissue in arms : legs.

Bone

The present work has been able to recognise that while in general, exercise has a very positive effect on the skeletal system, exercise which is weight supported, may be of no benefit or even detrimental to bone density. While traditional somatotype recognises a close relationship between muscular and bone development, data from the present studies illustrate that using the DXA morphotype, athletic groups can be outwardly very similar, but in skeletal terms, very different.

In female athletes, reduced spine bone density is associated with being light, slender and amenorrhoeic, and of the subjects investigated, particularly affected runners and dancers. Statistical analysis indicated that an average female athlete who was oestrogen sufficient, could afford to be 2.8kg lighter than one who was not, for the same spine T score or bone density. Data from the present study indicated that spine T

score was better predicted from BMI and oestrogen status, rather than existing % fat values suggested for minimum mass. Because the present study shows reduced spine bone density to be common in women athletes, the effective prediction of those at risk may be a useful way of triggering effective remedial action in this population.

The male athlete cohort had sufficient numbers in specific sports to enable comparisons to be made. These data suggested that impact exercise had a profoundly significant effect on bone density, while weight supported exercise had little or no effect. In cyclists, however, the light overall mass of athletes combined with the absence of impact reduced the bone density of the whole skeleton, including the legs, and compared with controls, this reached significance in the spine ($P = 0.05$) but not after controlling for mass. Using the manufacturer's reference data, these cyclists were seven times more likely to suffer osteopenia than controls, although the figure was 2.5 using study control data. Such a finding was not replicated in rowers - the only other weight-supported exercise group - who pursue considerable strength training of the upper body which is likely to have a positive influence on bone. The explanatory mechanism for reduced bone density in cyclists is unclear, and may involve several factors which interact. Possible contributors include the horizontal position of the spine, increased evaporative sweat (and calcium) loss, greater metabolic cost due to longer training than other athletic groups, and an endocrine consequence of this, or trauma to the testes affecting the influence of testosterone over bone. Whatever the mechanism, the finding would suggest that cyclists require to be cautious about pursuing cycle- based training to the exclusion of impact exercise.

Triathletes appear to obtain most of the skeletal benefits of running without the detrimental effect of cycling. Their greater arm BMD ($P < 0.01$) than the cyclists, runners or controls was apparently achieved with no impact loading of the arms apart from that arising as a result of muscular torque. This, in addition to the higher spine BMD in rowers, suggests different mechanisms may prevail in the arm and spine compared to those in the leg, where cycling has a neutral or negative influence on leg BMD.

7.2 CONCLUSIONS

The conclusions of this work are structured to reflect outcomes relating to the statement of aims as itemised in chapter 1.5, pp15-16.

1. Using DXA as the reference method, anthropometry appears better than BIA in predicting fat and fat-free content in male and female athletes.
2. Regional fat distribution is affected by four independent factors: gender, age, adiposity and exercise.
3. The type of exercise (i.e. local muscle involvement) appears unimportant in determining regional fat distribution, however exercise of any type appears to oppose the tendency for centralisation of fat with increasing age and adiposity.
4. Anthropometric data suggest the alteration in subcutaneous fat distribution with age is best described by the AMC ratio in both sexes, while the TE ratio is affected by exercise duration in men only.
5. Exercise appears to increase limb lean tissue to a greater extent than torso lean tissue.
6. BMC, lean and total regional mass are highly influenced by exercise type, however there appears no local usage of fat according to metabolic activity in nearby muscle.
7. Female anorexics are not simply 'thinner' equivalents of athletes, but have differences in fat and lean distribution relative to athletes and controls. In addition to having relatively less leg fat than athletes, anorexics exhibit a non-uniform loss of lean tissue and bone.
8. The DXA morphotype may be a useful adjunct to other methods of describing body composition, and may discriminate differences between groups shown to be similar in physique.
9. Data from the study show that, despite the anabolic effect of weight bearing exercise on bone, the incidence of reduced BMD in women athletes is more than double that of controls. Osteopenia is more than twice as likely to occur in athletes whose BMI + Oestrogen Status is less than 20.4.

10. In males, subdividing athletic groups into weight supported or impact sports exposes significant differences in BMD. In broad terms, weight-supported sport appears to have no significant effect on the skeleton, while impact exercise has a considerable effect.
11. The investigation of running and cycling concluded that while running had a particularly positive effect on bone, cycling had a slight negative effect, especially in the spine.
12. While impact appears crucial to bone density in the legs and spine, muscular torque may play a key role in influencing arm bone, and may contribute an additional effect at the spine.

7.3 FURTHER RESEARCH

As we begin a new millennium, the increasing threat of obesity and cultural pressure to be slim in the Western world, mean the frame of reference for body composition research is itself expanding. In this context, DXA has much to offer. Increased availability of hardware make scans more accessible, while decreased scan times using newer fan–beam technology will allow greater throughput. DXA thus holds the potential for future studies to involve greater numbers of subjects, and have enhanced power, which will enable greater accuracy of findings. Inter-scanner differences may be quantified by more focused research efforts, potentially enabling pooling of data, and reducing the effective barrier to collaborative work between different centres.

With regard to body composition, DXA offers a convenient means of establishing all three body composition compartments, and the DXA morphotype could develop into a paradigm for clinical and nutritional research. It is probable that future improvements in software will account for differences in soft tissue distribution, and more sophisticated algorithms may be available for men, women, obese, anorexic and athletic groups, which will render DXA more accurate at both extremes of the range of body fat, and lead to improvements in prediction of muscle mass. With regard to the expanding population of

athletes, the partitioning of fat between subcutaneous and internal depots is still unknown, yet remains a central question for the accurate prediction of recommended minimum mass.

With regard to bone, there is a need to identify the dose-response of impact loading for skeletal benefit. Future animal studies may be able to test effects of strain sensing with a more rigorous study design than is possible using human subjects. However, the present study has flagged concern that some women athletes and male cyclists may have bone density levels considerably below normal, and that they may inadvertently be denying the achievement of a peak bone mass, and bringing forward the onset of osteoporosis in later life. There is a need to investigate such athletes further, especially using endocrine evidence which may assist athletes determine if existing regimes constitute 'overtraining'.

Perhaps more than anything there is a need to educate athletes themselves that, in addition to the health of their cardiovascular and muscular systems, their skeletal system has the potential to be the 'Achilles heel' of any training programme if the quest for ever better performance is at the expense of skeletal health.

APPENDIX

APPENDIX A: ABBREVIATED TERMS

AMC ratio	Abdominal : medial calf ratio (skinfolds)
ANOVA	Analysis of variance
AT	Adipose tissue
BAL ratio	Ratio of BMC in arms : legs (DXA)
BIA	Bioelectrical impedance analysis
BMC	Bone mineral content (the same as bone mineral mass)
BMD	Bone mineral density (areal)
BMI	Body mass index
BTC ratio	Ratio of BMC in torso : legs (DXA)
CV	Co-efficient of variation
DPA	Dual photon absorptiometry
DXA	Dual X-ray absorptiometry
FAL ratio	Ratio of fat tissue in arms : legs (DXA)
FFM	Fat-free mass
FID	Free induction decay
FTL ratio	Ratio of fat tissue in torso : legs (DXA)
ISAK	International Society for the Advancement of Kinanthropometry
LAL ratio	Ratio of lean tissue in arms : legs (DXA)
LTL ratio	Ratio of lean tissue in torso : legs (DXA)
MRI	Magnetic resonance imaging
OS	Oestrogen status
RF	Radio frequency
SAT	Subcutaneous adipose tissue
SD	Standard deviation
SE	Standard error
SEE	Standard error of the estimate
SNK	Student Newman Keuls post hoc test
ST ratio	Subscapular : triceps ratio (skinfolds)
T_1	Relaxation time (nucleus – lattice interaction)
T_2	Relaxation time (nucleus – nucleus interaction)
TBK	Total body potassium (^{40}K)
TE ratio	Trunk : extremity ratio (skinfolds)
TEM	Technical error of measurement
TR	Time to repeat
UWW	Underwater weighing

APPENDIX B: REPRODUCIBILITY AND PRECISION CALCULATIONS

The **Reliability Coefficient** R is the dimensionless intra-class correlation coefficient of reliability which expresses the proportion of the observed variance accounted for by variance in true values (Himes, 1989). R is calculated from the results of random-effects ANOVA, or the technical errors of measurement (TEM).

Technical Error of Measurement (TEM) is an index of precision indicating that two thirds of the time a measurement should come within +/- the value of the TEM (Carter and Ackland, 1994).

$TEM = \{\Sigma(x_2 - x_1)^2 / 2n\}^{0.5}$

$\%TEM = 100*(TEM/M_1)$

where n is the number of pairs of measurements, M_1 is the mean of the initial measurements.

Precision is described as Coefficient of Variation / mean, or more commonly as a percentage:

Precision error = 100* CV / mean.

APPENDIX C: DXA TECHNICAL DATA

Scanner type: Hologic QDR 1000W:

Scan area: 71.82 x 24.82 inches (max length 75 inches) organised in 140 lines at 660 samples / line

Line spacing: 0.513 inches

Point resolution: 0.08063

Pixels: 700 (Y) x 330 (X)

Voltage: 140 / 70 kVp

Current: 2.0 mA (average)

Radiation doses:

Lothian background per day: 5.2 μSv (UK average 6.0 μSv; maximum 20.2μSv)

Whole body scan: approx. 15 - 20 μSv (equivalent to one TransAtlantic flight)

Lumbar spine L1 - L4: approx. 5 - 10 μSv (equivalent to 1 - 2 days background)

For both scans, this amounts to 3% of the annual allowable dosage, and constitute World Health Organisation category I risk of carcinogenesis (approx. 1×10^{-6})

Figure of Block Diagram of a Hologic DXA scanner

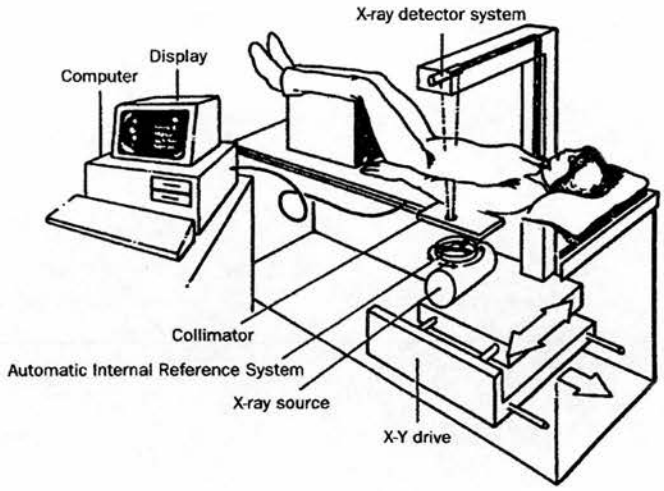


Figure Photograph of the Hologic DXA QDR 1000W in use

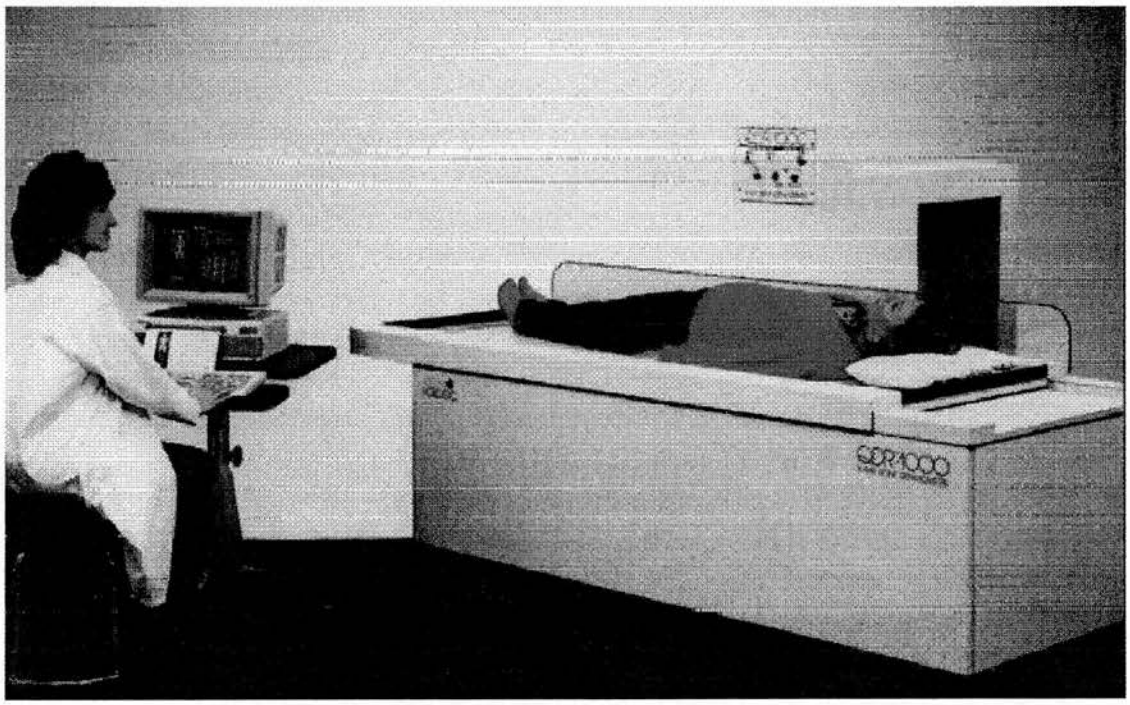
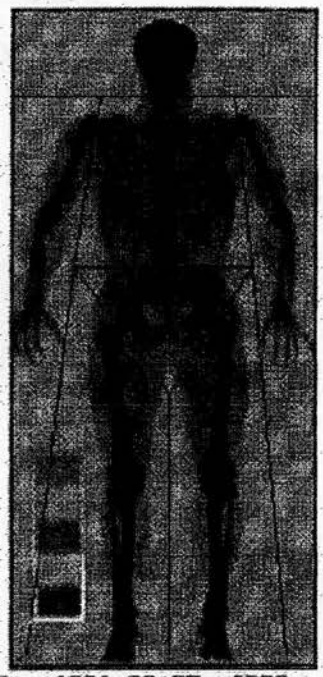


Figure of DXA scan output: body composition

Medical Physics, WGH. Edinburgh.



·17.Jun.1996 09:27 [330 x 146]
Hologic QDR-1000/W (S/N 967 P)
Enhanced Whole Body V5.55

U06149607 Fri 14.Jun.1996 15:10
Name:
Comment:
I.D.: R01A030 Sex: M
S.S.#: - - Ethnic: W
ZIPCode: C Height: 179.50 cm
Scan Code: SC Weight: 75.50 kg
BirthDate: Age: 21
Physician: STEWART
Image not for diagnostic use
TBAR231
F.S. 68.00% 0(10.00)%
Head assumes 17.0% brain fat
LBM 73.2% water

Region	Fat (grams)	Lean+BMC (grams)	% Fat (%)
L Arm	191.6	3899.2	4.7
R Arm	104.5	4551.4	2.2
Trunk	777.9	35175.7	2.2
L Leg	662.5	11503.0	5.4
R Leg	943.2	11619.5	7.5
SubTot	2679.8	66748.8	3.9
Head	790.5	4290.0	15.6
TOTAL	3470.3	71038.8	4.7

HOLOGIC

Medical Physics, WGH. Edinburgh.

Hologic QDR-1000/W (S/N 967 P)
Enhanced Whole Body V5.55
·17.Jun.1996 09:27

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Scan Code: SC Weight: 75.50 kg
BirthDate: Age: 21
Physician: STEWART

TBAR231
F.S. 68.00% 0(10.00)%

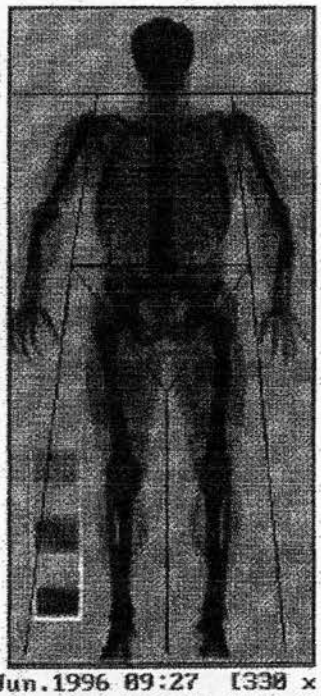
Region	BMC (grams)	Fat (grams)	Lean (grams)	Lean+BMC (grams)	Total (grams)	% Fat (%)
L Arm	209.8	191.6	3689.4	3899.2	4090.8	4.7
R Arm	261.5	104.5	4289.9	4551.4	4656.0	2.2
Trunk	1033.3	777.9	34142.4	35175.7	35953.6	2.2
L Leg	694.3	662.5	10808.7	11503.0	12165.5	5.4
R Leg	737.0	943.2	10882.5	11619.5	12562.7	7.5
SubTot	2935.9	2679.8	63812.9	66748.8	69428.6	3.9
~Head	536.8	790.5	3753.2	4290.0	5080.5	15.6
TOTAL	3472.7	3470.3	67566.1	71038.8	74509.1	4.7

~assumes 17.0% brain fat
LBM 73.2% water

HOLOGIC

Figure of DXA scan output: bone density

Medical Physics, WGH. Edinburgh.



·17.Jun.1996 09:27 [330 x 146]
Hologic QDR-1000/W (S/N 967 P)
Enhanced Whole Body V5.55

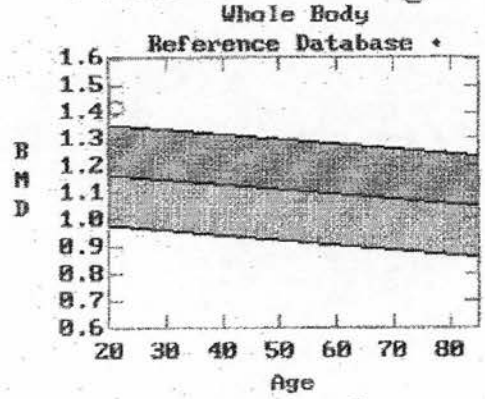
U06149607 Fri 14.Jun.1996 15:10
Name:
Comment:
I.D.: R01A030 Sex: M
S.S.#: - - Ethnic: W
ZIPCode: C Height: 179.50 cm
Scan Code: SC Weight: 75.50 kg
BirthDate: Age: 21
Physician: STEWART
Image not for diagnostic use

TOTAL BMC and BMD CV is < 1.0%
C.F. 1.014 1.090 1.000

Region	Area (cm2)	BMC (grams)	BMD (gms/cm2)
L Arm	229.60	209.80	0.914
R Arm	266.11	261.49	0.983
L Ribs	153.34	132.31	0.863
R Ribs	142.79	130.53	0.914
T Spine	159.02	198.20	1.246
L Spine	68.56	93.40	1.362
Pelvis	312.76	478.84	1.531
L Leg	433.24	694.32	1.603
R Leg	444.60	737.03	1.658
SubTot	2210.00	2935.93	1.328
Head	249.88	536.82	2.148
TOTAL	2459.89	3472.75	1.412

HOLOGIC

Medical Physics, WGH. Edinburgh.



BMD(WHOLE) = 1.412 g/cm^2

T(20.0)	Z
+2.68 122%	+2.71 122%

U06149607 Fri 14.Jun.1996 15:10
Name:
Comment:
I.D.: R01A030 Sex: M
S.S.#: - - Ethnic: W
ZIPCode: C Height: 179.50 cm
Scan Code: SC Weight: 75.50 kg
BirthDate: Age: 21
Physician: STEWART

* Age and sex matched
T = peak bone mass
Z = age matched PS 25 Oct 91

HOLOGIC

APPENDIX D: PUBLICATIONS OF THIS MATERIAL

Journal and abstract publications:

Stewart, A.D. and Hannan, W.J. Prediction of fat and fat-free mass in male athletes using dual X-ray absorptiometry (DXA) as the reference method. *Journal of Sports Sciences* (In Press).

Stewart, A.D. and Hannan, J. Total and regional bone density of runners, cyclists and controls. *Medicine and Science in Sports and Exercise* (In Press).

Stewart, A.D. and Hannan, W.J. The DXA Morphotype: A new approach to body composition (Abstract) *British Journal of Sports Medicine* (In Press).

Stewart, A.D. and Hannan, J. Sub-regional tissue morphometry in male athletes and controls using Dual X-ray Absorptiometry. *International Journal of Sports Nutrition* (In Press).

Brodie, D. and Stewart, A.D. Body Composition: A Hierarchy of Methods. *Journal of Paediatric Endocrinology and Metabolism* (In Press).

Stewart, A.D. (1999) Comparison of bone mineral density in athletes of impact and non-impact sports (Abstract) *Osteoporosis International,* **9** no 4: 377.

Stewart, A.D., Cowen, S. and Hannan J. (1999)Total and regional bone mineral content of cyclists, runners and controls (Abstract). *Osteoporosis International,* **9** no 4: 377 - 378.

Stewart, A., McLean, I., Cowen. S. and Hannan, J. (1998). The incidence of reduced bone density in recreational women athletes. (Abstract) *Current Research in Osteoporosis and Bone Mineral Measurement V,* 102 - 103.

McLean, I.P. and Stewart, A.D. (1998). The Female Athlete Triad: Converting anthropometry into clinical guidelines. (Abstract) *European College of Sports Science Congress Proceedings*, 157.

Stewart, A., Cowen, S. and Hannan, J. (1998). Regional body composition in women athletes, anorexics and controls using dual X-ray absorptiometry. (Abstract) *European College of Sports Science Congress Proceedings*, 462.

Stewart, A., Cowen, S. and Hannan, J. (1998). Bone mineral content of cyclists, runners and controls. (Abstract) *European College of Sports Science Congress Proceedings*, 461.

Douglas, L.N., Stewart, A. and McLean, I. (1998). Nutritional intake and the female athlete triad. (Abstract) *European College of Sports Science Congress Proceedings*, 111.

Stewart, A. and Eston, R. (1997). Skinfold Thickness Measurement. *British Journal of Nutrition* **78**, 1040 - 1042.

Stewart, A., Cowen, S. and Hannan J. (1997). Comparison of dual X-ray absorptiometry with bioelectrical impedance and anthropometry for predicting body composition in habitually active males. (Abstract) *J. Sports Sci.* **15**, 65 - 66.

All publications have been with the approval of Dr. J. Hannan, supervisor.

Presentations

Stewart, A. Bone density and body composition in women athletes. Scottish Universities Physical Education Association Annual Conference in Edinburgh, June 1977.

Stewart A. and McLean, I. Fatness and thinness - a discussion of the female athlete triad. Edinburgh Postgraduate Board for Medicine conference of Exercise and Sports Medicine, February 1998.

Stewart, A., Cowen, S. and Hannan, J. Total and regional bone mineral content of cyclists, runners and controls. Exercise and The Skeleton conference, University College London, November 1998.

Stewart, A. Comparison of bone mineral density in athletes of impact and non-impact sports. Exercise and The Skeleton conference, University College London, November 1998.

Stewart, A. Bones in male athletes. Musculo - skeletal interest group, University of Aberdeen Medical School, May 1999.

Stewart, A. Body composition and somatotyping in athletes. British Diatetic Association, East of Scotland Section Conference, Perth, May 1999.

Stewart, A.D. and Hannan, W.J. The DXA Morphotype: A new approach to body composition. Annual Congress of the British Association of Sports Medicine conference, Gosforth, October 1999.

Skinfold thickness measurement

With reference to the Technical Note, Skinfold thicknesses: is there a need to be very precise in their location? by Durnin *et al.* (1997), we wish to make the following points.

The authors reported that one of the most common methods of assessing fatness in adults is by measuring skinfold thickness using the equations of Durnin & Womersley (1974) and Jackson & Pollock (1978). Whilst reference is made to the generalized equations of Jackson & Pollock (1978) for men (and we assume by inference the Jackson *et al.* (1980) equation for women), there is no further reference to the use or application of these equations.

It is notable therefore that the selection of sites omits any from the lower body, which are among the strongest predictors of subcutaneous adiposity. The Brussels Cadaver Study (Martin *et al.* 1985) revealed that of the six best predictors of subcutaneous adipose tissue, all but one were in the lower limb, and four of these were situated in the thigh. The suprailiac, biceps, subscapular and triceps ranked 8th, 9th, 10th and 11th respectively, with the latter not reaching significance at the $P < 0{\cdot}05$ level. The thigh and calf were also among the best predictors of hydrostatically determined body density in a group of Chinese men and women (Eston *et al.* 1995). Other independent studies have also shown that ultrasound sections of the thigh region are highly correlated with body density (Eston *et al.* 1994) and studies using dual X-ray absorptiometry (Stewart *et al.* 1997) show higher correlations with the skinfold totals which incorporated the thigh site. It is a pity that the lower limb sites were not included in the paper.

Obviously, the conclusions drawn from this study are dependent on the selection of the subcutaneous fat tissue at specific sites which, as MRI studies show, vary considerably from site to site. Of the four sites measured, the suprailiac site is one of particular concern, because the exact location varies depending on the equation used. The commonly recognized suprailiac site, such as that used by Durnin & Womersley (1974), is a horizontal fold in the mid axillary line above the iliac crest. However, the data for the generalized equations of Jackson and colleagues (1978, 1980) were determined from a diagonal fold at the anterior axillary line. Studies show that inter-tester reliability is lowest at this site (Pollock & Jackson, 1984), and that anterior sites are normally 3–6 mm lower than sites on the mid-axillary line. This was observed in the Durnin *et al.* (1997) paper, although it does not appear that the exact site used in the Jackson *et al.* (1980) studies was included in the present paper.

On a slightly separate but related issue, the authors recognize that the skinfold technique may provide the most valid estimates of fatness, but we have observed that percentage body fat estimates from the Durnin & Womersley (1974) equations are systematically higher than the Jackson equations. This is an important point when one considers the frequency with which these equations are used to estimate body fat, particularly on lean individuals and athletes. As to which equation is the more valid, from our experience of skinfold topography and the evidence of studies which have included the lower limb we are of the opinion that the equations of Jackson & Pollock (1978) provide the more valid estimate.

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REFERENCES

Durnin, J. V. G. A., De Bruin, H. & Feunekes, G. I. J. (1997). Skinfold thicknesses: is there a need to be very precise in their location? (Technical Note). *British Journal of Nutrition* **77**, 3–7.

Durnin, J. V. G. A. & Womersley, J. (1974). Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *British Journal of Nutrition* **32**, 77–97.

Eston, R., Evans, R. & Fu, F. (1994). Estimation of body composition in Chinese and British men by ultrasonographic assessment of segmental adipose tissue volume. *British Journal of Sports Medicine* **28**, 9–13.

Eston, R., Fu, F. & Fung, L. (1995). Validity of conventional anthropometric techniques for predicting body composition in healthy Chinese adults. *British Journal of Sports Medicine* **29**, 52–56.

Jackson, A. S. & Pollock, M. L. (1978). Generalized equations for predicting body density of men. *British Journal of Nutrition* **40**, 497–504.

Jackson, A. S., Pollock, M. L. & Ward, A. (1980). Generalized equations for predicting body density of women. *Medicine and Science in Sports and Exercise* **12**, 175–181.

Martin, A. D., Ross, W. D., Drinkwater, D. T. & Clarys, J. P. (1985). Prediction of body fat by skinfold caliper: assumptions and cadaver evidence. *International Journal of Obesity* **9** Suppl. 1, 31–39.

Pollock, M. L. & Jackson, A. S. (1984). Research progress in validation of clinical methods of assessing body composition. *Medicine and Science in Sports and Exercise* **16**, 606–613.

Stewart, A., Cowen, S. & Hannan, J. (1997). Comparison of dual X-ray absorptiometry with bioelectrical impedance and anthropometry for predicting body composition in habitually active males. *Journal of Sports Science* **15**, 65–66.

Comparison of dual X-ray absorptiometry with bioelectrical impedance and anthropometry for predicting body composition in habitually active males

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While many studies predicting body composition have traditionally used densitometry as the criterion method, only relatively recently has dual X-ray absorptiometry (DXA) emerged as a reliable alternative. Dual X-ray absorptiometry provides a precise composition of fat, lean and bone mineral content, with a low X-ray exposure (Mazess *et al.*, 1990, *American Journal of Clinical Nutrition*, **51**, 1106–1112). The method avoids assumptions based on the density of bone and other tissues, difficulties in estimating body gas, and the need for subjects to be water-confident.

While this advantage may in particular suit youthful or elderly populations, this study examined body composition in a group of 17 habitually active Caucasian males aged 32.5 ± 8.0 years (mean ± S.D.). All subjects exercised for a minimum of 3 h per week, and most trained at least once per day. The group included participants in running, orienteering, badminton, swimming, rowing and cross-country skiing.

The subjects refrained from exhaustive exercise and diuretic drinks and endeavoured to rehydrate fully before the test. Two DXA scans were performed using a Hologic QDR-1000 whole-body scanner (Hologic, Inc., Waltham, MA, USA) and the results were averaged to provide % fat. Bioimpedance analysis (BIA) was performed using a RJL BIA 101 50 kHz analyser (RJL Systems, Detroit, MI, USA). Anthropometric measurements, including height, weight, waist and hip girths and 19 skinfolds, were made following the method of *The Anthropometric Definer* software (M. Hawes and A. Soucie, 1994, University of Calgary, Canada). The results of these measures are summarized in Table 1. Skinfolds were summed according to two prediction equations in common use. The sum of skinfolds at the biceps, triceps, subscapular and suprailiac sites, used by Durnin and Womersley (1974, *British Journal of Nutrition*, **32**, 77–97), gave a correlation of $r = 0.780$ ($P < 0.01$, S.E. = 4.01) with DXA %fat, while the sum of skinfolds at the triceps, axilla, subscapular, thigh, abdominal and suprailiac sites, used by Jackson and Pollock (1978, *British Journal of Nutrition*, **40**, 497–504), gave a correlation of $r = 0.921$ ($P < 0.0001$, S.E. = 2.49), marginally exceeding the correlation of the sum of all 19 skinfolds ($r = 0.914$, $P < 0.0001$, S.E. = 2.59).

There was no clear relationship between DXA %fat and BIA (r = 0.374, N.S.). Despite the controlling influences employed, individuals whose routine involves extensive and frequent exercise may be prone to prolonged fluid loss to which bioimpedance analysis is sensitive (Cumlea *et al.*, 1993: In *Human Body Composition*, edited by K.J. Ellis and J.D. Eastman. New York: Plenum Press). For such subjects, skinfolds are a better predictor of DXA %fat.

Table 1 Anthropometric, impedance and DXA measurements (n = 17)

	Mean ± S.D.	Range
Age (years)	32.5 ± 8.0	19–43
Body mass index (kg m^{-2})	23.4 ± 1.71	19.7–25.7
Waist to hip ratio	84.3 ± 2.83	79.8–90.1
Resistance (Ω)	514 ± 61.7	412–676
Σ 4 skinfolds (mm)	25.8 ± 8.03	15.7–48.2
Σ 7 skinfolds (mm)	62.9 ± 25.2	30.6–120
Σ 19 skinfolds (mm)	153 ± 52.9	78.3–268
DXA total %fat	12.6 ± 4.3	4.2–21.6

The incidence of reduced bone density in recreational women athletes

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Recreational or competitive women athletes are under pressure to minimize excess body fat, which is usually seen as detrimental to sports performance. While this pressure may allow such individuals to achieve an ideal weight and body composition, it has been reported to cause disorders which include osteoporosis [1]. The American College of Sports Medicine has suggested 12% fat as the minimum necessary for competitive female athletes to achieve optimal health [2]. This study aimed to explore the relationship between bone density and anthropometric, hormonal, and training variables in 26 recreational athletes (mostly runners, dancers and rowers).

Bone density and body composition were measured by dual X-ray absorptiometry using a Hologic QDR 1000W scanner in whole-body mode, and also spine mode (L1–L4). A total of 19 skinfolds, eight skeletal breadths and nine circumferences, together with height and weight, were measured, and training history recorded for 1 month. A hormonal questionnaire provided details of menstrual history and contraceptive pill use, and scored for oestrogen status as 0 for amenorrhoea or oligomenorrhoea, 1 for eumenorrhoea or pill use.

Of the 26 athletes, 11 had osteopenia, including one with osteoporosis (according to the WHO definition). While spine *T*-score showed a significant correlation with % fat ($r^2=0.15$, $p<0.05$) and corrected thigh circumference ($r^2=0.32$, $p<0.01$), stepwise regression analysis best predicted spine *T*-score from body mass index (BMI) and oestrogen according to the formula:
Spine *T*-score $=0.408$(BMI) $+0.65$(oestrogen status) $-$ 9.23 ($p<0.0001$, SE $=0.76$, $r^2=0.59$)
BMI alone explained 51% of the observed variation in spine BMD. Stepwise regression analysis predicted whole-body bone mineral content (BMC) from weight according to the formula:
Whole-body BMC (g) $=42.9$(weight in kg) -166.4 ($p<$ 0.0001, SE $=163.9$, $r^2=0.71$).

Conclusions. While all those athletes with under 12% fat had osteopenia, several others with higher percentages of fat also did. These data suggest that BMI and normal hormonal function best predict spine *T*-score and thus the risk of osteoporosis in this population.

References

1. Yeager K K, Agostini R, Nattiv A, Drinkwater B. The female athlete triad: disordered eating, amenorrhoea and osteoporosis. *Med Sci Sport Exercise*, 25, 775–7 (1993).
2. Cantu R C, Michell L J (eds) *American College of Sports Medicine: Guidelines for the Team Physician*. Philadelphia: Lea & Febiger (1991).

Stewart et al: Table 1

	Mean	SD	Minimum	Maximum
Age (years)	26.0	5.2	20.3	39.2
BMI (kg m^{-2})	20.0	1.9	16.7	25.2
Training (h/week)	9.2	3.0	4.0	15.0
% fat	16.1	6.2	3.9	32.1
% muscle	46.2	3.8	38.9	52.9
Total BMC (g)	2222.8	291.0	1689.2	2790.1
Spine BMD (g cm^{-2})	0.970	0.12	0.715	1.245

THE FEMALE ATHLETE TRIAD: CONVERTING ANTHROPOMETRY INTO CLINICAL GUIDELINES

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The female athlete triad of reduced bone mineral density, altered menstrual function and eating disorder is associated with considerable morbidity (Teitz, 1997). The American College of Sports Medicine has suggested that a body fat content of 12% is necessary for normal menstrual function and normal bone mineral density (Cantu and Micheli, 1991). This study was undertaken to check the usefulness of body fat content, and other clinical evidence which can be obtained more easily, in determining the relative risk of osteopenia amongst active young women.

Local ethical committee approval was obtained. Twenty-six women volunteers aged 20-39 years gave informed consent to take part in the study. Bone density and body composition were measured by dual X-ray absorptiometry (DXA) using a Hologic QDR 1000W scanner in whole body mode and in spine mode (L1-L4). Height, mass, 19 skinfolds, 8 skeletal breadths and 9 circumferences were measured. Body mass index (BMI) was calculated. Exercise diaries were kept for one month to assess exercise quantity. A questionnaire providing details of menstrual history and oral contraceptive use was completed to assign a score of 1 if menstrual function was normal or an oestrogen-containing preparation was being used regularly (positive oestrogen status) or 0 if the subject was amenorrhoeic or oligomenorrhoeic (negative oestrogen status). Stepwise regression analysis was used to find the best predictors of spine T score. The sensitivity, specificity and likelihood ratios of the two best predictors were calculated and used to give post-test probabilities of osteopenia (Bernstein, 1997). Relative risks were calculated for ranges of the two best predictors (Szabo, 1998).

The 26 subjects exercised for a mean of 9 hours per week (range 4 – 15), had a mean BMI of 20.0kg.m^{-2} (range 16.7 – 25.2) and a mean body fat content of 16.1% (range 3.9 – 32.1). Eleven of the volunteers had osteopenia (T score < -1), a prevalence of 0.42 (95% confidence limits 0.34 – 0.50). Stepwise regression analysis best predicted spine T score from BMI and oestrogen status according to the formula: Spine T score = (0.408 x BMI) + (0.65 x oestrogen status) – 9.23 ($p<0.0001$, SE = 0.76, $r^2 = 0.59$). Selected positive predictive values (PPV) and negative predictive values (NPV) with 95% confidence limits are shown in the table:

Factor	PPV (95% CI) with prevalence = 0.42	NPV (95% CI) with (1 - prevalence) = 0.58
BMI < 18	1 (1)	0.65 (0.53 - 0.77)
BMI < 21.5	0.5 (0.4 - 0.6)	1 (1)
Oestrogen status negative	0.75 (0.63 - 0.87)	0.72 (0.60 - 0.84)
BMI < 19 + negative oestrogen status	1	-
BMI < 20 + positive oestrogen status	-	1
Body fat content < 12%	1 (1)	0.68 (0.56 - 0.80)

Using body fat content of 12% as a risk factor for osteopenia produces too many false negative results (sensitivity = 0.36; 95% CI = 0.29 – 0.43). Combining BMI and oestrogen status provides a simple method for ruling osteopenia in or out in this group of athletic young women and could have removed the need for 10 out of the 26 DXA scans.

REFERENCES

Bernstein J.[1997]. *J. Bone Jt. Surg.* 79A, 1404-1414

Cantu RC, Micheli LJ [1991]. *American College of Sports Medicine Guidelines for the Team Physician.* Lea & Febiger.

Szabo RM. [1998]. *J. Bone Jt. Surg.* 80A, 111-120

Teitz CC. [1997]. In *The Female Athlete (ed. CC Teitz).* American Academy of Orthopaedic Surgeons. Rosemont, Illinois.

REGIONAL BODY COMPOSITION IN WOMEN ATHLETES, ANOREXICS AND CONTROLS USING DUAL X-RAY ABSORPTIOMETRY.

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While it is well recognised that female athletes and anorexics have less total mass and fat mass than healthy controls, the regional distribution of fat is poorly investigated. Dual X-ray absorptiometry offers a convenient method for examining regional tissue composition, which allows quantification of limb and torso masses separately. This information will show whether fat quantity alone varies between the groups, or relative fat distribution changes in addition.

30 anorexics, 30 athletes and 30 age matched controls were measured for total body composition using a Hologic QDR 1000W (Hologic Inc. Waltham, MA. USA) using software version 5.55. Anthropometric variables (Mean ± SD) and regional body composition data of the groups was as follows:

	Anorexics	Athletes	Controls
Age (yr.)	28.0 ± 6.4	25.9 ± 5.5	28.1 ± 4.6
Height (cm)	163.1 ± 5.4	168.1 ± 6.3	165.0 ± 6.9
Weight (kg.)	39.6 ± 4.5	57.1 ± 7.4	63.7 ± 9.1
BMI ($kg.m^{-2}$)	14.8 ± 1.2	20.2 ± 2.2	23.4 ± 2.8

	Anorexics	Athletes	Controls
Total fat %	10.7 ± 5.0	17.1 ± 6.4	29.2 ± 5.0
Torso fat mass (kg)	0.94 ± 0.74	2.67 ± 2.03	6.49 ± 2.73
Arm fat mass (kg)	0.60 ± 0.26	1.29 ± 0.62	2.78 ± 0.85
Leg fat mass (kg)	2.11 ± 1.17	5.25 ± 2.00	9.03 ± 2.15

Independent T tests showed no significant differences between any of the groups for age and height, but highly significant differences for weight, BMI, total fat %, arm fat, torso fat and leg fat ($p<0.001$). The distribution of fat varied between the groups. Because DXA is limited in its ability to predict fat content inside the skull, fat data are presented with the head excluded. The findings were not altered as a result.

Compared with controls, Anorexics had proportionately more fat on the arms ($p<0.05$), more on the legs ($p<0.001$) and less on the trunk ($p<0.001$). Compared with controls, athletes had similar proportions of fat on the arms, proportionately more on the legs ($p<0.001$) and less on the toso ($p<0.001$). Compared with anorexics, athletes had proportionately less fat on the arms ($p<0.01$) and similar proportions in the legs and torso.

These findings suggest fat topography to be different in the three groups, and that the metabolic influences of exercise and undernourishment induce different effects. This would suggest caution in applying % fat prediction equations using skinfold thickness derived on a control population to athletes or anorexic subjects, because the underlying fat topography is different.

BONE MINERAL CONTENT OF CYCLISTS, RUNNERS AND CONTROLS

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8 competitive racing cyclists, 8 distance runners, 8 who both cycle and run (mostly triathletes) and 12 age-matched controls were compared for whole body bone mineral density (BMD) and lumbar spine BMD using a Hologic QDR 1000W scanner (Hologic Inc. Waltham, MA, USA). Athletic subjects were matched for BMI, and body composition. All athletes had participated in their selected sports for a minimum of three years and trained for a minimum of six hours per week. Subjects with a family history of osteoporosis were excluded. Ethical permission for the study was provided by Lothian Health.

Subject data was as follows:

	Runners	Cyclists	Both	Controls
Age (yr.)	25.8 ± 5.4	29.0 ± 7.2	30.8 ± 7.0	29.8 ± 7.0
Height (cm)	180.7 ± 4.9	179.6 ± 5.8	176.4 ± 5.3	176.9 ± 6.6
Weight (kg)	70.6 ± 5.4	70.0 ± 6.0	69.5 ± 7.1	74.3 ± 8.7
BMI ($kg.m^{-2}$)	21.7 ± 1.4	21.5 ± 0.9	22.2 ± 1.8	23.7 ± 2.2
% fat	8.2 ± 3.5	8.3 ± 3.1	8.2 ± 2.0	22.7 ± 7.3
Training ($hrs.wk^{-1}$)	8.8 ± 2.6	11.3 ± 5.0	9.5 ± 3.8	none

The athletes trained for 9.5 + 3.8 hrs per week (mean ± SD), there being no difference between the various groups. The controls took no deliberate exercise. Results for the bone density of the whole body and lumbar spine were as follows:

	Runners	Cyclists	Both	Controls
WBBMD ($g.cm^{-2}$)	1.31 ± 0.07	1.19 ± 0.08	1.33 ± -.10	1.21 ± 0.11
Spine BMD ($g.cm^{-2}$)	1.09 ± 0.11	0.94 ± 0.11	1.16 ± 0.13	1.06 ± 0.15

Independent T tests showed significant differences between the groups . Runners showed no differences from the 'both' group, but had greater total BMD than controls and cyclists ($p<0.01$), and greater spine BMD than cyclists ($p<0.01$). Cyclists, by contrast, had lesser total BMD and spine BMD than the 'both' group ($p<0.01$) and lesser spine BMD than controls ($p<0.05$).

Impact-bearing exercise is generally considered to be beneficial to the skeleton because it presents sufficient strain for the remodeling cycle to respond to by increasing bone mass. While a high peak bone mass is desirable due to decreased fracture risk, cycling and swimming are not promoted as suitable activities for promoting increases in bone mass (ACSM, 1995, Skerry, 1997). Furthermore, strain magnitude and strain rate appear to be the major determinants, and frequency of strain is insignificant in remodeling bone (Frost, 1997, Skerry, 1997). Running produces much greater bone strains than cycling, but the reason cyclists in the present study have lower spine BMD than controls is unclear. Rico (1993) reported young cyclists as having lesser regional bone densities than controls, although the difference disappeared when a correction was made for weight. It is possible that a similar phenomenon exists in adult cyclists, who remain significantly lighter than controls.

These data support the theory that cycling may reduce spine BMD, but that running increases total BMD. Furthermore, the effect of running would appear to counteract the effect of cycling.

REFERENCES

ACSM Position Stand on Osteoporosis and Exercise (1995). *Med. Sci. Sports Exerc.* 27 no 4, i-vii.
Frost, H.M. (1997) *Bone* 20, 183-189
Rico, M., Revilla, M., Hernandez, E.R., Gomez, F. and Villa, L.F. (1993). *Bone* 14, 93-95.
Skerry, T. (1997) *Bone* 20, 179-181.

NUTRITIONAL INTAKE AND THE FEMALE ATHLETE TRIAD.

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Dietary factors have long been implicated in the development of the female athlete triad syndrome.Failure to meet energy and micronutrient requirements can lead to impaired oestrogen status, low body weight and poor bone mineralisation over time. Reduced bone mineral density (BMD) in exercising females has important long term health implications as it is associated with stress fractures and more severe hip and spine fractures in later years(Lindberg et al 1984).

In the present study 17 female athletes who trained for greater than 4 hours per week were assessed using Dual X Ray Absorptiometry for spine BMD (T- Score) and body fat. Dietary intake was assessed using 7 day weighed intake and analysed using Compeat dietary analysis package. World Health Organisation criteria for osteopenia (T score below -1) and osteoporosis (T score below -2.5) were used to classify athletes bone status.

Results revealed 9 athletes with normal BMD (group 1), 7 with T score below -1.0 and 1 with T score below -2.5 (group 2,). Median (range) % body fat was 17.6% (12.1-22.6) and 12.6% (7.7-19.9) P=0.03 for normal and low BMD groups respectively . No significant difference was found between energy, protein,fat carbohydrate, calcium, vit D or sodium intakes between groups. Within the low BMD group 62.5% of athletes were amenorrheic or oligoamenorrheic and had impaired oestrogen status, 37.5% had positive oestrogen status ie, eumenorrheic or supplemented with oral contraceptive pill.

Of subjects with low BMD (n=8) 50% did not meet the recommended intake of 1500mg calcium per day (Otis 1992) for bone mineral accretion. Interestingly 50% of the normal BMD group did not meet the present UK Reference Nutrient Intake of 700mg for calcium.

The results of this study show no correlation between bone mineral density and macro or micronutrient intake in normal or osteopenic athletes. Calcium intakes however, were lower than recommended values in 50% of both osteopenic and non osteopenic subjects. This study suggests that calcium supplementation is justified in women suspected of having the female athlete triad.

REFERENCES

Lindberg. J.S., Fears, W.B and Hunt, M.M. [1984]. *Ann.Int. Med. 101:647-648.*
Otis. C.L. [1992]. *Clin. Sports Med. 11:351-361.*

APPENDIX E: GLOSSARY

2 compartment model A whole body model comprising fat and fat-free mass

4 compartment model A whole body model comprising fat, water, bone mineral and lean tissue which is neither bone nor water

Anorexia athletica A variety of anorexia associated with compulsive exercise and other disorders

Anorexia nervosa A disordered eating behaviour associated with chronic undernourishment leading to deliberate and sustained weight loss, and a distortion of body image involving a dread of fatness

Areal bone density Bone mineral content (mass) divided by unit area, in g. cm^{-2}

Bland and Altman plot A scatterplot of the mean of two methods for measuring or predicting the same variable plotted against the difference between the methods, used to assess agreement between methods

Body mass index The mass of a person in kg divided by the square of stature in m.

Bone density - see Areal bone density. A true volumetric density is bone mineral content per unit volume.

Bonferroni correction In statistical comparison, a manipulation of required significance levels in multiple T tests to ensure significant differences do not arise through pure chance

Student Newman Keuls post hoc test A method of grouping data after analysis of variance into groups which are significantly different from one another

Corrected girth An anatomical circumference of the limb or torso which allows for the thickness of subcutaneous adipose tissue, by subtracting π multiplied by skinfold thickness from total girth

Cortical bone The dense, crystalline architecture of bone which forms the skull and the 'shell' of many other bones

Cross-sectional design A study which compares different groups with one another at a single point in time

Dummy variable A variable inserted into a statistical regression, which can score 0 or 1 according to the absence or presence of a factor

DXA morphotype A statistical standard score based on observed departure from mean values for DXA variables

DXA subregional morphometry Comparison or analysis of sub-regions of the body using DXA data of bone, lean and fat mass, or ratios of these

Ectomorphy The somatotype component of relative fragility arising from low total body mass in relation to height

Endomorphy The somatotype component of relative fatness or 'roundness'

Fit-fat distribution A pattern of fat distribution which includes less torso or abdominal fat than non-exercising individuals

Homeostasis Normal internal biologic functioning of an organism

Intra-scanner differences Differences in tissue mass, density or area arising from DXA scanners from different manufacturers

Kinanthropometry The science which describes the relationship between structure and function of the human body, especially within the context of movement.

Lipid fraction The fractional mass of adipose tissue which is lipid

Mechanostat The descriptive paradigm which summarises adjustments to skeletal architecture arising from mechanical strain

Mesomorphy The somatotype component of musculo-skeletal robustness

Morphological prototype An anthropometric descriptor of athletes' physique adjustment over time, especially with respect to training status and proximity to major competition

Muscular torque The effective rotation about a joint arising from muscular contraction

Normal bone density Bone density of not more than 1 SD below the young adult mean

Oestrogen status Classification of menstrual status according to the frequency of menstrual periods and the presence or absence of supplementary oestrogen

Osteogenic effect An increase in bone mass

Osteopenia Reduced bone density (between 1 and 2.5 SD below the young adult mean bone density) defined by the World Health Organisation

Osteoporosis Significantly reduced bone density (2.5 SD or more below young adult mean bone density) defined by the World Health Organisation

Peak bone mass The maximum skeletal or bone mineral mass attained during life, commonly achieved at or around age 30

Ponderal index Height in metres divided by the cube root of mass in kg

Receiver operating characteristic A method of analysing a predictive test with a dichotomous result, by using sensitivity and specificity in a graphical plot

Reference method The method viewed as the criterion against which other methods or predictions are compared

Region of interest A highlighted area of a DXA scan analysed separately

Remodelling Adjustment of skeletal architecture arising from turnover of bone cells which repair microdamage

Reproducibility The precision of a repeated measurement (see Appendix B)

Sensitivity The proportion of subjects who have a condition and test positive for that condition

Sitting body mass index Body mass index (see above) calculated by using sitting height instead of total height

Skelic index The ratio of sitting height : leg length

Specificity The proportion of subjects who do not have a condition who test negative for that condition

Strain The change in shape or dimensions of bone arising from mechanical forces. Strain magnitude, and its rate of change appear to be osteogenic stimuli

T-score The number of standard deviations above or below a young adult mean bone density, usually predicted between 20 and 35yr

Total error The prediction error of a formula calculated from the sum of the squares of the residuals (between measured and predicted) divided by the number of observations

Trabecular bone The plate-like 'honeycomb' architecture of bone

Z score A calculated departure from a standard or mean (measured value minus population mean divided by population standard deviation) – also referred to as standardised deviate score, or standard score.

REFERENCES

1. Abrahamsen, B., Gram, J., Hansen, T.B. and Beck-Nielsen, H. (1995). Cross calibration of QDR-2000 and QDR-1000 dual-energy X-ray densitometers for bone mineral and soft tissue measurements. *Bone* **16**, 385-390.
2. Abrahamsen, B., Hansen, T.B., Hogsberg, I.M., Pedersen, F.B. and Beck-Nielsen, H. (1996). Impact of hemodialysis on dial X-ray absorptiometry, bioelectrical impedance measurements, and anthropometry. *Am. J. Clin. Nutr.* **63**, 80–86.
3. Ackmann, J. and Seitz, M. (1984). Methods of complex impedance measurements in biologic tissue. *Crit. Rev. Biomed. Eng.* **11**, 281-311.
4. Adams, J., Mottola, M., Bagnell, K.M. and McFadden, K.D. (1982) Total body fat content in a group of professional football players. *Can. J. Appl. Sport Sci.* **7**, 36–40.
5. Allemann, Y., Horber, F.F., Columbo, M., Ferrari, P., Shaw, S., Jaeger, P. and Weidman, P. (1993). Insulin sensitivity and body fat distribution in normotensive offspring of hypotensive parents. *Lancet* **341**, 327-331.
6. Aloia, J.F. (1993). *A colour atlas of osteoporosis*. London, Wolfe.
7. American College of Sports Medicine. Position stand on osteoporosis and exercise (1995). *Med. Sci. Sports Exerc.* **27**, i–vii.
8. Anderson, E.C. and Langham, W. (1959). Average potassium concentration of the human body as a function of age. *Science*, **130**, 713–714.
9. Bailey, D.A. and McCulloch, R.G. (1990). Bone tissue and physical activity. *Can. J. Sport. Sci.* **15**, 229-239,.
10. Baldwin K.M., White, T.P., Arnaud, S.B., Edgerton, V.R., Kraemer, W.J., Kran, R., Raab-Cullen, D. and Snow, C.M. (1996). Musculo-skeletal adaptations to weightlessness and development of effective countermeasures. *Med. Sci. Sports Exerc.* **28**, 1247-1253.
11. Bale P, Rowell D. Colley E. (1985). Anthropometric and training characteristics of female marathon runners as determinants of distance running performance. *J. Sports Sci.* **3**, 115–126.
12. Ballor, D.L. and Keesey, R.E. (1991). A meta-analysis of the factors affecting exercise-induced changes in body mass, fat mass and fat-free mass in males and females. *Int. J. Obes.* **15**, 717–726.
13. Bamman, M.M., Hunter, G.R., Newton, L.E., Roney, R.K. and Khaled, M.A. (1993). Changes in body composition, diet and strength of bodybuilders during the 12 weeks prior to competition. *J. Sports Med. Phys. Fit.* **33**, 383–391.

14. Baumgartmer, , R.N., Rhyne, R.L., Troup, C., Wayne, S. and Garry, P.J. (1992). Appendicular skeletal muscle areas assessed by magnetic resonance imaging in older persons. *J. Gerontol.* **47**, M67–72.
15. Baumgartner, R. (1996). Electrical impedance and total body electrical conductivity. In *Human Body Composition* edited by A. Roche, S. Heymsfield and T. Lohman, Illinois, Human Kinetics, 79-107.
16. Baumgartner, R., Chumlea, W. and Roche, A. (1988). Bioelectric impedance phase angle and body composition. *Am. J. Clin. Nutr.* **48**, 16-23.
17. Baumgartner, R., Chumlea, W. and Roche, A. (1989). Estimation of body composition from segmental impedance. *Am. J. Clin. Nutr*, **50**, 221-226.
18. Baumgartner, R., Chumlea, W. and Roche, A. (1990). Bioelectric impedance for body composition. *Ex. Sports Sci. Rev.* **18**, 193-224.
19. Baumgartner, R.N., Roche, A.F., Guo, S., Lohman, T., Boileau, R.A. and Slaughter, M.H. (1986). Adipose tissue distribution: the stability of principal components by sex, ethnicity and maturation stage. *Hum. Biol.* **60**, 549–567.
20. Behnke, A. R. and Wilmore, J.H. (1974). Evaluation and regulation of body build and composition. Englewood Cliffs, NJ. Prentice-Hall.
21. Behnke, A.R. 1959. The estimation of lean body weight from 'skeletal' measurements. *Hum. Biol.* **32**, 259–315.
22. Bennell K, Malcolm, S., Khan, K. Thomas, S.A., Reid, S.J., Brukner, P.D., Ebeling, P.R. and Wark, J.D. (1997). Bone mass and bone turnover in power athletes, endurance athletes and controls: A 12 month longitudinal study. *Bone* **20**, 477-484.
23. Bergsma-Kadijk, J.A., Baumeister, B. and Deurenberg, P. (1996). Measurement of body fat in young and elderly women: comparison between a four-compartment model and widely used reference methods. *Brit. J. Nutr.* **75**, 649-657.
24. Bernstein J. (1997). Current concepts review: Decision analysis. *J. Bone Joint Surj.* **79**A, 1404-414.
25. Bjorntorp, P. 1978. Physical training in the treatment of obesity. *Int. J. Obes.* **2**, 149–156.
26. Bland, J.M. and Altman, D.G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*, **i**, 307 - 310.
27. Boddy, K., King, P.C., Womersley, J. and Durnin, J.V.G.A. (1973). Body potassium and fat-free mass. *Clin. Sci.* **44**, 622–625.
28. Boileau, R.A., Lohman, T.G., Slaughter, M.H., Ball, T.E., Going, S.B. and Hendrix, M.K. (1984). Hydration of the fat-free body in children during maturation. *Hum. Biol.* **56**, 651-666.

29. Boot, A.M., Bouquet, J., de Ridder, M.A., Krenning, E.P. and de Munick Keizer-Schrama, S.M. (1997) Determinants of body composition measured by dual X-ray absorptiometry in Dutch children and adolescents. *Am. J. Clin. Nutr.* **66,** 232-238.

30. Borkan, G.A., Hults, D.E., Gerzof, S.G. Robbins, A.H. and Silbert, C.K. (1983). Age changes in body composition revealed by computed tomography. *J. Gerontol.* **38**, 673–677.

31. Bouchard, C and Pérusse, L. (1988). Heredity and body fat. *Ann. Rev. Nutr.* **8**, 259–277.

32. Bouchard, C. (1991). Genetic aspects of anthropometric dimensions relevant to assessment of nutritional status. In *Anthropometric assessment of nutritional status,* edited by J. Himes, New York, Alan R Liss, 213–231.

33. Bouchard, C., Pérusse, L, Leblanc, C. Tremblay, A. and Theriault, G. (1988). Inheritance of the amount and distribution of human body fat. *Int. J. Obes.* **12**, 205–215.

34. Bouchard, C., Shepherd, R.J. and Stephens, T. (Eds.) (1993). *Physical activity, fitness and health: Consensus statement.* Champaign IL., Human Kinetics, 46-47.

35. Brodie, D., Moscrip, V. and Hutcheon, R. (1998). Body composition measurement: A review of hydrodensitometry, anthropometry and impedance methods. *Nutrition*, **14**, 296-310.

36. Brodie, D.A. (1988). Techniques of measurement of body composition: Part 1. *Sports Med* **5**, 11-40.

37. Brodie, D.A., Eston, R.G., Coxon, A.Y., Kreitzman, S.N., Stockdale, H.R. and Howard, A.N. (1991). Effect of changes of water and electrolytes on the validity of conventional methods of measuring fat-free mass. *Ann. Nutr. Metab.* **35**, 89-97.

38. Buschang, P.H. (1980). Growth status and rate in schoolchildren 6 to 13 years of age in a rural Zatopec-speaking community in the valley of Oaxaca, Mexico. Unpublished doctoral dissertation: University of Texas, Austin, USA.

39. Cameron, J.R. and Sorenson, J. (1963). Measurement of bone mineral in vivo. *Science*, **42,** 230–232.

40. Cameron, N. 1978. The methods of auxological anthropometry. In *Human Growth: Vol. 2, Post natal growth*, edited by F. Falkner and J.M. Tanner, New York, Plenum Press, 35–90.

41. Cantu, R.C. and Micheli, L.J. (eds.) (1991). *American College of Sports Medicine: Guidelines for the team physician.* Philidelphia, USA. Lea & Febiger.

42. Carter, J.E.L. (1980). The Heath – Carter somatotype method, San Diego Statue University Syllabus Service, San Diego.

43. Carter, J.E.L. (1999). What is "essential" about "essential fat"? *Kinanthreport* **X11:2**, 6-9.

44. Carter, J.E.L. and Ackland, T.R. (1994). *Kinanthropometry in aquatic sports. A study of world class athletes.* Champaign, IL. USA, Human Kinetics.

45. Carter, J.E.L. and Heath, B.H. (1990). Somatotyping: Development and applications. Cambridge, UK, Cambridge University Press.

46. Chowdhury, B., Sjostrom, L., Alpsten, M., Kostanty, J., Kvist, H. and Lofgren, R. (1994). A multicompartment body composition technique based on computerized tomography. *Int. J. Obes.* **18**, 219-234.

47. Chumlea W.C. and Roche, A.F. (1979). Unpublished data. Wright State University School of Medicine, Department of Pediatrics, Yellow Springs, Ohio, USA

48. Chumlea, W., Baumgartner, R and Guo, S. (1993). Segmental bioelectric impedance, subcutaneous adipose tissue, and fat patterns in adults. In. *Recent developments in body composition analysis: Methods and applications*, edited by J. Kral and T. Van Itallie, London, Smith-Gordon, 49-60.

49. Chumlea, W., Baumgartner, R and Roche, A. (1988). The use of specific resistivity to estimate fat-free mass from segmental body measures of bioelectric impedance. *Am. J. Clin. Nutr*, **48**, 7-15.

50. Chumlea, W.C., Roche, A.F. and Rogers, E. (1984). Replicability for anthropometry in the elderly. *Hum. Biol.* **56**, 329–337.

51. Claessens, A.L., Veer, F.M., Stijnen, V. Lefevre, J. Maes, H., Steens, G. and Beunen, G. (1991). Anthropometric characteristics of outstanding male and female gymnasts. *J. Sports Sci.* **9**, 53–74.

52. Clark, R.R., Kuta, J.M. and Sullivan, J.C. (1993). Prediction of percent body fat in adult males using dual-energy x-ray absorptiometry, skinfolds and hydrostatic weighing. *Med. Sci. Sports. Exerc.* **25**, 528-535.

53. Clasey, J.L., Hartman, M.L., Kanaley, J., Wideman, L., Teates, C.D., Bouchard, C. and Weltman, A. (1997). Body composition by DEXA in older adults: accuracy and influence of scan mode. *Med. Sci. Sports. Exerc.* **29**, 560-567.

54. Clasey, J.L., Kanaley, J.A., Wideman, L., Heymsfield, S.B., Teates, C.D., Gutgesell, M.E., Thorner, M.O., Hartman, M.L. and Weltman, A. (1999). Validity of methods of body composition assessment in young and older men and women. *J. Appl. Physiol.* **86**, 1728-1738.

55. Cohen, J. 1992. Quantitative methods in Psychology: A Power Primer. *Psychol. Bull.* **112**, 155–159.

56. Cohn, S.H., Abesamis, C., Zanzi, I., Aloia, J.F., Yasumura, S. and Ellis, K. J. (1977). Body elemental composition: Comparison between black and white adults. *Am. J. Physiol.* **232**, E419 – E422.

57. Cote, K.D. and Adams, W.C. (1993) Effect of bone density on body composition estimates in young adult black and white women. *Med. Sci. Sports Exerc.* **25**, 290-296.

58. De Lorenzo, A., Sorge, S.P., Iacopino, L., Andreoli, A., de Luca, P.P. and Sasso, G.F. (1998). Fat-free mass by bioelectrical impedance vs dual-energy X-ray absorptiometry (DXA). *Appl. Radiat. Isot.* **49**, 739-741.

59. Deprés, J,P., Moorjani, S., Lupien, P.J., Tremblay, A., Nadeau, A. and Bouchard, C. (1990). Regional distribution of body fat, plasma lipo-proteins, and cardiovascular disease. *Arteriosclerosis* **10**, 497–511.

60. Deprés, J,P., Prud'homme, D., Tremblay, A. and Bouchard, C. (1991). Estimation of deep abdominal adipose tissue anthropometric measurements in men. *Am. J. Clin. Nutr.* **54**, 471–477.

61. Deprés, J,P., Ross, R and Lemieux, S. (1996). Imaging techniques applied to the measurement of human body composition. In *Human Body Composition*, edited by A. Roche, S. Heymsfield and T. Lohman, Illinois, Human Kinetics, 149-166.

62. Deurenberg, P., Kusters, C. and Smit, H. (1990). Assessment of body composition by bioelectric impedance in children and young adults is strongly age-dependent. *Eur. J. Clin. Nutr.* **44**, 261-268.

63. Diamond , J. (1991). *The rise and fall of the third Chimpanzee*. U.K. Random House.

64. Drinkwater, B.L., Bruemner, B. and Chesnut, C.H. (1990). Menstrual history as a determinant of current bone density in young athletes. *J.A.M.A.* **263**, 545–548.

65. Drinkwater, B.L., Nilson, K., Chesnut, C.H., Bremner, W.J., Shainholtz, S. and Southworth, M.B.(1984). Bone mineral content of amenorrheic and eumenorrheic athletes. *New Eng. J. Med.* **311**, 277–281.

66. Drinkwater, B.L., Nilson, K., Ott, s. and Chesnut, C.H. (1986)b. Bone mineral density after resumption of menses in amenorrheic athletes. *J.A.M.A.* **256**, 380–382.

67. Drinkwater, D.T., Martin, A.D., Ross, W.D. and Clarys, J.P. (1986). Validation by cadaver dissection of Matiegka's equations for the anthropometric estimation of anatomical body composition in adult humans. In *The 1984 Olympic Scientific Congress Proceedings: Perspectives in Kinanthropometry* edited by J.A.P. Day, Champaign, IL, Human Kinetics, 221–227.

68. Duquet, W. and Carter, J.E.L. (1996). Somatotyping. In: *Kinanthropometry and Exercise Physiology Manual*, Edited by R. Eston and T. Reilly, London, E & FN Spon, 35–50.

69. Durnin, J.V.G.A. and Womersley, J. (1974). Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 mean and women aged from 16 to 72 years. *Brit. J. Nutr.* **32**, 77-97.

70. Eckerson, J., Moore, G., Stout, J., Noonan, D., Cullen, D. Yee, J., Baumgartner, D. and Johnson, D. (1997). Prediction of % fat using DXA, BIA and NIR. *Med. Sci. Sports Exerc.* **29**, S53.

71. Economos, C.D., Nelson, M.E., Fiatarone, M.A., Dallal, G.E., Heymsfield, S.B., Wang, J., Yasumura, S., Ma, R., Vaswani, A.N., Russel-Aulet, M. and Pierson, R.N. (1997). A multi-center comparison of dual energy X-ray absorptiometers: In vivo and in vitro soft tissue measurement. *Eur. J. Clin. Nutr.* **51**, 312-317.

72. Ellis, K.J. (1996). Measuring body fatness in children and young adults: comparison of bioelectric impedance analysis, total body electrical conductivity, and dual X-ray absorptiometry. *Int. J. Obes.* **20**, 866-873.

73. Ellis, K.J., Shypailo, R.J., Pratt, J.A. and Pond, W.G. (1994). Accuracy of dual-energy x-ray absorptiometry for body composition measurements in children. *Am. J. Clin. Nutr.* **60**, 660-665.

74. Eriksen EF. (1986). Normal and pathological remodeling of human trabecular bone: three dimensional construction of the remodeling sequence in normals and in metabolic bone disease. *Endocr. Rev.* 7 , 379–408.

75. Feldesman, M.R. and Fountain, R.L. (1996). "Race" specificity and the femur/stature ratio. *Am. J. Phys. Anthropol.* **100**, 207–224,.

76. Ferland, M, Després, J.P., Tremblay, A., Pinault, S., Nadeau, A., Moorjani, S., Lupien, P.J., Theriault, G. and Bouchard, C. (1989). Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. *Brit. J. Nutr.* **61**, 139–148.

77. Fogelholm, G.M., Kukkonen-Harjula, T.K., Sievanen, H.T., Oja, P. and Vuori, I. (1996). Body composition assessment in lean and normal-weight young women. *Brit. J. Nutr.* **75**, 793-802.

78. Fogelholm, G.M., Sievanen, H.T., van Marken Lichtenbelt, W.D. and Westerterp, K.R. (1997). Assessment of fat mass loss during weight reduction in obese women. *Metab: Clin. & Exp.* **46**, 968-975.

79. Fogelman, I and Blake, G.M. (1998). Clinical use of DXA measurements. In *Current research in osteoporosis and bone mineral measurement V,* edited by E.F.J. Ring, D.M. Elvins and A.K. Bhalla, London, British Institute of Radiology, 74-75.

80. Fomon, S.J., Haschke, F., Ziegler, E.E. and Nelson, S.E. (1982). Body composition of reference children from birth to age 10 years. *Am. J. Clin. Nutr.* **35**, 1169-1175.

81. Forbes, G.B. and Lewis, A. (1956). Total sodium, potassium and chloride in adult man. *J. Clin. Invest.* **35**, 596–600.

82. Forbes, G.B., Gallup, J. and Hursh, J. (1961). Estimation of total body fat from potassium 40 content. *Science*, **133**, 101–102.

83. Formica, C., Atkinson, M.G., Nyulasi, I., McKay, J., Heale, W. and Seeman, E. (1993). Body composition following hemodialysis: studies using dual-energy X-ray absorptiometry and bioelectrical impedance analysis. *Osteoporosis Int.* **3**, 192-197.

84. Forslund, A.H., Johansson, A.G., Sjodin, A., Bryding, G., Ljunghall, S. and Hambraeus, L. (1996). Evaluation of modified multicomponent models to calculate body composition in healthy males. *Am. J. Clin. Nutr.* **63**, 856-862.

85. Fowler, P.A., Fuller, M.F., Glasbey, C.A., Foster, M.A., Cameron, G.G., McNeill, G. and Maughan, R.J. (1991). Total and subcutaneous adipose tissue in women: the measurement of distribution and accurate prediction of quantity by using magnetic resonance imaging. *Am. J. Clin. Nutr.* **54**, 18–25.

86. Friedl, K.E., DeLuca, J.P., Marchitelli, L.J. and Vogel, J.A. (1992). Reliability of body fat estimations from a four-compartment model using density, body water, and bone mineral measurements. *Am. J. Clin. Nutr.* **55**, 764-770.

87. Friedlander, J.S., Costa, P.T., Bosse, R., Ellis, E., Rhoads, J.G. and Stoudt, H.W. (1977). Longidutinal physique changes among healthy white veterns of Boston. *Hum. Biol.* **49**, 541–558.

88. Frisch, R.E. and McArthur, J. W. (1974). Menstrual cycles: fatness as a determinant of minimum weight for height necessary for their maintenance or onset. *Science* **185**, 949–951.

89. Frost, H. (1987). Bone mass and the mechanostat: a proposal. *Anat. Rec.* **219**, 1-9.

90. Frost, H. (1997). Why do marathon runners have less bone than weight lifters? A vital biomechanical view and explanation. *Bone* **20**, 183–189.

91. Fry, A.C., Ryan, A.J., Schwab, R.J., Powell, D.R., and Kraemer, W.J. (1991). Anthropometric characteristics as discriminators of body-building success. *J. Sports Sci.* **9**, 23–32.

92. Fullarton, G.D. (1982). Basic concepts for nuclear magnetic resonance imaging. *Magn. Reson. Imag.* **1**, 39–55.

93. Fuller, N.J., Jebb, S.A., Laskey, M.A., Coward, W.A. and Elia, M. (1992). Four component model for the assessment of body composition in humans: comparison with alternative methods, and evaluation of the density and hydration of fat-free mass. *Clin. Sci.* **82**, 687-693.

94. Fuller, N.J., Laskey, M.A. and Elia, M. (1992)b. Assessment of the composition of major body regions by dual-energy X-ray absorptiometry (DEXA), with special reference to limb muscle mass. *Clin. Physiol.* **12**, 253-266.

95. Fuller, N.J., Sawyer, M.B., Laskey, M.A., Paxton, P. and Elia, M. (1996). Prediction of body composition in elderly men over 75 years of age. *Ann. Hum. Biol.* **23**, 127-147.

96. Geddes, L. and Baker, L. (1967). The specific resistance of biological material: A compendium of data for the biomedical engineer and physiologist. *Med. Biol. Eng. Comput.* **5**, 271-293.

97. Goodsitt, M.M. (1992). Evaluation of a new set of calibration standards for the measurement of fat content via DPA and DXA. *Med. Phys.* **19**, 35-44.

98. Goran, M.I. (1997). Energy expenditure, body composition, and disease risk in children and adolescents. *Proc. Nutr. Soc.* **56**, 195-209.

99. Goran, M.I., Toth, M.J. and Poehlman, E.T. (1998). Assessment of research-based body composition techniques in healthy elderly men and women using the 4-compartment model as a criterion method. *Int. J. Obes.* **22**, 135-142.

100. Goran. M.I., Driscoll, P., Johnson, R., Nagy, T.R. and Hunter, G. (1996). Cross-calibration of body composition techniques against dual-energy X-ray absorptiometry in young children. *Am. J. Clin. Nutr.* **63**, 299-305.

101. Gotfredsen A.J., Borg, J., Christiansen, C. and Mazess, R.B. (1984). Total bone mineral in vivo by photon absorptiometry, II: Accuracy. *Clin. Physiol.* **4**, 357-362.

102. Gotfredsen, A. Jensen, J., Borg, J. and Christiansen, C. (1986). Measurement of lean body mass and total body fat using dual photon absorptiometry. *Metabolism* **35**, 88-93.

103. Gray, D., Bray, G., Gemayel, N. and Kaplan, K. (1989). Effect of obesity on bioelectrical impedance. *Am. J. Clin. Nutr*, **50**, 255-260.

104. Grier, S.J., Turner, A.S. and Alvis, M.R. (1996). The use of dual-energy x-ray absorptiometry in animals. *Invest. Radiol.* **31**, 50-62.

105. Gueguen, R., Jouanny, P., Guillemin, F., Kuntz, C., Pourel, J. and Siest, G. (1995). Segregation analysis and variance components analysis of bone mineral density in healthy families. *J. Bone Miner. Res.* **12**, 2017–2022.

106. Gutin, B., Litaker, M., Islam, S., Manos, T., Smith, C. and Treiber, F. (1996). Body composition measurement in 9-11-y-old children by dual-energy X-ray absorptiometry, skinfold thickness measurements and bioimpedance analysis. *Am. J. Clin. Nutr.* **63**, 287-292.

107. Haas, J.D. and Flegal, K.M. (1981). Anthropometric measurements. In *Progress in cancer research: Vol. 17. Nutrition and cancer: Etiology and treatment*, edited by G.R. Newell and N.M. Ellison, New York, Raven Press, 123– 40.

108. Hammond, W.H. (1955). Measurement and interpretation of subcutaneous fat, with norms for children and young adult males. *Brit. J. Prevent. Soc. Med.* **9**, 201–211.

109. Hannan, J. 1990. Unpublished data on Total Body Potassium. Personal communication.

110. Hannan, W. J., Cowen, S.J., Freeman, C.P., Wrate, R.M. and Barton, J. (1993). Evaluation of dual-energy x-ray absorptiometry for the assessment of body composition in anorexic females. In *Human Body Composition*, Edited by. K.J. Ellis and J.D. Eastman, New York, Plenum Press, 169-172.

111. Hansen, N.J., Lohman, T.G., Going, S.B., Hall, M.C., Pamenter, R.W., Bare, L.A., Boyden, T.W. and Houtkooper, L.B. (1993). Prediction of body composition in premenopausal females from dual-energy x-ray absorptiometry. *J. Appl. Physiol.* **75**, 1637-1641.

112. Hansen, R.D., Raja, C., Aslani, A., Smith, R.C. and Allen, B.J. (1999 – in press). Determination of skeletal muscle and fat-free mass by nuclear and dual energy x-ray methods in men and women aged 51 to 84. *Am. J. Clin. Nutr.*

113. Hart, P.D., Wilkie, M.E., Edwards, A. and Cunningham, J. (1993). Dual energy X-ray absorptiometry versus skinfold measurements in the assessment of total fat in renal transplant recipients. *Eur. J. Clin. Nutr.* **47**, 347-352.

114. Hartz, A.J., Rupley, D.C., and Rimm, A.A. (1984). The association of girth measurements with disease in 32856 women. *Am. J. Epidemiol.* **119**, 71–80.

115. Hawes, M.R. (1977). Systematic Human Anatomy Manual, with notes on the Rhesus Monkey. Toronto, Canada, Burgess Publishing Co.

116. Hawes, M.R. (1996). Human Body Composition. In: Kinanthropometry and Exercise Physiology Manual, edited by R. Eston and T. Reilly, London, E & FN Spon, 5–33.

117. Hawes, M.R. and Soucie, A. (1993). *Anthropometric Definer* software, Athletic Computer Systems, Calgary, Canada.

118. Hawes, M.R. and Sovak, D. (1994). Morphological prototypes, assessment and change in elite athletes. *J. Sports Sci.* **12**, 235–242.

119. Hayward, J.S., Eckerson, J.D., and Dawson, B.T. (1986). Effect of mesomorphy of hypothermia during exercise in a warm, humid environment. *Am. J. Phys. Anthropol.* **70**, 11–17.

120. Heath, B.H. and Carter, J.E.L. 1967. A modified somatotype method. *Am. J. Phys. Anthropol.* **27**, 57–74.

121. Heinonen, A., Kannus, P., Sievanen, H., Oja, P. and Vuori, I. (1996). Randomized controlled trial of effect of high impact exercise on selected risk factors for osteoporotic fractures. *Lancet* **348**,1343-1347.

122. Heinonen, A., Oja, P., Kannus, P., Sievanen, H., Manttari, A. and Vuori, I. (1993). Bone mineral density of female athletes in different sports. *Bone Miner.* **23**, 1-14.

123. Heymsfield S.B., McManus C., Smith, J., Stevens, V. and Nixon, D.W. (1982). Anthropometric measurement of muscle mass: revised equations for calculating bone-free arm muscle area. *Am. J. Clin. Nutr.* **36**, 680–690.

124. Heymsfield, S.B., Smith, R., Aulet, M., Bensen, B. Lichtman, S.L., Wang, J. and Pierson, R.N. Jr. (1990). Appendicular skeletal muscle mass: measurement by dual photon absorptiometry. *Am. J. Clin. Nutr.* **52**, 241–218.

125. Heyward, V.H. and Stolarczyk, L.M. (1996). Body composition and ethnicity. In *Applied Body Composition Assessment*, Champaign, IL. USA, Human Kinetics, 106–134.

126. Hillam, R.A., Jackson, M., Goodship, A.E. and Skerry, T.M. (1995). Regional differences in human bone strain in vivo. *J. Bone Miner. Res.* **10**, suppl. 443.

127. Himes, J.H. Reliability of anthropometric methods and replicate measurements. (1989). *Am. J. Phys. Anthropol.* **79**, 77–80.

128. Himes, J.H. and Bouchard, C. (1985). Do the new Metropolitan life assurance weight-height tables correctly assess body frame and body fat relationships? *Am. J. Publ. Health* **75**, 1076–1079.

129. Himes, J.H. Racial variation in physique and body composition. (1988). *Can. J. Sport Sci.* **13**, 117–126.

130. Hoffer, E., Meador, C. and Simpson, D. (1969). Correlation of whole body impedance with total body water volume. *J. Appl. Physiol.* **27**, 531-534.

131. Horber, F.F., Thomi, F., Casez, J.P., Fonteille, J., and Jaeger, P. (1992). Impact of hydration status on body composition as measured by dual-energy x-ray absorptiometry in normal volunteers and patients on haemodialysis. *Brit. J. Radiol.* **65**, 895 -900.

132. Horswill, C.A., Lohman, T.G., Slaughter, M.H., Boileau, R.A. and Wilmore, , J.H. (1990). Estimation of minimal weight of adolescent males using multicomponent models. *Med. Sci. Sports Exerc.* **22**, 528–532.

133. Hubbell, J.H. (1969). Photon cross sections, attenuation coefficients and energy absorption coefficients from 10 keV to 100 GeV. Washington DC. USA National Bureau of Standards, 1–85.

134. Hubbell, J.H. (1982). Photon mass attenuation and energy absorption coefficients from 1 KeV to 20 MeV. *J. Appl. Radiat. Isot.* **33**, 1269–1290.

135. Hughes-Fulford, M. and Lewis, M. (1996). Effects of microgravity on osteoblast growth activation. *Exp. Cell Res.* **224**, 103-109.

136. Jackson, A.S. and Pollock, M.L. (1978). Generalized equations for predicting body density of men. *Brit. J. Nutr.* **40**, 497–504.

137. Jackson, A.S., Pollock, M.L. and Ward, A. (1980). Generalized equations for predicting body density of women. *Med. Sci. Sports Exerc.* **12**, 175–182.

138. Jebb, S.A., Goldberg, G.R. and Elia, M. (1993). DXA measurements of fat and bone mineral density in relation to depth and adiposity. *Human Body Composition* edited by K.J. Ellis and J.D. Eastman, New York, Plenum Press.

139. Jensen, M.D., Kanaley, J.A., Reed, J.E. and Sheedy, P.F. (1995). Measurement of abdominal and visceral fat with computed tomography and dual-energy x-ray absorptiometry. *Am. J. Clin. Nutr.* **61**, 274-278.

140. Jensen, M.D., Kanaley, J.A., Roust, L.R., O'Brien, P.C., Braun, J.S., Dunn, W.L. and Wahner, H.W. (1993). Assessment of body composition with the use of dual-energy X-ray absorptiometry: evaluation and comparison with other methods. *Mayo Clinic Proc.* **68**, 867-873.

141. Johnston, F.E. and Mack, R.W. (1985). Interobserver reliability of skinfold measurements in infants and young children. *Am. J. Phys. Anthropol.* **67**, 285–290.

142. Johnston, F.E., Hamill, P.V.V. and Lemeshow, S. (1974). Skinfold thickness of children 12 - 17 years, United States, 1966 – 1970 (Vital and Health Statistics, Series 11, no. 132, Department of Health, Education and Welfare). Washington DC: US Government Printing Office.

143. Johnston, F.E., Hamill, P.V.V. and Lemeshow, S. (1972). Skinfold thickness of children 6 – 11 years, United States, 1963 – 1965 (Vital and Health Statistics, Series 11, no. 120, Department of Health, Education and Welfare). Washington DC: US Government Printing Office.

144. Jones, P. R. and Pearson, J. (1969). Anthropometric determination of leg fat and muscle plus bone volumes in young male and female adults. *J. Physiol.* **204**, 63P-66P

145. Kanai, H., Haeno, M. and Sakamoto, K. (1987). Electrical measurement of fluid distribution in legs and arms. *Med. Prog. Technol.* **12**, 159-170.

146. Kanis JA and the World Health Organisation Study Group. (1994). Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. *Osteoporosis Int.* **4**, 368-381.

147. Katzmarzyk, P.T. and Leonard, W.R. (1998). Climatic influences on human body size and proportions: ecological adaptations and secular trends. *Am. J. Phys. Anthropol.* **106**, 483–503.

148. Khaled, M., McCutcheon, M., Reddy, S. Pearman, P., Hunter, G.R. and Weinsier, R.L. (1988). Electrical impedance in assessing body composition: The BIA method. *Am. J. Clin. Nutr*, **47**, 789-792.

149. Kistorp, C.N. and Svendsen, O.L. (1997). Body composition analysis by dual energy X-ray absorptiometry in female diabetics differ between manufacturers. *Eur. J. Clin. Nutr.* **51**, 449-454.

150. Klesges, R.C., Eck, L.H., Isbell, T.R., Fulliton, W. and Hanson, C.L. (1991). Physical activity, body composition, and blood pressure: A multicomponent approach. *Med. Sci. Sports Exerc.* **23**, 759–765.

151. Knapik, J.J., Staab, J.S. and Harman, E.A. (1996). Validity of an anthropometric estimate of thigh muscle cross-sectional area. *Med. Sci. Sports Exerc.* **28**, 1523-1530.

152. Kohrt, W.M. (1993). Body composition by DXA: tried and true? *Med. Sci. Sports Exerc.* **27**, 1349 - 1353.

153. Kohrt, W.M., (1998). Preliminary evidence that DEXA provides an accurate assessment of body composition. *J. Appl. Physiol.* **84**, 372-377.

154. Kohrt, W.M., Obert, K.A. and Holloszy, J.O. (1992). Exercise improves fat distribution patterns in

60 to 70 year old men and women. *J.Gerontol.: Med. Sci.* **47**, M99-M105.

155.Kulwich, R., Feinstein, L. and Anderson, E.C. (1958). Correlation of potassium 40 concentration and fat-free lean content of hams. *Science*, **127**, 338–339.

156.Kushner, R. (1992). Bioelectric impedance analysis: A review of principles and applications. *J. Am. Col. Nutr.* **11**, 199-209.

157.Lands, L.., Hornby, L., Hohenkerk, J.. and Glorieux, F. (1996). Accuracy of measurements of small changes in soft tissue mass by dual energy x-ray absorptiometry. *Clin. Invest. Med.* **19**, 279-285.

158.Lanyon, L. (1998). Functional load bearing as the controlling stimulus for bone strength. *Proceedings of the Exercise and the Skeleton Conference*, University of London.

159.Laskey, M.A. (1996). Dual-energy x-ray absorptiometry and body composition. *Nutrition.* **12** , 45-51.

160.Leake, C.N. and Carter, J.E.L. (1991). Comparison of body composition and somatotype of trained female triathletes. *J. Sports Sci.* **9**, 125-135.

161.Lohman, T.G. (1996). Dual energy X-ray absorptiometry. In *Human Body Composition* edited by A. Roche, S. Heymsfield and T. Lohman, Champaign, IL, Human Kinetics 63 – 78.

162.Lohman, T.G. (1981). Skinfolds and body density and their relation to body fatness: A review. *Hum. Biol.* **53**, 181–225.

163.Lohman, T.G. (1992). *Advances in body composition assessment. Current issues in exercise science series monograph no.3.* Champaign, IL. Human Kinetics.

164.Lohman, T.G., Roche, A.F. and Martorell, R. (Eds.) (1988). *Anthropometric standardization reference manual.* Champaign, IL. Human Kinetics.

165.Lohman, T.G., Slaughter, M.H., Boileau, R.A., Bunt, J. and Lussier, L. (1984). Bone mineral measurements and their relation to body density in children, youth and adults. *Hum. Biol.* **56**, 667-679.

166.Lukaski, H.C. and Bolonchuk, W.W. (1987). Theory and validation of the tetrapolar bioelectrical impedance method to assess human body composition. In *In vivo body composition studies*, edited by K.J. Ellis, Yasamura and W.D. Morgan, London: The institute of physical sciences in medicine, 410–414.

167.Lukaski, H.C. (1987). Methods for the assessment of human body composition: Traditional and new. *Am. J. Clin. Nutr.* **46**, 537-556.

168.Ma, K., Kotler, D.P., Wang, J., Thornton, J.C., Ma, R.. and Pierson, R.N. Jr. (1996). Reliability of in vivo neutron activation analysis for measuring body composition: comparisons with tracer dilution and dual-energy x-ray absorptiometry. *J. Lab. Clin. Med.* **127**, 420-427.

169.Madsen, O.R., Lauridsen, U.B., Hartkopp, A. and Sorensen, O.H. (1997). Muscle strength and soft

tissue composition as measured by dual energy x-ray absorptiometry in women aged 18-87 years. *Eur. J. Appl. Physiol.* **75**, 239–245.

170. Malina R.M. and Bouchard, C. (1988). Subcutaneous fat distribution during growth. In *Fat distribution during growth and later outcomes,* edited by C. Bouchard and F.E. Johnston, New York, Plenum Press, 63–84.

171. Malina R.M. and Bouchard, C. (1991). *Growth, maturation and physical activity*, Champaign, Illinois, Human Kinetics.

172. Malina, R.M. (1968). Growth, maturation and performance of Philadelphia Negro and white elementary school children. Unpublished doctoral dissertation, University of Pennsylvania, Philadelphia, USA.

173. Malina, R.M. (1996). Regional Body Composition: Age, sex and ethnic variation. In *Human Body Composition* edited by. A.F. Roche, S.B. Heymsfield and T.G. Lohman, Champaign, Illinois, Human Kinetics, 217–255.

174. Malina, R.M. and Buschang, P.H. (1984). Anthropometric asymmetry in normal and mentally retarded males. *Ann. Hum. Biol.* **11**, 515–531.

175. Malina, R.M., Hamill, P.V.V. and Lemeshow, S. (1973). Selected body measurements of children 6 – 11 years, United States (Vital and Health Statistics, Series 11, no. 123. Department of Health, Education and Welfare). Washington DC: U.S. Government Printing Office.

176. Manzoni, P., Brambilla, P., Pietrobelli, A., Beccaria, L., Mora, S. and Chiumello, G. (1996). Influence of body composition on bone mineral content in children and adolescents. *Am. J. Clin. Nutr.* **64**, 603-607.

177. Marcus R., Cann C., Madvig P., Minkoff, J., Goddard, M., Bayer, M., Martin, M., Gaudiani, L., Haskell, W. and Genant, H. (1985). Menstrual function and bone mass in elite women distance runners. *Ann Int Med.* **102**, 158–163.

178. Marcus, R. (1996). Skeletal "impact" of exercise. *Lancet* **348**, 1326-1327.

179. Martin, A.D. (1986). Unpublished data, Simon Fraser University, Burnaby, Canada, in Lohman, T.G., Roche, A.F. and Martorell, R. (Eds.) (1988). *Anthropometric standardization reference manual*. Champaign, Illinois, Human Kinetics.

180. Martin, A.D., Daniel, M.Z., Drinkwater, D.T., and Clarys, P. (1994). Adipose tissue density, estimated adipose lipid fraction and whole body adiposity in male cadavers. *Int. J. Obes.* **18**, 79–83.

181. Martin, A.D., Drinkwater, D.T., Clarys, J.P. and Ross, W.D. (1986). The inconstancy of the fat-free mass: a reappraisal with implications for densitometry. In *Kinanthropomtery III*, edited by T. Reilly, J. Watkins and J. Borms, London, E.& F.N. Spon, 92-97.

182. Martin, A.D., Spenst, L.F., Drinkwater, D.T. and Clarys, J.P. (1990). Anthropometric estimation of

muscle mass in men. *Med. Sci. Sports Exerc.* **22**, 729–733.

183. Martorell, R., Habicht, J.P., Yarbrough, C., Guzman, G. and Klein, R.E. (1975). The identification and evaluation of measurement variability in the anthropometry of preschool children. *Am. J. Phys. Anthropol.* **43**, 347–352.

184. Mateigka, J. (1921). The testing of physical efficiency. *Am. J. Phys. Anthropol*, **4**, 223–230.

185. Mazess, R.B., Barden, H.S. Bisek, J.P. and Hanson, J. (1990). Dual-energy x-ray absorptiometry for total and regional bone mineral and soft tissue composition. *Am. J. Clin. Nutr.* **51**, 1106-1112.

186. Mazess, R.B., Peppler, W.W. and Gibbons, M. (1984). Total body composition by dual photon (153Gd) absorptiometry. *Am. J. Clin. Nutr.* **40**, 834-839.

187. Mazzetti, S.A., Nindl, B.C., Gotshalk, L.A., Duncan, N.D., Kim, S., Emmert, W. and Kraemer, W.J. (1997). Comparison of body composition assessment among lean black and white male collegiate athletes. *Med. Sci. Sports Exerc.* **29**, S54.

188. McLean, B.D. and Parker, A.W. (1989). An anthropometric analysis of elite Australian track cyclists. *J. Sports Sci.* 7, 247–255.

189. Meleski, B.W. (1980). Growth, maturity, body composition and familial characteristics of competitive swimmers 8 to 18 years of age. Unpublished doctoral dissertation, University of Texas, Austin, USA.

190. Milliken, L.A., Greaves, K.A., Going, S.B. and Lohman, T.G. (1997). Reliability of dual energy X-ray absorptiometry for measurement of abdominal soft tissue. *Med. Sci. Sports Exerc.* **29**, S37.

191. Mitchell, A.D., Conway, J.M. and Potts, W.J.E. (1996a). Body composition analysis of pigs by dual-energy X-ray absorptiometry. *J. Animal. Sci.* **74**, 2663–2671.

192. Mitchell, A.D., Conway, J.M. and Scholz, A.M. (1996b). Incremental changes in total and regional body composition of growing pigs measured by dual-energy x-ray absorptiometry. *Growth, Dev. Aging* **60**, 95-105.

193. Modlesky, C.M., Lewis, R.D., Yetman, K.A., Rose, B., Rosskopf, L.B., Snow, T.K. and Sparling, P.B. (1996). Comparison of body composition and bone mineral measurements from two DXA instruments in young men. *Am. J. Clin. Nutr.* **64**, 669-676.

194. Morris, F.L. and Payne, W.R. (1996). Seasonal variations in the body composition of lightweight rowers. *Brit. J. Sports Med.* **30**, 301-304.

195. Nichols, D.L., Sanborn, C.F., Bonnick, S.L., Gench, B. and DiMarco, N. (1995). Relationship of regional body composition to bone density in college females. *Med. Sci. Sports Exerc.* **27**, 178-182.

196. Nindl, B.C., Friedl, K.E., Marchitelli, L.J., Shippee, R.L., Thomas, C.D. and Patton, J.F. (1996). Regional fat placement in physically fit males and changes with weight loss. *Med. Sci. Sports Exerc.* **28**, 786–793.

197. Nord, R.H. and Payne, R.K. (1995). Body composition by dual energy X-ray absorptiometry - a review of the technology. *Asian Pac. J. Clin. Nutr.* **4**, 167-171.

198. Nyboer, J. (1981). Percent body fat by four terminal bio-electrical impedance and body density in college freshmen. In *Proceedings of the Vth International Conference on Electrical Bio-Impedance*, Business for Academic Societies, Japan.

199. O'Connor, J., Lanyon, L. and MacFie, H. (1982). The influence of strain rate on adaptive bone remodeling. *J. Biomech.* **15**, 767-781.

200. Ogle, G.D., Allen, J.R., Humphries, I.R., Lu, P.W., Briody, J.N., Morley, K. Howman-Giles, R. and Cowell, C.T. (1995). Body composition assessment by dual energy x-ray absorptiometry in subjects aged 4 - 26y. *Am. J. Clin. Nutr.* **61**, 746-753.

201. Olds, T. (1999). Foresnsic Anthropometry. *Kinanthreport **VII*: 1**, 16–17.

202. Oppliger, R.A., Nielsen, D.H., Hoegh, J.E. and Vance, C.G. (1991). Bioelectrical impedance prediction of fat-free mass for high school wrestlers validated. *Med. Sci. Sports Exerc.* **23**, S73.

203. Orphanidou, C.I., McCargar, L.J., Birmingham, C.L. and Belzberg, A.S. (1997). Changes in body composition and fat distribution after short term weight gain in patients with anorexia nervosa. *Am. J. Clin. Nutr.* **65**, 1034-1041.

204. Orvanova, E. (1987). Physical structure of winter sports athletes. *J. Sports Sci.* **5**, 197–248.

205. Otis C.L., Drinkwater, B., Johnson, M., Loucks, A. and Wilmore, J. (1997). American College of Sports Medicine position stand. The female athlete triad. *Med. Sci. Sports Exerc.* **29**, i-ix.

206. Overend, T.J., Cunningham, D.A., Paterson, D.H. and Lefcoe, M.S. (1993). Anthropometric and Computed Tomographic Assessment of the Thigh in Young and Old Men. *Can. J. Appl. Physiol.* **18**, 263–273.

207. Oxford Classical Dictionary (1996) 3rd ed., Oxford University Press, 1211-1212.

208. Pawan, G.L.S. and Clode, M. (1960). The gross chemical composition of subcutaneous adipose tissue in the lean and obese human subject. *J. Biol. Chem.* **74**, 9P.

209. Peters, D.M. and Eston, R. (1993). Prediction and measurement of frame size in young adult males. *J. Sports Sci.* **11**, 9–15.

210. Pierson, R.N. Jr., Wang, J., Thornton, J.C., Kother, D.P., Heymsfield, S.B., Weber, D.H. and Ma, R.M. (1995). Bone mineral and body fat measurements by two absorptiometry systems: comparisons with neutron activation analysis. *Calc. Tiss. Int.* **56**, 93-98.

211. Pietrobelli, A., Faith, M.S., Allison. D.B., Gallagher, D., Chiumello, G. and Heymsfield, S.B. (1998). Body mass index as a measure of adiposity among children and adolescents: a validation study. *J. Pediatr.* **132**, 204-210.

212. Pietrobelli, A., Formica, C., Wang, Z. and Heymsfield, S. (1996). Dual-energy X-ray absorptiometry

body composition model : review of physical concepts. *Am. J. Physiol.* **271** (Endocrinol. Metab. 34), E 941-E951.

213. Pietrobelli, A., Wang, Z., Formica, C. and Heymsfield, S.B. (1998). Dual-energy x-ray absorptiometry: fat estimation errors due to variation in soft tissue hydration. *Am. J. Physiol.* **274**, E808-E816.

214. Pintauro, S.J., Nagy, T.R., Duthie, C.M. and Goran, M.I (1996). Cross-calibration of fat and lean measurements by dual-energy X-ray absorptiometry to pig carcass analysis in the pediatric body weight range. *Am. J. Clin. Nutr.* **63**, 293-298.

215. Pocock, N.A., Eisman, J.A., Hopper, J.L., Yeats. M.G., Sambrook, P.N. and Eberl, S. (1987). Genetic determinants of bone mass in adults: A twin study. *J. Clin. Invest.* **80**, 706–710.

216. Pollock, M.L. (1985). Unpublished data, University of Florida, Department of Exercise Science, Gainesville, USA. In *Anthropometric standardization reference manual* edited by Lohman, T.G., Roche, A.F. and Martorell, R. (1988). Champaign, Illinois, Human Kinetics.

217. Pollock, M.L. (1986). Unpublished data, University of Florida, Department of Exercise Science, Gainesville, USA. In *Anthropometric standardization reference manual* edited by Lohman, T.G., Roche, A.F. and Martorell, R. (1988). Champaign, Illinois, Human Kinetics.

218. Pollock, M.L., Gettman, L.R., Jackson, A., Ayres, J. Ward, A. and Linnerud, A.C. (1977). Body composition of elite class distance runners. *Ann. N.Y. Acad. Sci.* **301**, 361–370.

219. Pollock, M.L., Hickman, T., Kendrick, Z., Jackson, A., Linnerud, A.C. and Dawson, G. (1976). Prediction of body density in young and middle-aged men. *J. Appl. Physiol.* **40**, 300–304.

220. Pollock, M.L., Laughridge, E.E., Coleman, B., Linnerud, A.C., and Jackson, A.C. (1975). *J. Appl. Physiol.* **38**, 745–749.

221. Prior, B.M., Cureton, K.J., Modelsky, C.M., Evans, E.M., Slonogeer, M.A., Saunders, M. and Lewis, R.D. (1997). In vivo validation of whole body composition estimates from dual-energy X-ray absorptiometry. *J. Appl. Physiol.* **83**, 623–630.

222. Rao, P.S. and Gregg, E.C. (1975). Attenuation of monoenergetic gamma rays in tissues. *Am. J. Roentgenol.* **123**, 631–637.

223. Remenchik, A.P. and Bernsohn, J. (1963). Relationships between the composition of adipose tissue and the total fat content of the body. *Ann. N.Y. Acad. Sci.* **110**, 754–759.

224. Reynolds, E.L. (1950). The distribution of subcutaneous fat in childhood and adolescence. *Monographs of the society for research in child development*, **15**, 1–189.

225. Rico, H., Revilla, M., Hernandez, F., Gomez-Castresana ,F. and Villa, L. (1993). Bone mineral content and body composition in postpubertal cyclist boys. *Bone* **14**, 93-95.

226. Roberts, N., Cruz-Orive, L.M., Reid, M.K., Brodie, D.A., Bourne, M. and Edwards, R.H.T. (1993).

Unbiased estimation of human body composition by the Cavalieri method using magnetic resonance imaging. *J. Microscopy* **171**, 239-253.

227. Rosenfalck, A.M., Almdal, T., Gotfredsen, A., Hojgaard, L.L. and Hilsted, J. (1995). Validity of dual x-ray absorptiometry scanning for determination of body composition in IDDM patients. *Scand. J. Clin. Lab. Invest.* **55**, 691-699.

228. Ross, R., Shaw, K.D., Rissanen, J., Martel, Y., de Guise, J. and Avrush, L. (1994). Sex differences in lean and adipose tissue distribution by magnetic resonance imaging: anthropometric relationships. *Am. J. Clin. Nutr.* **59**, 1277–1285.

229. Ross, W.D. (1999). The BMI – Just how useful is it? *Kinanthreport* **XII**:**1**, 5–9.

230. Ross, W.D. and Ward, R. (1986). Scaling anthropometric data for size and proportionality. In *Kinanthropometry III*, Edited by T. Reilly, J. Watkins, J. and J. Borms, J., London, E. & F.N. Spon.

231. Ross, W.D., Drinkwater, D.T., Bailey, D.A., Marshall, G.R. and Leahy, R.M. (1980). Kinanthropometry: traditions and new perspectives. In *Kinanthropometry II*, edited by M. Ostyn, G. Beunen, J. Simons, Baltimore, University Park Press, 3–27.

232. Roubenoff, R., Kehayais, J.J., Dawson-Hughes, B. and Heymsfield, S.B. (1993). Use of dual-energy x-ray absorptiometry in body composition studies: Not yet a "gold standard". *Am. J. Clin. Nutr.* **58**, 589-591.

233. Rubin, C. and Lanyon, L. (1984). Regulation of bone formation by applied dynamic loads. *J. Bone Joint Surg.* **66**, 411-417.

234. Rubin, C. and Lanyon, L. (1987). Osteoregulatory nature of mechanical stimulii: Function as a determinant for adaptive remodeling in bone. *J. Orthop. Res.* **5**,300–310.

235. Rush, S., Abildskov, J. and McFee, R. (1963). Resistivity of body tissues at low frequencies. *Circulation Res.* **12**, 40-50.

236. Ryde, S.J., Eston, R., Laskey, M.A., Evans, C.J. and Hancock, D.A. (1998). Changes in body fat: measurements by neutron activation, densitometry and dual X-ray absorptiometry. *Appl. Radiat. Isot.* **49**, 507-509

237. Sanborn CF, Albrecht BH, Wagner WW. Jr. (1987). Athletic amenorrhea: lack of association with body fat. *Med. Sci. Sports Exerc.* **19**, 207–212.

238. Schoeller, D.A. (1989). Changes in total body water with age. *Am. J. Clin. Nutr.* **50**, 1176-1181.

239. Schwartz, R.S., Shuman, W,P., Bradbury, V.L., Cain, K.C., Fellingham, G.W., Beard, J.C., Kahn, S.E., Stratton, J.R., Cerqueira, M.D. and Abrass, I.B. (1990). Body fat distribution in healthy young and older men. *J. Gerontol.* **45** , M181–M185.

240. Segal. K.R., Van Loan, M. Fitzgerald, P.I., Hodgdon, J.A. and Van Itallie, T.B. (1988). Lean body mass estimation by bioelectrical impedance analysis: A four-site cross-validation study. *Am. J. Clin.*

272. Tothill, P., Avenell, A., Love, J. and Reid, D.M. (1994b). Comparisons between Hologic, Lunar and Norland dual-energy X-ray absorptiometers and other techniques used for whole body soft tissue measurements. *Eur. J. Clin. Nutr.* **48**, 781-794.

273. Tothill, P., Han, T.S., Avenell, A., McNeill, G. and Reid, D.M. (1996). Comparisons between fat measurements by dual-energy X-ray absorptiometry, underwater weighing and magnetic resonance imaging in healthy women. *Eur. J. Clin. Nutr.* **50** , 747-752.

274. Tremblay A., Després, J.P. and Bouchard, C. (1988). Alteration in body fat and fat distribution with exercise. In *Fat distribution during growth and later health outcomes*, edited by C. Bouchard and F.E. Johnston, New York, Alan Liss Inc. 297–312.

275. Treuth, M.S., Ryan, A.S., Pratley, R.E., Rubin. M.A., Miller, J.P., Nicklas, B.J., Sorkin, J., Harman, S.M., Goldberg, A.P. and Hurley, B.F. (1994). Effects of strength training on total and regional body composition in older men. *J. Appl. Physiol.* **77**, 614-620.

276. Tsunenari , T., Tsutsumi, M., Ohno, K., Yamamoto, Y., Kawakatsu, M., Shimogaki, K., Negiski, H., Sugimoto, T., Fukase, M. and Fujita, T. (1993). Age and gender-related changes in body composition in Japanese subjects. *J. Bone Miner. Res.* **8**, 397-402.

277. Van Loan, M.D., Keim, N.L., Berg, K. and Mayclin, P.L. (1995). Evaluation of body composition by dual energy x-ray absorptiometry and two software packages. *Med. Sci. Sports Exerc.* **27**, 587-591.

278. Van Marken Lichenbelt, W.D., Fogelholm, M., Ottenheijm, R. and Westerterp, K.R. (1995). Physical activity, body composition and bone density in ballet dancers. *Brit. J. Nutr.* **74**, 439-451.

279. Wahner, H.W. and Fogelman, I. (1994). *The evaluation of osteoporosis: Dual energy X-ray absorptiometry in clinical practice*. London: Dunitz.

280. Wang , J., Heymsfield, S.B., Aulet, M., Thornton, J.C. and Pierson, R.N. jr. (1989). Body fat from body density: Underwater weighing vs dual-photon absorptiometry. *Am. J. Physiol.* **256**, E829-E834.

281. Wang, Z.M., Visser, M., Ma, R., Baumgartner, R.N., Kotler, D., Gallagher, D. and Heymsfield, S.B. (1996). Skeletal muscle mass: evaluation of neutron activation and dual-energy X-ray absorptiometry. *J. Appl. Physiol.* **80**, 824–831.

282. Warner, S.E. and Dalsky, G.P. (1997). Bone mineral density of elite male cyclists. *Med. Sci. Sports Exerc.* **29**, S5.

283. Wellens, R., Chumlea, W.C., Guo, S., Roche, A.F., Reo, N.V. and Siervogel, R.M. (1994). Body composition in white adults by dual-energy x-ray absorptiometry, densitometry and total body water. *Am. J. Clin. Nutr.* **59**, 547-555.

284. Weltman, A. and Katch, V. (1975). Preferential use of casing (girth) measures for estimating body volume and density. *J. Appl. Physiol.*. **38**, 560–563.

285. White, D.R., Peaple, L.H.J. and Crosby, T.J. (1980). Measured attenuation coefficients at low photon energies for 44 materials and tissues. *Radiat. Res.* **84**, 239–252.

286. Wilmore, J.H. and Behnke, A.R. (1969). An anthropometric estimation of body density and lean body weight in young men. *J. Appl. Physiol.* **27**, 25–31.

287. Winter, E.M., Brookes, F.B.C. and Hamley, E.J. (1991). Maximal exercise performance and lean leg volume in men and women. *J. Sports Sci.* **9**, 3 – 13.

288. Woodrow, G., Oldroyd, B., Smith, M.A. and Turney, J.H. (1996). Measurement of body composition in chronic renal failure: comparison of skinfold anthropometry and bioelectrical imedance with dual X-ray absorptiometry. *Eur. J. Clin. Nutr.* **50**, 295-301.

289. Yaeger, K.L., Agostini, R., Nattiv, A and Drinkwater, B. (1993). The female athlete triad: disordered eating, amenorrhea, osteoporosis. *Med. Sci. Sports Exerc.* **25**, 775–777.

290. Zamboni, M., Armellini, F., Turcato, E., Todisco, P., Gallagher, D., Dalle Grave, R., Heymsfield, S., and Bosello, O. (1997). Body fat distribution before and after weight gain in anorexia nervosa. *Int. J. Obes.* **21**, 33-36.

291. Zavaleta, A,N. (1976). Densitometric estimates of body composition in Mexican Americans. Unpublished doctoral dissertation, University of Texas, Austin, USA.

292. Zavaleta, A.N. and Malina, R.M. (1982). Growth and body composition of Mexican –American boys 9 through 14 years of age. *Am. J. Phys. Anthropol.* **57**, 261–271.

293. Zuti, W.B. and Golding, L.A. (1973). Equations for estimating percent body fat and body density in active adults. *Med. Sci. Sports Exerc.* **5**, 262–266.