STUDIES ON THE VARIABILITY OF HUMAN BASAL METABOLIC RATE .

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by

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"Much more research lies ahead before we can begin to understand why one person can live on half the calories of another".

Dr. Elsie Widdowson (1947).

I hope the work contained in this thesis takes us a little closer to the answer.

SUMMARY.

The studies presented in this thesis examine some of the factors responsible for variation in basal metabolic rate (BMR, defined as the energy expended by an individual lying quietly at rest in a thermoneutral environment, at least 12 hours postabsorptive). They are particularly concerned with the parts played by differences in body composition and cellular metabolic activity, under the influence of thyroid hormones and catechloamines, in explaining variability in BMR.

In the great majority of people, and certainly most of those living in an industrialised society, BMR accounts for the largest part of daily energy expenditure, often making up more than two thirds of the total. Study of the factors that effect BMR is therefore central to our understanding of the causes of variation in daily energy needs.

The first study undertaken sought to explore some of the general relationships between BMR and body composition in a group of 97 healthy women. BMR was measured using the Douglas bag technique, and body composition assessed by measurements of weight, height, body fat content (skinfold and densitometry estimates), circumferences and diameters. Differences in BMR between the women were found to be large (CV = 11.8%, standard deviation 159 kcal/day) and could be best explained by differences in FFM, accounting for 45% of the total variance. The relationship between BMR and FFM was unaffected by body fatness or age. However, at a given FFM considerable variation in the BMR of individual women was evident. Moreover, for the purposes of predicting an individual's BMR, FFM was found to be no better than body weight. Simple differences in the weight of the FFM therefore, could only partially explain the variation in BMR between the women. A further observation from the study was that BMR expressed per kg body weight or per kg FFM tended to decline form light to heavy individuals. This finding has implications for the

use of FFM as a metabolic reference standard, and it is suggested that it may relate to differences in the composition of the FFM.

The role of the catecholamines was considered in a study which investigated the effect of ß-adrenergic blockade on basal metabolic rate. The BMRs (measured using a ventilated hood system) of a group of 18 patients receiving beta blocker drugs in the treatment of cardiovascular disorders were compared to those of 28 healthy control subjects. In relation to the FFM (estimated from skinfold thicknesses) the BMRs of the ß-blocker patients were found to 8% lower than that of the controls, equivalent to 136 kcal/day. The study revealed a potentially important side effect of this widely prescribed group of drugs and moreover, suggested that BMR has an adrenergically mediated component.

A further study was undertaken with the aim of elucidating the causes of the marked variation in BMR relative to the FFM observed in the initial investigation. Two groups of women characterised by particularly high or low BMRs in relation to their FFM were selected for further study. Repeat measurements of BMR suggested that part of the differences between the groups, and by extension the initial study also, resulted from within-subject variation in BMR. Error in measurement of the FFM (estimated by skinfolds, total body water and densitometry) was found to be small and its potential contribution considered minor. It was estimated that genuine inter-individual variation in BMR in relation to the FFM was in the region of 100 kcal/day. Thyroid hormone levels were found to be significantly greater in the high BMR group than in the low and it was postulated that these differences were likely to be responsible for at least part of the variance in BMR relative to FFM. Thyroid status did not however, provide the complete explanation, a residual standard deviation approximately 70 kcal/day remained. It was considered likely that differences in the composition of the FFM were involved in explaining the remaining variance. Urinary catecholamine levels were comparable in both groups, however the possibility that differences in an

adrenergically mediated process may have contributed to the differences in BMR could not be ruled out.

Traditionally, differences in BMR have been ascribed to differences in body size, age, sex, race, climate and nutritional status. The studies presented in this thesis suggest however, that that these may have a common basis in that they relate to differences in one or both of the major determinants of BMR; to differences in body composition - primarily to the mass of fat-free tissue and to the relative proportions of its component parts - and to hormonally induced metabolic activity of the tissues. Moreover, it has been possible to make some assessment of the relative importance of these factors in explaining variations in BMR: 45% of the variance observed in BMR in the original cross-sectional study was found to result from differences in FFM. It was estimated that around 15% of the variance was attributable to within-subject differences and a further 20% to differences in thyroid hormone levels. The remaining 20% can probably be accredited to differences in the size of the metabolically active organs.

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CHAPTER 1

INTRODUCTION.

DEFINITION OF BMR.

Basal metabolic rate is the energy output of an individual under standardised resting conditions. A detailed statement of the criteria to be met for a measurement to be considered basal was given by Benedict in 1938. The conditions laid down are rigorous, but necessarily so if comparisons are to be made either within or between individuals or groups. The subject should be lying awake in a state of complete physical repose, gross muscular activity should be absent. He or she should be in a post-absorptive state, at least 12 hours after the last meal. The environment should be thermoneutral, eliciting no thermoregulatory effect on heat production. Emotional disturbance should be minimal. Fever and disease should be absent from the individual.

The term 'basal' is perhaps a misnomer since it may imply that energy expenditure measured under these conditions corresponds to a minimum level. This is not the case. Metabolic rate has been shown to fall below basal (as defined above) during sleep (Mason & Benedict, 1934; Passmore & Durnin, 1967), anaesthesia (Mitchell, 1962) and transcendental meditation (Farrell, 1980). It is clearly not therefore, as it has sometimes been interpreted (Mitchell, 1962), the absolute minimum level of energy expenditure compatible with life. The specific conditions imposed for measurement of BMR are not so much to secure minimum rates of metabolism as to secure comparable rates.

For the purposes of this thesis BMR is taken to be a measure of the energy utilised by the metabolic activities of all the cells in the body under the resting conditions specified above.

FUNCTIONAL BASIS OF BMR.

BMR represents the energy expended by the body on the performance of internal work - by definition no work is performed on the environment. Oxidation

of nutrients in the body provides ATP which is utilised to accomplish this work. Internal work can be subdivided into three components:

(1) Mechanical work, which is mostly the pressure-volume work of propulsion, subsequently dissipated as fluid friction to appear as heat. Mechanical work includes the work of the heart, lungs and gut.

(2) Work done in the synthesis of new chemical compounds from monomers and their subsequent breakdown. A typical example is that of protein turnover. Also included are the substrate or 'futile' cycles which exist at certain key points in intermediary metabolism. Here the actions of pairs of enzymes lead to substrate / product cycles resulting in the expenditure of energy with little net metabolism of the substrate. The interconversions of glucose and pyruvate, glucose and glucose 6-P, glucose phosphorylation and triglyceride turnover in adipose tissue are all examples of so-called futile cycles.

(3) Work done in the transport of ions and molecules in secretion, absorption and the maintenance of electrochemical gradients in cells. For example sodium and other ion pumps and all processes of active transport. The contribution of mechanical work to basal energy expenditure is thought to be very small, probably accounting for only 3% of the total (Agriculture Research Council & Medical Research Council, 1974). Work done in biosynthetic and ion transport processes are less easy to quantify. Various reports, summarised by Reeds *et al.* (1985), have estimated the contribution of protein turnover to BMR to be somewhere in the region of 11-15%. In general the work done in ion transport is considered to be of greater importance. To date the contribution of the Na^+/K^+pump has received most attention. Sims (1987) believes that a large part of our metabolic energy, perhaps up to 50%, 'is still devoted to keeping the primordial brine out of our cells'. Other investigators give more conservative estimates. Sestoft (1980) for example, suggests that the Na^+/K^+ pump is unlikely to account for more than 5-10% of BMR. The discrepancies seem to arise from differences in the cellular integrity of

the various preparations used. Similarly, the precise contribution of the energy costs of substrate cycles to basal expenditure is not known; Reeds *et al.* (1985) estimate that it may well be in the region of about 15%, other investigators put the potential contribution substantially higher (Newsholme, 1980). Clearly, the amount of internal work performed determines an individual's basal energy requirements. Differences in work will be manifest in differences in BMR. This thesis will consider some of the factors responsible for the differences in the amount of internal work performed and consequently therefore, for variations in BMR.

For the great majority of individuals BMR makes up the largest part of daily energy expenditure, accounting in the sedentary for around two thirds of the total. Consequently, differences in BMR are likely to be the most important determinant of differences in daily energy expenditure. In view of this, the study of factors affecting BMR would seem essential to our understanding of the causes of inter-individual variability in daily energy needs.

AN OVERVIEW OF THE FACTORS THOUGHT TO AFFECT BMR:

The purpose of the following section is to briefly review some of the factors thought to affect BMR. It is not intended to be fully comprehensive but rather to provide some background to the subject and to introduce concepts and ideas which will be expanded upon in subsequent sections.

(1) **Body Size.**

From the first, investigators have acknowledged that the size of the body will influence BMR and have made attempts to take this into account when making comparative measurements. It was recognised early on that some form of 'normalisation' for differences in size was required if the effects on metabolism of

factors other than body size, for example race, disease and nutritional status, were to be considered.

For many years it was customary to express BMR on a surface area basis. This practice stems from the observation of Rubner in 1883 that the fasting metabolism of seven dogs varying in body weight from 3 to 31kg was approximately constant when expressed per unit area of body surface. Subsequently Rubner's pupil, Voit, published results in 1901 which showed that the fasting metabolisms of a number of different species were also proportional to their surface areas (Kleiber, 1947). It was largely on the basis of these inter-specific results that surface area became the attribute of body size to which basal metabolism of humans was referred. This in itself is a potential criticism of the use of surface area as a metabolic reference standard in man. However, perhaps more fundamental is the assumption implicit in the 'surface area law' that in a basal state an individual is producing heat primarily to keep the body warm. On this basis, the greater the surface area the greater the heat loss to the environment and consequently the greater the metabolic rate must be to generate sufficient heat to maintain body temperature. This is not so. One of the prerequisites for measurement of BMR is a thermoneutral environment. Energy is produced for physiological work, in the accomplishment of which heat is produced and dissipated. It is the magnitude of the work to be performed that determines BMR, not the need to maintain body temperature. As Garrow (1978) points out, if BMR was determined by the rate of heat loss energy expenditure would be higher if an individual was lying spread-eagled, and thus presenting the maximum area for heat loss than if curled up in a small a volume as possible, but this is not so. Another criticism of the use of surface area as a reference standard has been the questionable accuracy of its estimation. Most commonly surface area is calculated from weight and height using the formula of Du Bois & Du Bois (1916). Mitchell *et al.* (1971) showed that the Du Bois' formula underestimates surface area by about 7% relative

to the true area measured by a photometric technique. Heusner (1985) believes that the surface area law has become an example of Cantor's Law of the Conservation of Ignorance: "A false conclusion once arrived at and widely accepted is not easily dislodged and the less it is understood the more tenaciously it is held"!

An alternative way of taking into account differences in body size is simply to express BMR in relation to weight. This also has its problems however, in that BMR is not constant per unit of weight; rather there is a tendency for BMR/kg to decline as weight increases (Scofield, 1985a; Owen *et al.*, 1986; Lawrence *et al.*, 1988). Consequently, BMR has sometimes been related to a power function of body weight. Between species of animals Kleiber (1947) observed BMR was constant when expressed in relation to weight$^{0.75}$. The 'three-quarters power rule' has also been applied to man, but with seemingly little justification. The relationship is an empirical one, based on observation and not on theoretical considerations. The explanation for the observed decline in BMR/kg probably lies in differences in body composition between light and heavy individuals. This relationship will be discussed in some depth in a later section.

(2) **Age.**

The way in which age affects BMR is not the same at all stages of life. The metabolism of the new born infant is characteristically low but increases rapidly to reach a peak in relation to body weight in the first year of life (Widdowson, 1981). When expressed per unit weight, BMR is higher in children than at maturity. This can partly be attributed to the energy cost of growth (Spady *et al.*, 1976; Millward *et al.*, 1976) however, differences in body composition between adults and infants in relation to their weight may also play a part.

Once adulthood is reached the general view is that basal metabolism tends to fall. On the basis of cross-sectional data Shock (1972) has estimated that between the ages of 30 and 80 years BMR/day declines by about 200 kcal in men. Before

the age of 60 the fall in BMR in relation to weight is thought to be comparatively small, only about 1-2% per decade, but to become more pronounced thereafter (FAO/WHO/UNU, 1985). Much of the decline in BMR with age has been attributed to changes in body composition, (Keys *et al.*, 1973; Cunningham, 1980). There is still some conjecture however, as to whether age-related changes in the metabolic rates of the tissues themselves also occur - slowing as we become older. The respective roles of changes in the body composition and of tissue metabolism will be discussed in some depth later in this thesis.

(3) **Sex.**

When expressed in relation to body weight women have lower BMRs than men. The difference between the sexes becomes apparent in early adolescence and remains throughout adult life. It is thought to be largely attributable to differences in body composition - at a given weight women are on average fatter and less muscular than men - and not to reflect any inherant metabolic differences between men and women (Webb, 1981; Cunningham, 1980 & 1982).

(4) **Race.**

A number of studies have attempted to assess the possibility of ethnic differences in BMR. Results have sometimes been conflicting but several reports have suggested that in relation to body weight, Asian subjects have lower BMRs than their North American or North European counterparts (Quenouille *et al.*, 1951; Shetty, 1984; Scofield, 1985a; McNeill *et al.*, 1987; Drummond, 1988). It is often difficult to decide whether this is a result of genuine genetic differences between Asians and other racial groups or relates to, for example, differences in nutritional status, diet, climate or body composition. Recently Lawrence *et al.* (1988) have shown that differences in the BMRs of Scottish, Gambian and Thai women could be explained by differences in the amount of fat free tissue in the body and found

race, climate and nutritional status to have little effect. The results of this study suggest that differences in body composition may play an important part in explaining ethnic variations in BMR, but the issue has yet to be conclusively resolved.

(5) Climate.

It is clear that ambient temperature affects metabolic rate. Exposure to severe cold can double resting metabolism (Buskirk *et al.*, 1963; Wyndham *et al.*, 1968 & Rochelle & Horvath 1969) and even moderate cold exposure, such as might occur in normal life, has been shown to increase metabolic rate. Dauncey (1981) found a 6% rise in metabolic rate with a fall in environmental room temperature from 28°C to 22°C. A similar drop was reported by Blaza and Garrow (1983) for women at the lower end of their 'thermal comfort zone'. The converse would also seem to apply, an increase in ambient temperature has been reported to reduce metabolic rate (Mason & Jacob, 1972).

It should be remembered however, that one of the stipulations of a proper BMR determination is thermo-neutralility. From the available data it is unclear whether BMR is affected by climate if the measurement condition of a thermoneutral room temperature is strictly adhered to. Buskirk *et al.* (1957) studied soldiers at military bases with ambient temperatures ranging from -25°C to 34°C and found no significant relationship between BMR and temperature. On the other hand Mason and Jacob (1972) report data from individuals whose BMRs were altered by a change from a tropical to a temperate climate or vice versa. Recent FAO/WHO committees on energy requirements have not made an allowance for climatic factors in predictions of BMR, but concede that further investigation is required before it can be concluded for certain that temperature and humidity have no important affect on BMR (FAO/WHO, 1973; FAO/WHO/UNU, 1985). The question of whether BMR in its strict sense is affected by climate may be only of academic interest.

Durnin (1981) has argued that differences in metabolic rate at the individual's usual environmental temperature may be of greater practical significance.

(6) Nutritional Status.

BMR is reduced by under-nutrition. This was clearly shown by Benedict *et al.* (1919) in their classical study of calorie restriction in normal young men, and confirmed in the Minnesota experiment undertaken by Keys and his colleagues 30 years later (Keys *et al.*, 1950). It has been demonstrated on numerous occasions since.

Much of the drop in BMR can be explained by loss of the fat free tissue which accompanies negative energy balance. Many authors however, have also reported a decrease in BMR over and above that expected from tissue loss alone (Keys *et al.*, 1950; Grande *et al.*, 1958; James *et al.*, 1978; Bessard *et al.*, 1983; Finer *et al.*, 1986; Barrows & Snook, 1987). Two explanations for this reduction have been offered: (1) There may be an alteration in the composition of the lean tissue mass. There is some evidence that during energy restriction tissues with high metabolic rates such as the liver are initially lost at a proportionately greater rate than other less active tissues (Grande *et al.*, 1958). If this were the case a fall in the overall metabolic rate of the fat-free tissue would result. (2) The alternative proposal is that the metabolic activity of the individual tissues themselves fall (Keys *et al.*, 1950; James *et al.*, 1978). The decrease in thyroid hormone and catecholamines levels associated with energy restriction have been suggested to bring about such a change (Jung *et al.*, 1980; Shetty *et al.*, 1979). The above hypotheses are not mutually exclusive and it may well be that the decrease in BMR per unit weight of tissue is the result of a combination of them both.

The effect of over-feeding on BMR is a contentious issue. Chronic over-nutrition undoubtedly leads to an increase in body weight - the mass of both fat and fat-free components of body weight increase. BMR rises as a result. The area of

dispute lies however, in the observation by some investigators that weight gain is not always as great as would be predicted from the energy surplus. Consequently it has been postulated that over-feeding stimulates an increase in BMR over and above (and preceding) that resulting from changes in body composition (Sims, 1976; Schutz *et al.*, 1982). Again it has been suggested that this occurs through hormonally-induced alterations in the metabolic activity of the tissues. The existence of a such a mechanism to limit the consequences of overeating, so-called luxus consumption, is by no means universally accepted.

Traditionally then, differences in BMR have been attributed to the factors outlined above - to differences in body size, age, sex, race, climate and nutritional status. It should have become clear in the course of the preceding review however, that much of the variance seems to have a common basis, in that it appears to relate to differences in just two factors; either to differences in body composition - to the relative amounts of fat and lean tissue in the body and to the composition of the lean tissue - or to differences in the metabolic activity of the tissues - to differences in the basic energy demanding processes at cellular level. Much of this thesis is devoted to investigating the parts played by these two factors in explaining variability in BMR.

EFFECT OF BODY COMPOSITION ON BMR.

In human biology the term body composition has come primarily to refer to the fat and fat free components of body mass. Fat is defined as the ether-extractable constituent of the body, fat free mass (FFM) as the mass of all the tissues and fluids in the body less fat. This distinction is a chemical one, its anatomical analogue is the division of body weight into adipose tissue and lean body mass (LBM). Adipose tissue is made up not only of fat (roughly 80%) but also of about 2%

protein and the remainder water. Clearly, fat and adipose tissue are not the same, in the literature however, the terms are often used synonymously.

It is desirable to know how much fat is present in the body for a number of reasons and various techniques have been developed to quantify fat and FFM in living subjects. Body fat content influences morbidity and mortality, alters the efficacy of drug and anaesthetic action and influences the tolerance to cold and starvation. It is also recognised that the degree of body fatness will affect metabolic rate, such that at a given weight the greater the fat content the lower the metabolic rate. To make some allowance for differences in body fatness it has become common practice to express BMR in relation to the FFM. The adoption of FFM as a metabolic reference standard can be traced back to the work of Miller and Blyth (1950). In a series of experiments with 48 college students who had fat contents, estimated from body density, varying from 3% to 44%, they found better relations of basal oxygen consumption to FFM than to either body weight or surface area. Subsequent studies (discussed below) have reported that differences in BMR in relation to age, sex, obesity and ethnic origin largely disappear when differences in FFM are taken into account.

Between-Group Differences in BMR.

Sex

Several studies have reached the conclusion that differences in BMR between the sexes are, for the most part, attributable to differences in FFM (Bernstein *et al.*, 1983; Ravussin *et al.*, 1986; Owen *et al.*, 1987; Weststrate, 1989). Employing regression analysis to data derived from relatively large numbers of men and women these workers all found that once differences in FFM had been taken into account, sex had no significant influence on BMR. In other words, men and women with a similar FFM had a similar BMR. In a re-analysis of data from some 200 subjects, which provided the basis for the Harris-Benedict

equations (1919), Cunningham (1980) also found FFM to be the best single predictor of BMR, the influence of sex adding little to the estimation. As evidence against a characteristically 'masculine' or 'feminine metabolism' this study is perhaps the most widely quoted, it is however, open to criticism, in that the FFM of the subjects was not measured. Rather, using the equations developed by Moore *et al.* (1962), Cunningham estimated total body water from age and body weight and used this to predict FFM. The main body of evidence does however, seem to support Cunningham's conclusion that differences in BMR between the sexes are a reflection of differences in FFM - no investigation has found to the contrary. At a given weight women are on average fatter and have a lighter FFM than men, and consequently, it is suggested, a lower metabolic rate. On this basis there have not been thought to be any inherant differences in the metabolic activity of the tissues themselves between men and women. The observation that athletic training which negates differences in body fatness and FFM between the sexes also reduces differences in oxygen consumption at a given body weight is consistent with this idea (Cunningham, 1982). However, in one obvious way the 'metabolisms' of the sexes do differ; in women BMR may be affected by the menstrual cycle (Soloman *et al.*, 1982; Bisdee *et al.*, 1989), no such cyclical changes are apparent in men.

Age

Keys *et al.* (1973) suggest that almost all of the decrease in BMR they observed in longitudinal studies of aging can be explained by changes in the relative proportions of fat and FFM making up body weight. They estimated that the reduction in BMR attributable to age per se was only about 1% per decade over the age range 20-75 years. Results of the Baltimore Longitudinal Study of Aging (Shock, 1972) also appear to support the conclusion that age-related changes in BMR are a consequence of changes in the mass of fat-free tissue. Both these analysis rely on the assumption that the FFM has a relatively constant composition

and a fairly uniform metabolic rate, ie. a 10% reduction in the FFM will be accompanied by a 10% reduction in BMR. This assumption is almost certainly not justified (see later). However, the results of several cross-sectional studies would seem to add weight to the general conclusions drawn from the above investigations. Using regression analysis Cunningham (1980), Webb (1982), Bernstein *et al.* (1983), Ravussin *et al.* (1986) and Owen *et al.* (1987) have all found that once differences in FFM have been taken into account age has no significant affect on BMR. The findings of two groups of workers however, are contrary to the main body of opinion. In a group of 58 Indian men, McNeill *et al.* (1987) found that in relation to the FFM, BMR was not constant with age, but in fact showed a small but significant decline with increasing years. Similarly Doré *et al.* (1982) report that in a group of obese women resting energy expenditure decreased with age even after adjusting for differences in weight of the FFM.

Race

There is some evidence to suggest that variation in BMR between ethnic groups may be explained by differences in FFM. Recently, Lawrence *et al.* (1988) reported that differences in the BMRs of Scottish, Gambian and Thai women were largely eliminated when differences in the FFM were taken into account; women from all three countries with a similar FFM were found to have a similar BMR . In this investigation however, FFM was estimated from skinfold thicknesses using the equations of Durnin & Womersley (1974). A possible criticism of this approach is that these prediction equations (derived from studies on Scottish subjects) may not be applicable to Gambian and Thai women. Whether the lower BMR/kg body weight of Indian subjects compared to their North European and North American counterparts (Shetty, 1984; Schofield, 1985; McNeill *et al.*, 1987; Drummond, 1988) is also a consequence of differences in FFM relative to weight has yet to be established.

Obesity.

The majority of investigations which have examined the effect of body fatness on metabolic rate have found no significant difference in the relation between BMR and FFM in lean and obese subjects; individuals with the same FFM have a similar BMR no matter what their body fat content (James *et al.*, 1978; Halliday *et al.*, 1979; Doré *et al.*, 1982; Ravussin *et al.*, 1982; Garrow & Webster, 1985; Lawrence *et al.*, 1987; Weststrate, 1989).

For the most part, the above data would seem to suggest that much of the variation in BMR between groups, of different age, sex, body fatness and race may be largely explained by differences in FFM. On this basis, with some reservations (see below), FFM may serve as a useful reference standard. It probably represents a suitable 'normalisation' factor to allow comparison of metabolic rate between the young and elderly, men and women, lean and obese and those of differing ethnic origin.

In contrast however, differences in the mass of fat-free tissue appear to explain comparatively little of the difference in BMR apparent within groups of individuals.

Within-Group Variation in BMR.

Within a relatively homogeneous group, comparable in terms of age, sex, race and body fatness, correlations between BMR and FFM are relatively low (Bernstein *et al.*, 1983; Lawrence *et al.*, 1988). Rarely is FFM able to account for more than about half of the total variance. Moreover, in such groups BMR is not, in general, more highly correlated with FFM than it is with body weight (Bernstein *et al.*, 1983; Lawrence *et al..*, 1988; Owen *et al.*, 1986 & 1987). For example, in a group of 60 male subjects Owen *et al.* (1987) report a correlation between BMR

and FFM estimated from skinfolds of 0.78, the correlation between BMR and body weight was only marginally lower, 0.75. For the purposes of predicting of an individual's BMR, the two variables are comparable. It has therefore been argued the ease with which weight can be measured makes it preferable to FFM in the estimation of BMR within a group.

Individual Variations in BMR in Relation to the FFM.

At a given FFM, BMR may vary considerably between individuals. The literature reveals many examples of physically very similar individuals with widely divergent basal or resting metabolic rates. Garrow (1985) cites the case of two female students who had almost the same mass of fat free tissue (45.07kg and 43.56kg respectively) yet whose BMRs differed by some 35%. Similarly Durnin (1988) found a standard deviation of about 10% in the BMR of a group of thirty men selected to have a similar FFM. This implies that 15% of the men at the lower end of the range had BMRs which were about 450-500 kcal/day less than the BMRs of the 15% at the top end of the range. Individual variations in BMR in relation to the FFM of a similar order of magnitude are also indicated by the residual standard deviations of regression equations of BMR against FFM. For instance, Lawrence *et al.* (1988) correlated BMR with FFM for groups of women in three countries, Scotland, The Gambia and Thailand, and found that the residual standard deviations around the regression lines ranged from 95 kcal/day (7% of the mean) to 152 kcal/day (10% of the mean). Bogardus *et al.* (1986) reported a residual standard deviation of 141 kcal/day for the equation they derived for predicting BMR from FFM in North American Indians. It would seem that 500 kcals or more a day may separate the BMRs of people with the same measured FFM.

Systematic Differences in BMR/kg FFM.

Lawrence *et al.* (1988) have observed that BMR/kg FFM is not constant with weight but tends to be lower in heavier compared to lighter individuals. In their study they report a 15% difference in BMR/kg FFM between women with a FFM of 33kg compared to those with a FFM of 50kg. Miller & Blyth's data (1953) also showed that BMR/kg FFM was not constant in individuals of different body weight but declined as weight increased, although they did not specifically comment on this. A recent investigation by Weststrate (1989) found that BMR/kg FFM was significantly lower in the heavier men who took part in the study compared to the lighter women (27.3 kcal/kg FFM/day and 30.0 kcal/kg FFM/day respectively, $p < 0.001$). Observations on undergraduate students in our own laboratory have also suggested that BMR/kg FFM is higher in women than in men. Superficially this may seem at odds with the assertion that there are no differences in the BMR of men and women once FFM has been taken into account. The two findings are not however contradictory. Weststrate's results also confirmed that at a given FFM men and women have a similar BMR. Since the difference in BMR/kg FFM is not related to sex per se, it may simply be a reflection that BMR/kg FFM is lower in those individuals with a relatively large FFM - men compared to women. By the same reasoning the lower BMR/kg FFM found in some obese subjects may be a consequence of a relatively enlarged FFM (Ravussin *et al.*, 1982; James, 1985; Weststrate, 1989).

As Lawrence *et al.* (1988) have pointed out, the variation observed in BMR/kg FFM between light and heavy individuals has implications for the use of FFM as a metabolic reference standard, in that it may not be appropriate to express BMR 'per kg FFM'. In their own study they calculated that BMR was approximately constant when divided by the square root of FFM (BMR/kg $FFM^{0.5}$) and suggest that as a standard of reference it might be more appropriate to use $FFM^{0.5}$ rather than FFM itself.

Clearly, simple differences in the mass of fat-free tissue between individuals fall far short of explaining all the variation evident in BMR. It would appear that several hundred kcal/day may separate individuals with the same FFM. Moreover, BMR/kg FFM seems to show systematic changes with body weight. The following sections are concerned with examining the causes of such variation. The possible roles of intra-individual variation, erroneous estimates of FFM, differences in the composition of the FFM and differences in metabolic activity of the tissues will be discussed. For the sake of clarity they are considered separately, it is likely however, that differences in BMR in relation to the FFM may result from a combination of them all.

Intra-individual Variation.

A component of the large individual differences observed in BMR relative to FFM is likely to arise from differences in basal metabolic rate within subjects - intra-individual variation. Differences in the preceding day's energy intake, level of exercise and - for women - the stage of the menstrual cycle are all suggested to contribute to day to day or short-term fluctuations in BMR and therefore to intra-individual variation. A portion of within-subject variation is also likely to be methodological, resulting from errors in measurement of energy expenditure. Making repeat measurements of BMR in the same individual allows some quantification of the extent of intra-individual differences and of their potential contribution to between-subject variation. The results of some of the studies which have made an attempt at this are summarised in Table 1.1. The conditions under which the investigations were performed differed markedly. In some cases antecedent diet and exercise were extremely carefully controlled to look, for example, at the effect of the menstrual cycle on BMR. In others, subjects could be described as 'free-living' with no fixed dietary or exercise regimes. Methods of measuring BMR also varied, and included the Douglas bag technique, ventilated

Table 1.1

Studies which have investigated intra-individual differences in BMR.

Reference	n	Sex	CV (%)	Apparatus	Conditions : Diet & Activity
Berkson & Boothby (1938)	10 23	F M	4.7 3.5	Douglas bag	Controlled
Mahedeva *et al.* (1953)	2	?	7.5	Douglas bag	Unspecified
Jequier & Schultz (1981)	14	?	2.0	Calorimeter	Controlled
Soloman *et al.* (1982)	6	F	9.0	Douglas bag	Controlled
Garby *et al.* (1984)	8	M	4.3	Douglas bag	Habitual
Garby & Lammert (1984)	23	M	3.4	Douglas bag	No control
Soares & Shetty (1986)	5	M	3.2	H.B. Metabolator	No control
Ravussin *et al.* (1986)	12	M F	6.0	Ventilated Hood	No control
Bogardus *et al.* (1986)	26	?	4.0	Ventilated Hood	No control
Murgatroyd *et al.* (1987)	4	M	6.0	Calorimeter	Strict control
Bisdee *et al.* (1989)	8	F	6.4	Calorimeter	Strict control
Weststrate (1989)	49 54	M F	6.0 6.0	Ventilated Hood	No control

CV, coefficient of intra-individual variation

hood system and respiratory chamber. Coefficients of intra-individual variation in BMR ranged from about 3% up to 6% or 7%. The estimated 'method-free' variation (total variance less that estimated to be attributable to measurement error) from about 2% to 6% (not shown in Table 1.1). There appears to be no clear cut distinction in the extent of variation between those studies where conditions were carefully controlled and those in which regimes were much less strict. In general the results suggest that intra-individual variation in BMR is smaller than the large inter-individual differences that have been reported. The indications are therefore, that a significant proportion of variation observed in BMR relative to the FFM is the result of genuine differences between individuals.

Error in measurement of the FFM.

It is also possible however, that a component of the variation observed in BMR relative to FFM will be the result of errors in measurement of FFM; under- or over-estimation of fat-free tissue will necessarily introduce a degree of variation with methodological rather than biological cause. Erroneous estimates of FFM could result from experimental error, for example not measuring a skinfold at exactly the correct sight, but will also arise if the fundamental assumptions upon which the techniques are based - for skinfolds that the ratio of subcutaneous to internal fat is relatively constant, for density, that the density of the FFM is 1100 kg/m^{-3}, and so on - are violated. Analysis by Womersely & Durnin (1977) suggests that both densitometry and the skinfold technique are associated with errors of around ±2% - 3% body weight. Errors of this magnitude are perhaps unlikely to account for a large proportion of variation in BMR relative to the FFM. No attempt however, seems to have been made to quantify their contribution more exactly.

Composition of the FFM.

The FFM is very much heterogeneous in composition. By definition it comprises all the 'non-fat' tissues and fluids in the body and therefore includes tissues as structually and functionally diverse as the visceral organs, muscle, bone, blood and connective tissue. The metabolic rates of the various components of the FFM differ widely (see Table 1.2). The viscera, notably the liver, kidneys and the heart and brain have very high energy requirements. It has been estimated that the metabolism of these four organs alone, accounts for around 60% of resting oxygen consumption yet they represent only about 6% of the FFM in terms of weight (Brokek & Grande, 1955). Skeletal muscle on the other hand, making up about half the weight of the FFM, has a relatively low metabolic rate at rest and contributes less than 25% to basal metabolism (Table 1.2). The picture is one of a small mass of organs with high energy requirements under resting conditions, and a much greater mass with a relatively low metabolic rate, mainly constituted by bone and skeletal musculature (Brozek & Grande, 1955).

The heterogeneity of the FFM in terms of the metabolic rates of its components seems to have been lost sight of by some investigators. Conceptually there is tendency to regard the fat-free body as comprising of a mass of uniformly active tissues. Clearly this is erroneous. Yet the temptation to extrapolate the chemical division of fat and FFM into an all encompassing functional one has been too great for some. Clearly the amount of active tissue in the body will influence BMR. To view this however, solely in terms of fat compared to fat-free tissue is restrictive. FFM may well provide a useful metabolic reference standard but to gain a greater understanding of the nature of variation that exists in BMR it may be necessary to look at the relative amounts of 'active' and 'inactive' tissue within the FFM.

Table 1.2

Contribution of organ and tissue metabolic rates to BMR in man.*

Organ	Weight	BMR kcal/day	% of whole body BMR
Liver	1.60	482	27
Brain	1.40	338	19
Heart	0.32	122	7
Kidney	0.29	187	10
Muscle	30.00	324	18
Remainder by difference			19
Total	70.00	1800	

* 70 kg adult male

Adapted from table complied by Durnin, FAO/WHO/UNU (1985)

Possible Influence of Differences in Composition of the FFM on BMR.

Clearly, since FFM is made up of tissues of very different metabolic rates differences in the relative proportions of 'active' compared to 'inactive' tissues could produce differences in the average metabolic rate of the FFM. It is therefore conceivable that some of the differences observed in BMR are a result of differences in the composition of the FFM. Lawrence *et al.*, (1988) have suggested that systematic differences in the composition of the FFM may provide an explanation for the finding that BMR/kg FFM tends to be lower in heavier compared to lighter individuals. The observed decrease in BMR/kg FFM with increasing weight could feasibly be produced if, as weight increases, the proportion of the FFM occupied by metabolically active organs declined and concurrently the proportion of relatively inactive tissue such as muscle increased.

In individuals who are heavy because they are obese it has been suggested that their relatively enlarged FFM contains a greater proportion of muscle than that of their lighter counterparts - presumably occasioned by the greater physical effort required in moving the obese body around (James, 1985; Durnin, 1988). If this is the case it could provide at least part of the explanation for the low BMR/kg FFM found in some obese subjects (Ravussin *et al.*, 1982; James, 1985; Weststrate, 1989). Similarly, Weststrate's (1989) finding that BMR/kg FFM is lower in heavier men compared to lighter women could result from a greater proportion of muscle making up the larger FFM of men.

The potential importance of differences in the contribution of muscle in determining BMR per kg of lean tissue is illustrated by the high value for BMR/kg in patients who have undergone substantial weight loss in illness where preferential muscle wasting is common (Roza & Shizgal, 1984). Similarly, Montgomery (1963) suggested that the high BMR in children recovering from protein-energy

malnutrition (PEM) may be partially due to the relatively large proportion of internal organs compared to muscle making up the FFM.

The finding by Doré *et al.* (1982) and McNeill *et al.* (1987) that in relation to the FFM, BMR is not constant with age could also result from differences in the composition of the FFM. Cohn *et al.* (1980) have suggested that the muscular component of the FFM is preferentially reduced in the ageing process. Differential rates of atrophy of the components of the fat free tissue could produce changes in the composition of the FFM and consequently in BMR in relation to FFM.

Differences in BMR of individuals with a similar mass of fat free tissue might also result from differences in the proportions of active and inactive tissue making up the FFM.

The above hypotheses are based on the premise that the composition of the FFM in healthy adults is variable. In first making the case for the utility of FFM as a metabolic reference standard, Miller and Blyth (1950) suggested that the FFM "should be relatively constant in composition and incorporate, as a constant fraction, the 'active tissue masses'". Most common techniques for estimating body fat and FFM, including densitometry, whole body counting and total body water measurement, also rely on the assumption that the composition of the FFM does not alter between individuals. It therefore becomes necessary to ask the question, does the composition of the FFM vary, and if so does it vary in such a way as to explain some of the differences observed in BMR ?

Is the Composition of the FFM Constant ?

Direct evidence from cadaver analysis is extremely scarce. The literature reveals only four reliable adult human dissections for which the composition of the FFM is known (Mitchell *et al.*, 1945; Forbes *et al.*, 1953, 1956). Nevertheless even such limited data shows quite clearly that the relative proportions of the tissues and organs making up the FFM are far from fixed (Table 1.3).

Table 1.3

Variations in the proportions of the organs and tissues occupying the FFM.

	% of FFM Range	CV (%)
Muscle	34.9% - 50.6%	15.9%
Bone	14.1% - 16.4%	6.7%
Liver	2.9% - 3.9%	12.1%
Kidneys	0.58% - 0.72%	9.8%
Heart	0.65% - 0.89%	13.0%

CV, coefficient of variation.

Complied from data of Mitchell *et al* .(1945) & Forbes *et al* .(1953), (1956)

Although the values for the heart should be viewed with some caution since three of the four men died of cardiovascular related illness there is no reason to suppose that the proportions of the other tissues and organs were abnormal. In these four men the muscular component of the FFM would seem particularly variable.

In 1984 Clarys, Martin & Drinkwater (the Brussels study) shed further light on the variability of the lean compartment of the human body with the dissection and analysis of a further twenty-five cadavers (thirteen females and twelve males). The Brussels study did not include fat determinations and therefore provides no direct information on the composition of the FFM. They considered instead however, the adipose tissue-free mass (ATFM) which consists of body mass less all dissectible adipose tissue (analogous to the LBM). While the composition of the ATFM and FFM are not identical, compositional changes in one will clearly be reflected in the other. Again, variability in the relative proportions of muscle and bone between individuals was very much in evidence (Table 1.4)

The cadavers analysed in the Brussels study were elderly (mean age, 76 years) and this may have some bearing on the variability of ATFM. The high coefficient of variation of bone in the female subjects (16.8%) may reflect differential degrees of osteoporosis; a relatively common condition which affects women primarily after the menopause. It is possible that in a younger population the variation in the proportion of bone may not have been so pronounced. However, this does suggest that ageing may have an important effect on the composition of the FFM and potentially therefore on BMR in relation to the FFM.

Clarys & Martin (1985) also present data from twelve 19th century dissections (three female, nine male) for which gross tissue weights were reported and hence the composition of the ATFM known. Muscle was found to range from 33.1% to 54.0% of the ATFM (CV 11.5%), bone from 19.4% to 23.0% (CV 6.2%).

Table 1.4

Variations in the proportion of muscle and bone occupying the adipose tissue-free mass (ATFM) of the Brussels study cadavers.*

		% of ATFM Range	CV (%)
Female cadavers	muscle,	41.9% - 54.8%	10.0%
	bone,	17.4% - 25.7%	16.8%
Male cadavers	muscle,	45.3% - 59.4%	8.3%
	bone,	16.3% - 24.8%	12.0%
Overall range	muscle,	41.9% - 59.4%	8.8%
	bone,	16.3% - 25.7%	12.6%

CV, coefficient of variation

* Clarys, Martin & Drinkwater (1984).

When all the above data are combined the coefficient of variations for the components of the ATFM are as follows:

Muscle,	female	8.3%
	male	11.6%
Bone,	female	11.7%
	male	10.0%

Clarys *et al.* (1985) suggest that variability in the proportion of muscle and bone is likely to be even greater. They echo Bakker and Struikenkamp's (1977) comment that the amount of direct cadaver data available can "merely suggest an order of magnitude for the inter-individual variation that may be expected".

Further reports in the literature regarding the extent of the variability in the proportions of bone and muscle making up the FFM are essentially limited to indirect measurements. Again these suggest that variation is large - further analysis of some data presented by Bisdee *et al.* (1989) suggested that the percentage of the FFM occupied by muscle in women (estimated by creatinine excretion) ranged from 33% to 63% - these will however, obviously include a component related to errors in estimation.

The above, allbeit limited data, suggests that bone may account for between about 14% to 26% of the FFM and muscle from 30% to 60%.

Blehnke (1958) reports that the coefficient of variation for the weights of organs such as the liver, heart and kidneys is usually in the range 10% to 14%. Greenwood & Brown (1913) suggest that the viscera are even more variable than this, reporting coefficients of variation for liver, kidney and heart weight of some 20%. Data on organ weight in relation to the FFM however, is extremely sparse and essentially seems to be limited to the four cadavers discussed above (Mitchell *et*

al.. 1945; Forbes *et al.*, 1953, 1956). These would suggest that organ size does vary in relation to the FFM, perhaps by around 10%.

Some evidence for systematic variations in the composition of the FFM:

As discussed above, Lawrence *et al.* (1988) have proposed that the differences they observed in BMR/kg FFM between light and heavy individuals may result form systematic differences in the composition of the FFM; a decline in 'active' tissue and a concurrent increase in 'inactive' tissue. Further analysis of the cadaver data presented by Clarys *et al.* (1985) provided the opportunity to investigate the possibility that the muscle and bone content of the ATFM, might vary in a systematic way.

Figure 1.1A clearly shows that as ATFM increased so too did the proportion of muscle it contained ($r = 0.76$, $p < 0.001$, $n = 25$) lending some support to Lawrence *et al.'s* suggestion that individuals with a large FFM are more muscular. However, in an elderly population such as this, the tendency may be exaggerated, since muscle lost as part of the ageing process (Forbes & Reina, 1970; Tzankoff & Norris, 1978; Cohn *et al.*, 1980) is likely to result in a reduction in the fat-free or adipose tissue-free mass. The male cadavers generally had larger ATFMs than the females and therefore proportionately more muscle. This may go someway to explaining the lower BMR/kg FFM Weststrate (1989) reported in males compared to females. However, the regression analysis revealed that once differences in ATFM had been taken into account sex had no influence on the percentage of muscle. In other words, the single regression line shown described the relationship between ATFM and percentage muscle in both males and females. This implies that at a given ATFM men and women had the same amount of muscle. Intuitively one might have expected men to have more, but no adequate data exist against which to compare this observation. It should perhaps be

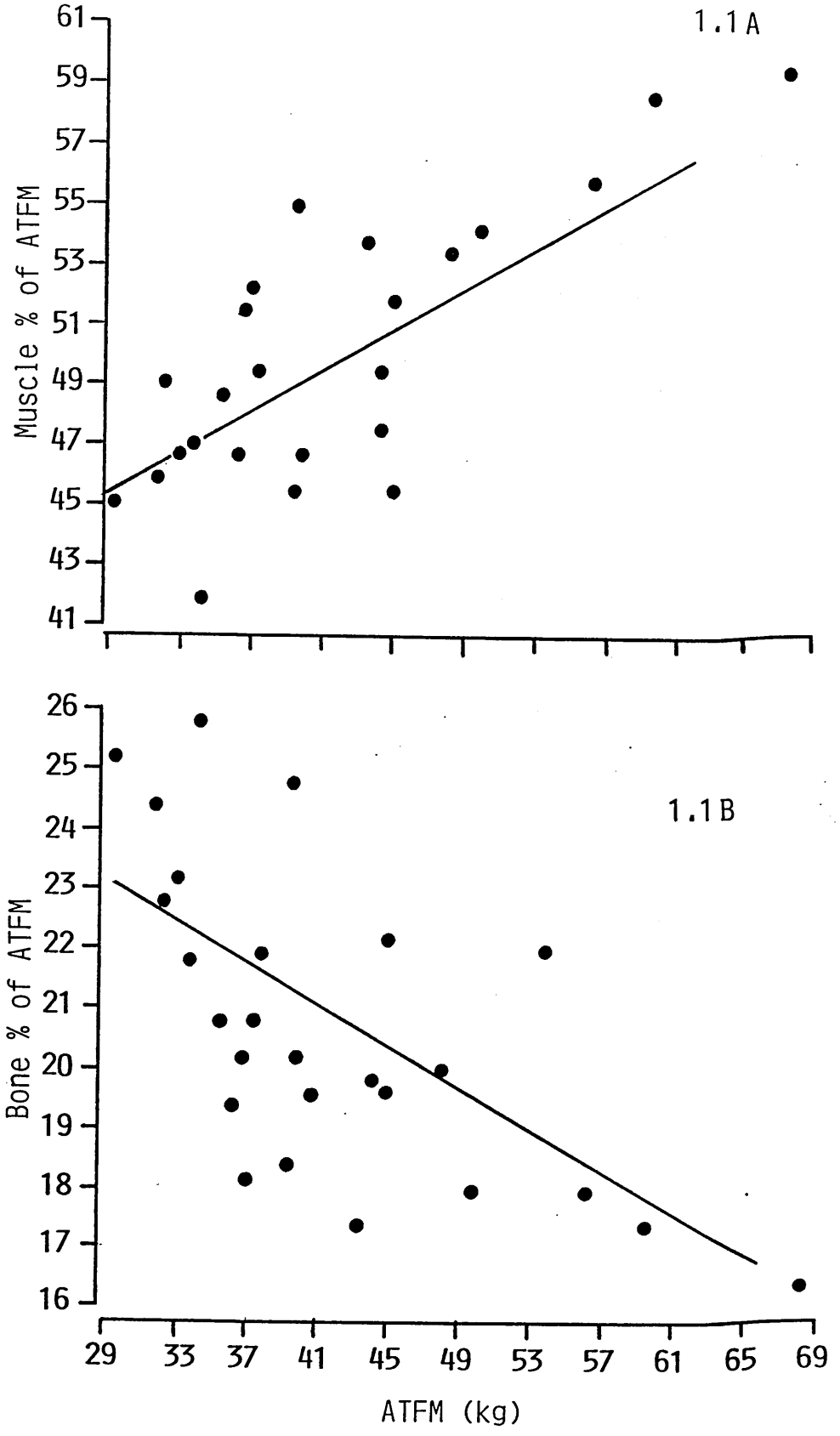

Figure 1.1
Scatterplots relating the percentages of muscle and bone in the adipose tissue-free mass (ATFM) of the Brussels study* cadavers with ATFM.

* Clarys et al. (1984)

remembered that the advanced ages of the cadavers may have some bearing on this and also the numbers involved are relatively small.

In contrast to muscle, the skeleton was found to form proportionately less of the ATFM as ATFM increased ($r = - 0.65$, $p < 0.001$, $n = 25$) (See Figure 1.1B). Once again however, the relationship did not differ between males and females. Since both muscle and bone have relatively low metabolisms these changes may tend to offset one another other with respect to average metabolic rate of the FFM.

The literature does not appear to contain any data regarding the possibility that organ size may vary in a systematic way with FFM in man. Some early data from a study by Greenwood and Brown (1913) however, suggests that in relation to body weight at least, the viscera account for a proportionately smaller fraction in heavy compared to light individuals. Greenwood and Brown recorded body mass and liver, kidney, heart and brain weight in seventy nine male cadavers between the ages of twenty-five and fifty-five years. In general the men were in good health before death, in most cases due to accident, and body composition would therefore be expected to be relatively normal. Further analysis of the data presented by these workers reveals a clear decline in the proportion of body weight occupied by the four organs as weight increases ($r = - 0.74$, $p < 0.001$) (Figure 1.2). However, since no fat determinations were included in Greenwood and Brown's study the possibility that changes in organ size relative to body weight were a reflection of concomitant changes in percentage fat cannot be ruled out. Indeed in cattle, Kraybill *et al.* (1954) have established that heart, liver and kidney weight increase in direct proportion to lean body mass.

Ho *et al.* (1980) investigated the relationship between brain and body weight in 1,261 adult cadavers. They found a positive relationship between the two. However, proportionately brain weight made up a smaller fraction of total body mass as weight increased. Again, increases in weight may reflect increases in

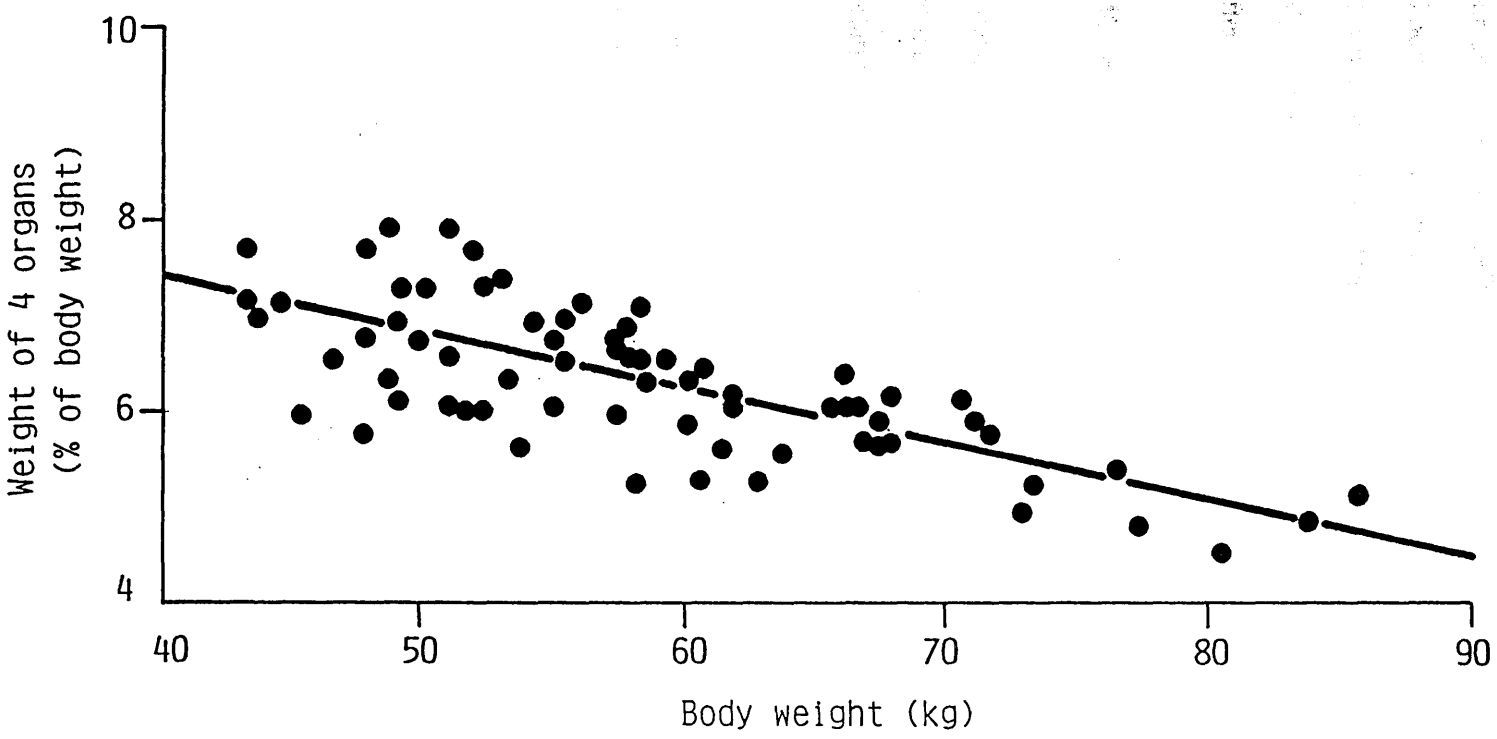

Figure 1.2
Scatterplot relating organ weight (heart, liver, kidney & brain) to body weight in the Greenwood & Brown (1913) cadavers.

body fatness, and the relative decrease in brain weight can not necessarily be construed to mean that the brain forms a smaller proportion of the lean part of the body. Dekaban and Sadowsky (1978) have also looked at the relationship between brain size and body weight. Their data, derived from almost 5000 cadavers, seems to show that for both males and females up until about 55 years the ratio of brain weight to body weight decreases very slightly but has a tendency toward lower values thereafter.

METABOLIC ACTIVITY OF THE TISSUES.

It has been proposed that in addition to depending on the mass and relative proportions of the various organs and tissues in the body, BMR has a second major component relating to the metabolic activity of the tissues, to the rate of energy utilisation by the basic energy demanding processes at cellular level (James, Dauncey & Davis, 1979). Differences in this component will be manifest in differences in metabolic rate per unit weight of tissue. The idea that BMR is, in part, dependant on the metabolic activity of the tissue stems largely from observations of the apparant metabolic changes which accompany under- or over-feeding. A fall in BMR is an early response to energy shortage (Keys *et al.*, 1950; Ferro-Luzzi *et al.*,1990). The decline precedes any measureable change in body weight and this has been taken to indicate that in response to the energy deficit a change in the rate of cellular thermogenesis has occured; the metabolic activity of the tissues has declined (Shetty *et al.*, 1979). Moreover, as energy restriction proceeds and weight is lost, a decline in BMR has been reported which exceeds that predicted from loss of lean tissue alone (eg. Keys *et al.*, 1950; James *et al.*, 1978; Bessard *et al.*, 1983; Barrows & Snook, 1987). Whilst it is possible that this observation and the initial fall in BMR may to some extent reflect a change in the composition of the FFM, for example a more rapid loss of metabolically active

tissue, it is generally accepted that some degree of metabolic adaptation does occur in response to energy restriction (James, 1987). Much more controversially, it has also been suggested that an alteration in the metabolic activity of the tissues takes place with over-feeding, whereby energy expenditure increases over and above that predicted from the increased tissue mass to promote the dissipation of excess calories (Sims *et al.*, 1976; Schutz *et al.*, 1982).

The metabolic activity of the tissues is to some extent thought to be under hormonal control. Both thyroid hormones and catecholamines have been implicated in the regulation of cellular thermogenesis.

Certainly, there can be little doubt that thyroid status affects metabolic rate. A classical symptom of hyperthyroidism is of course an elevated BMR, in hypothyroidism the converse is true and BMR is depressed. Until relatively recently in fact, measurement of basal oxygen consumption was an integral part of the diagnosis of thyroid disease. 3,5,3'-triiodothyronine (T_3), mainly produced by peripheral deiodination of thyroxine (T_4), (the inter-relationships between the thyroid hormones are shown in Figure 1.3) is the most biologically active of the thyroid hormones and is known to be a thermogenic agent (Himmus-Hagen, 1983; Danforth & Burger, 1984; Gelfand *et al.*, 1987). The decline in BMR which accompanies energy restriction is associated with a fall in the serum concentration of T_3 and an increase in its inactive analogue, reverse 3,3',5-triiodothyronine (rT_3), thyroxine levels show little change (Bray, 1969; Vagenakis *et al.*, 1977; Jung *et al.*, 1978; O'Dea *et al.*, 1982; Acheson & Burger, 1980 & Mathieson *et al.*, 1986; Mansell & Macdonald, 1988). Consequently, it has been suggested that the decline in BMR evident in semi-starvation may be mediated by the reduction in T_3 levels. This is supported by the observation that physiological doses of exogenous T_3 are able to prevent the fall (Bray *et al.*, 1973; Shetty *et al.*, 1979; Rozen *et al.*, 1986). The role for T_3 in the regulation of metabolic rate is not entirely clear cut however. For example Acheson and Burger (1980) induced a fall in T_3 in

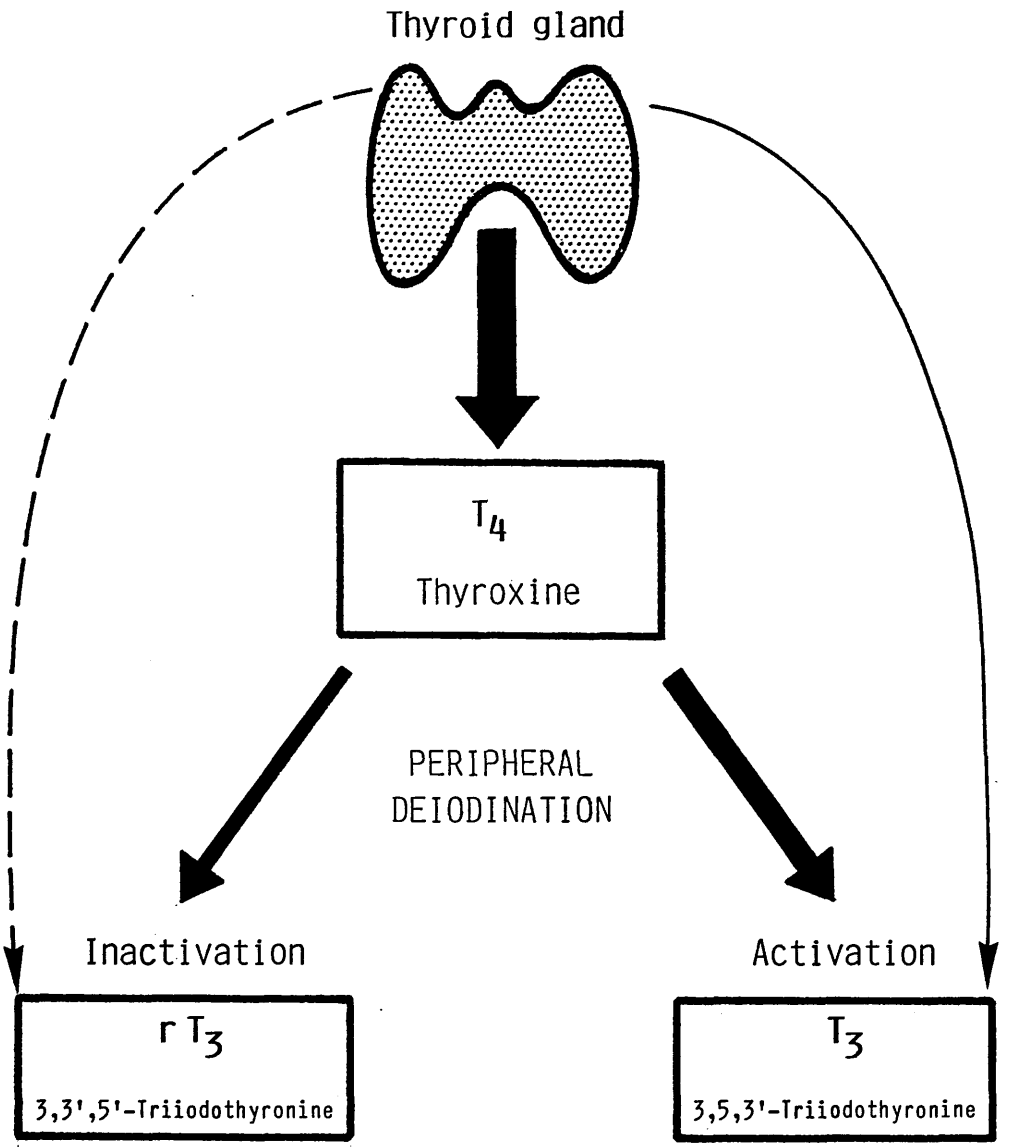

Figure 1.3
Thyroid hormone production and metabolism.

euthyroid subjects by administration of iopanoic acid which partially blocks the conversion of T4 to T3 but failed to observe a fall in metabolic rate. Similarly Serog *et al.* (1982) produced changes in T3 levels by manipulation of carbohydrate intake in a group of nine subjects but report no significant change in oxygen consumption. Despite a decline in T3 levels, Shetty *et al.* (1979) were able to prevent RMR falling on semi-starvation by administration of the catecholamine precursor, levodopa.

As alluded to earlier, catecholamines are also thought to play a regulatory role in cellular thermogenesis. Much research effort has been directed at the ability of catecholamines to produce an increase in metabolic rate. There is little doubt that catecholamines are thermogenic. Numerous studies in animals and now also in man have shown that acute or long-term administration of adrenaline, noradrenaline or adrenergic agonists produce an increase in metabolic rate (Steinberg *et al.*, 1964; Havel *et al.*, 1964; Sjostrum *et al.*, 1983; Scheidegger et al., 1984; Fellows *et al.*, 1985; Staten *et al.*, 1987; Connacher, 1988). Increased sympathetic nervous system activity is also associated with an elevated metabolism (Engelmen *et al.*, 1964; Welle *et al.*, 1980; Landsberg & Young, 1983). As a consequence of their calorigenic action, catecholamines have been implicated in the thermic response to cold exposure and to food, although their role in the latter is not without contention (for review see Landsberg & Young, 1983). Like T3, levels of plasma or urinary noradrenaline, and so by implication sympathetic nervous system activity, have been found by most investigators to fall on energy restriction in association with the decline in BMR (Shetty *et al.*, 1979; DeHaven *et al.*, 1980; O'Dea *et al.*, 1982; Sowers *et al.*, 1982). The exception being a study by Mansell & MacDonald (1988), which reported considerable metabolic adaptation to underfeeding but could find no evidence that these changes were associated with a decrease in sympathetic nervous system activity. Urinary excretion of 4-hydroxy-3-methoxy mandelic acid (HMMA), the principle metabolite of catecholamines and indicative of the rate of

catecholamine turnover, has also been found to be reduced in conditions of energy deprivation (Jung *et al.*, 1979 & 1980; Shetty *et al.*, 1979). Moreover, administration of the catecholamine precursor, levodopa can prevent the decline in BMR associated with semi-starvation (Shetty *et al.*, 1979). This would certainly suggest a role for the catecholamines in the determination of BMR. Further evidence for their involvement was provided by Jung and colleagues in 1980. These workers found that administration of the ß-adrenergic antagonist, propranolol, to obese subjects on a weight maintenance diet resulted in a significant reduction in BMR. This suggested to Jung and co-workers that BMR has a component which is adrenergically mediated. Scheidegger *et al.* (1984) also found a reduction in resting energy expenditure with acute propranolol administration. However, several groups have reported that acute ß-adrenergic blockade with propranolol has no effect on BMR (Acheson *et al.*, 1983; Welle & Campbell, 1983; Defronzo *et al.*, 1984; Seaton *et al.*, 1984; Vernet *et al.* 1987; Gelfand *et al.*, 1987).

It is suggested that T3 and noradrenaline probably interact to influence metabolic rate. At least part of the thermogenic effect of the catecholamines is thought to be due to their ability to increase circulating levels of T3 by stimulating the peripheral conversion of thyroxine to T3 (Galton, 1965; Rothwell *et al.*, 1982; Scheidegger *et al.*,1984). Propranolol reduces this conversion and results in a decline in T3 concentrations (Lotti *et al.*, 1977; Eisenstein *et al.*, 1978; Jung *et al.*, 1980; Jones *et al.*, 1981). However, it is likely that noradrenaline also has a direct action on a thermogenic mechanism. The results of Shetty *et al.'s* study (1980) suggests that a major action of propranolol in lowering RMR occured by direct inhibition of an energy requiring mechanism normally responsive to catecholamines rather than by its effects on peripheral thyroid metabolism. Catecholamines and thyroid hormones are also known to interact at cellular level, where T3 may modulate a thermogenic action of noradrenaline most probably through regulation of the catecholamine stimulated adenylate cyclase - cAMP system (see below).

The precise mechanisms by which thyroid hormones and catecholamines may regulate metabolic rate are not entirely understood. Presumably they must act on one or more of the energy requiring processes at cellular or molecular level. Alterations in the rate of protein turnover, substrate cycling and ionic pumping have all been implicated. Certainly, all the above systems are sensitive to thyroid hormones. Changes in the activity of the Na^+/K^+ pump in response to alterations in thyroid status have been particularly well documented. An increase in thyroid hormone produces an increase in both Na^+/K^+ pump number and activity in a variety of tissues including skeletal muscle, liver and kidney (Edelman & Ismail-Beigi, 1974; Clausen, 1986; Simmons *et al.*, 1986; Everts & Clausen, 1988). Thyroid hormones have also been shown to increase the rate of membrane Ca^+pumping, at least in skeletal muscle (Everts *et al.*, 1989). Both protein turnover (Brown & Millward, 1980) and some substrate - 'futile' - cycles increase in association with increased levels of thyroid hormones (Sestoft, 1980; Shulman *et al.*, 1985). The triglyceride-fatty acid cycle for example, is accelerated in several different tissues taken from hyperthyroid animals (Sestoft, 1980) and Shulman *et al.* (1985) have demonstrated that substrate cycling between glucose and glucose-6-phosphate and between fructose-6-phosphate and fructose-1,6-diphosphate is decreased in hypothyroidism and elevated in hyperthyroidism compared to the euthyroid state.

Another potential site of metabolic regulation by thyroid hormones is at the level of the mitochondria themselves. An increase in thyroid status is thought to bring about a change in the properties of the mitochondria such that their rate of respiration increases even though they remain in a coupled state (Himmus-Hagen, 1983; Brand & Murphy, 1987; Dauncey, 1990). This may be brought about through a thyroid induced change in membrane fatty acid or polypeptide composition or by a direct interaction of T_3 with receptors in the mitochondria (Himmus-Hagen, 1983; Brand & Murphy, 1987). In addition, thyroid hormones

are also reported to increase total mitochondrial mass and thereby increase oxygen consumption, although the way in which mitochondrial proliferation is brought about is not clear (Himmus-Hagen, 1983; Brand & Murphy, 1987).

Like thyroid hormones, catecholamines are also known to influence substrate cycling (Newsholme, 1985) and have a direct action on the Na^+/K^+pump (Phillis & Wu, 1981; Himmus-Hagen, 1983; Clausen, 1986). In addition, it has been suggested that at least part of the calorigenic effects of the catecholamines are linked to their lipolytic actions. The catecholamines are potent lipolytic agents, activating the principle lipolytic enzyme - hormone-sensitive lipase - via interaction with ß-1 adrenergic receptors and stimulation of the adenylate cyclase - cAMP system, and thereby inducing a prompt rise in plasma free fatty acids (FFA). As circulating FFA levels rise, fatty acid uptake and oxidation by the tissues are increased since these events in part depend on blood levels (Eisenstein & Singh, 1980). Increased rates of FFA mobilisation and oxidition have been shown by several investigators to be associated with an increase in metabolic rate (Havel *et al.*, 1964; Steinberg *et al.*, 1964; Englemen *et al.*, 1964; Jung *et al.*, 1981; Scheidegger *et al.*, 1984). However, Havel *et al.* (1964) observed that reducing levels of FFA by injections of nicotinic acid produced no change in BMR, which casts some doubt on the importance of FFA availability in determination of metabolic rate under ordinary resting conditions. Moreover, in starvation lipolysis is increased (Engfeldt *et al.*, 1982) and yet BMR declines. Thyroid hormones are known to modulate the adenylate cyclase - cAMP system at several different sites - including alteration of ß-receptor numbers, augmentation of receptor coupling to the adenylate cyclase system and direct modulation of the activity of regulatory and catalytic components of the cyclase - and thereby have the potential to influence catecholamine stimulated lipolysis and FFA oxidation - and indeed other adrenergic effects mediated through this system (Williams *et al.*, 1977; Tsai *et al.*, 1978; Jung *et al.*, 1979; Kunos, 1981; Malbon & Greenberg, 1982; Lansberg & Young, 1983;

Lefkowitz *et al.*, 1984). Allied to this, is the observation of accelerated FFA turnover in hyperthyroidism and a reduced rate in hypothyroidism (Hagenfeldt *et al.*, 1981; Kunos, 1981).

At the present time there is little evidence in man that either thyroid hormones or catecholamines can regulate energy expenditure by altering the efficiency of mitochondrial oxidative phosporylation, so called uncoupling (Danforth & Burger, 1984; Dauncey, 1990).

Assuming that BMR is to some extent dependant on metabolic activity at celluar level, and the evidence presented above would seem to point to this, differences in this component between individuals represent a potential source of variation in BMR. The extent to which subtle differences in the control of celluar thermogenesis are able to explain differences in BMR at a given FFM however, does not appear to have been investigated.

Bogardus *et al.* (1986) found that in Pima Indians 11% of the variance in RMR, independent of the effects of FFM, sex and age, could be attributed to family membership. The cause of these familial differences was not elucidated, but Bogardus and co-workers suggest that they may relate to differences in the efficiency of energy requiring processes between families. The possibilities suggested include variation in the activities of the sympathetic nervous system, cellular pumping of Na^+ and K^+, rates of protein turnover and gluconeogenesis.

Since the metabolic activity of the tissues is thought to be under hormonal control it seems reasonable to postulate that some of the differences in BMR in relation to the FFM may be related to differences in the levels of thermogenic hormones between individuals or in tissue responsiveness to them.

Data regarding the effect on BMR of differences in thyroid status between normal, clinically euthyroid, individuals is extremely scanty. However, for the most part, the limited reports that are available do seem to indicate that variation in thyroid hormone levels within the normal physiological range may play a part in

explaining differences in BMR between individuals. Bernstein *et al.* (1983) measured free T3 (FT3) levels in a group of 154 women and observed a weak but significant positive correlation ($r = 0.2$, $p < 0.05$) between this index and resting metabolic rate. MacRitchie (1988) found a similar relationship between BMR and both serum T3 and T4 in a small group of euthyroid control subjects. BMR was expressed as the % deviation from standard values predicted using the Mayo Clinic equations (Boothby, Berkson & Dunn, 1936); the greater the positive deviation between the actual and predicted BMR, the greater the serum T3 or T4 concentration ($r = 0.47$, $p < 0.01$ & $r = 0.5$, $p < 0.01$ respectively). This study is particularly interesting in that by expressing the results in relation to values predicted on the basis of surface area some degree of 'normalisation' for body size was achieved. In this group then, there is an indication that at a given body size the higher the T3 or T4 levels, the higher the BMR. Danforth (1983) reports a positive correlation ($r = 0.59$, $p < 0.01$) between free T3 serum concentrations and the number of calories per kg FFM required a day for 16 subjects to maintain weight during a three week stay in a metabolic unit. Since under the circumstances imposed on the metabolic ward BMR is likely to make up the largest proportion of daily energy expenditure it may well be that the correlation is also illustrative of the relationship between T3 and BMR/kg FFM. If this is indeed the case, it would seem to suggest that high levels of T3 are associated with a high BMR/kg FFM. In a group of patients following a 1000 kcal/day diet Moore *et al.* (1980) observed that the patients who lost the most weight were, not surprisingly, those who had the highest RMRs, but that these individuals also had the highest T3 levels. They took this to imply that in these patients T3 had a role in the regulation of RMR.

Data regarding differences in sympathetic nervous system activity and catecholamine levels between individuals and the part they might play in explaining some of the variability in BMR is even more scanty. Weststrate (1989) observed that high BMRs/kg FFM were associated with high rates of fatty acid oxidation.

Since fatty acid oxidation is enhanced by increased catecholamine levels he suggests that this may reflect a greater degree of sympathetic activity in these individuals and provide a possible explanation for the high BMR/kg FFM.

AIMS

The studies presented in this thesis sought to examine some of the causes of variation in basal metabolic rate. The respective roles of differences in body composition and hormonally mediated metabolic activity were explored.

CHAPTER 2

GENERAL METHODOLOGY

MEASUREMENT OF BODY COMPOSITION.

All measurements were made by one observer, the author. They were all performed during the morning when the subjects were in a fasted state.

Body Weight.

Weight was recorded using an Avery beam balance (model no. 3302). The subjects were clothed only in underwear, a swim suit or a light dressing gown (the weight of which was subsequently deducted) and were weighed after emptying their bladders. Readings were taken to the nearest 0.1 kg. The weighing scales were calibrated frequently.

Height.

Height was measured using a wall stadiometer (Holtain Ltd. Grymych, Dyfed, UK). Each subject stood (without shoes) on the horizontal platform of the stadiometer, with heels together and arms by their side, stretching upward to their fullest extent. The subject's back was as straight as possible against the vertical bar and the so-called Frankfort plane (Weiner & Lourie, 1981) was checked to be horizontal. The subjects were asked to breathe in deeply to make them stretch up, and the head-bar brought down gently on to the head. The subject's heels were always watched to make sure they remained on the floor. Readings were taken to the nearest mm.

Circumferences.

Measurement of circumferences in various parts of the body give an indication of muscle mass. All circumference measurements were made using a

flexible steel tape (Harpenden Anthropometry tape (2m) Holtain Ltd. Grymych, Dyfed, UK). The tape was placed firmly around the position of measurement and the reading was recorded to the nearest mm.

1. Upper Arm: - the subjects arm hung relaxed, just away from their side. The circumference was measured horizontally at the same level as the triceps skinfold thickness (see below).

2. Calf: - the subject sat on the table with their legs hanging freely and the back of the knee touching the table. By moving the tape up and down the leg the maximum horizontal circumference was located and measured.

3. Buttocks: - the maximum circumference over the buttocks was measured with the subject standing with their feet together.

4. Thigh: - the subject stood with their feet slightly apart and with their weight distributed evenly on both feet. The measurement was taken with the tape placed around the thigh horizontally with its top edge just under the gluteal fold.

5. Waist: - this was measured midway between the lower rib margin and the iliac crest. The subjects were asked to breathe out gently at the time of the measurement to prevent them from contracting their muscles or holding their breath.

Bone Diameters.

Bone diameter measures may give some indication of 'frame size'. Wrist and knee breadths were measured using a sliding caliper (Holtain Ltd., Grymych, Dyfed, UK.). Biacromial and bi-iliac diameter measurements were made using a

long arm anthropometer (Holtain Ltd., Grymych, Dyfed, UK.). In all cases pressure was exerted to compress the tissues overlying the bone. Measurements were recorded to the nearest mm.

1. Ulna or wrist breadth: - the breadth was taken across the styloid processes (oblique to the long axis of the arm).

2. Bicondylar femur or knee breadth: - the subject sat on a table with knees bent to a right angle, and the width across the outermost parts of the lower end of the femur was measured.

3. Biacromial diameter: - to give maximum shoulder width the subject stood with their shoulders relaxed. Standing behind the subject, the outside edges of the acromion processes were located which could be felt as ridges just above the shoulder joints. The two arms of the anthropometer were then placed along the lateral boarders of the acromion processes and the measurement taken.

4. Bi-iliac diameter: - the subject stood with their heels together and the anthropometer arms were brought into contact with the iliac crests which gave the maximum diameter. Strong pressure was applied to the anthropometer blades to push aside any fat covering the bone. This measurement was taken standing behind the subject.

Methods of Measuring Body Fat Content:

All the methods which will be described for estimating body fat content rely on one or more assumptions, usually with regard to the constancy of composition of the FFM. As already discussed the contention that the FFM is of constant composition is not entirely justified. However, the validity and

consequences of these suppositions will not be discussed in great depth here, when describing the techniques, but rather in a subsequent chapter in the light of the results the various methods yielded.

(1) Densitometry.

The basic assumption made when estimating body fat by densitometry is that the body consists of two compartments, fat and FFM, which have distinctly different and constant densities. At body temperature FFM has been estimated to have an average density of about 1100 $kg.m^{-3}$ (Behnke et al, 1942), while fat (lipid) has a significantly lower density of 900 $kg.m^{-3}$ (Keys & Brozek, 1953). As Benhke first realised, a determination of body density can therefore provide an estimate of the relative proportions of fat and FFM.

$$\text{Whole body density} = \frac{\text{whole body mass}}{\text{total body volume}}$$

$$\text{Total body volume} = \frac{\text{fat mass}}{\text{fat density}} + \frac{\text{fat-free mass}}{\text{fat-free density}}$$

There are several methods available for measuring whole body density. Probably the most widely used is the underwater weighing technique, and this was the method employed in the studies presented here. Since the density of the body is equal to its weight per unit volume, the object of this method is really to determine body volume (weight is easily measured). Archimedes' principle is employed, which states that the volume of an object submerged in water equals the volume of water the object displaces. The subject is weighed in air and then weighed again when totally submerged in water. The difference between the two, corrected for the density of the water, is equal to the volume of the body.

$$\text{Volume of the body} = \frac{\text{weight in air - weight in water}}{\text{density in water}}$$

However, this volume will include the volume of gas present in the lungs and gastrointestinal tract at the time of submersion. The subject is usually asked to take a maximal expiration before going underwater, however, the contribution from residual lung volume is still sizeable (1-2 litres) and it is therefore necessary to measure and deduct this component. The volume of gastrointestinal gas is usually considerably smaller (about 100 ml) and is not measured.

$$\text{Body density} = \frac{\text{weight in air}}{\text{body volume - residual volume}}$$

Body fat content can then be estimated according to Siri's equation (1956):

$$\text{Body fat (kg)} = \frac{4.95}{\text{density}} - 4.50 \times \text{weight}$$

or

$$\%\ \text{fat} = \frac{495}{\text{density}} - 450$$

To recap, determination of density by the underwater weighing method requires three measurements; weight in air, weight underwater and residual lung volume.

Weight in air:

The subject was weighed on an Avery beam balance as described above.

Underwater weight:

The apparatus used for this part of the procedure consists of a tank (1.38m x 1.19m x 1.19m) containing water at 36.5°C. A canvas seat on a metal frame is suspended in the tank by means of nylon cords, attached first to a wheatstone bridge resistance type strain gauge (Western Load Cell Co. Ltd. Scotland) and

then to the ceiling. The strain gauge produces a signal according to the weight applied to it (ie. the weight of the subject sitting in the chair). This signal is diverted to a load cell digital display, calibrated to read the weight in kg.

After being weighed in air, the subject (wearing a swim suit) climbed into the tank and positioned themselves on the chair. The subject held on to the sides of the chair and their feet rested on a cross bar just below the seat. Their heads were above water at this stage. (See Figure 2.1). The procedure was explained to them and once they felt relaxed and confident they began the following protocol:

1. A nose clip was fitted.
2. The subject was asked to make a full expiration.
3. With the mouth firmly closed and still holding their breath, the subject bent gently forwards until the head was completely immersed in water. (See Figure 2.2).
4. This positon was maintained, keeping as still as possible, until the digital display on the load cell stabilised (about 10-15 seconds). The underwater weight was recorded at this point.
5. The subject was then given a signal, banging on the side of the tank, to slowly surface.

Residual lung volume:

This was determined using the nitrogen wash-out technique (Durnin & Rahaman, 1967; Durnin & Womersley, 1974).

1. After the subject had surfaced a mouth piece, attached to one limb of a three way tap, was placed in between the lips. The other end of the tap was connected to a rubber anaesthetic bag containing a known volume of pure oxygen (measured using a spirometer). The subject continued to hold their breath until the lips were tightly over the mouth piece.

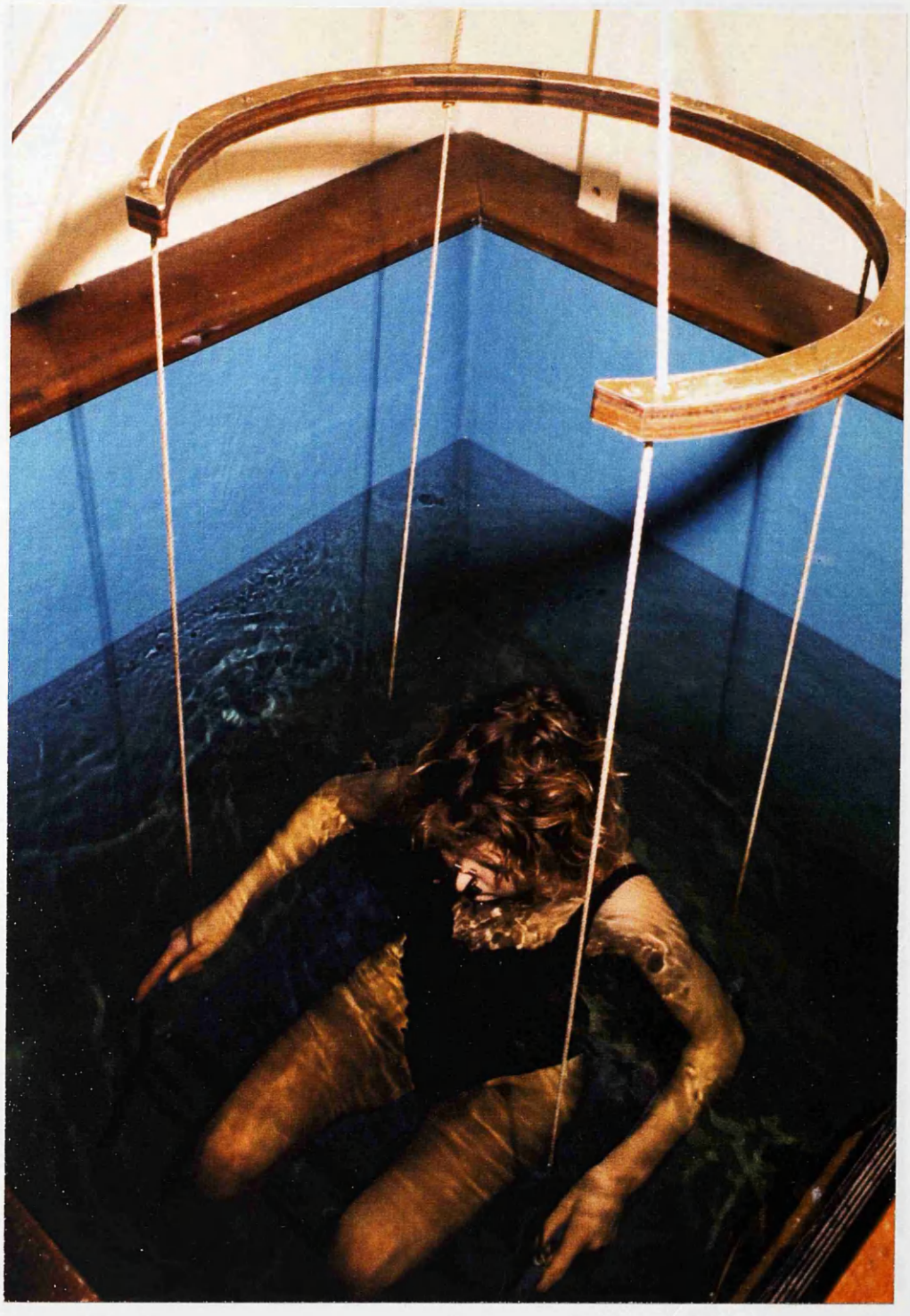

Figure 2.1

Body density by underwater weighing: the subject is shown with her head above the water before the procedure begins

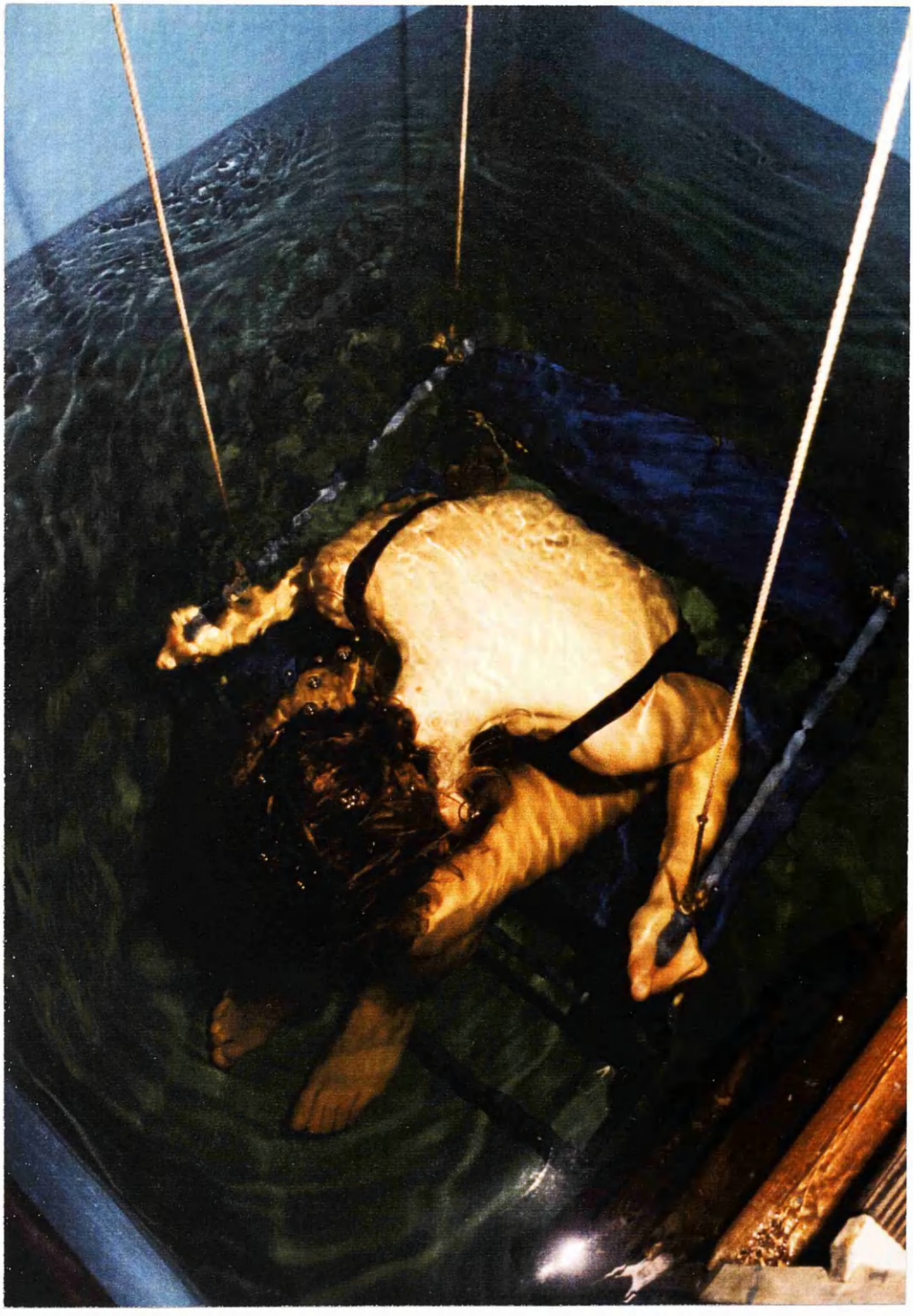

Figure 2.2

Underwater weighing: the subject's whole body is completely submerged

2. The tap was then opened and the subject inhaled deeply from the bag, followed by a large exhalation back into the bag. This was repeated twice more, thereby allowing the nitrogen in the lungs to mix with the oxygen in the bag. At the end of the third expiration the tap was closed.

3. The expired air was then analysed for oxygen and carbon dioxide content, as described later, and nitrogen content determined by difference (ie. % N_2 = 100% - % O_2 + % CO_2).

According to Rhan *et al.* (1949) at the end of the third expiration the gases in the respiratory passages and the lungs are almost in complete equilibrium with those in the anaesthetic bag. The total volume of the system will therefore be equal to residual volume in the lungs (R) plus the volume in the anaesthetic bag (V). The volume of the bag before rebreathing and the amount of nitrogen contained in the bag after rebreathing are known and can be utilised to compute residual lung volume as follows:

Nitrogen content of lungs and bag before rebreathing. = Nitrogen content of whole system after rebreathing.

$$\frac{80}{100} + (R + 25) + n.\frac{V}{100} = \frac{N.}{100}(R + V + 25)$$

'25' is the volume of the three-way tap and 'n' is the volume of nitrogen contaminating the oxygen in the anaesthetic bag. The equation condenses to:

$$\text{Residual lung volume} = \frac{F.N.(V + 25) - 2000 - (V.n)}{80 - N}$$

'F' is a correction factor to account for atmospheric pressure, body temperature, the saturated vapour pressure of air in the lungs and in the spirometer, and for the temperature of the spirometer.

Calculations of residual volume, whole body density and subsequently body fat content were all made using a BBC microcomputer (model B). This ensured that the results of the underwater weighing procedure were obtained quickly while the subject was still in the tank. Routinely two measurements (plus an initial practice run) were made. On the occasions that the body fat content calculated from these measurements differed by more than 3% of body weight a third measurement was made before the subject was allowed out of the tank. The mean of the two, or three, measurements was taken.

This technique obviously requires an extremely high degree of co-operation from the subject. Nevertheless we found that almost all the individuals recruited (95 out of 97) were able to complete the test in a satisfactory manner, even those who were unable to swim.

(2) Total body water (TBW).

The total body water method is based on the finding that stored triglyceride is essentially anhydrous, whereas water occupies a relatively fixed fraction (73%) of the FFM (Pace & Rathburn, 1945). Measurement of TBW will therefore yield an estimate of the relative proportions of fat and FFM.

$$FFM = TBW \times \frac{100}{73}$$

TBW is measured by the administration of a tracer which will mix throughout the total body water pool. The subsequent concentration of the tracer in a sample of body water (eg. saliva, urine or plasma) once equilibrium has been

reached (or the increase in concentration if the tracer is already present in the body) provides an estimate of TBW.

$$C = \frac{D}{TBW} \quad \text{and} \quad TBW = \frac{D}{C}$$

Where 'C' is the concentration or increase in concentration of the tracer, and 'D' the dose given. Several tracers are available, including the isotopes deuterium (D_2), tritium (^2H) and ^{18}O, a stable heavy isotope of oxygen. Tritium has the advantage of being easy to measure by scintillation counting. It is however, radioactive which precludes its general use. $D_2$and ^{18}O on the other hand are naturally occuring, stable isotopes and are therefore preferable for use in man. Deuterium oxide (D_20) was chosen as the tracer for this investigation (Sigma Chemical Co., Poole, Dorset, England).

Dose of deuterium oxide.

The dose of the isotope given to the subjects must be large enough to produce a readily measurable increase in the deuterium oxide concentration of the body, ie. 'C' must be large in relation to the precision with which it can be measured. It was estimated that for analytical error to be < ±1%, an increase in D_2O concentration of at least 180ppm was necessary, this is equivalent to a dose rate of 0.1g D_2O/kg TBW. In practice, to allow a slightly greater margin of error, a dose of 0.12g of D_20/kg TBW was utilised.

To determine the appropriate dose for each subject it was therefore necessary to first make an estimate of their TBW. This was done by using body weight and skinfold thicknesses to estimate FFM, (see below) and assuming TBW to represent 73% of the FFM.

In advance of the study a 40% solution of deuterium oxide had been made up (ie. two parts D_2O to three parts tap water). This had then been used to prepare

individual doses corresponding to range of FFMs. These were sealed and refrigerated until required. This procedure was necessary as deuterium oxide is hygroscopic and as such tends to absorb water vapour from the air. Repeated opening of the stock solution to make up individual doses on a daily basis would have changed the composition of the solution during the course of the study.

On the basis of the subjects estimated TBW the appropriate diluted dose of deuterium was selected and administered to the subject.

Pre-dose saliva sample.

As deuterium is naturally present in body water it is necessary to establish the background level of the isotope before any additional dose is given. This was done by determining the concentration of deuterium in a sample of saliva. Throughout the investigation saliva was taken as the representative body fluid. It was chosen in preference to other examples of body water such as urine, plasma, tears etc as its collection is the least inconvenient and stressful for the subjects. In addition the equilibriation of deuterium with saliva is achieved more rapidly than it is with urine - 2-3 hours compared to 4-6 hours - minimising the time the volunteers were required to spend in the laboratory.

The saliva was collected by wrapping a small piece of cotton wool around a stick and asking the subjects to work this around their mouths until it was 'soggy'. The cotton wool was then transfered, using tweezers to avoid contamination, to a 2ml plastic syringe and the saliva squeezed out into an appropriately labelled 1.5ml sample tube. This procedure was repeated until sufficient saliva had been collected, about 1ml. The sample was then sealed and frozen at -20°C until analysis.

When using saliva it is important to ensure that it is not contaminated with water from either food or drink. This did not present a problem in this study however, as all the subjects were in a fasted state.

Administration of the dose.

1. The dose selected for the subject (inclusive of bottle and lid) was weighed on a balance accurate to 0.01g.

2. The subject then drank as much of the isotope as possible using a plastic drinking straw (the weight of the straw had previously been recorded).

3. The straw was pushed down inside the bottle and the lid put back on. The whole thing was then reweighed.

4. It was then possible to calculate exactly how much of the diluted isotope the subject had drunk. From which, according to the dilution factor and the percentage of deuterium in dosing solution, the amount of deuterium oxide ingested could be computed (see below).

Post-dose saliva samples.

Saliva samples were collected two and three hours after the administration of the dose using the same procedure as described above. Complete equilibration of the deuterium with the body water pool should have been achieved after about two hours. The concentration of D_2O at plateau however, was taken as the mean of the two samples. This approach to some extent allowed for any random fluctuations in D_2O concentration at equilibrium and further, provided confirmation that the plateau stage had infact been reached.

Measurement of deuterium oxide in saliva.

The deuterium oxide concentrations of the saliva samples were determined by isotope ratio mass spectometry at the Scottish Universities Research and Reactor Centre, East Kilbride. This measurement is based on the principle that the

hydrogen atoms in deuterium oxide (^2H_2O) have a different mass from those of normal water (H_2O). Consequently mass spectrometry can elucidate the relative proportions of the two forms of hydrogen in a sample of fluid, in this case saliva. Water from the sample is reduced to hydrogen gas before measurement. Because relatively little deuterium is present in the samples (<500 ppm) there is very little chance of any D_2 molecules forming and it is the ratio of DH/H_2 that is measured. It is then necessary to convert the isotope ratio (in ppm) into a concentration.

Calculation of total body water.

(1) Conversion of ratio to concentration:

R = result in ppm obtained from the mass spectrometer.

$$R \times 10^{-6} = \frac{\text{parts DH}}{\text{parts } H_2}$$

and

$$0.5 \times R \times 10^{-6} = \frac{\text{parts } D_2}{\text{parts } H_2}$$

$$= \frac{\text{parts } D_2O}{\text{parts } H_2O}$$

For measurement of TBW R<600, and ratios can be considered equivalent, ie. the concentration of D_2O in parts D_2O/parts of water can be taken to be $0.5 \times R \times 10^{-6}$.

(2) Conversion of parts to weights:

mol wt $H_2O = 18$, Mol wt $D_2O = 20$

therefore,

$$g\ D_2O = \text{parts } D_2O \times 20$$

$$g\ H_2O = \text{parts } H_2O \times 18$$

$$= \frac{0.5 \times R \times 10^{-6}}{0.9}$$

and the concentration of D_2O in water, in g/kg

$$= \frac{R}{1800}$$

(3) Calculation of TBW:

$$TBW = \frac{d}{C}$$

$$C = \frac{Rs - Rp}{1800}$$

where p and s denote pre- and post-dose samples respectively and

$$TBW = \frac{1800d}{Rs - Rp} \quad \text{equation 1}$$

Calculation of dose.

The deuterium oxide content of the dose solution was measured at the same time as the samples. A sample of the dose ('a' g) was diluted to a total weight of 'W' g with tap water.

From equation 1,

$$W = \frac{1800y}{Ra - Rt}$$

where 'y' is the amount of D_2O in a grams of dose, 'Ra' is the concentration of D_2O in the diluted dose and 'Rt' the concentration of D_2O in the tap water used to prepare the diluted dose.

Re-arranging,

$$y = W \frac{(Ra - Rt)}{1800}$$

and % D_2O in the dose

$$= 100 \times \frac{y}{a} = \frac{W \times (Ra - Rt)}{a \times 18} \quad \text{equation 2}$$

If 'A' g dose are administered, then the weight of the D_2O administered.

$$d = A \times \frac{W \times (Ra - Rt)}{a \times 1800} \quad \text{equation 3}$$

Calculation of results.

From equation 1,

$$TBW = \frac{1800d}{Rs - Rp}$$

The dose, d, was calculated from the weight of the diluted dose consumed and the % D_2O in the dose.

$$\text{Body fat (kg)} = Wt - \frac{TBW}{0.73}$$

$$\% \text{ Body fat} = 100 - \frac{TBW}{0.0073 \times Wt}$$

The use of deuterium oxide as a tracer results in the over-estimation of TBW by about 4%, because some of the deuterium in the dose exchanges with hydrogen atoms in protein and fat molecules. Therefore,

$$\text{True TBW} = \frac{\text{calculated TBW}}{1.04}$$

In the calculation of % fat the correction factor 1.04 was therefore applied,

$$\text{Body fat (kg)} = \text{Wt} - \frac{\text{TBW}}{0.759}$$

$$\% \text{ Body fat} = 100 - \frac{\text{TBW}}{0.00759 \times \text{Wt}}$$

(3) Skinfold Thickness.

Measuring the thickness of folds of skin at various sites on the body gives an indication of the amount of fat located just beneath the skin's surface. Since most of the fat in the body is suggested to be stored subcutaneously (Edwards, 1950) it is therefore possible to make an estimate of total body fat content. Equations for the prediction of body fat from skinfold thicknesses have been developed and would appear to give a relatively good measure of fat content, at least in the populations from which they were derived (Durnin & Womersley, 1974; Jackson & Pollock, 1978; Jackson *et al.*, 1980). The skinfold technique relies on three assumptions. The first is that the ratio of subcutaneous to total body fat is relatively constant. The second, that the sites selected for measurement represent the average thickness of subcutaneous adipose. Thirdly, it is assumed

that the compressibility of the skinfolds is constant. Errors in estimation of body fat arise if these assumptions are violated. Possibly the best established prediction equations are those of Durnin and Womersley (1974). These authors developed regression equations to predict body density, and hence body fat, using the logarithmic transformation of the sum of four skinfolds (biceps, triceps, subscapula and supra-iliac), age and sex. The mathematical transformation of the sum of the skinfold thicknesses is needed because body density is not linearly related to subcutaneous fat. Inclusion of age and gender takes into account differences in fat distribution related to these factors.

The skinfold technique is inexpensive and requires the minimum of equipment, its relatively simple and quick to perform and not difficult to master, all of which make it particularly suitable for use in a field situation. Moreover, in the hands of a trained observer the error is estimating body fat is reportedly only about ±3% of body weight (Durnin & Womersley, 1974).

Measurement of skinfolds in the studies presented in this thesis were made as follows:

Harpenden calipers (Holtain Ltd. Grymych, Dyfed, UK), calibrated to exert a constant pressure of $10g/mm^2$, were used throughout the study. Skinfold thicknesses were always measured on the right side of the body while the subject was standing in a relaxed fashion. The skinfold was picked up between the thumb and forefinger and pulled gently away from the underlying muscle. The calipers were applied to the the fold a little below the point where the skinfold was being held, at exactly the sites described below. The caliper jaws were allowed to exert their full pressure on the skinfold and the reading taken after 2-3 seconds when the measurement began to stabilize. The values were recorded to the nearest 0.2mm. Each skinfold was measured and recorded in triplicate and average value to the nearest 0.5mm taken. A total of four skinfold thicknesses were measured and

body fat content estimated from the sum of the four, using the equations of Durnin and Womersley (1974).

1. Biceps: - the skinfold was picked up on the front of the arm directly above the centre of the cubital fosa. The calipers were applied to the skinfold at the midpoint (or the "belly") of the muscle.

2. Triceps: - the skinfold was taken at the back of the arm halfway between the inferior border of the acromian process and the tip of the olecranon process, directly in line with the point of the elbow and the acromian process. (The site was marked on every subject.)

3. Subscapular: - the skinfold was picked up just below the tip of the right scapula at an angle of about 45° to vertical, and with the fingers touching the bone.

4. Supra-iliac: - the vertical skinfold was picked up immediately above the anterior iliac spine in the mid axillary line. On the rare occasions that this proved too difficult, in a very obese subject for example, the horizontal skinfold was taken at the same site.

Reproducibility of skinfold measurements.

A preliminary study was carried out to assess the reproducibility of repeat measurements taken by the observer. Skinfold thicknesses (biceps, triceps, subscapula and supra-iliac) were measured in a group of 13 undergraduate students, 7 males and 6 females, and body fat content calculated using the Durnin and Womersely equations (1974). A week later the measurements were repeated. The average difference in % fat measured on the two occasions was found to be only 0.2% (SD ±1.0) suggesting that the reproducibility of the repeat measurements taken by the observer was high.

MEASUREMENT OF ENERGY EXPENDITURE.

Resting energy expenditure can be assessed by direct or indirect calorimetry.

Direct Calorimetry:

Direct calorimetry involves the measurement of heat loss from the body. It is based on the principle that the sum of the heat lost - by radiation, conduction, convection and as latent heat arising from the vapourisation of water - equals, in the long-run, the heat released by metabolism in the body. It is the oldest of the techniques for measuring energy expenditure, dating back to the time of Lavoiser at the end of the 18th century. Today three types of calorimeters are in use to assess heat loss in man:- the isothermal calorimeter, pioneered by Atwater and Benedict (1903), the gradient layer calorimeter (Benzinger & Kitzinger, 1949) and a water cooled garment developed by Webb *et al.* (1972).

Direct calorimetry is considered to be extremely accurate for measurements of energy expenditure over relatively long periods of time (a day or more). However, because of the body's capacity to store heat energy and the consequent delayed response between heat production and heat loss, it is not suitable for short-term assessment of energy expenditure such as measurement of BMR, the thermic effect of food or exercise. Nor is it appropriate for measurement of energy expenditure in large numbers of free living subjects. Moreover, the equipment is, on the whole, complex and expensive to construct.

Indirect calorimetry:

The term indirect calorimetry is employed to describe those methods of estimating heat production or energy expenditure which are based on determinations of gaseous exchange; oxygen consumption and carbon dioxide production. If it is assumed that all the oxygen consumed by an individual is used to oxidise degradable fuels and that all the carbon dioxide so liberated is recovered, it is possible to calculate the total amount of energy 'produced'. When the rate of nitrogen excretion is also known, the type and rate of fuel utilisation can also be deduced.

Indirect calorimetry has a short response time due to the body's inability to store oxygen. It is therefore suitable, and indeed widely used, to assess the acute effects on metabolic rate of stimuli such as food or exercise and for measurement of BMR.

Indirect calorimetry techniques fall into one of two categories, they are either open or closed-circuit.

(1) **Closed-circuit**

The subject breaths pure oxygen which is, as the name suggests, circulated around a closed system. Expired air is passed through soda lime to remove carbon dioxide and the remaining oxygen returns to the system. The decrease in the volume of oxygen over a set time gives a measure of oxygen consumption. By using appropriate conversion factors the metabolic rate (kcal/min) of the individual can be estimated.

(2) **Open-circuit**

In open-circuit indirect calorimetry the subject breaths normal atmospheric air and their expired gases are collected and analysed for oxygen and carbon dioxide content.

Several open-circuit methods are available (for review see McLean & Tobin, 1987). These range from sophisticated respiration chambers suitable for the measurement of energy expenditure over several days, to the simple Douglas bag system (Douglas, 1911) which being light, portable and inexpensive is often the method of choice in a field situation.

In the studies presented here, energy expenditure was measured by open-circuit indirect calorimetry in one of two ways, either using the Douglas bag technique or using a ventilated hood system.

The Douglas Bag Technique.

Apparatus.

The apparatus consists of a large gas impermeable plastic bag - the Douglas bag - of either 100 or 200 litres capacity (Cranlea & Co., Birmingham, UK). This is connected via a three-way aluminium tap to a length of flexible corrugated plastic tubing, which in turn attaches to a two-way Rudolf valve (Kansas City MO. USA). A rubber mouth piece is fitted onto the Rudolf valve.

Collection of expired air.

(See Figure 2.3)

The subject's nose is closed off with a nose clip, so they are able to breath only through the mouth piece. The Rudolf valve, to which the mouth piece is attached, allows the volunteer to draw air from the atmosphere but all expired gas is directed down the tubing toward the Douglas bag. Depending on the position of the three-way tap, the expired air can either enter the collection bag or pass back to the atmosphere. For the first few minutes of a measurement (usually 3-5 depending on the specific protocol) the tap is in the latter position and expired air returns to the room. This allows the subject to 'settle down' and become used to the apparatus before the actual collection begins. After the appropriate run-in

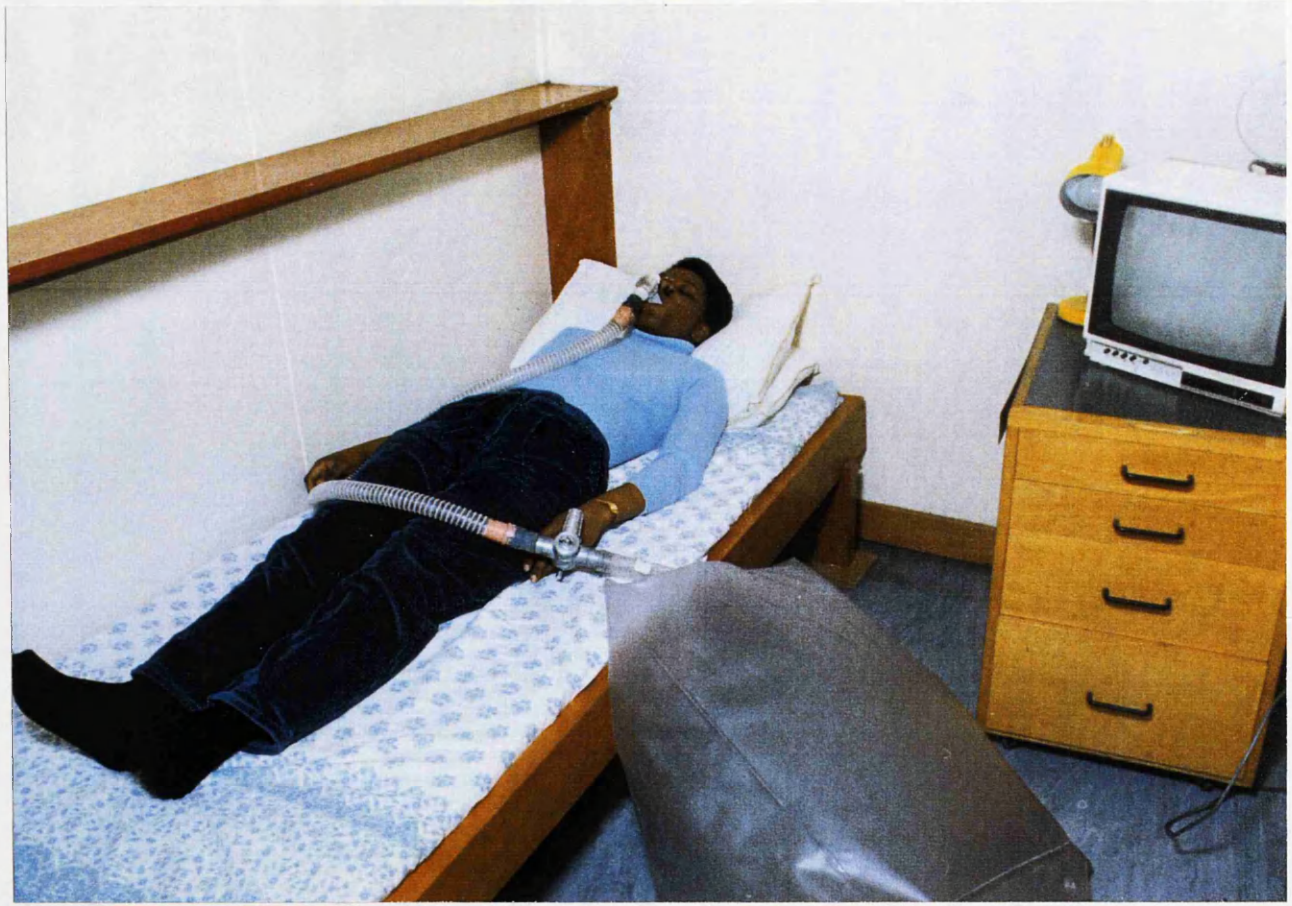

Figure 2.3

Collection of expired air using the Douglas bag system

period the tap is opened and expired air collected into the Douglas bag for a specified time. The tap is then closed off once again, the bag disconnected from the breathing system and taken away for gaseous analysis.

Analysis of expired air.

A paramagnetic oxygen analyser (Servomex model 570 SYBRON, Servomex Ltd., Crowbridge, Sussex, England) and an infrared carbon dioxide analyser (PK Morgan Ltd., Chatham, Kent, England) were used for the analysis of the subject's expired air. A sample of expired air was introduced into the analysers through a side tube attached to the Douglas bag. One minute was allowed for the readings on the analysers to stabilise (equivalent to 0.5 litres of gas passing from the bag) and the carbon dioxide and oxygen contents recorded. The side tube was closed off and the volume of the expired air then measured using a gas meter (Parkinson-Cowan Ltd., London, England), taking into account the 0.5 litres already used for analysis. The temperature of the air passing through the meter was recorded by an attached thermister. The volume of expired air was corrected to standard temperature, pressure and saturation (STPD) using the appropriate 'atmospheric correction factor' obtained from a nomogram on the basis barometric pressure and temperature (Consolazio *et al.*, 1963).

Calibration of the gas analysers.

The oxygen and carbon dioxide analysers were calibrated each morning prior to the start of the experiments. They were first set at zero by introducing oxygen-free nitrogen and then 'spanned' using standard gas mixtures containing either 4.05% CO2 : 16.30% O_2 or 6.06% CO_2 : 15.62% O_2. The span of the oxygen analyser was checked to be 20.93% using atmospheric air. Oxygen-free nitrogen was then reintroduced to check the zero setting of the analysers.

All gases were supplied by the British Oxygen Co. Ltd., Brentford, England.

Calculation of Metabolic Rate.

Metabolic rate was calculated according to the following equation:

$$\text{Metabolic rate (kcal/min)} = O_2 \text{ consumption (l/min)} \times \text{calorific equivalent } O_2 \text{ (kcal/l)}$$

(1) Oxygen consumption

$$O_2 \text{ consumption} = \text{'true' oxygen} \times \text{ventilation rate}$$

Ventilation rate is equal to the total volume of air expired per minute, and is usually expressed as litres of dry air at standard temperature and pressure (STPD). It is obtained by multiplying the metered volume of expired air by the 'atmospheric correction factor' (see above), and dividing this by the duration of the sample collection:

$$\text{Ventilation rate (l/min at STPD)} = \frac{\text{metered volume (l)} \times \text{correction factor}}{\text{sample duration (mins)}}$$

If the volume of the inspired air is equal to the volume of expired air, then oxygen consumption can be obtained simply from the difference between the volume of O_2 inspired and that expired:

$$O_2 \text{ consumption} = \text{vol } O_2 \text{ insp} - \text{vol } O_2 \text{ exp}$$

$$= \frac{20.93}{100} \times Vi - \frac{\%O_2e}{100} \times Ve$$

$$= \frac{20.93 - \%O_2e}{100} \times Ve \quad \text{(equation 1)}$$

Where Vi = vol air inspired

Ve = vol air expired

20.93 = $\%O_2$ in inspired air

%O2e = $\%O_2$ in expired air

However, when Vi and Ve are not equal, as is the case when the RQ is less than 1, an adjustment is required to derive the 'true' value for the oxygen difference. This computation is based on the fact that the volume of nitrogen breathed in (Ni) will always equal the volume of nitrogen breathed out (Ne):

$$Vi \times \frac{Ni}{100} = Ve \times \frac{\%Ne}{100}$$

and

$$Vi = Ve \times \frac{\%Ne}{79.1} \qquad (Ni = 79.1\%)$$

Taking equation 1 and substituting:

$$O_2 \text{ consumption} = \frac{20.93}{100} \times Ve \frac{\%Ne}{79.1} - \frac{\%O_2e}{100} \times Ve$$

$$= Ve \times \frac{20.93 \times \%Ne}{100 \times 79.1} - \frac{\%O_2e}{100}$$

'true' oxygen

Thus the 'true' oxygen value can be derived, and when multiplied by ventilation gives a measure of oxygen consumption. In this study for speed and

ease of calculation 'true' oxygen, was obtained using a nomogram (Consolazio *et al.*, 1963).

(2) Metabolic rate.

In this study metabolic rate was calculated according to Weir's equation (1949). Previously the estimation of metabolic rate by indirect calorimetry involved measuring urinary nitrogen excretion, in addition to gaseous exchange, in order to determine the proportions of the different nutrients oxidised in the body. The calculations involved were so cumbersome that the effect of protein metabolism was commonly ignored. In 1949 however, Weir developed an equation which took into account the effect of protein metabolism, without the necessity of having to measure nitrogen excretion. The equation is based on the assumption that a fixed percentage (12.5%) of the total calories expended by the body arise from protein metabolism and of an RQ equal to 1. If this is the case, Weir calculated that the amount of heat released for every litre of O_2 used, the calorific equivalent of O_2, would be 5 kcal/l.

Thus,

$$\text{Metabolic rate} = 20.93 - \%O_2e \times Ve \times 5$$

$$= \frac{20.93 - \%O_2e}{20} \times Ve$$

If however, the RQ is less than 1 (and consequently Ve is less than Vi - see above) the volume of oxygen inspired and therefore the metabolic rate calculated according to this equation, will be under-estimated. However, as RQ falls the calorific equivalent of oxygen also falls tending to over-estimate metabolic rate. Under normal circumstances these two errors cancel out and the Weir equation gives an accurate assessment of metabolic rate.

The Ventilated Hood.

In studies investigating the effect of ß-adrenergic blockade on energy expenditure (see later for details) BMR was measured using a ventilated hood system. It was used in preference to the Douglas bag technique because measurements were usually carried out away from the laboratory (in hospital or the subjects' place of work) and no gas meter was available. In addition, hospital staff reported that, in their experience, the group of patients involved were ill at ease using a mouthpiece and noseclip. Like the Douglas bag technique, the ventilated hood is an example of open-circuit indirect calorimetry.

The principle of the ventilated hood system is that atmospheric air is drawn past the subject's face at a controlled rate, mixing with and collecting any expired gases as it does so. The concentration of O_2 in the mixture of room and expired air leaving the hood is measured. With a knowledge of the O_2 content of inspired air and the rate of air flow through the hood (ie. ventilation rate) the volume of oxygen utilised can be calculated and metabolic rate determined as above.

Apparatus and Procedure (See Figure 2.4)

The ventilated hood system used in these studies was designed and built in the Institute of Physiology by Dr. Mark Lawrence. It consists of a flexible clear plastic hood, of the type used in the chemical industry (Wavelock protecting hood, Plysu Industrial Ltd., Milton Keynes, England) which was placed over the subject's head and shoulders. Room air was drawn through the hood, and up past the volunteer's face by a negative pressure gradient created by a centrifugal exhauster pump located 'downstream' of the hood (Air Control Instruments (Chard) Ltd., Chard, Somerset, England). Interposed between the hood and exhauster pump are a flow meter and flow controller; the former was connected to

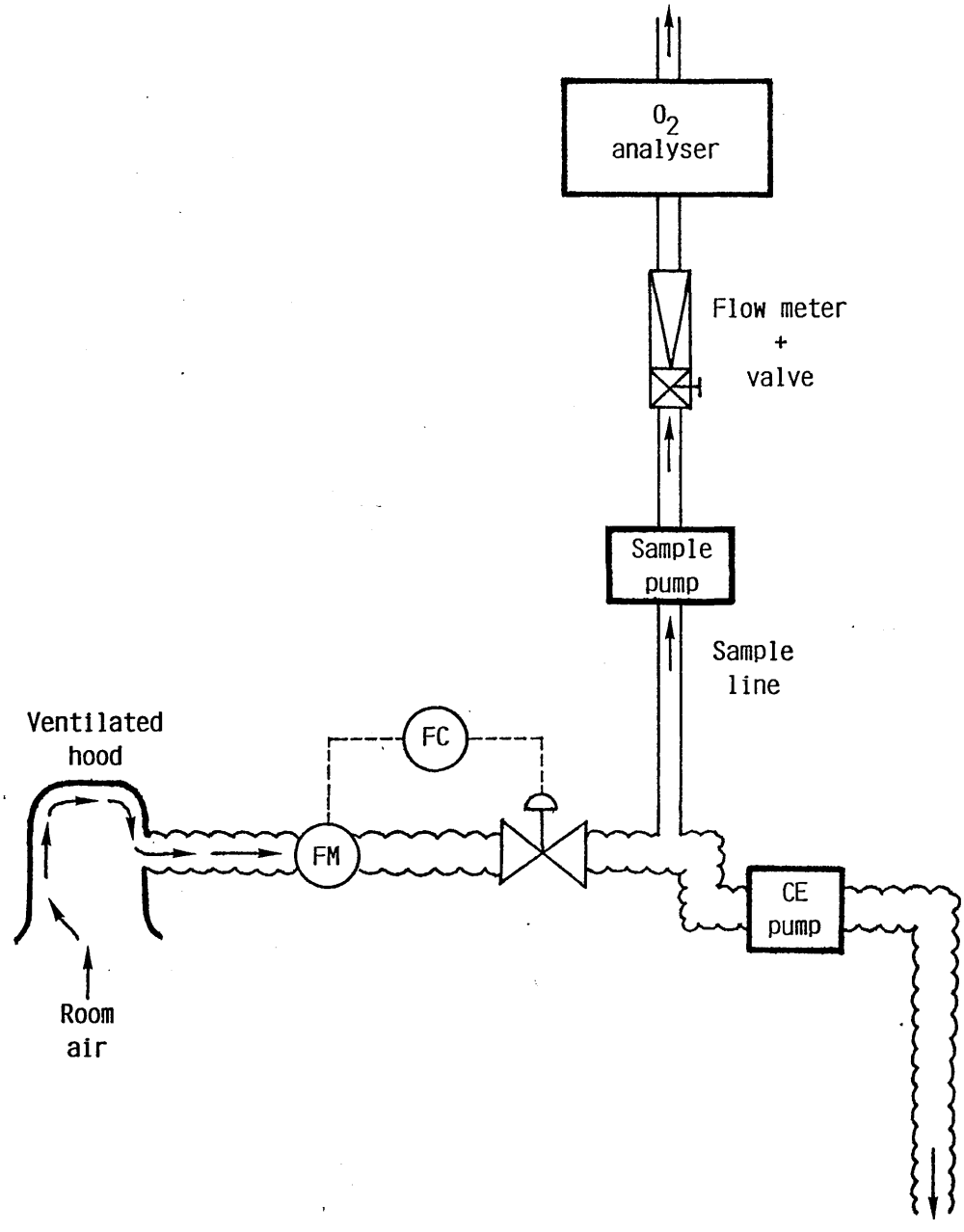

Figure 2.4
Schematic representation of ventilated hood system.

FM = Flowmeter, FC = Flow Controller, CE pump = Centrifugal Exhauster Pump.

the hood via a length of corrugated plastic tubing. Air was drawn through the hood at a rate of approximately 1 litre per kilogram of body weight per minute. This rate was chosen so as to to prevent stimulation of ventilation by an increased CO_2, and yet not produce excessive dilution of the subject's expired air to a level that exceeded the resolution of the gas analysers. The exact rate at the beginning and end of each measurement run was recorded and the mean taken. This was multiplied by the appropriate atmospheric correction factor to derive the ventilation rate at STPD (see above). After the flow meter, a small and constant quantity, (0.4 l/min) of the air leaving the hood was drawn off by means of an airtight pump (The Analytical Development Co. Ltd., Hoddeson, England) for gas analysis. The oxygen content of the air, a mixture of room air and the subject's expired gases, was determined using a paramagnetic oxygen analyser (Servomex model 570 SYBRON, Servomex Ltd., Crowbridge, Sussex, England). The gas was dried on route to the analyser by passing it through silica gel. Calibration of the O_2 analyser took place each morning in the same way as described above. During a measurement run a simultaneous collection of room air was always made (by means of an airtight pump and 1-litre gas-impermeable plastic bag) and later analysed to provide a mean estimate of the oxygen content of inspired air.

Calculation of metabolic rate.

As with the Douglas bag studies (see above), metabolic rate was calculated using the Weir equation (Weir, 1949):

$$\text{Metabolic rate} = V \times \frac{\%O_2\text{in} - \%O_2\text{out}}{20}$$

Where,

V = Ventilation rate litres/min at STPD (air flow through the hood)

$\%O_2$in = % oxygen in inspired (room) air

$\%O_2$out = % oxygen in air leaving the hood (room air + expired air)

As the air leaving the hood is a mixture of both room air and expired gases, the difference in oxygen content between it and inspired air will be small. The potential impact of fluctuations in inspired O_2 content on the calculated metabolic rate will therefore be great. For this reason the O_2 concentration of inspired air was always measured rather than assumed to be 20.93%

Unfortunately no carbon dioxide analyser was available for use with the ventilated hood system and consequently RQs could not be determined.

Calibration of the ventilated hood system:

All the component parts of the ventilated hood system, including tubing and connections, were deamed to be airtight. However, the system was checked for leaks and calibrated, both before and after the study, by a series of gas recovery tests. These involved introducing nitrogen into the system at a measured rate and comparing this value to the rate calculated from the reduction in O_2 content of the air leaving the hood.

A flow rate of nitrogen approximately one fortieth of the flow rate of room air through the hood was used, since this produced a drop in O2 concentration of similar magnitude to that encountered with a subject under the hood. The rate of nitrogen flow into the hood was measured by means of a wet gas meter (Alexander Wright & Co. (Westminster) Ltd., London, England) and corrected to STPD. Simultaneously, samples of the air leaving the hood and room air entering it were collected and analysed for O_2 content. Nitrogen flow was calculated as follows:

$$N_2 \text{ flow rate} = \text{Ventilation rate} \times \frac{\%O_2\text{in} - \%O_2\text{out}}{\%O_2\text{in}}$$

This procedure was carried out a total of twenty times using a number of different flow rates over the range used for the subjects. The mean difference

between the measured and calculated flow rates was found to be 0.42% ± 1.78. Thus it was evident that the system did not contain any leaks and had an error which compares favourably to that of other indirect calorimetry techniques - suggested by Garrow (1978) to be in the range 2-5%.

MEASUREMENT OF BASAL METABOLIC RATE.

Conditions for measurement

As discussed in the introduction, in this thesis BMR is defined as the rate of energy produced under the standardised resting conditions outlined by Benedict (1938). To recap, the subject should be:

- lying awake in a supine position, at complete physical rest (immobile).
- postabsorptive, at least 12 hours after the last meal.
- in a thermoneutral state.
- emotionally undistrubed.
- without disease or fever.

In all measurements of BMR every attempt was made to meet each of the above criteria.

In the recent past the impression seems to have arisen that for a measurement to be properly defined as basal it must be made just after wakening, prior to the subject getting out of bed (Schutz, 1984). Whatever the merits of imposing these conditions they are certainly not the ones under which almost all the fundamental work on BMR was carried out (Benedict, 1915; Du Bois, 1927; Boothby *et al.*, 1936; Benedict, 1938). In all these cases, subjects were not required to stay overnight before a BMR measurement. Similarly in our studies subjects arrived at the laboratory, or other place of measurement, early in the

morning (about 9.00am) after spending the night in their own homes. The exception was the ß-blockade study, where some of the volunteers were hospital inpatients (the specific details are discussed later). In all cases however, subjects were required to lie quietly at rest, for at least thirty minutes before any measurements began.

In order that they should be in a post-absorptive state, the subjects were instructed not to eat or drink anything after 9.00pm on the day preceding their measurements and to continue the fast on the morning of the test. There is some dispute however, as to how long the thermic effect of food (TEF) lasts. Dauncey (1980) has shown that on a diet supplying only 3.7 MJ/day, an elevation of metabolism of 6% was present 15 hours after the last meal. Similarly Schutz *et al.* (1985) report a study in which the thermic effect of a large meal was still evident the following morning. It is likely that the duration of TEF will depend to some extent on the size and composition of the meal; the effect on metabolic rate of a large, high calorie meal will likely last longer than that of a light snack. With this in mind, the subjects were asked not 'stock up' with an extra large meal just prior to the 9.00pm deadline. They were also asked to consume their usual quantity of food on the day before the measurement, to avoid the effects that acute overfeeding may arguably have on BMR.

A similar cautionary policy was adopted with regard to the previous day's exercise. Again, reports of the after-effects of exercise on metabolic rate are conflicting. However, most of the studies which report a carry-over effect to the following day have generally involved prolonged periods of high intensity activity (Passmore & Johnson, 1960; Hermansen *et al.*, 1984; Bielinski *et al.*, 1985). It seems doubtful whether a moderate degree of activity, such as that likely to be undertaken by volunteers in our studies, would affect BMR on the morning following the exercise session (Pacy *et al.*, 1985; Freedman-Akabas *et al.*, 1985; Shah *et al.*, 1988; Weststrate, 1989; Bingham *et al.*, 1989; Buckley *et al.*, 1989).

Subjects were therefore requested to refrain only from strenuous exercise on the preceding day. (In reality it is likely that most of the subjects took no exercise at all on the day before their tests, since a questionnaire (see later) revealed that over two thirds of the participating volunteers could be classified as sedentary). On the test day itself, all subjects either walked to the lab or came by car or bus - none cycled or jogged - in which case the 30 minute rest period prior to the measurement (see above) should have been sufficiently long for metabolic rates to return to basal levels.

The entire measurement procedure was always explained to the subjects very carefully at the outset, and they were shown and familiarised themselves with the equipment. This helped to ensure that they were as relaxed and confident as possible. It was impressed upon the volunteers that they must remain very still throughout the measurements. With the studies involving the ventilated hood the observer stayed in the room with the subject and was able to ensure that they did indeed remain immobile. This was not possible with the Douglas bag studies however, where measurements on two or three subjects were often being made simultaneously.

In all studies a thermoneutral room temperature of between 18°C and 22°C, depending on what felt most comfortable to the subject, was maintained.

Measurement procedure.

(1) Douglas bag.

After a half hour rest period the subject was fitted with the nose clip and mouth piece. A 5 minute run-in period followed, allowing the subject to become accustomed to breathing through the apparatus. Two consecutive 10 minute collections of expired air were then made. After the second collection the subject was allowed to take out the mouth piece and nose clip and rest for a few minutes before a third and final 10 minute measurement got underway. This last bag was

preceded by a 3 minute run-in. At the end of each collection heart rate was measured using the radial pulse. Bags were analysed and metabolic rate calculated as described above. Unless an obvious malfunction had occured, the average of all three bags was taken to represent the BMR of the subject.

(2) Ventilated hood.

A 30 minute rest period was followed by a 30 minute measurement run. During the rest period the subjects wore the ventilated hood. This allowed them to become used to the apparatus and also provided the opportunity for the observer to monitor when oxygen consumption had reached a steady state. This was usually achieved after 20 minutes or so, routinely however, the acclimatisation period was allowed to proceed for the full half an hour. The measurement run followed on without a break. The oxygen concentration of the air leaving the hood was recorded at 30 second intervals and the mean concentration per 10 minute period calculated. A sample of room air was collected simultaneously and analysed at the end of each measurement period to provide a mean estimate of inspired of content. Flow rate at the beginning and end of each 10 minutes was recorded and the mean taken. This data was then used to calculate metabolic rate for each of the three 10 minutes periods. BMR was taken to be the mean of the three measurements.

CHAPTER 3

BMR AND BODY COMPOSITION.

INTRODUCTION

In healthy adults BMR can vary over a three fold range (Harris & Benedict, 1919; Boothby & Sandiford, 1929). These differences have traditionally been attributed to differences in body size, age, sex, race, climate and nutritional status. More recently however, several studies have observed that differences in BMR between groups of individuals, of differing age, sex, race and so on, are largely eliminated when the size of the FFM is taken into account (eg. Cunningham, 1980; Bernstein *et al.*, 1983; Ravussin *et al..*, 1986; Lawrence *et al..*, 1988). This has led to the widespread use of FFM as a metabolic reference standard and, by extension, to the tendency by some investigators to regard simple differences in body composition - the mass of fat-free tissue - as the genesis of all variation in BMR. More critical evaluation of the data would suggest that this is not so. At a given FFM for example, BMR can vary considerably between individuals (Bogardus *et al.*, 1986; Ravussin *et al.*, 1986; Lawrence *et al.*, 1988) and there is some suggestion that in relation to the FFM, BMR may decline with age (Doré *et al.*, 1983; McNeill *et al.*, 1987). Moreover, it has been observed that BMR/kg FFM is not constant with weight but tends decline from light to heavy individuals, bringing into question the validity of expressing results in this way when groups of differing body size are to be compared (Lawrence *et al.*, 1988).

The aim of the present study was to investigate the part played by differences in body composition, assessed primarily in terms of weight, height, fat and FFM, in explaining variability in BMR in a group of healthy women. In view of the important role generally accredited to differences in the mass of fat-free tissue it was of particular interest to determine how much of the differences between the women could be attributed to differences in FFM. Could for example, any age related changes in BMR - an area not well documented in women - be explained entirely in terms of FFM (Cunningham, 1980) or, as

observed by Doré *et al.* (1983) and McNeill *et al.* (1987), are there age differences even when the size of the FFM has been taken into account ? Past work has tended to concentrate on women in their twenties and thirties, it was intended therefore to extend observations on BMR and FFM over a wider age range.

As noted above, Lawrence *et al.* (1988) have found that BMR/kg FFM has a tendency to fall from light to heavy individuals. They concede however, that this observation may to some extent have a statistical, rather than physiological, basis, relating to error in estimation of FFM using the skinfold technique; measurement error will have the effect of reducing the slope of the regression line relating BMR to FFM and exaggerate any tendency for BMR/kg FFM to fall as weight increases. Since there is some uncertainty as to the error involved in the skinfold method - one analysis has suggested 2% to 3% of body weight (Womersley & Durnin, 1977) - in the present study it was decided to also measure FFM using densitometry. This technique is generally thought to be more accurate than the skinfold method and there is a greater degree of confidence as to the error involved (Lukaski, 1987). It was postulated that if error in skinfold method was an important factor in explaining the decrease in BMR/kg FFM from light to heavy individuals one might expect a less of a fall in BMR/kg FFM when FFM was measured by densitometry.

It has been suggested that variation observed in BMR in relation to the FFM and to the apparant fall in BMR/kg FFM with increasing weight may result from differences in the composition of the FFM, to the relative proportions of 'active' organs compared to 'inactive' tissues such as muscle. Circumference and diameter measurements were therefore made on the women in the expectation that these might provide an estimate of relative muscularity and skeletal size, allowing this to be investigated further.

SUBJECTS AND METHODS.

Subjects.

97 women living in and around Glasgow participated in the study. Volunteers were members of the general public and were largely recruited through the distribution of leaflets (see Appendix 1) to libraries and commercial organisations. It was hoped that this approach would yield a broad cross section of volunteers. Most of the subjects were employees of Strathclyde Regional Council and British Telecom.

All the women were in apparant good health and reported no previous history of diabetes mellitus, thyroid disease or other metabolic disorders. None were receiving any drugs or medication apart from the contraceptive pill and, in the case of two older women, hormone replacement therapy. Stage of the menstrual cycle was recorded or note taken if the women had reached the menopause. None of the women were on reducing diets at the time of the measurements.

Details were also obtained of the women's usual activity and exercise habits. On the basis of this information volunteers were very roughly classified as either very active - participating in a sporting activity or exercise session 4 or more times a week; moderately active - exercising 2 to 3 times a week; or sedentary - undertaking no additional activity or exercising only infrequently, once a week or less. None of the women had physically active jobs - most were office workers - so classifications were based solely on leisure time activities.

Measurement of BMR.

BMR was determined using the Douglas bag technique under the standardised conditions described in Chapter 2.

Body Composition.

Body weight, height, circumference and diameter measurements were made as described in Chapter 2. Body fat content was estimated from the sum of four skinfold thicknesses (biceps, triceps, subscapular and supra-iliac) according to the equations of Durnin & Womersley (1974) and from body density determined by underwater weighing. (Details of procedures are given in Chapter 2).

There was no significant difference in mean fat content of the group assessed by the two techniques:

$$\% \text{ fat skinfolds} = 30.3\ \% (\pm 5.8)$$

$$\% \text{ fat density} = 30.2\ \% (\pm 7.3)$$

A good correlation ($r = 0.86$, $p < 0.001$) was found between the methods, with the slope of the regression line being close to one (Figure 3.1). Furthermore, differences between the measurements were found not to be related to the degree of body fatness. In the light of these observations the results for body fat content, and consequently FFM, are reported throughout the study as the mean of the two estimates. The implications of adopting this approach with respect to the correlation between BMR/kg FFM and FFM will be discussed in the appropriate section below.

Statistical methods.

The linear relationships between BMR and the various body composition variables were assessed using univariate regression analysis. A stepwise regression analysis was performed to determine which of the body composition variables best explained differences in BMR. This yielded the regression equation relating BMR to FFM. In addition a multiple regression analysis was carried out to derive the regression equation relating BMR to body weight. The statistical significance of the difference in the residual standard deviations of the regression

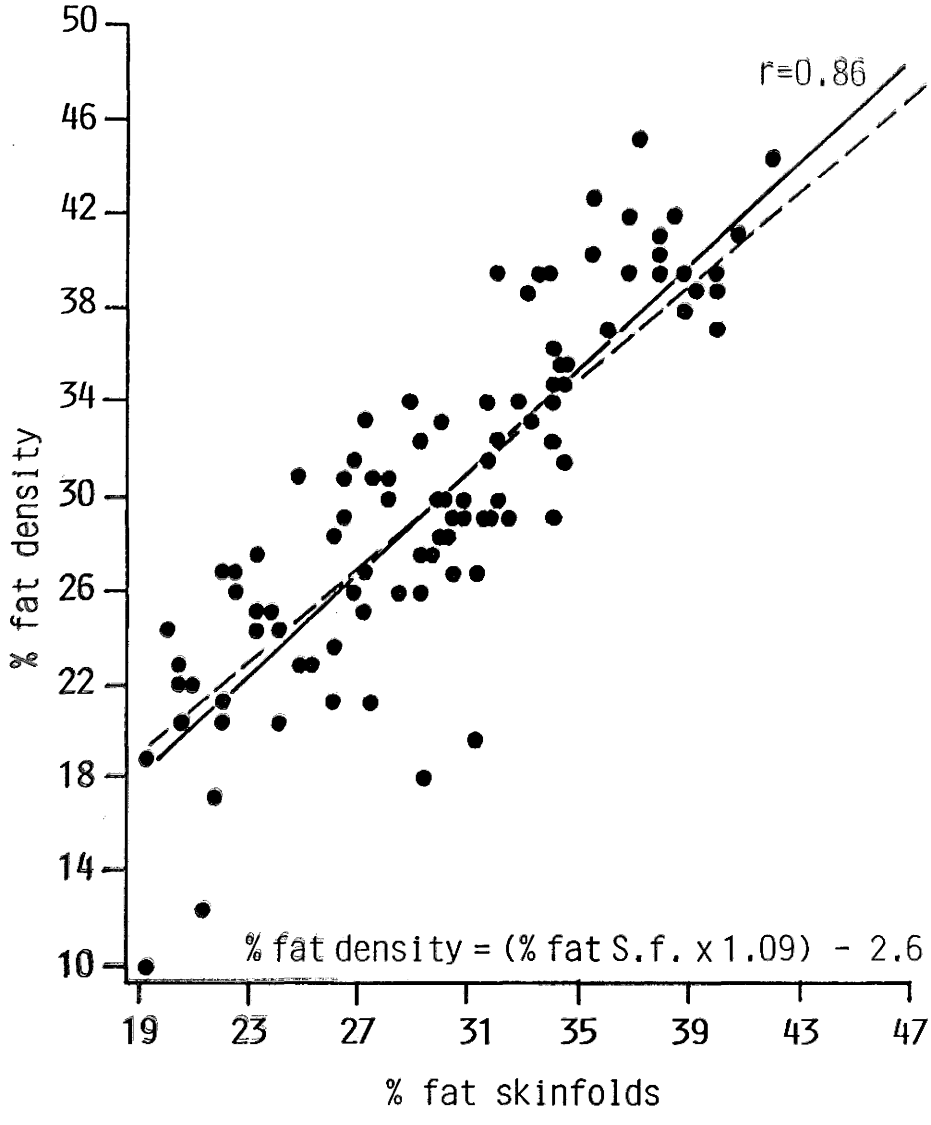

Figure 3.1
Relationship between percentage fat estimated by densitometry and percentage fat estimated by skinfolds.

equations derived between BMR and FFM and BMR and weight and also between BMR and calf circumference was assessed using an F-test. Finally, univariate regression analysis was used to derive the linear relationships between between age and body weight, height, % fat and FFM. All procedures were performed using the SPSSx statistical language.

RESULTS.

The anthropometric data and BMR results of the women who took part in the study are shown in Table 3.1.

Variation in BMR between individual women, either in absolute terms or when expressed per kg of body weight or per kg FFM, proved large, with a coefficients of variation of 11.8%, 13.6% and 9.6% respectively.

In order to determine which of the variables, or combination of variables, listed in Table 3.1 were best able to explain these differences in BMR, a stepwise regression analysis was performed on the data. The following equation was derived:

$$\text{BMR (kcal/day)} = (\text{FFM} \times 22.4) + 403$$

$$r = 0.67,\ r^2 = 0.45,\ p < 0.001 \quad \text{(residual SD 118)}$$

Differences in FFM accounted for 45% of the total variance in BMR. Once differences in FFM had been taken into account, none of the other variables were able to explain a significant portion of the remaining variance. In other words the relationship between BMR and FFM (see Figure 3.2) was independent of age, weight, height, differences in body fatness, circumferences, bone diameters and so on. The residual standard deviation about the line of best fit (118 kcal/day, 8.7% of the mean) indicated however, that in relation to the FFM there was considerable variation in the BMR of individual women.

Table 3.1

Anthropometric data and BMR results of the 97 women.

	Mean	(SD)	Range
Age (yrs)	34.5	11.4	17 - 66
Body weight (kg)	61.1	9.8	42.2 - 91.8
Height (cm)	162.4	5.8	150.2 - 174.7
BMI (kg/m2)	23.1	3.5	17.0 - 33.3
% Body fat	30.2	6.5	15.0 - 44.0
FFM (kg)	42.0	4.7	34.0 - 55.8
Circumferences :			
Upper arm (cm)	27.4	3.2	20.4 - 36.6
Buttocks (cm)	96.8	6.9	84.5 - 117.8
Thigh (cm)	55.6	5.2	45.0 - 70.0
Calf (cm)	35.9	2.8	29.2 - 42.0
Diameters:			
Biacromial (cm)	37.0	1.7	32.0 - 41.1
Bi-iliac (cm)	30.7	2.3	22.0 - 36.0
Ulna (cm)	5.2	0.3	4.6 - 5.8
Knee (cm)	9.4	0.5	8.5 - 10.8
BMR:			
kcal/day	1346	157	950 - 1670
kcal / Wt kg / day	22.3	3.0	16.4 - 29.7
kcal / FFM kg /day	32.2	3.1	24.8 - 40.2

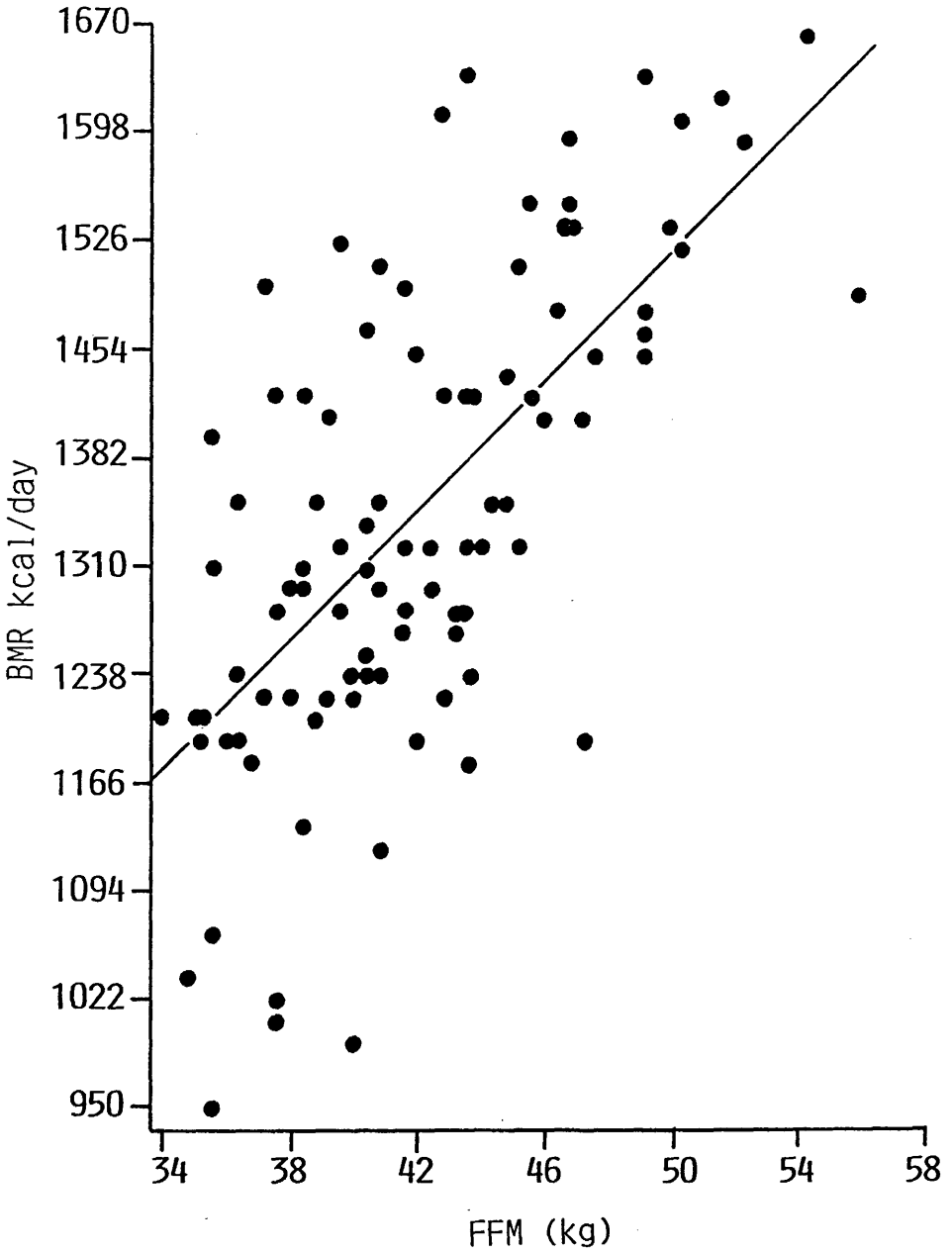

Figure 3.2
Relationship between BMR and FFM.

Univariate correlations were also performed between BMR and the other body composition characteristics of the women. The results are presented in Table 3.2.

There was no significant correlation between BMR and age. All other variables however were positively correlated with BMR. Although, as might be expected from the results of the stepwise analysis, FFM showed the highest correlation with BMR, correlation coefficients for other anthropometric variables, in particular body weight and calf circumference, were not substantially lower. The regression equations for these two variables are shown below:

(1) BMR (kcal/day) = (Weight x 9.0) + 797

$r = 0.55$, $r^2 = 0.31$, $p < 0.001$. (residual SD = 132)

(See Figure 3.3 for scatterplot)

(2) BMR (kcal/day) = (Calf Circ. x 31.7) + 205

$r = 0.57$, $r^2 = 0.32$, $p < 0.001$ (residual SD = 130)

An F-test revealed that the residual standard deviations of the three equations - BMR with FFM, weight and calf circumference - were not significantly different, indicating that in these women FFM was no better a predictor of BMR than weight or calf circumference.

However, in contrast to FFM, multiple linear regression analysis of BMR on weight, showed that the inclusion of age significantly increased the proportion of variance accounted for, from 31% to 41%. In relation to weight therefore, BMR was lower in older women. Addition of height significantly improved the correlation still further, explaining 46% of the total variance in BMR. The equation is shown below:

BMR (kcal/day) = (Weight x 9.2) - (Age x 4.2) + (Ht x 657) - 144

$r = 0.68$, $r^2 = 0.46$

Table 3.2

Univariate correlations for BMR with age and body composition variables.

Independent variables	Correlation coefficients (r)	Statistical significance (p <)
Age (yrs)	- 0.11	
Body weight (kg)	0.55	0.001
Height (cm)	0.45	0.001
BMI (kg/m^2)	0.37	0.001
Body fat (%)	0.37	0.001
FFM (kg)	0.67	0.001
Circumferences :		
Upper arm (cm)	0.32	0.001
Buttocks (cm)	0.51	0.001
Thigh (cm)	0.52	0.001
Calf (cm)	0.57	0.001
Diameters :		
Biacromial (cm)	0.26	0.01
Bi-iliac (cm)	0.37	0.001
Ulna (cm)	0.34	0.001
Knee (cm)	0.48	0.001

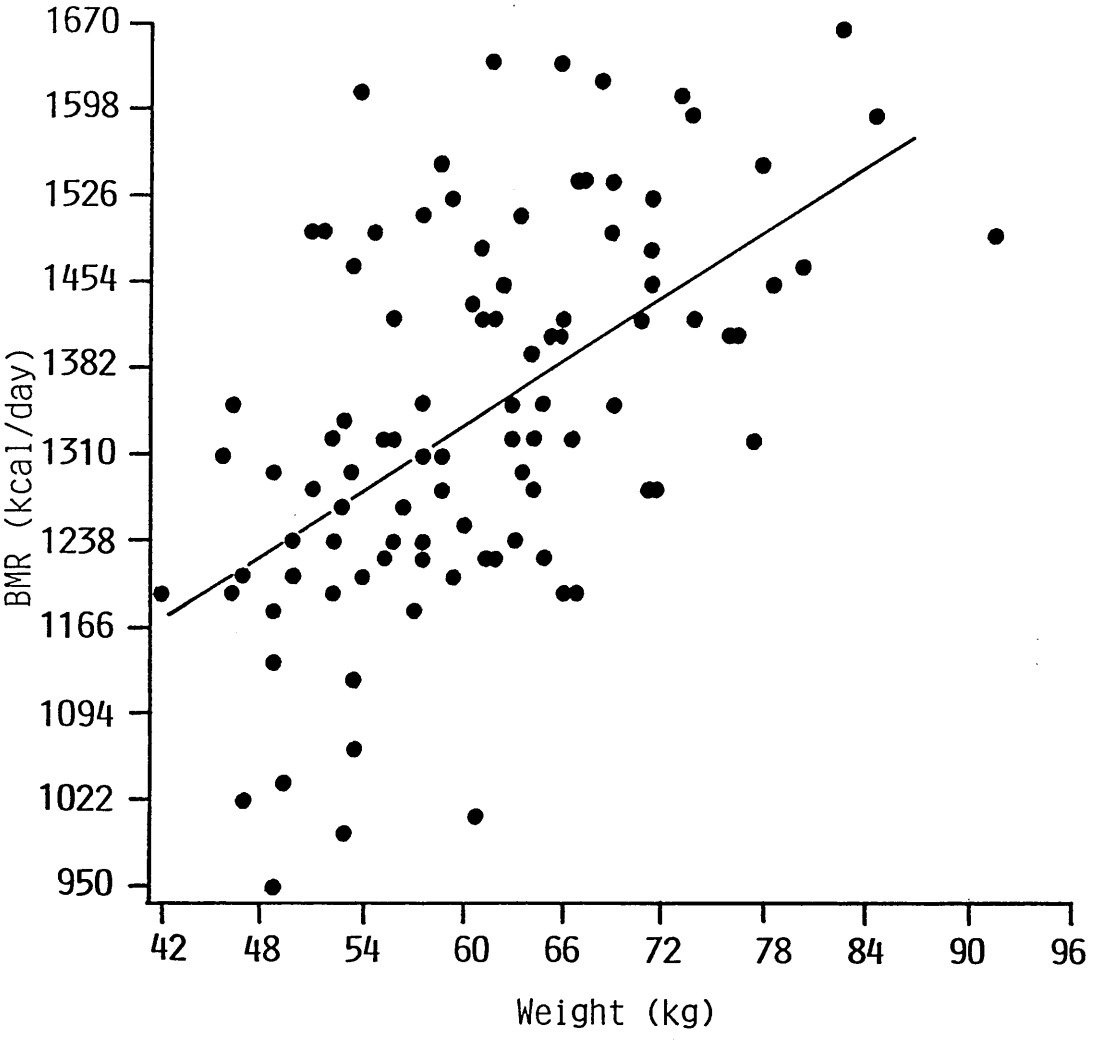

Figure 3.3
Relationship between BMR and body weight.

Lawrence *et al.* (1988) have observed that BMR expressed per kg body weight or per kg FFM tends to be lower in heavier compared to lighter women. To investigate whether this was also true in these women BMR/kg body weight was correlated with weight and BMR/kg FFM with FFM. The scatterplots are presented in Figure 3.4A & Figure 3.4B. It is evident that as the weight of the women increased so BMR/kg declined. Subjects weighing 55kg had a BMR/kg of about 24.6 kcal/day, a value which fell by about 17% to approximately 20.5 kcal/day in women weighing 70kg. Similarly, BMR/kg FFM declined as FFM increased, such that women with a FFM of 55kg had a BMR/kg FFM 15% lower than women with a FFM of 35kg.

The univariate correlations between age and weight, height, FFM, %fat and the BMR of the subjects are shown in Table 3.3. Height, FFM and BMR did not change significantly as the women became older. There was however, a clear increase, in both body weight and body fat content with increasing years.

Details obtained on the volunteers' activity and exercise habits revealed that the majority (56 out of 97) could be classed as sedentary, exercising infrequently or not at all. 32 of the women were moderately active and only 9 fell into the very active classification.

DISCUSSION

As alluded to in the Introduction, BMR can vary enormously between individuals. Three fold differences in the BMR of healthy adults - ranging from under 1000 kcal/day to over a 3000 - have been reported in studies which have encompassed different races, both sexes and extended across the spectrum of body size and age (eg. Harris & Benedict, 1919; Boothby & Sandiford, 1929; Ravussin *et al.*, 1986). With respect to the diversity of the participating subjects, the present study differs from many of its predecessors. The women who took part were not

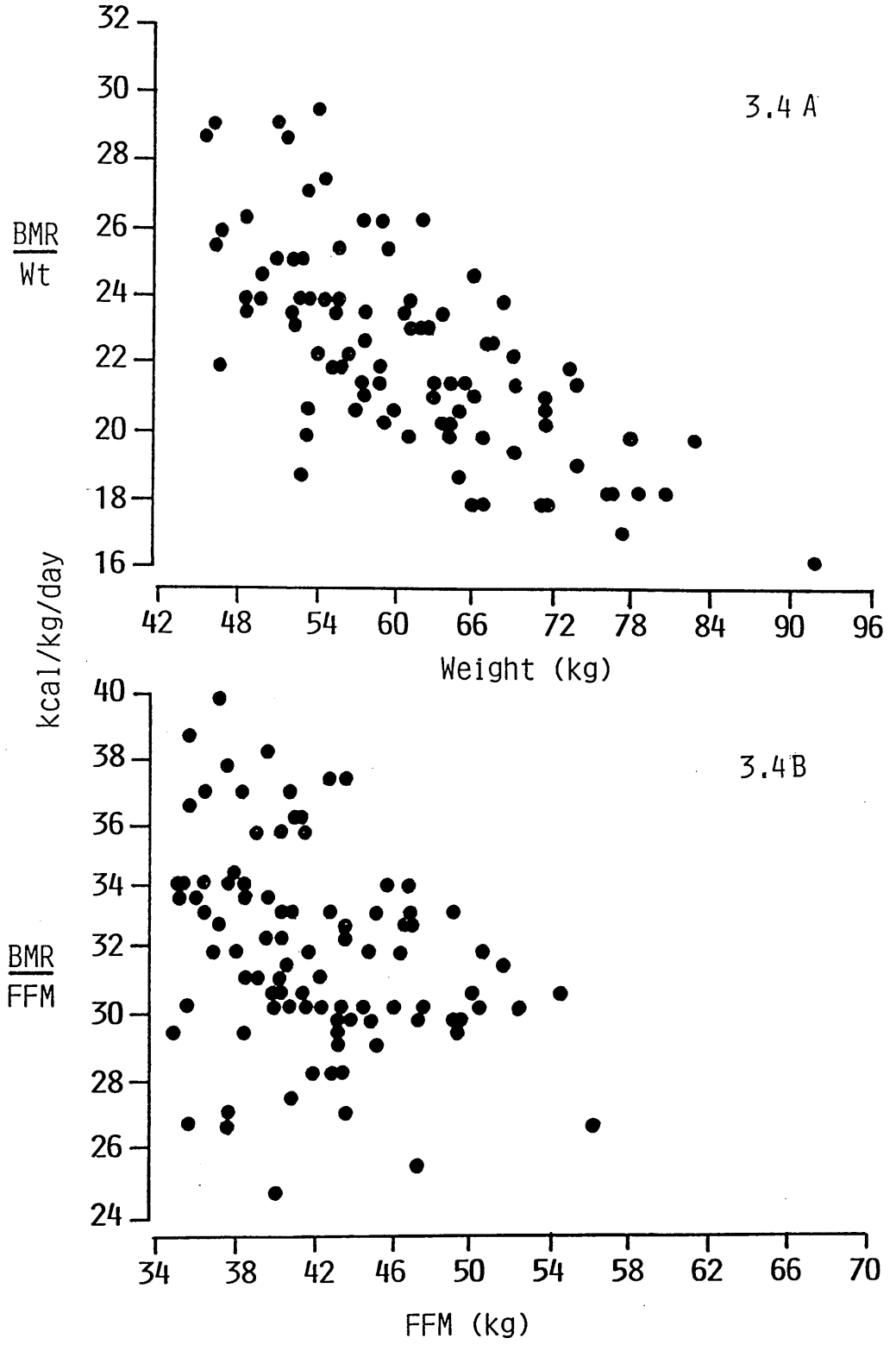

Figure 3.4
Scatterplots of BMR/kg weight against weight and BMR/kg FFM against FFM.

Table 3.3

Univariate correlations between age and weight, height, FFM, % fat and BMR.

Independent variables	Correlation coefficients (r)	Statistical significance (p <)
Weight (kg)	0.34	0.001
Height (cm)	-0.03	NS
% Body fat	0.56	0.001
FFM (kg)	0.01	NS
BMR (kcal/day)	-0.11	NS

selected for extremes of body size or age, rather it was hoped they would represent a relatively normal cross-section of healthy Glaswegian women, with the aim of quantifying, and hopefully subsequently explaining, differences in BMR in such a group.

Variation in BMR among the women proved large, to the extent that some 500 kcal/day separated the 15% of the women at the top end of the range from the 15% at the bottom. Over recent years we have become happy to accept that a large part of such variation can be attributed to differences in body composition, in particular to differences in the mass of fat-free tissue. Studies which have extended across groups of individuals, incorporating in their analysis data from both men and women, different races and extremes of age and body size, generally report high correlations between BMR and FFM, with FFM explaining upwards of 65% of variability in BMR (Cunningham, 1980; Webb, 1981; Ravussin *et al.*, 1982; Bernstein *et al.*, 1983; Ravussin *et al.*, 1986; Bogardus *et al.*, 1986; Weststrate, 1989). In such investigations BMR is usually found to be more highly correlated with FFM than with body weight. Moreover, differences in BMR which are evident between groups in relation to weight, are largely eliminated when differences in FFM are taken into account. For instance, Owen *et al.* (1987) found that when weight was used to predict BMR, the regression equations derived for men and women were significantly different. In contrast, the relationship between BMR and FFM was found to be statistically indistinguishable between the sexes. Similarly, at a given weight, BMR is usually found to differ according to age but at a given FFM, most groups have reported that BMR is independent of age (eg, Keys *et al.*, 1973; Bernstein *et al.*, 1983; Ravussin *et al.*, 1986; Owen *et al.*, 1987) (see below). Much of the variation in BMR between groups of individuals, of different age, sex, body fatness and race can, this would suggest, be largely explained by differences in the FFM.

In the present study it was also observed that once differences in the FFM of the women had been taken into account, none of the other variables measured had any significant influence on BMR. The relationship between FFM and BMR was independent of age, weight, height, differences in body fatness, circumference or diameter measurements. The effect of age on the relationship between BMR and FFM will be discussed below. However, with regard to body fatness, the observation that individuals with a similar FFM had a similar BMR irrespective of differences in body fat content is in accord with several previous investigations (James *et al.*, 1978; Halliday *et al.*, 1979; Doré *et al.*, 1982; Ravussin *et al.*, 1982; Garrow & Webster, 1985; Lawrence *et al.*, 1988; Weststrate, 1989). In those women with the same mass of fat-free tissue yet a different percentage fat, in one respect at least, the composition of the FFM will differ - the fatter individual will have a greater proportion of nonfat adipose tissue occupying the FFM. This would appear to have little impact on BMR. A possible explanation may be that the metabolic rate of fat-free component of adipose tissue approximates to the average metabolic rate of the rest of the FFM, and as such differences in the relative proportion it occupies have no discernible effect on BMR. In its entirety adipose tissue has a relatively low metabolic rate per unit weight. The metabolic rate of the nonfat component - constituted by cytoplasm of the adipose cell - is however, likely to be somewhat greater since it is involved in the energy transformations which occur in adipose tissue including the constant cycle of breakdown and re-esterfication of triglycerides. Its rate of energy expenditure may be sufficient to equate to the average metabolic rate of the rest of the FFM.

Although a single equation could be used to predict BMR from FFM - age, body fatness and so on having little additional effect once the amount of fat-free tissue had been taken into account - differences in FFM were able to explain less than half of the total variance observed in the basal metabolic rate of the women,

some 45%. Other investigations which have also looked at relatively homogeneous groups - of single sex and race and over a narrower age range - have similarly observed lower correlations with FFM than those reported between divergent groups of individuals (Bernstein *et al.*, 1983; Lawrence *et al.*, 1988). For example, in Scottish women aged between 20 and 35 years Lawrence *et al.* (1988) found a correlation coefficient for BMR against FFM of 0.31; only 10% of the variance in BMR could be attributed to differences in FFM. Within groups of individuals, and in contrast to observations between groups, it would seem that differences in FFM are able to explain relatively little of the variation in BMR, usually no more than about half.

Furthermore, the residual standard deviation of the regression equation describing the relationship between BMR and FFM in this study (118kcal/day, 8.7% of the mean) indicates that at a given FFM considerable variation exists in the BMR of individual women. Two women with the same mass of fat-free tissue may have BMRs that differ by as much as 400 kcal/day. Other studies have reported differences in BMR in relation to the FFM of a similar order of magnitude (Bogardus *et al.*, 1986; Lawrence *et al.*, 1988; Durnin, 1988). Assuming a sedentary lifestyle, (activity data suggested that most of the women were indeed relatively inactive) this translates to a difference in total daily energy expenditure in the region of 600 kcal/day. Clearly, this has practical implications for the women. The individual with the low level of energy expenditure must match this with a corresponding low intake if she is to remain in energy balance and avoid gaining weight. For many years the debate has raged as to whether those with a low level of energy expenditure are predisposed to obesity. Unfortunately, study in this area has often been limited to comparing the metabolic rates of the lean with those of the obese. This approach is fraught with difficulties, not least because it gives no indication of the metabolic rates of obese subjects before they gained weight

Recently however, Ravussin and colleagues (1988) conducted a series of prospective studies to determine the relative rates of weight gain in persons with low metabolic rates (after adjusting for age, sex and differences in FFM) compared to those with high metabolisms. The results showed that those with low adjusted energy expenditures were at significantly greater risk of gaining weight. It must of course, be perfectly possible to have a low level of energy expenditure relative to FFM and remain lean, but in an affluent Western society where food is abundant it is not too difficult to envisage how this may prove a problem for some. In contrast, in a situation where food is in relatively short supply, as is the case in many developing countries today, a relatively low level of energy expenditure may well be advantageous.

Clearly, the large differences observed in BMR in relation to the FFM have potentially important practical implications. To look in more depth at the possible causes of such variation two groups of women, characterised by particular high or low BMRs relative to FFM, were selected from this study for further investigation. The results are presented in Chapter 5.

Although FFM was found to have highest correlation with BMR, the correlation coefficients of some other body composition variables, in particular body weight and calf circumference, were only slightly lower (Table 3.2). Indeed, as indicated by the 'F' ratios of the residual standard deviations, for the purposes of predicting the BMR of an individual, FFM was no better than body weight or calf circumference. The predictive ability of the circumference measurements, exemplified by the calf, probably results from the fact that they are highly correlated to FFM ($r = 0.71$, $p < 0.001$, for the calf) and are essentially providing an estimate of FFM. The parity of FFM and body weight in predicting BMR may be a consequence of two factors. The first may relate to the heterogeneity of the FFM in terms of the metabolic rates of its component organs

and tissues. If, as suggested, the composition of the FFM - the relative proportions of 'active' and 'inactive' tissue - can vary between individuals and potentially result in very different metabolic rates for a given mass of fat-free tissue, it is perhaps not surprising that in a group of individuals of relatively normal body size, the use of whole body mass gives as good an estimate of BMR as does FFM. As alluded to above however, when groups of very different body size, particularly of extremes of body fatness, are compared this is not found to be the case. A second possible reason, linked with the first to a certain extent, may relate to differences in measurement error. No technique of estimating FFM is free from error. In addition to experimental error, any deviation for the assumptions upon which the techniques rely - a difference in the composition of the FFM for example - will lead to a spurious result. Body weight on the other hand can be determined extremely accurately. Day to day fluctuations in weight do occur - for most women these probably do not exceed 0.5kg (Garrow, 1978) - however, any error resulting from deviations from the 'true' mean will apply equally to an estimate of FFM derived from body weight as to weight itself. It could be argued that the ease and accuracy with which body weight can be measured makes a good case for the use of this variable to predict BMR in preference to FFM. The observation that, as a predictor of BMR, body weight is little, if any, better than FFM is not an isolated one, Owen *et al.* (1986, 1987) and Lawrence *et al.* (1988) have also found that within a group of individuals the use of weight was comparable to the use of FFM. However, the large residual standard deviations for both FFM and weight casts doubt on the usefulness of either variable in predicting an individual's BMR (this is discussed subsequently in Chapter 6).

As noted by Scofield (1985a), Owen *et al.* (1987) and Lawrence *et al.* (1988), BMR/kg body weight was found to decline as the weight of the women increased. This may be partially explained by the relative increase in adipose

tissue, with its low metabolic rate, from light to heavy individuals. However, in accord with Lawrence *et al's* (1988) study and the earlier work of Miller & Blyth (1953) BMR expressed per kg FFM also tended to be lower in heavier compared to lighter women, suggesting that the increase in body fat content may be be only part of the explanation.

It was pointed out in the Introduction that part of the reason for the decline in BMR/kg FFM may be statistical rather than physiological. If FFM is either under- or over-estimated it follows that BMR expressed per kg FFM will appear either higher or lower than in reality. Outlying points have a disproportionate effect on the slope of the regression line, and in the regression of BMR/kg FFM against FFM these outlying points will tend to be from small subjects whose FFM is under-estimated (high BMR/kg) or from large subjects whose FFM is over-estimated (low BMR/kg). Any tendency for the BMR/kg FFM to fall as the weight of the FFM increases will therefore be exaggerated by measurement error. Lawrence *et al.* (1988) acknowledge that the decline in BMR/kg FFM from light to heavy women observed in their study may be partly a consequence of this sort of statistical artifact, resulting from errors in estimation of FFM using the skinfold technique. In the present study FFM was measured using both the skinfold method and densitometry. It was postulated that if the error in the skinfold method was an important factor in explaining the decrease in BMR/kg FFM, the slope for the regression line relating BMR to FFM estimated by densitometry would be steeper than that relating BMR to FFM estimated from skinfolds. In the event however, the slopes of the lines were not found to be statistically different:

$$\text{Skinfold, BMR (kcal/day)} = \mathbf{23.0}\text{FFM} + 375$$

$$\text{Density, BMR (kcal/day)} = \mathbf{20.9}\text{FFM} + 462$$

There are two possible explanations for this. The first is that both methods were equally inaccurate, each having an error of ± 10% of body weight - using data simulations it is possible to show that the error in measuring FFM would have to be in the region of ± 10% to account for the 15% difference in BMR/kg FFM between light and heavy women. The alternative possibility is that the decline in BMR/kg FFM with increasing weight of the FFM is the result of physiological variation. It seems very unlikely that the skinfold and density techniques are both associated with a 10% error. The residual standard deviation of regression equation relating skinfolds to density was 3.7%, suggesting in fact that the error was much less, around ± 2-3% of body weight. This is consistent with the analysis of Womersley & Durnin (1977) and would seem to indicate much of the fall in BMR/kg FFM with increasing weight of the FFM must have a physiological explanation.

It might have been expected that the approach adopted in this study of taking the average of the two estimates of FFM would result in a regression line with a steeper slope than for that for the skinfold or densitometry estimates alone since taking the mean of the estimates should reduce measurement error. This was not found to be the case.

Skinfold + Density, BMR (kcal/day) = **22.4**FFM + 403

There were no significant differences in the three slopes. However, it is likely that if the measurement errors associated with the two techniques were small, taking an average would not result in very much change in the slope. This again suggests that errors in estimation were small and unlikely to be responsible for the fall in BMR/kg FFM. The indications are therefore, that the observed decline has a physiological basis.

A decrease in BMR/kg FFM from light to heavy individuals could feasibly be produced if, as weight increased, the proportion of the FFM occupied by the metabolically 'active' tissues such as the liver, kidneys, heart and brain declined and concurrently the proportion of tissues with comparatively low metabolic rates increased. In this group of women the fat-free component of adipose tissue would certainly have accounted for a greater proportion of the FFM in heavy compared to light individuals, since those with a large FFM also tended to be fatter. However, it is debatable, as discussed above, whether nonfat adipose tissue can be considered to have a low metabolic rate. Furthermore, regression analysis has shown that, once differences in FFM have been taken into account there is little correlation between percentage fat and BMR. This would indicate that the increasing fraction of the FFM made up of nonfat adipose tissue can not explain the observed fall in BMR/kg FFM. It is therefore necessary to look for other differences in the composition of the FFM between light and heavy women which may be responsible for this decline.

Lawrence *et al.* (1988) have suggested that perhaps the most likely difference would be the proportion of skeletal muscle. They contend that it would not be too surprising if heavier individuals were more muscular, as well as fatter, than their lighter counterparts. The literature contains very little data from which to assess this possibility. The only direct evidence appears to be that from the study of Clarys, Martin & Drinkwater (1984). The Brussels group examined the composition of the adipose tissue-free mass (ATFM, body mass less all dissectible adipose tissue) in twenty five elderly Belgian cadavers. A further analysis of the data presented by these authors clearly showed that as the ATFM increased so too did the proportion of muscle it contained (see Introduction for details), adding some credence to the suggestion that heavy individuals are more muscular than their lighter counterparts. However, because of the small numbers involved in this

investigation and the advanced years of the cadavers, the results must be interpreted with some caution and can not necessarily be taken as evidence that this sort of relationship exists in other groups.

In the present study the circumference measurements unfortunately proved too crude an index of muscularity to test the hypothesis that the heavy women had a greater proportion of muscle making up their FFM than the light individuals - a possible explanation for their lower BMR/kg FFM. As pointed out above, circumferences are highly correlated with FFM, since the larger the FFM, the greater the absolute amount of muscle, it follows that the greater the circumference the greater the muscle mass. They are not however, a measure of muscle which is independent of the size of the FFM and cannot therefore be utilised to determine the proportion of muscle occupying the FFM of the light and heavy women.

As discussed above, it has been shown in this and previous studies that in relation to the FFM, BMR is not affected by body fatness (eg. James *et al.*, 1978; Halliday *et al.*, 1979; Lawrence *et al.*, 1988) nor does the relationship appear to differ between the sexes (eg. Cunningham, 1980; Bernstein *et al.*, 1983; Owen *et al.*, 1987; Weststrate, 1989). In other words, those with a similar FFM will have a similar BMR whether they are male or female and whatever their body fat content. However, it has been noted that when expressed 'per kg' of FFM men have a higher BMR than women (Weststrate, 1989) and some groups of obese subjects a higher BMR than their lean counterparts (Ravussin *et al.*, 1982; James, 1985; Weststrate, 1989). Since men generally have a greater FFM than women and the obese a relatively enlarged FFM compared to the lean it may be that these observations fit into a general picture of a decline in BMR/kg FFM as the weight of the FFM increases. Data from several studies (n = 15), including the present one, where FFM and BMR are given were collated and the mean BMR/kg FFM for each group plotted against the mean FFM (Figure 3.5). The data are diverse,

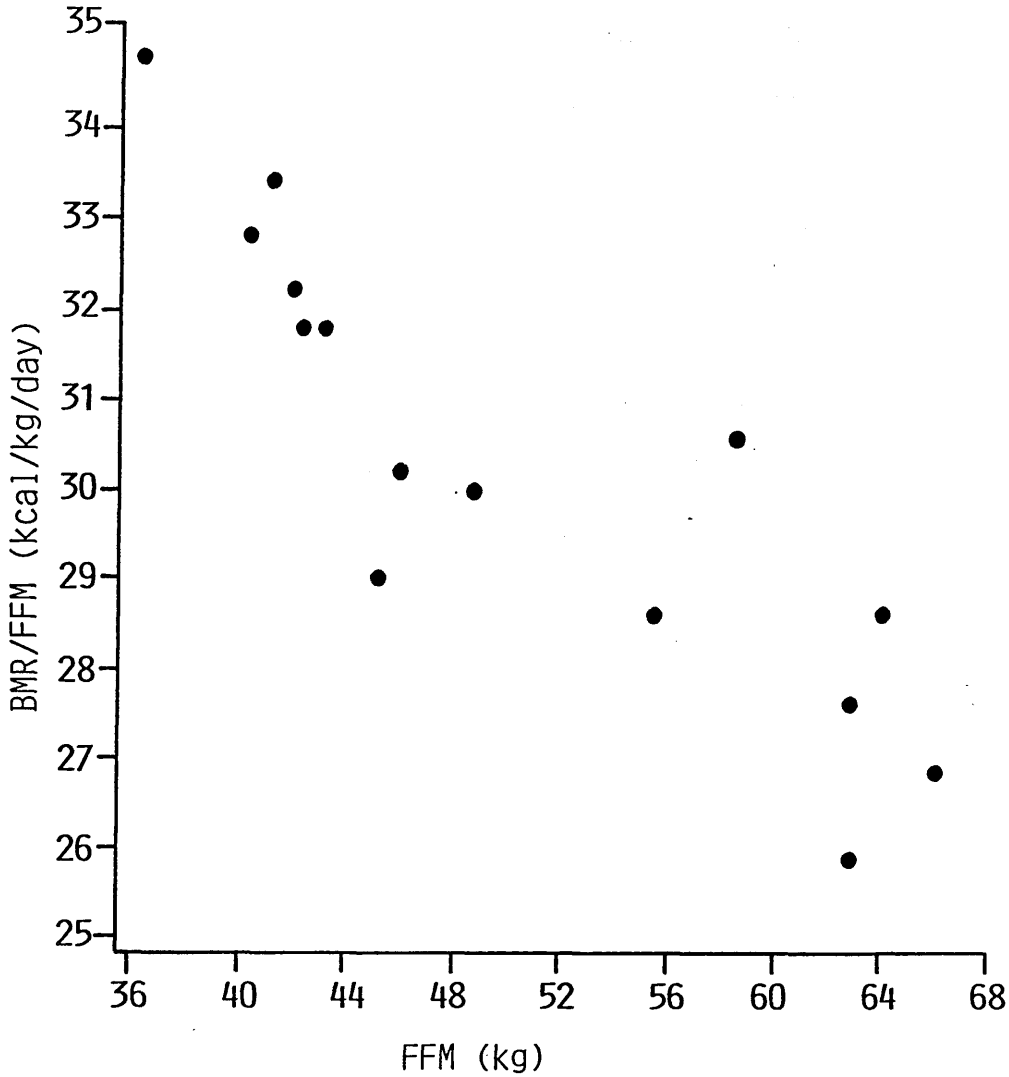

Figure 3.5
Scatterplot of BMR/kg FFM against FFM: some group data from the literature.*

*Ravussin et al (1982); Ravussin et al (1986); Owen et al (1986); Bogardus et al (1986); McNeill et al (1987); Owen et al (1987); Lawrence et al (1988); Weststrate (1989).

including groups of predominantly obese subjects, single sex studies, groups described as 'living at a low plane of nutrition' and groups of the same race, as well as those extending across the entire range. FFM was measured by a number of different techniques. The use of the mean values for FFM should largely eliminate the statistical problems associated with errors in estimation of FFM, which can affect this type of correlation when performed within a group (see above).

A strong negative correlation between BMR/kg FFM and FFM was apparant ($r = - 0.86$, $p < 0.001$); BMR/kg FFM declined as the weight of the FFM increased. This is consistent with the relationship observed for the women in the present study. It seems to suggest that irrespective of why the FFM is a particular size - whether the result of obesity, sex, nutritional status or ethnic origin - there is a general trend for BMR/kg FFM to decline as the weight of the FFM increases.

This observation has important implications for the use of FFM as a metabolic reference standard. Expressing BMR 'per kg FFM' could potentially result in misinterpretation of data when the groups or individuals concerned are of different body size. Superficially for example, it might suggest that the metabolic rate of the obese is lower than the lean or the BMR of women is greater than men, when this is apparently not the case if differences in the weight of the FFM are taken into account. 'Normalising' BMR data in this way could be a particular hazard when investigating the possibility of metabolic adaptation in those exposed to chronically low energy intakes since studies in this area often involve comparing groups of markedly different body size (eg. Shetty, 1984). Certainly in those groups with different weights of fat-free tissue a difference in BMR/kg FFM cannot necessarily be taken as an indication of differences in metabolic activity at cellular level.

Miller & Blyth's data (1953) indicated that in the college students they studied BMR was not constant when divided by FFM itself but rather by FFM $^{0.64}$

By correlating log BMR against log FFM in the same way, Lawrence *et al.* (1988) found BMR in their groups of women to be approximately constant when divided by the square root of FFM (BMR/kg $FFM^{0.5}$). Adopting the same approach in the present study revealed that in these women BMR was constant when the demoninator was $FFM^{0.7}$. In the combined studies BMR was similar in all groups when divided by $FFM^{0.48}$. These data would seem to suggest that for use as a reference standard it might be more appropriate to relate BMR to a power function of FFM rather than to simply express metabolic rate per kg FFM.

Age Related Changes in BMR and Body Composition.

Age related changes in the BMR of women have not been well documented. The present study provided the opportunity to investigate the effect of age on BMR, particularly in relation to body composition, in a relatively large number of healthy women aged between 16 and 66 years.

No significant change in BMR per day was observed in the women as age increased. Other cross-sectional investigations covering a comparable age range have obtained similar results. Data presented by Harris & Benedict (1919) from 103 women aged between 15 and 74 years suggests little decline in basal heat production before about 50 to 60. Mackay & Patton (1936) reached a similar conclusion. In a study of 73 women aged between 35 and 70 years they found that basal metabolism remained relatively constant until the age of about 50 and declined thereafter. More recently, in a group of 44 women aged 18 to 65, Owen et al. (1986) report age to have little influence on RMR. Doré et al. (1982) did find a decrease in BMR with age in a group of obese women, the numbers involved were small however, and the relation weak ($r = - 0.22$). From the limited data available the indications are that an appreciable decrease in the BMR of

women does not take place before about 50 to 60 years. This may to some extent reflect maintenance of the FFM until this time.

Over the age range of the women in this study FFM showed no significant decrease with age. A large scale anthropometric assessment of almost 1200 women undertaken by Durnin *et al.* (1985) similarly reports no obvious difference in the size of the FFM over the age range 20 to 60 years, a finding supported by Owen *et al's* study (1986) and by that of Cohn *et al.* (1980). Forbes & Reina (1970), who analysed data from over 3,000 women investigated by four groups of workers, suggest that an appreciable reduction in the size of the FFM does not occur in women until after the menopause, around 50 to 60 years. The numbers of women in the present study in this higher age bracket was relatively small; 13 out of 97 were over 50 and of these only 7 were post-menopausal. On this basis, it may have been surprising if FFM had shown a significant decrease with age. Forbes & Reina (1970) based their conclusions on the finding that the potassium content of the women changed little before the age of about 50. This suggests that little muscle (rich in potassium) had been lost. Womersley *et al.* (1976), comparing a group of young sedentary women with a group of older nonobese women (mean age 58), also report only a small decreament in body potassium with advancing years. Cohn *et al.* (1980) reach a similar conclusion. Using total body potassium and nitrogen measurements to parcel out muscle and non-muscle components of the FFM, these workers did not find an appreciable decrease in muscle mass before the age of about 60 in women. In the present investigation circumference measurements made on the women suggest that here too, muscle mass was little different in the older compared to the younger subjects. The interpretation of circumference data as an index of muscularity is complicated by the effect that body fatness has on the measurement; any circumference will necessarily be the product of both muscle and fat. For a given muscle mass the greater the amount of overlying fat, the greater the circumference. By the same

reasoning, for a given amount of fat, the more muscle present the greater the circumference. Using regression analysis we were able to show that at a given skinfold thickness, - an estimate of subcutaneous fat - age had no effect on the circumference measurement. If muscle mass had declined with age one would have expected a smaller circumference for a given amount of fat in older compared to younger women but this was not the case. This implies that muscle mass did not decline in these women.

The other component of FFM which might have been expected to show some loss with increasing years is bone. There is some controversy as to the onset of bone demineralisation in women; as early as 20 years has been suggested (Riggs *et al.*, 1982), others put the figure closer to 40 or 45 years (Vaughan, 1970; Nordin *et al.*, 1972; Cohn *et al.*, 1980; Munro, 1988). It is generally agreed however, that loss is accelerated, and therefore most significant, after the menopause. Durnin & Womersley (1974) suggest that it is probable that between the ages of 45 and 75 years women lose between 18% and 30% of their total mineral content. These workers report a fall in the density of the FFM from about 1,100 kg m^{-3} in young women to between 1,092 and 1,095 kg m^{-3} by the age of about 60 which they attribute primarily to bone mineral depletion (Womersley *et al.*, 1976). Some loss of bone may have occured in the women in the present study. However, bearing in mind the relatively small number of older - fifty plus - women and the even smaller number of post-menopausal volunteers this is unlikely to be substantial and would probably have little effect on the mass of fat-free tissue.

One component of the FFM is however, likely to have shown significant change with age. As the the women became older they also became fatter and consequently the nonfat component of adipose tissue would have increased. Rough calculations based on weight gain over the age range suggest that this probably equates to around 0.5kg per decade. All other things being equal FFM

would have been expected to increase. It did not appear to however, suggesting that some other component of the FFM must have decreased. Muscle and bone might have been thought the most likely candidates. As discussed above however, it seems unlikely that any appreciable loss of muscle occured over the age range. Similarly, although some demineralisation of the skeleton may have occured, it would be very surprising if this could match the calculated gain in nonfat adipose tissue. Indeed it is difficult to envisage which component of the FFM could have declined sufficiently to have offset this apparently large gain in adipose tissue and by so doing allowed the mass of fat free tissue to remain relatively constant.

Several studies have reported that once differences in FFM have been taken into account age has little influence on BMR (eg. Cunningham, 1980; Bernstein *et al.*, 1983; Owen *et al.*, 1986, 1987). In the present investigation FFM remained constant with increasing years and so too did BMR, supporting the contention that the relationship between the two is not affected by age; women with a similar FFM had a similar BMR, no matter how old they were. It would seem that the increasing proportion of nonfat adipose tissue occupying the FFM of the older women had little impact. This is consistent with the general observation that differences in body fatness have no effect on BMR once differences in FFM have been taken into account (see above).

In contrast to the findings of this study, McNeill *et al.* (1987) and Doré *et al.* (1982) both report a small but significant decline in BMR in relation to FFM with increasing years; at a given FFM older individuals have a lower BMR. The differential results are not readily explained but may be associated with differences in age-related changes in body composition or perhaps even the metabolic activity of the individual tissues in the groups considered by these workers compared to our own; McNeill *et al.* (1987) studied Indian men, Doré *et al.* (1982) obese women.

The present study gives no indication of a fall with age in metabolic rate per unit of tissue - a 'slowing of metabolism' at cellular level. However since little age-related change in body composition is obvious in these women (apart from progressive fat accumulation) it would have perhaps been surprising if the data had indicated otherwise.

The lack of an affect of age on the relationship between BMR and FFM would suggest that over the age range of the women in this study at least, a single equation could be used to predict BMR from FFM. Using body weight in a similar way would not be appropriate. In relation to weight BMR was found to decline with increasing years. For a given weight BMR was some 12% lower in a women of 50 compared to a 20 year old; equivalent to a decline in relation to weight of some 4% per decade. This fall is in excess of the 1% per decade cited in the FAO/WHO/UNU report (1985) but in relatively good agreement with the 5% reported for women in Harris & Benedict's analysis (1919). The decline is probably the result of changes with age in the proportion of the body weight made up as fat. Over the age range of the women in this study weight was found to increase. As already discussed, this is essentially a consequence of an increase in body fat content, FFM remaining relatively constant. At a given weight therefore, an older woman is likely to have a greater proportion of body fat and less FFM than her younger counterpart, and consequently her BMR is likely to be lower.

The decrease observed in BMR in relation to body weight of women between the ages of 20 and 60 in this study brings into question the age categories chosen for the FAO/WHO/UNU equations (1985). A single equation is used to predict BMR from body weight for women between 30 and 60 years. Since this study has shown that over these years BMR falls significantly in relation to weight, the equation might not be equally applicable over the entire range. To test this contention the percentage differences between the measured BMR for women of 30 to 60 and that predicted using the appropriate WHO equation were plotted

against age (Figure 3.6). A significant positive correlation was obtained ($r = 0.32$, $p = 0.01$) indicating that the error associated with prediction did indeed change with age. Overall, the equation over-estimated the BMR of the women by some 4%. At 30 years the error was small; as age increased, the over-estimation became progressively greater. This would suggest that the equation may be more applicable to women at the younger end of the age range rather than to older individuals. A review of the relevant literature revealed that over half of the measurements from which the 30 to 60 years equation had been derived (Schofield, 1985b) were made on women between the ages of 30 and 40. This inclusion of a disproportionate number of younger women by Scofield may explain the over-estimation of BMR in older women in the present study therefore.

It appears that age related changes in the proportion of body weight made up of fat and FFM may also affect the usefulness for older males of the WHO equation for 30 to 60 year old men, see Chapter 4.

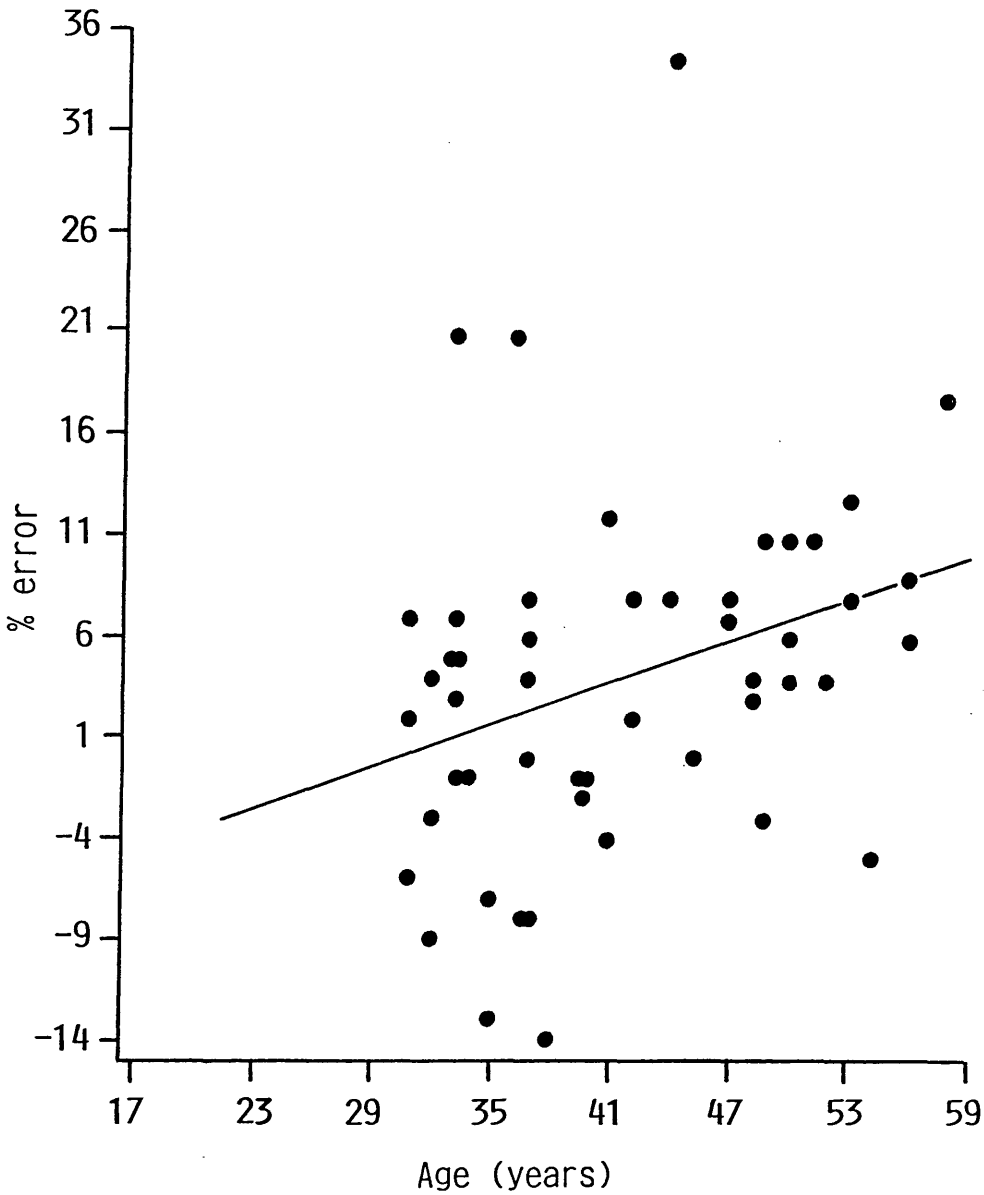

Figure 3.6
Error in estimating the BMR of 30 to 60 year old women in the present study using the FAO/WHO/UNU equation (1985).

CHAPTER 4

THE EFFECT OF ß-ADRENERGIC BLOCKADE THERAPY ON THE BMR OF PATIENTS WITH CARDIOVASCULAR DISORDERS.

INTRODUCTION.

It has been suggested that BMR has two major components: one that relates simply to the mass of the individual organs and tissues in the body, and one to the metabolic activity of those tissues - to the energy demanding processes at cellular level (James *et al.*, 1979). Together these will determine BMR and differences in either component will be manifest in differences in metabolic rate, thus representing potential sources of variation between individuals. Jung *et al.* (1980) have suggested that the metabolic activity of the tissues is at least partly under the control of the sympathetic nervous system. They found that administration of the ß-adrenergic blocker propranolol over the course of a week to a group of obese women on a weight maintenance diet resulted in a fall in RMR of almost 9%. They took this to imply that BMR has a component which is adrenergically mediated. Scheidegger *et al.* (1984) also report a decrease in BMR after acute ß-adrenergic blockade in lean men. However, the effect of ß-blockade and the part played by catecholamines in determining BMR is controversial. Several groups have found acute administration of propranolol to have no significant effect on basal or resting metabolic rate (Acheson *et al.* ,1983; Welle & Campbell, 1983; Defronzo *et al.*, 1984; Seaton *et al.*, 1984; Vernet *et al.*, 1987; Gelfand *et al.*, 1987). In Vernet *et al's* study a trend for BMR to decline was observed but the decrease just failed to reach statistical significance. The reason for the discrepancy between the various studies is not clear. It may relate to the doses of ß-blocker given or to the manner in which they were administered. In the studies where no effect of propranolol was observed the dose was generally - with the exception of Vernet *et al's* study (1987) - lower than that given by Jung *et al.* (1980) who found an effect. In addition, propranolol was administered by IV infusion over relatively short periods of time, typically 3-4 hours, in contrast with the oral doses given over the course of a week

in Jung's study. It may be that longer term administration is necessary to produce a change in BMR.

Since ß-adrenergic blockers are routinely prescribed in the treatment of a number of common cardiovascular complaints, including hypertension, angina and cardiac arrhythmias, the question arises as to whether the doses of ß-blockers used in the management of such disorders could cause a reduction in BMR. A significant lowering of basal metabolism could have important implications regarding energy balance in patients taking ß-blockers. Since somewhere in the region of 10% of the male population of the UK over the age of 40 are estimated to be receiving ß-blockade therapy this is an issue of some importance.

The doses of propranolol and the manner of administration employed in Jung *et al's* study (1980) are probably most akin to those used clinically, although their doses maybe somewhat higher. In this case RMR was reduced, but the issue is far from resolved.

The aim of the present study therefore, was to investigate the effect on BMR of clinically prescribed doses of ß-adrenergic blockers. The BMRs of a group of patients undergoing ß-blockade treatment - with propranolol or, more commonly, another ß-blocker - were compared to those of control subjects. As well as examining a potentially important and apparently over-looked side effect of this widely used group of drugs, the study provided the opportunity to investigate the contention that BMR has an adrenergically mediated component, possibly involved in the regulation of the metabolic activity of the tissues.

SUBJECTS AND METHODS.

Subjects.

18 male subjects receiving ß-blockade therapy and 28 healthy controls participated in the investigation. The study took place in collaboration with the Department of Cardiac Surgery at Glasgow's Royal Infirmary and all ß-blockade

subjects were inpatients at the hospital. Most of the patients were being treated for angina pectoris. Two, however, were in hospital for more serious cardiovascular complaints and subsequently underwent bipass operations. The majority of patients were receiving cardio-selective ß-1 adrenergic blockers such as metropolol (Lopressor) and atenolol (Tenrmin). Less commonly, propranolol (Inderal), a ß-1 & 2 antagonist, had been prescribed. Unfortunately, information on the specific drug and dose received by each patient is not available.

The control subjects were male employees with Strathclyde Regional Council. All had recently been screened as part of Greater Glasgow Health Board's 'Good Hearted Glasgow' campaign and were free from cardiovascular disease, receiving no medication and deemed in generally good health.

Measurement of BMR.

In all subjects BMR was determined using the ventilated hood system, as described in Chapter 2. ß-blockade patients were measured at the hospital where they had spent the night - all had been 'up and about' for sometime before their measurements - control subjects at their place of work, having arrived from home early in the morning. In both cases a quiet room was set aside specifically for the purpose of the study. All subjects were in a fasted state and rested for half an hour before the measurements began.

Anthropometry.

Subjects were weighed after emptying their bladders and when wearing only pyjamas or underwear. Their weight was recorded to the nearest 0.1 kg. Height was measured to the nearest mm. (See Chapter 2). BMI (Wt (kg) / Ht $^2(m^2)$ was determined from these two variables.

Body fat content was assessed from the sum of four skinfold thicknesses (biceps, triceps, subscapular and supra-iliac) according to the equations of Durnin

& Womersely (1974), see Chapter 2. Other methods of determining percentage body fat were not considered practical in this study.

Statistical methods.

Two tailed Student's t-tests were used to evaluate differences between ß-blockade and control subjects. Analysis of variance and co-variance were used to compare the BMR of the groups, adjusted for differences in FFM or weight. Linear regression analysis was used to derive the regression equations relating BMR to weight, and to FFM. All procedures were performed using the SPSSx statistical language

RESULTS.

The physical characteristics of the ß-blockade and control subjects are presented in Table 4.1. Although attempts were made to match the controls as closely as possible with the patients, in the event the ß-blockade patients proved to be significantly heavier and had a greater BMI and FFM than the controls. There were no significant differences in the ages, heights or body fat contents of the two groups.

The absolute BMRs of the groups were not different. However, when expressed per kg of body weight or per kg FFM, the BMR of the ß-blockade subjects was found to be 10% lower than that of the controls (Table 4.1). In Chapter 3 we have shown that as weight increases BMR/kg tends to fall, similarly BMR/kg FFM declines as the FFM increases. It may not be appropriate therefore, to compare the BMRs of groups who differ in weight or FFM. To overcome this problem an analysis of variance was carried out using weight or FFM as covariables. The results, (Table 4.2), demonstrate that after adjusting for differences in weight or FFM in this way, there was a significant difference

Table 4.1

Physical characteristics and BMR results of ß-blockade and control subjects.

	ß-blockade (n = 18)		Control (n = 28)		
	mean	(SD)	mean	(SD)	P
Age (yrs)	53.6	6.3	53.3	6.6	0.9
Weight (kg)	81.9	8.9	74.1	8.5	0.005
Height (cm)	173.7	3.6	173.4	5.2	0.8
BMI (kg/m^2)	27.2	2.7	24.5	2.5	0.002
% Body fat	26.4	4.3	26.1	2.9	0.78
FFM (kg)	60.1	4.6	54.6	5.4	0.001
BMR:					
kcal/day	1544	199	1546	177	0.97
kcal/kg Wt//day	18.9	1.9	21.0	2.3	0.002
kcal/kg FFM/day	25.6	2.3	28.4	2.6	0.001
% diff WHO*	-14.2	8.5	-9.6	8.5	0.08

* % difference from BMR predicted according to the equations of the FAO/WHO/UNU (1985).

Table 4.2

The effect of weight or FFM on the relationship between BMR and ß-blockade therapy assessed by analysis of variance.

Dependent variable : BMR

(Residual df = 44)

A. Source of variation:	df	F-ratio	
Weight	1	22.2	**
ß-blockade therapy	1	3.9	*

B. Source of variation:	df	F-ratio	
FFM	1	33.5	***
ß-blockade therapy	1	7.8	*

df = degrees of freedom.

Statistical significance of F-ratio, * P < 0.005, ** P < 0.01, *** P < 0.001

between the BMR of the ß-blockade and control subjects. Using linear regression analysis the following equations were derived which best described the relationship between BMR and weight, and BMR and FFM in the two groups:

$$BMR = (Weight \times 12.6) + 615 - (Gp \times 100)$$

$$BMR = (FFM \times 24.3) + 216 - (Gp \times 136)$$

Where Gp = 1 for the ß-blockade patients and Gp = 0 for the controls.

These indicate that in relation to weight the BMR of the ß-blockade patients was 100 kcals/day lower than that of the controls, in relation to FFM, 136 kcal/day less. At a weight of 75kg and a FFM of 57kg this is equivalent to a difference of 6% and 8% respectively.

In comparison with standard values predicted from FAO/WHO/UNU equations (1985) the BMR of the ß-blockade patients was some 14% lower than expected (Table 4.1). The BMR of the control subjects was also less than predicted, by about 10%. The difference between the groups just failed to reach statistical significance.

DISCUSSION.

The results of this study indicate that clinically prescribed doses of ß-adrenergic blockers reduce BMR. When adjusted for differences in FFM the BMR of the ß-blockade patients was some 8% lower than that of the control subjects. Both Jung *et al.* (1980) and Scheidegger *et al.* (1984) have observed that ß-blockade with propranolol reduces BMR, this study suggests however, that ß-

blockers other than propranolol can produce this effect. The majority of patients in the study were receiving ß-1 blockers such as atenolol and metroprolol. This reflects the general trend away from the clinical use of propranolol - a ß-1 & 2 antagonist - because of the increase in airway resistance which can accompany its cardiovascular effects, a potential danger to asthmatics. The inhibitory effects of ß-1 drugs, unlike propranolol, are generally limited to the cardiovascular system, although none are entirely cardio-selective (Weiner 1987).

It would seem then, that a reduction in BMR may be a side effect of both propranolol and, of greater clinical relevance, the more usually administered ß-1 antagonists. The usefulness of ß-blockade therapy in the management of cardiovascular disease is undisputed, however, even when set against the undoubted beneficial effects of the drugs, a depressed BMR is a potentially important side effect. The quantitative importance of BMR to the total daily energy expenditure (TEE) of subjects such as the ß-blockade patients who, because of the nature of their cardiovascular disorders, are essentially sedentary means that a reduced BMR will necessarily result in a reduced TEE. If the energy intake of the patients remains unchanged in the face of a reduced energy expenditure positive energy balance and weight gain will ensue. Some of the patients did in fact report that they had put on weight, but this may not necessarily be the result of ß-blockade therapy. If however, through a reduction in BMR and TEE, ß-blockers did lead to weight gain this could potentially exacerbate the very conditions these drugs are prescribed to treat. Obesity is recognised to be a complicating factor in cardiovascular disease.

It is possible that BMR may not be the only component of TEE to be affected by ß-blockade, the thermic effect of food (TEF) might also be reduced; there is some evidence to suggest that it too has an adrenergically mediated component (Robertson & Porte, 1974; Welle *et al.*, 1980; Acheson *et al.*, 1983; Le

Blanc *et al.*, 1984; DeFronzo *et al.*, 1984). If this is the case, the result would be to lower TEE still further and increase the potential risk of weight gain.

Whether the result of ß-blockade therapy or not, the patients in this study could certainly be classified, from either body fat content or BMI, as moderately obese and would probably benefit from weight loss. It is well established that weight loss is useful means of reducing blood pressure (Ramsey *et al.*, 1978; Reisen *et al.*, 1978; Jung *et al.*, 1979a; Sowers *et al.*, 1982) and other cardiovascular conditions may also become more manageable with weight reduction (Jung *et al.*, 1980). Moreover, a very obese individual might be required to slim down prior to surgery. Jung *et al's* data (1980) suggest that should these patients decide to diet their weight loss will not be slowed by ß-blockade therapy. The rate of weight loss during energy restriction depends on the extent of the energy deficit incurred. On a given intake the lower the BMR and therefore TEE, the smaller the deficit and the slower the rate of weight loss. A fall in BMR would therefore be expected to reduce the efficacy of a slimming diet. However, Jung *et al.* (1980) found propranolol to have no effect on the BMR of obese women already following a semi-starvation diet, presumably because the adrenergic component of BMR was already minimal.

To date, the fact that ß-blockers have any effect on metabolic rate does not appear to have been recognised. Certainly, the authoritative text, Goodman and Gilman's 'The Pharmacological Basis of Therapeutics' makes no mention of a depression in BMR when discussing the possible untoward effects of ß-blockade treatment (Weiner, 1987).

Aside from the clinical relevance of a reduction in BMR with ß-blockade therapy, the results of this study support the view that BMR has an adrenergically mediated component (Jung *et al.*, 1980). They provide no clue however, as to the precise way in which the sympathetic nervous system is involved in the determination of BMR. The ß-blockade may have inhibited a direct action of the

catecholamines on an energy requiring process at cellular level - the Na^+/K^+pump and certain substrate cycles are known to be sensitive to catecholamine levels (Newsholme, 1985; Himus-Hagen, 1983; Clausen, 1986). It is also possible that the depression in BMR resulted from inhibition of catecholamine stimulated lipolysis and FFA oxidation. Several investigators have suggested that at least part of the calorigenic response to catecholamine infusion or increased sympathetic nervous system activity results from the concomitant increase in FFA mobilisation rate (Havel *et al.*, 1964; Steinberg *et al.*, 1964; Eisenstein & Singh, 1980; Scheidegger *et al.*, 1984). Since the lipolytic effects of the catecholamines are thought to be mediated through ß-1 receptor stimulation (Eisenstein & Singh, 1980; Kunos, 1981) both propranolol, a ß-1 & 2 antagonist, and the ß-1 antagonists administered to the patients in this study are likely to have reduced the rate of FFA mobilisation. In retrospect it would have been interesting to compare the rates of substrate oxidation between ß-blocker and control subjects. However, a lower rate of fat oxidation in the ß-blockade patients would not necessarily be indicative of a cause and effect relationship between inhibition of lipolysis/fat oxidation and the reduced BMRs. Havel *et al.* (1964) found that a reduction in plasma FFA levels caused by nicotinic acid injections had no effect on BMR, suggesting that the availability of FFA as a fuel may not be an appreciable influence on resting metabolic rate under normal circumstances. Weststrate (1989) noted that high BMRs/kg FFM were associated with high rates of fat oxidation but whether the high metabolic rates were caused by the high rates of fat oxidation or by another manifestation of increased sympathetic activity is unclear.

It has been suggested that at least part of the thermogenic effect of the catecholamines results from their actions on peripheral thyroid metabolism. Catecholamines are able to increase circulating levels of T_3 by stimulating the peripheral conversion of T_4 to T_3 (Galton, 1965; Rothwell *et al.*, 1982; Scheidegger *et al.*, 1984). Propranolol has been shown to inhibit this conversion

and so reduce circulating levels of T3 (Lotti *et al.*, 1977; Eisenstein *et al.*, 1978; Jung *et al.*, 1980; Jones *et al.*, 1981). However, inhibitory effects on peripheral T3 production may vary from one ß-antagonists to another. Atenolol for instance, has been shown to have no significant effect on T3 levels (Jones *et al.*, 1981). In retrospect it would have been informative to measure, and compare, T3 levels in ß-blocker and control subjects. If the reduction in BMR with ß-blockade is caused, at least in part, by its actions on peripheral thyroid metabolism this may explain the lack of any appreciable effect in previous investigations where propranolol was administered acutely (Acheson *et al.*, 1983; Welle & Campbell, 1983; Seaton *et al.*, 1984; DeFronzo *et al.*, 1984; Vernet *et al.*, 1987; Gelfand *et al.*, 1987) compared to the long-term administration in this and Jung *et al's* study (1980). Most of the effects of thyroid hormones appear to be mediated by activation of nuclear receptors that lead to formation of RNA and subsequent protein synthesis, for example formation of Na^+/K^+ATPase (Dauncey, 1990). Consequently, a time lag can be expected between changes in thyroid status and the resultant metabolic effects. Any depression of metabolic rate caused by a ß-blocker reduction in T3 levels is unlikely therefore, to have become evident in experiments lasting only a few hours.

The BMR of the ß-blockade patients was found to be 14% below standard values predicted on the basis of age and weight by FAO/WHO/UNU equations (1985). However, the BMR of control subjects was also lower than expected, by some 10%, which seemed to suggest that WHO equations may not be applicable to this group of men.

The control subjects were all aged between 41 and 64 years, BMR was therefore predicted from body weight according to one of two equations:

(1) 30 - 60 years BMR (kcal/day) = (11.6 x WT) + 879

or

(2) Over 60 BMR (kcal/day) = (13.5 x WT) + 487

Since the majority of the men, 25 of the 28, were covered by the 30 - 60 year equation the results are essentially reflecting a tendency for this equation to over-estimate BMR. In view of the findings for the female subjects in Chapter 3, it was suspected that this may again suggest that the 30 - 60 year equation might be more applicable to men at the younger end of the age range rather than to our older, 40 plus, group. An examination of the relevant literature provided confirmation; it was found that only about half of the measurements from which the equation was derived (Scofield, 1985b) were made on men over 40. Assuming that for men too, as in the female subjects, there is an increase in the proportion of the body weight made up as fat with increasing years (eg. Keys *et al.*, 1953; Shock *et al.*, 1973) at a given weight the over 40s are likely to have more fat and a lower FFM than their younger counterparts and consequently a lower BMR. This could well explain the over estimation of BMR in the control subjects, especially as most were near the upper end of the 30 to 60 range (mean age 53).

Since the WHO equation for men over 60s gives a lower estimate for BMR in relation to weight it may be a better predictor of BMR in those between 40 and 60. Indeed when using this equation to estimate the BMR of all the control subjects - both those above and below 60 - a mean difference of only + 4.1% was found between the actual and predicted values, this did not prove to be statistically significant ($p > 0.05$). However, aside from the apparent merits of this equation, the results certainly suggest that the equation for 30 to 60 year olds may not be applicable to those at the upper end of this age range and this is likely due to the preponderance of measurements which were made on younger individuals.

CHAPTER 5

ANALYSIS OF FACTORS RESPONSIBLE FOR VARIATION IN BMR IN RELATION TO FFM.

INTRODUCTION.

Over recent years differences in 'body composition' - generally taken to mean differences in the mass of fat-free tissue - has become the standard, 'text book', explanation for variation in BMR. The study described in Chapter 3 however, showed that even when differences in the weight of the FFM had been taken into account there was still considerable variation in the BMR of individual women. At a given FFM, BMR could differ by as much as 400 kcal/day. This observation is by no means isolated, others studies have found the residual standard deviations of regression equations relating BMR to FFM to be of a similar magnitude (Bogardus *et al.*, 1982; Lawrence *et al.*, 1988) and several authors have pointed to apparently comparable individuals with widely divergent BMRs (Garrow, 1985; Durnin, 1988). The nature of such variation has not however, been comprehensively studied and is largely speculative. The aim of the present investigation was therefore to elucidate more fully the reasons why, at a given FFM, BMR can apparently vary between individuals by several hundred kcal/day.

Two groups of women, characterised by particularly high or low BMRs in relation to their FFM, were selected from the initial study for further investigation (see Figure 5.1). As shown in Table 5.1, on average the two groups were relatively closely matched in terms of body weight, FFM and percentage body fat, yet differed in basal energy requirements by almost 300 kcals a day. The contribution of the above factors to the differences between the groups was examined. It was hoped that understanding the causes of the very marked differences evident in these women would shed some light on the inter-individual variation in BMR relative to FFM evident in the initial study.

It was postulated that part of the variance may be the result of intra-individual differences in BMR; day to day or short-term fluctuations in a subject's BMR; day to day or short-term fluctuations in an individual's BMR, affected by

Table 5.1

Anthropometric data and BMR results for the high and low BMR groups.

	High (n = 9)	**Low** (n = 10)	
Age (yrs)	28 (12)	32 (13)	NS
Weight (kg)	57.8 (5.5)	62.7 (13.5)	NS
Height (cm)	160.3 (4.0)	164.9 (4.6)	0.05
BMI (kg/m^2)	22.4 (2.6)	23.0 (1.8)	NS
% Body fat :			
Skinfolds	29.3 (6.8)	29.1 (29.1)	NS
Density	30.6 (6.3)	27.3 (7.1)	NS
TBW	33.1 (6.2)	32.8 (8.3)	NS
FFM (kg) :			
Skinfolds	40.6 (2.7)	44.2 (6.6)	NS
Density	39.9 (1.9)	44.9 (6.0)	0.05
TBW	38.9 (6.2)	42.1 (11.1)	NS
Circumferences:			
Upper arm (cm)	27.1 (2.3)	26.9 (3.4)	NS
Buttocks (cm)	94.6 (5.1)	96.5 (9.6)	NS
Upper thigh (cm)	55.5 (3.7)	55.8 (7.8)	NS
Calf (cm)	36.8 (1.8)	36.5 (4.0)	NS
Diameters :			
Biacromial (cm)	35.7 (0.9)	38.2 (1.5)	0.001
Bi-iliac (cm)	29.3 (1.6)	30.1 (3.7)	NS
Wrist (cm)	5.2 (0.2)	5.3 (0.3)	NS
Knee (cm)	9.3 (0.3)	9.5 (0.6)	NS
BMR			
kcal/day	1509 (75)	1227 (64)	NS
kcal/Wt kg/day	26.3 (2.6)	19.8 (1.8)	NS
kcal/FFM kg/day *	37.5 (1.3)	27.5 (1.7)	NS

* FFM, mean of the skinfold and density estimates, see text for explanation.

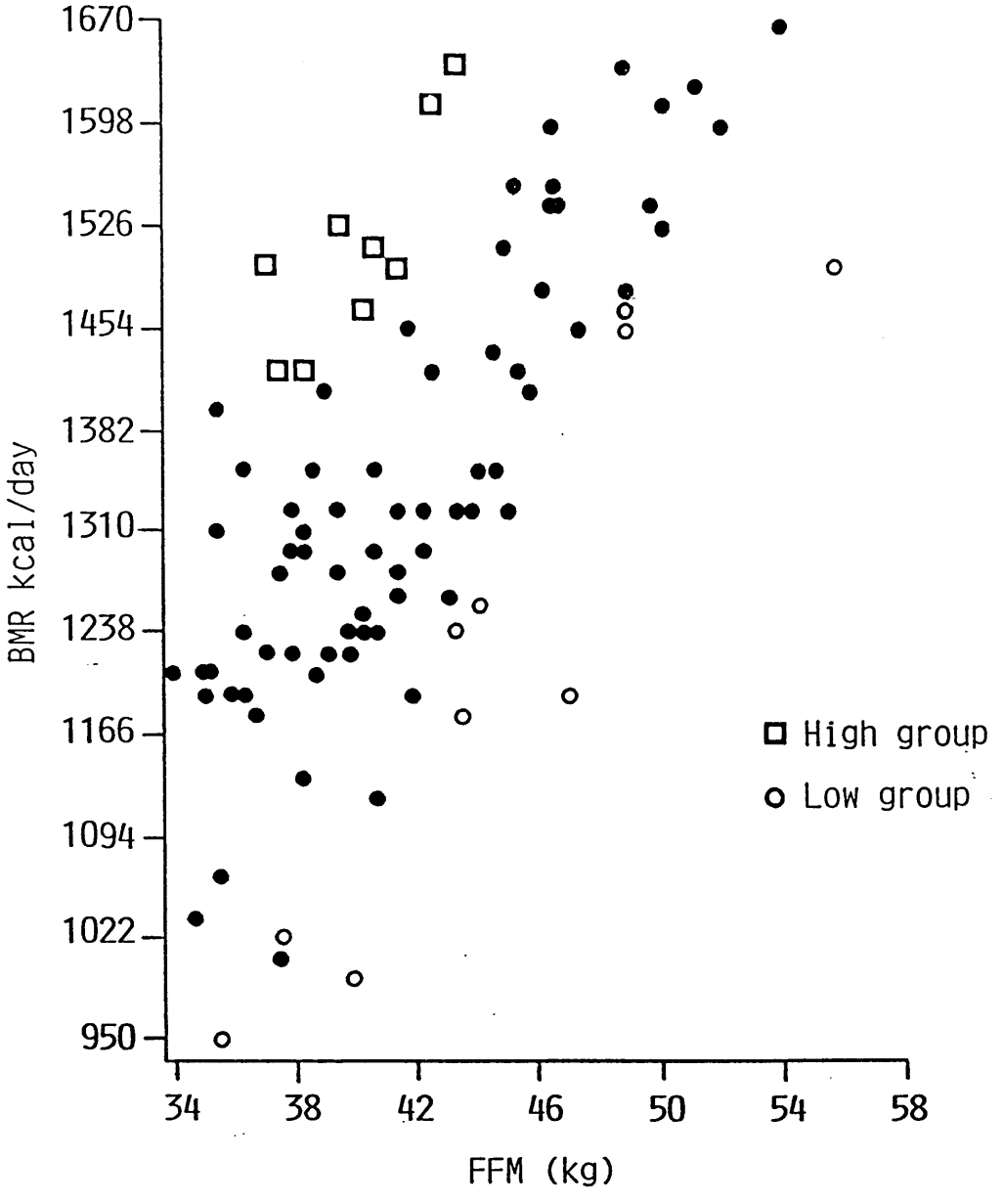

Figure 5.1
Scatterplot of BMR against FFM showing the 19 women selected for further study.

factors such as the preceding day's energy intake, level of exercise and - for women - stage of the menstrual cycle. To make some assessment of the extent to which intra-individual variation in BMR contributed to the differences between the groups, the BMRs of the women were remeasured. FFM was also measured again, on the premise that any error in its initial estimation could also have contributed to the variance. In the first study the FFM of the women had been estimated by TBW, densitometry and skinfold techniques, it was hoped that comparison of the three methods would also provide information as to the possible error involved in measurement of FFM. It seemed reasonable to assume that at least some portion of the variance would be due to genuine differences in the relationship between BMR and FFM. Two possible, hypothesis were proposed. The first relates to composition of the FFM. Since FFM is made up of tissues and organs with very different metabolic rates it is possible that differences between the groups could have arisen through differences in the relative proportions of 'active' tissue, such as the liver,kidney, heart and brain, compared to 'inactive' tissue, much for example, making up the FFM. Alternatively, there may have been differences in the metabolic activity of the tissues between the groups - differences in the rate of energy demanding processes at cellular level. The metabolic activity of the tissues is thought to some extent to be under hormonal control; the results of the ß-blockade study, Chapter 4, certainly suggested BMR has an catecholamine mediated component and thyroid hormones are also implicated in the control of celluar thermogenesis. It was postulated that differences in the levels of these hormones may a play at part in explaining variation between the groups. To test this hypothesis plasma levels of the thyroid hormones and urinary catecholamine concentrations were determined in the women.

SUBJECTS AND METHODS.

Subjects.

The nineteen women selected for study (see above) made a return visit to the laboratory and the measurements detailed below were carried out. The time interval between the first and second visits was variable, ranging from 2 to 9 months, the majority of the repeat measurements however, took place within 3 months of the first visit.

It is evident from Figure 5.1 that the women chosen were not necessarily those in the group with the absolute highest and lowest BMRs in relation to their FFM (this is particularly true with regard to the low group). They were however, as close as possible to this ideal within the practical constraints of the subjects being willing and able to return for a second visit.

Measurement of BMR.

To make some assessment of the contribution of intra-individual variation to the differences observed between the groups, the BMRs of the nineteen women were remeasured. As on the first occasion the Douglas bag technique was employed and metabolic rate calculated as described in Chapter 2. All the conditions prerequisite for determination of BMR were again carefully adhered to.

Body Composition.

The body weight and body fat content of the women were also remeasured. Body fat was estimated from the sum of four skinfolds (biceps, triceps, subscapular and supra-iliac) according to the equations of Durnin & Womersley (1974) and from body density determined by underwater weighing. Details of procedures are given in Chapter 2.

On the first visit to the laboratory percentage body fat had also been estimated from measurement of total body water (TBW). (See Chapter 2 for details).

Hormonal Status.

Serum Thyroid Hormones:

Following measurements of BMR and body composition, and whilst the subject was still in a fasted state, a 10ml sample of blood was taken from a brachial vein. The blood was allowed to coagulate, spun down and the serum drawn off. Aliquots of the serum were then frozen until required for analysis. Assays were subsequently performed for total serum thyroxine (T_4), free T_4 (FT_4), tri-iodothyronine (T_3) and thyroid stimulating hormone (TSH) concentrations. T_4 and T_3 were measured by 'in house' radioimmunoassay (RIA) using SAPU antibody (Scottish Anitibody Production Unit); TSH, by 'in house' two-site RIA using SAPU; FT_4, by 'in house' RIA using microencapsulated antibody. All analyses were carried out by the Department of Clinical Biochemistry at Glasgow's Royal Infirmary.

Urinary Catecholamines:

Each of the women performed a 24 hour urine collection, usually beginning on the morning following the visit to the laboratory. The subjects were instructed to empty their bladders soon after getting up (this urine was discarded) and to begin timing the collection from this point. All subsequent urine was collected until the same time the following morning when the subjects again voided their bladders, this time adding the urine to that already collected. 2-litre plastic bottles were provided for this purpose, which contained 50 ml hydrochloric acid to act as a preservative. The bottles were subsequently uplifted from the subjects' homes by

the investigator. The total volume of the urine was carefully measured and small aliquots kept frozen ready for analysis.

Urinary adrenaline (A) and noradrenaline (NA) concentrations were subsequently determined using high performance liquid chromotography (HPLC) and excretion per 24 hours calculated. The analyses were again carried out at the Department of Clinical Biochemistry, Glasgow Royal Infirmary. Urinary levels of the catecholamines were measured in preference to plasma concentrations since the latter tend to be susceptible to short term fluctuations, affected by factors such as posture, stress and even the physical discomfort involved in the collection of blood samples (Barrand & Callingham, 1983).

Ethical permission for this study was granted by the Ethical Committee of the Greater Glasgow Health Board.

Statistical Methods.

Differences within-subjects in the estimate of percentage body fat obtained by the skinfold, density and TBW methods were evaluated using paired Student's t-tests. Two tailed Student's t-tests were used to evaluate differences in body composition, BMR and hormonal levels between the 'high' and 'low' BMR groups. Analysis of variance and co-variance were used to compare the BMR of the two groups, adjusted for differences in FFM. Linear regression analysis was used to derive regression equations relating BMR to FFM. Coefficients of variation were calculated for the repeat measurements of BMR and FFM. All the procedures were performed using the SPSSx statistical language.

RESULTS.

As pointed out in the Introduction, on the basis of the first set of measurements the two groups of women appeared physically quite similar (Table 5.1). There was a tendency however, for the low BMR group to be somewhat 'bigger' than the high. They were both taller and, on the basis of shoulder width, broader than their high BMR counterparts. They also tended to be slightly heavier, although the difference failed to reach statistical significance. Consequently, the FFM of the low BMR group was slightly larger; significantly so when measured by densitometry but not for the skinfold and TBW estimates. None of the three techniques revealed any significant difference between the % body fat contents of the groups. Age, BMI, circumferences and diameters - with the exception of the biacromial - were also comparable.

In absolute terms or when expressed per kg body weight or per kg FFM, the BMRs of the groups differed markedly (Table 5.1). As pointed out in Chapter 3 however, expressing BMR/kg FFM may not be an appropriate way to compare groups of differing FFM. In view of the tendency for the low BMR group to have a slightly larger FFM than the high, an analysis of variance was carried out using FFM as a covariable. The results, (Table 5.2A), indicated that in relation to the FFM there was a significant difference in BMR between the two groups.

In this instance, and in the BMR 'per kg' FFM computation, FFM was taken as the mean of the skinfold and densitometry estimates since this was the way the results were presented in the first study and the basis on which the subjects were selected. However, analysis of variance indicated that whether in relation to FFM measured by TBW, skinfolds, densitometry or the mean of the estimates, the BMRs of the two groups still differed significantly.

Using multiple linear regression analysis, the following equation was derived which best described the relationship between BMR and FFM in the two groups:

Table 5.2A

Initial measurements : between - group differences in the relationship between BMR and FFM assessed by analysis of variance.

Dependant variable : BMR (Residual df = 16)			
Source of variation :	df	F - ratio	
FFM	1	89.7	***
Group	1	168.0	***

Statistical significance of F - ratio, *** $p < 0.001$

Table 5.2B

Repeat measurements : between - group differences in the relationship between BMR and FFM assessed by analysis of variation.

Dependant variable : BMR (Residual df = 16)			
Source of variation :	df	F - ratio	
FFM	1	19.7	***
Group	1	13.6	**

Statistical significance of F - ratio, *** $p < 0.001$, ** $p < 0.01$

$$BMR\ (kcal/day) = (FFM \times 30.1) + 295 - (Gp \times 411)$$

Gp = 0 for the high BMR group and Gp = 1 for the low BMR group.

This indicates that at a given FFM, the BMR of the low group was some 400 kcal/day less than that of the high, equivalent to a difference of 26% at a FFM of 42kg.

The results of the duplicate measurements of BMR and FFM are shown in Figure 5.2. Very little difference was apparant in the FFM of either of the groups on the two occasions, with a coefficient of variation of only 1.3% for the high and 1.0% for the low group. BMR however, was more variable. In the high group it tended to fall from the first to the second measurement, on average by some 9.0%. In contrast, the BMRs of the low group tended to rise, by an average of 6%. The intra-individual coefficient of variation for BMR in the whole group was 6.5%. This is equivalent to a within-subject variation (standard deviation) of 96 kcal/day.

The new relationship between BMR and FFM for each of the women is shown in Figure 5.3. Clearly, some regression towards the mean has occured. For the most part this is a consequence of differences in BMR on the two occasions rather than to differences in FFM. The average BMR of the groups was no longer significantly different, 1385 kcal/day in the high group compared to 1305 kcal/day in the low ($p = 0.2$). An analysis of variance of the repeat measurements indicated however, that in relation to the FFM the BMRs of the two groups were still significantly different (Table 5.2B). The difference had however, approximately halved. The regression equation describing the relationship between BMR and FFM (see below) indicated that at a FFM of 42kg, the BMR of the low group was now 12% lower than that of the high compared to a 26% difference with the initial measurements.

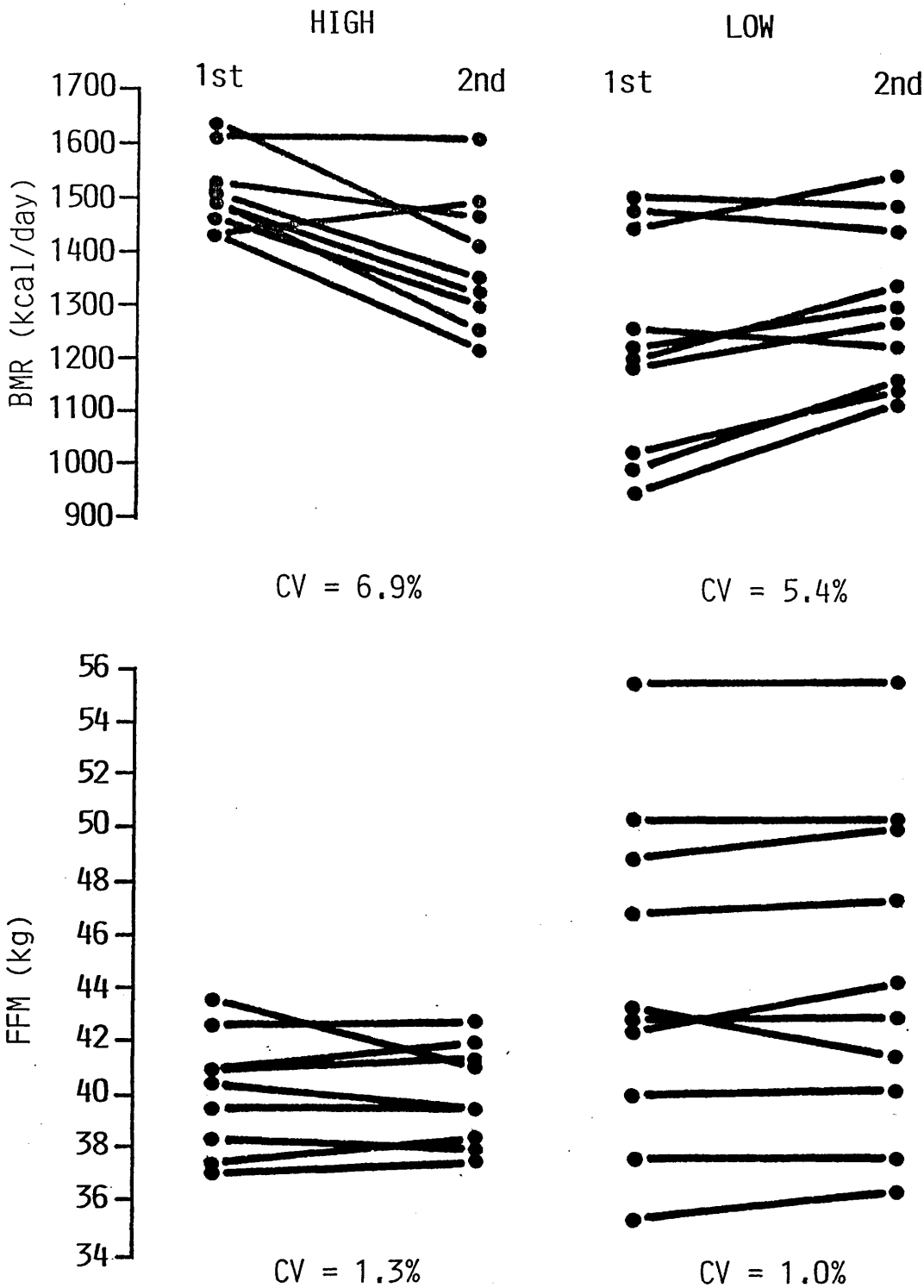

Figure 5.2
Duplicate measurements of BMR and FFM in the high and low BMR groups.

Table 5.3

Hormonal status of high and low BMR groups.

	High (n = 9)		Low (n = 10)		
	mean	(SD)	Mean	(SD)	P >
Thyroid Hormones:					
T4 (nmol/l)	126.2	27.7	91.0	14.8	0.001
T3 (nmol/l)	2.5	0.56	2.0	0.47	0.05
TSH (mU/l)	1.32	0.77	1.51	0.63	NS
FT4 (pmol/l)	15.11	2.47	12.44	2.35	0.05
Catecholamines:					
NA (n.mol/24 hr)	222.5	84.7	236.6	126.3	NS
A (n.mol/24 hr)	78.8	64.9	155.6	98.8	NS

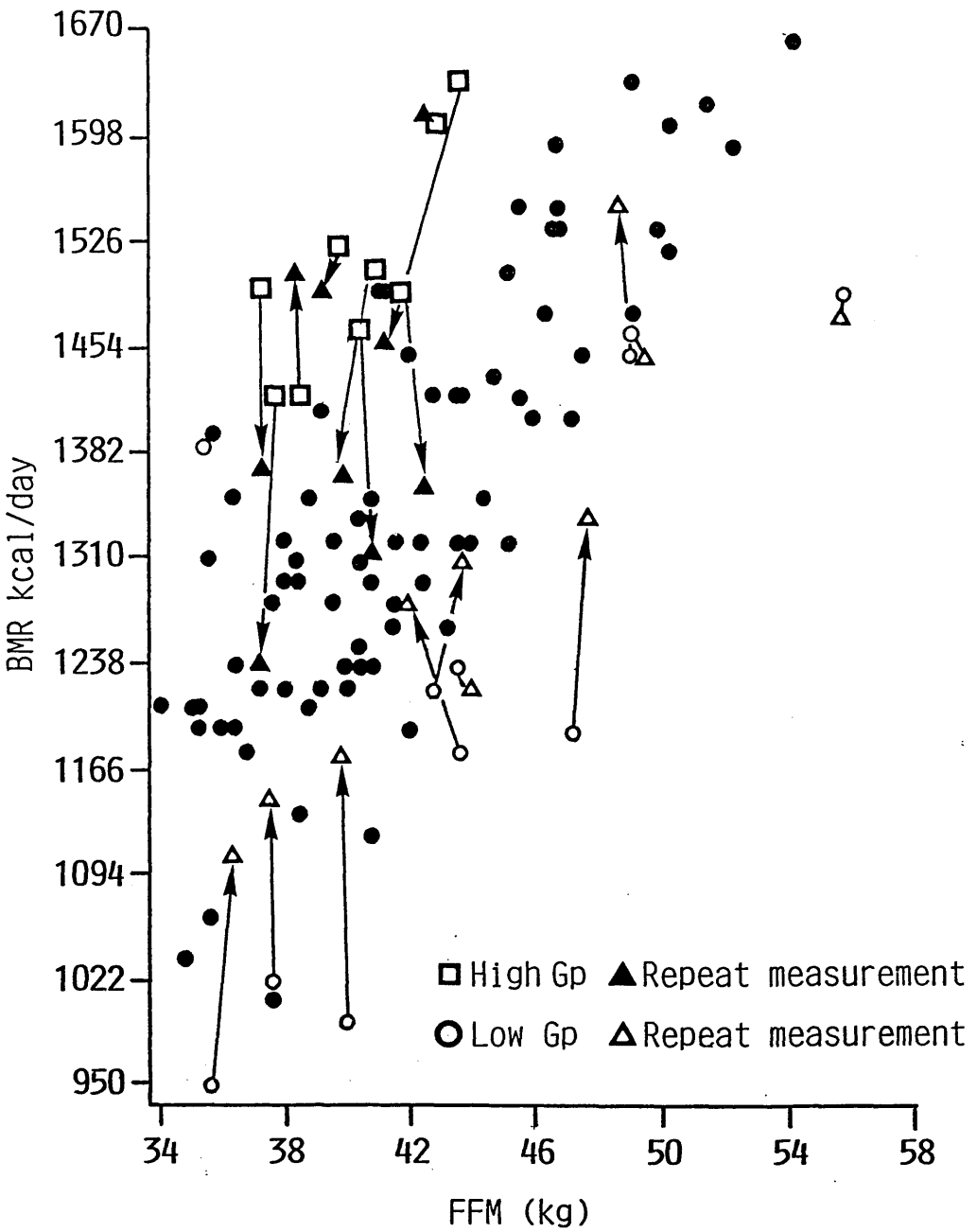

Figure 5.3
Scatterplot of BMR against FFM showing the new positions of the 19 women following repeat measurements.

$$BMR\ (kcal/day) = (FFM \times 22.5) + 480 - (Gp \times 185)$$

Gp = 0 for the high BMR group, and 1 for the low BMR group.

The levels of catecholamines and thyroid hormones in the two groups are shown in Table 5.3. In all the women concentrations were within the normal reference ranges. Significant differences were apparant in the plasma levels of T_4, T_3 and FT_4 between the groups; the women in the high BMR group had significantly higher levels of these hormones than the low BMR group. There was no significant differences in the concentrations of TSH or of the catecholamines - noradrenaline and adrenaline - between the groups.

DISCUSSION.

Intra-individual Variation:

It is apparant from the duplicate measurements of BMR that part of the large difference in basal metabolic rates between the two groups of women was a consequence of intra-individual variation, a within-subject variance of 96 kcal/day was observed. A regression to the mean at the second measurement - a tendency for the BMR of the high group to drop and the low group to increase - was clearly evident. This is perhaps not too surprising. Simply by virtue of the fact that the BMRs of these women were extreme, particularly high or low, it is likely that on the first occasion when measured BMR was, with respect to any short-term fluctuations (ie intra-individual variations), also at an extreme; at a peak for the high group and at a trough for the low group. Consequently when remeasured, the BMR of the low group is likely to increase to some extent, the BMR of the high group to fall.

On both occasions when BMR was determined, the measurement was made under strictly controlled conditions. As standard procedure the room temperature

was thermoneutral, the women had fasted for at least 12 hours prior to the measurement and rested for 30 minutes before the test began. On both occasions it was impressed upon the volunteers that they must remain quiet and immobile throughout the procedure. However, in most instances it was not possible to stay with the subject during the measurement to ensure that they complied. It may be, therefore, that part of the intra-individual variation observed resulted from differences in a volunteer's behaviour on the two occasions; perhaps restless during one measurement compared to the other. Some indication of the subject's state of relaxation on a particular day can be gleaned from the variability of the three 10 minute determinations; big differences between bags might suggest the subject was restless. However, as none of the women showed significantly more variation in metabolic rate during one set of measurements compared to the other this seems to suggest that with regard to small movements or fidgeting the subjects' behaviour did not differ markedly between days and is therefore unlikely to contribute significantly to the intra-individual variability. In those women whose BMR was lower on the second occasion compared to the first (particularly noticeable in the 'high' group) it is possible that they were initially nervous or anxious and less so at the subsequent measurement when they had became more familiar with the technique. Stress could have induced an elevated metabolic rate through stimulation of the sympathetic nervous system or possibly even through an increase in muscle tone. One might have expected increased sympathetic nervous system activity to be characterised by an elevated heart rate. Certainly in some subjects whose BMR was higher on the first occasion heart rate was also relatively increased (5 out of 10), but this was by no means universal. Furthermore, in three women whose heart rate fell from the first to the second occasion BMR increased or remained the same.

It has also been suggested that the preceding day's energy intake and level of exercise can affect BMR and contribute to differences within a subject. In recognition of this, diet and exercise were, to some extent, controlled on the two

occasions. As mentioned above, prerequisite for any BMR measurement was a 12 hour fast, in addition subjects were always asked to avoid 'stocking up' with an extra large meal just prior to the start of their fast and to try to consume their usual quantity of food on the day before the measurement - factors thought most likely to affect BMR on the following morning (Dauncey, 1980; Schutz *et al.*, 1985). To avoid any possible carry-over effects of exercise, the women were requested to refrain from strenuous activity on the day preceding the measurement, light exercise was permitted, although usually not undertaken. Since these were the limits to the control exercised over antecedent diet and activity it is conceivable that some differences could have occured on the two occasions. However, their contribution to the between-day differences would probably, at most, have been small. Weststrate (1989) found no marked difference in intra-individual variation in BMR between a group of 'free-living' subjects and a group following a carefully controlled dietary and activity regime. Furthermore, a review of the literature reveals no clearcut difference in the extent of between-subject variation in those studies where diet and exercise have been rigorously controlled (Jequier & Schutz, 1981; Murgtroyd *et al.*, 1987; Soloman *et al.*, 1982; Bisdee *et al.*, 1989) compared to those where energy intake and activity varied (Garby & Lammert, 1984; Garby *et al.*, 1984; Soares & Shetty, 1986; Weststrate, 1989) (also see Introduction, Table 1.1).

Reports of the influence of the menstrual cycle on BMR are conflicting. Several studies have found no effect (Blunt & Dye, 1921; Wiltshire, 1921; Weststrate, 1989), others however, have reported cyclical changes in BMR corresponding to the stage of the menstrual cycle - with BMR peaking just prior to menstruation and falling to a low point before ovulation (Snell *et al.*, 1920; Wakeman, 1923; Soloman *et al.*, 1982; Bisdee *et al.*, 1989). In studies made under carefully controlled conditions where energy intake and physical activity of the women were held constant, coefficients of intra-individual variation in the region of

6% or 7% have been reported (Soloman *et al.*, 1982; Bisdee *et al.*, 1989). Assuming only small contributions from measurement noise and perhaps stress, the authors have attributed most of this variation to differences in the stage of the menstrual cycle. If this is the case, it is possible that a relatively large proportion of within-subject variation in the present study could result from this source - no attempt was made to standardise for day or stage of the cycle between first and second measurements and consequently several of the women differed in this respect on the two occasions. Measurements of the author's BMR during the course of several menstrual cycles revealed a coefficient of variation of 3.4% which could not be readily attributed to any factor other than the stage of the cycle. This would again seem to suggest that the affect of the menstrual cycle on BMR could potentially account for a sizeable portion of the differences found within the women.

Some portion of the variation is also likely to have been methodological, sometimes termed measurement noise, resulting from errors in determination of energy expenditure. For some types of calorimetry the contribution of measurement noise to intra-individual variation is relatively easy to quantify. The measurement error of a respiratory chamber or ventilated hood system for instance, can be assessed by means of simple gas recovery tests or alcohol combustion. Studies which have used such techniques, have generally found measurement noise to be a small component of the total within-subject variance, somewhere between about 10% and 20% (Ravussin *et al.*, 1986; Murgatroyd *et al.*, 1987; Weststrate, 1989). The error incurred in measuring metabolic rate using the Douglas bag technique (the method employed in the present study) is much more difficult to assess. Garby & Lammert (1984) suggest that an estimate can be derived from the variance of multiple determinations of BMR made on the same day. However, this approach fails to take into account differences between measurements due to the subject and not to the method itself (see above). In reality, quantification of

measurement error using the Douglas bag technique is probably impossible. However, there seems little reason to suppose that when used competently, the error incurred is markedly greater than that with the respiratory chamber or ventilated hood. Studies by both Segal (1987) and Owen *et al.* (1986) have found the Douglas bag and ventilated hood techniques to give very comparable estimates of resting metabolic rate. It therefore seems unlikely that in the present study measurement noise would make up a large part of the difference in BMR on the two occasions.

For some of the women there was a relatively long interval between the first and second measurement, up to 9 months in one case. Over the intervening period changes in body weight were evident in some of the volunteers. This was particularly marked in one woman who had managed to lose over 6kg - she had ceased dieting some weeks before the second measurement. Changes in body weight and body composition would have affected BMR and can be expected to account for part of the intra-individual variability.

The overall coefficient of within-subject variation in this study, 6.5% (corresponding to a standard deviation of 96 kcal/day) is, perhaps not surprisingly considering the nature of the investigation, at the upper end of the individual fluctuations reported in previous studies; coefficients of intra-individual variation have ranged from 2% up to about 7% (eg. Garby & Lammert, 1984; Soares & Shetty, 1986; Murgatroyd *et al.*, 1987; See Introduction, Table 1.1 for details). As pointed out above, in selecting subjects with particularly high or low BMRs it is also likely that we selected those individuals whose BMRs were, with respect to daily fluctuations, also particularly high or low. Consequently an estimate of intra-individual variation obtained from measuring BMR a second time is likely to be high. In addition, the relatively long time interval between the first and measurements, sometimes months rather than days or weeks, is likely to increase the estimate of within-subject variance. Relating the measure of intra-individual

variation obtained from these highly selected groups to the original study is therefore problematic. However, on the assumption that the within-subject variance in the 97 women is the same as that observed in the 19 (ie. 96 kcal/day) one can calculate the relative contributions of inter- and intra-individual differences to the total variance observed in BMR in relation to the FFM (118 kcal/day):

$$SD^2 \text{ total} = \sqrt{SD^2 \text{ intra} + SD^2 \text{ inter}}$$

$$118^2 = \sqrt{96^2 + SD^2 \text{ inter}}$$

$$SD^2 \text{ inter} = \sqrt{118^2 + 96^2}$$

$$SD \text{ inter} = 70 \text{ kcal/day}$$

For the reasons given above, the degree of intra-individual variance is almost certainly an over-estimate, and as such inter-individual variation will probably be somewhat greater than this.

An alternative approach is to assume that within-subject variance in the initial study represents the same proportion of the total variance it does in the 19 women, as shown below:

In the 19 women:

$$203^2 \text{ total} = \sqrt{96^2 \text{ intra} + 180^2 \text{ inter}}$$

In the 97 women:

$$118^2 \text{ total} = \sqrt{SD^2 \text{ intra} + SD^2 \text{ inter}}$$

$$SD^2 \text{ inter} = \sqrt{118^2 - 96^2 \times \frac{118^2}{203^2}}$$

$$SD \text{ inter} = 104 \text{ kcal/day}$$

$$SD^2 \text{ intra} = \sqrt{(203^2 \text{ total} - 104^2 \text{ inter.}}$$

$$SD \text{ intra} = \underline{56 \text{ kcal/day}}$$

Clearly neither estimate of intra-individual variance is entirely satisfactory, but it seems reasonable to assume that the real value probably lies somewhere between the two - between 60 and 100 kcal/day and correspondingly, inter-individual variance between 70 and 100 kcal/day. The literature in this area suggests the intra-individual variation may be closer to the lower estimate. An average figure for the coefficient of within-subject variance seems to be around 3% to 4%, compared to our value of 6.4%. The relative contributions of intra- and inter-individual variance to the total variance in BMR in relation to the FFM has important implications for the usefulness of FFM in predicting an individual's BMR. This will be discused in Chapter 6.

Since intra-individual differences in BMR fall short of explaining all the differences in BMR in relation to the FFM other factors must therefore be involved in explaining inter-individual differences in BMR in relation to the FFM. One possibility that could potentially cause variation is an error in estimation of the FFM.

Errors in the measurement of FFM.

Any error in the measurement of the FFM will necessarily distort the true relationship between BMR and FFM introducing a degree of variation with

technical rather than biological cause. With respect to the differences between the two groups, it is possible that high BMRs relative to FFM may be partially the result of under-estimation of the fat-free tissue, conversely relatively low BMRs, from an over-estimation of FFM. The very low coefficients of variation, only 1.3% and 1.0% for the high and low groups respectively, suggest that reproducibility of the estimation of FFM was high, especially since a component of this will have resulted from genuine differences in the FFM - as mentioned above, the body weight of some women had changed. However, this in itself tells us relatively little about actual error of the measurement. It is possible that with respect to the true value, FFM could have been consistently over- or under-estimated.

Both the TBW and densitometry techniques for measuring body fat and FFM rely on assumptions regarding the constant composition of the fat-free tissue; for TBW that the FFM is 73% water, for densitometry that the density of the FFM 1100 kg/m^3 and so on. If these assumptions are not always justified, and limited cadaver analysis (see Introduction) suggests they are not, some degree of error in estimating FFM will be incurred. It is possible that deviations from the assumed composition could have resulted in erroneous estimates of FFM in the two groups.

However, <u>within</u> each group, both densitometry and TBW techniques employed gave a similar estimate of FFM (as did the skinfold method). Since the assumptions involved in each case are essentially independent - a deviation from the assumed density of the FFM will not necessarily be accompanied by a change in the proportion of water it contains and the converse is also true - this implies that the composition of the FFM did not differ substantially from the 'norm' in either of the groups and on this basis suggests error in the estimation of FFM is on average likely to be small. The similarity of the skinfold estimates adds further weight to this argument. It is difficult to envisage how within each group all three methods could produce the same spurious result - under-estimation for the high group, over-estimation for the low.

The observation that in relation to any one of the three estimates of FFM the BMRs of the two groups were significantly different also suggests that the differences between the groups were not wholly attributable to measurement error. The comparability of the three methods within each group takes this further however, suggesting that error in estimation of FFM was small and its contribution to the differences between the groups therefore, likely to be minor. It seems reasonable to suggest that the same would apply to the initial study.

Having then, on the basis of the data from the two groups, made some estimate of the contribution of intra-individual variance to the total variance observed in BMR in the initial study and assuming error in measurement of FFM to be very small, the remaining variance - in the region of 100 kcal/day - is likely to be to genuine differences in BMR in relation to the FFM. Two explanations are possible. The first relates to differences in the levels of thermogenic hormones. Again these possibilities were investigated in the high and low BMR groups and the results related back to the original cross-sectional study of the 97 women.

Composition of the FFM.

It is possible that differences in BMR between the two groups are a consequence of differences in the composition of the FFM. As alluded to above, the parity of the three methods of estimating FFM within each group would to some extent suggest that the composition of the FFM did not differ markedly between the groups. However, differences in composition would probably not have to be particularly large to account for the differences observed. It has been estimated that the metabolic rates of just four organs - the heart, brain, liver and kidneys - are responsible for about 60% of BMR, yet in terms of weight they make up only about 6% of the FFM (Brozek & Grande, 1958; also see Table 1.2). Skeletal muscle on the other hand constitutes about half the weight of the FFM but has a relatively low

resting metabolism and accounts for less than 25% of the BMR (Brozek & Grande, 1955). Relatively subtle variations in the relative proportions of 'active' compared to 'inactive' tissues therefore, could potentially have a major impact on BMR. The difference between the two groups could theoretically arise through a difference of about 3% in the proportion of the FFM accounted for by the liver, heart, kidneys and brain. However, whether or not the sizes of these organs are sufficiently variable in relation to FFM to explain these sorts of differences is difficult to ascertain from the literature since available data essentially seems to be limited to only four cadavers (Mitchell *et al.*, 1945; Forbes *et al.*, 1953, 1956). Calculations based on data presented by Greenwood & Brown (1913) however, do suggest that in relation to body weight at least, quite a large proportion of variation in BMR could potentially be explained by differences in organ size. Greenwood & Brown recorded body mass, liver, kidney, heart and brain weight in 79 male cadavers, aged between 25 and 55 years. In general the men were in good health at the time of death, in most cases due to accident, and body composition would therefore be expected to be relatively normal. Using this information and the literature value for resting energy expenditure per unit weight of each of the organs (see Table 1.2), it was possible to make an estimate of the overall daily metabolic rate of the four organs.

Taking the example of two cadavers:

	Cadaver 1		Cadaver 2	
	age 44, body wt 66.7kg		age 45, body wt 65.3kg	
Organ	Weight	Contribution to BMR	Weight	Contribution to BMR
	(kg)	(kcal/day)	(kg)	(kcal/day)
Heart	0.28	108	0.38	145
Brain	1.46	352	1.40	338
Kidneys	0.22	142	0.31	206
Liver	1.79	539	1.96	590
Total		1141		1279

In this example the cadavers were closely matched in terms of age and body weight yet the estimated BMR of the four organs differed by some 140 kcal/day (10%). This would seem to suggest that differences in organ size at a given weight could potentially account for quite large differences in BMR. This point is further illustrated when the estimated metabolic rate of the four organs is correlated with the weight of the cadaver (Figure 5.4). The residual standard deviation around the line of best fit is 100 kcal/day. This is of a similar magnitude to the residual standard deviations observed when BMR is correlated with body weight. In the study presented in Chapter 3 for example, the residual standard deviation for BMR against body weight was 132 kcal/day; for the FAO/WHO/UNU (1985) equations the figure is around 110 kcal/day in females, 150 kcal/day in males and so on. This does seem to suggest that differences in organ size could potentially explain a relatively large portion of the variation in BMR at a given weight.

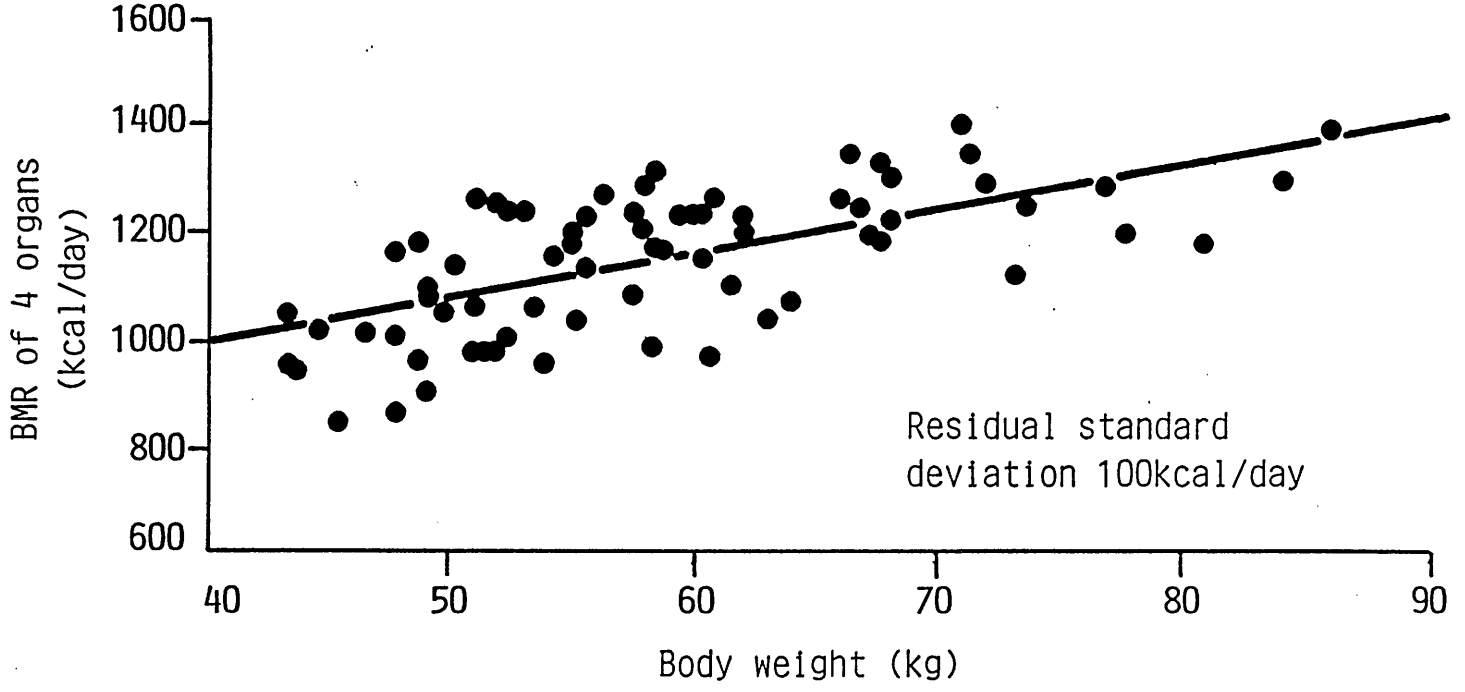

Figure 5.4
Scatterplot of estimated organ BMR (liver, kidney, heart & brain) against body weight.

The potential importance of organ size is further illustrated by a series of animal studies reported by Koong & Ferrell (1990). These workers observed that the large differences (up to 40%) in BMR (fasting heat production) between animals of the same weight and age but who had been subject to different nutritional regimes could be almost entirely explained by differences in the mass of the metabolically active organs making up body weight. Correlations between BMR and the weights of organs such as the liver and kidneys had 'r' values in the region of 0.98 and 0.99.

In the present study we have no indication of relative organ size in the two groups of women; initially it had been hoped to make some estimation of this. However, the available techniques for in vivo measurement, proved either insufficiently accurate for our purposes, eg. ultra sound, or were prohibitively expensive, eg. nuclear magnetic resonance (NMR). This is certainly an area with much potential for future investigation.

Assuming however, the low BMR group did indeed have a proportionately smaller mass of 'active' organs, it follows that they must have had more 'inactive' tissue; most probably more muscle. Superficially, circumference data would suggest that this was not the case since there were no significant differences in the measurements between the groups. As discussed previously however, the tendency for groups to have a slightly different FFM makes this sort of inference unreliable. Moreover, even with the same mass of fat-free tissue circumferences measurements are probably too crude an index of muscle mass to discriminate the relatively small differences which may have occured.

Hormonal Status

Thyroid hormone levels were found to differ significantly between the two groups of women - the high BMR group had significantly greater levels of T_3, T_4 and FT_4 and the low group. It seems reasonable to postulate that these differences

in thyroid status had apart to play in explaining differences in BMR in relation to the FFM between the two groups. None of the women had thyroid disease; T_3, T_4 and FT_4 were within the normal reference ranges for all the individuals and euthyroid status was confirmed by the normal TSH levels which did not differ significantly between the groups. Two reports are available which lend support to the inferences of the present study. In a group of 12 healthy volunteers MacRitchie (1988) found a significant positive correlation between BMR, expressed as the % deviation from standard values predicted using the Mayo Clinic equations (Boothby, Berkson & Dunn, 1936), and both serum T_3 and T_4 levels ($r = 0.47$, $p < 0.01$ & $r = 0.5$, $p < 0.01$, respectively). In expressing the results in relation to values predicted on the basis of surface area some degree of 'normalisation' for body size was achieved. In this group then, there is an indication that at a given body size, if not specifically FFM, the higher the T_3 of T_4 levels the higher the BMR. Danforth (1983) reports a positive correlation ($r = 0.59$, $p < 0.01$) between free T_3 serum concentrations and the number of calories per kg FFM required for 16 subjects to maintain weight during a three week stay in a metabolic unit. Since under the circumstances imposed on the metabolic ward BMR is likely to make up the largest proportion of daily energy expenditure it may well be that the correlation is also illustrative of the relationship between BMR/kg FFM. If this is the case, it implies that high levels of T_3 are associated with a high BMR/kg FFM.

In line with our own work, the studies by Danforth (1983) and MacRitchie (1988), certainly suggest that differences in thyroid status are involved in explaining differences in BMR in relation to the FFM, the results of the present investigation allow us to go further however, and make some quantitative estimate of their contribution.

When residual BMR (the difference between the measured BMR and that predicted from FFM according to the regression equation derived in the original study) is correlated with T_3 levels in the 19 women, the residual standard deviation

catecholamine levels in urine or plasma (Landsberg & Young, 1983). The lack of a significant difference in 24 hour urinary adrenaline and noradrenaline levels between the two groups in this study would seem to indicate that sympathoadrenal activity did not differ. However, these results should probably be interpreted with some caution. Their reliability depends upon an accurately timed and complete urine collection. Since the onus for this was placed upon the subject and not vindicated by means of an independent check (eg.PABA) we cannot be certain that this was achieved in every case. The large variability in the results suggests it may not have been. In addition, it has been suggested that whilst urinary (or plasma) catecholamine levels are a relatively good index of sympathoadrenal activity within an individual they may be of less use for inter-individual comparisons (Barrand & Callingham, 1983). However, even accepting that the catecholamine results genuinely reflect comparable sympathetic nervous system activity in the two groups, this does not necessarily rule out the possibility that differences in an adrenergically mediated process may have contributed to the differences in BMR - the groups may have differed in their responsiveness to catecholamines. There is certainly some evidence to suggest that catecholamine sensitivity can differ between individuals. Jung *et al.* (1979b) for example, have reported a lower thermogenic response to noradrenaline infusion in groups of obese and post-obese subjects compared to lean. Sjostrom *et al.* (1983) have also found noradrenaline sensitivity, assessed by changes in parameters such as metabolic rate, respiratory frequency and blood pressure, to differ between individuals. Interestingly, thyroid status is known to alter adrenergic reactivity; elevated levels of thyroid hormones increase catecholamine sensitivity, reduced levels have the opposite effect. The lipolytic response to noradrenaline for example, is decreased in both hypothyroidism and fasting (Kunos, 1981). Gelfand *et al.* (1987) found noradrenaline infusion to produce a proportionately greater increase in metabolic rate in volunteers with experimentally induced thyrotoxicosis than it did when they were in a euthyroid

about the regression line was 94 kcal/day. Making an allowance for the component of this variance likely to be attributable to within-subject differences (60 kcal/day, see above) we are left with a residual standard deviation of 70 kcal/day ($94^2 - 60^2 = 72^2$). This then, is an estimate of the variance remaining when FFM, within-subject differences and T3 levels have been taken into account and compares to the estimated 100 kcal/day residual standard deviation when allowance has been made for FFM and within-subject variance alone. This is obviously a rather rough estimation based on the limited data available, it would clearly have been more satisfactory to measure thyroid hormone levels in all 97 women. It does however, give us some idea of the relative importance of differences in thyroid status in explaining differences in BMR in euthyroid individuals - an area which has received little investigative attention to date.

The variance remaining when differences in thyroid status have been taken into account may be attributable to differences in a catecholamine mediated component of BMR, acting alone or perhaps in concert with thyroid hormones to determine the metabolic activity of the tissues (see below). Alternatively we are again brought to the possibility that differences in the composition of the FFM may be involved.

The lowered BMR of those undergoing ß-blockade therapy reported in this thesis and by other workers (Jung *et al.*, 1980; Scheidegger *et al.*, 1984) suggests that BMR has an adrenergically mediated component. Potentially therefore, differences in BMR could arise through differences in adrenal medulla or sympathetic nervous system activity - collectively termed the sympathoadrenal system. Weststrate (1989) has suggested that the differential rates of fatty acid oxidation he observed between two groups characterised by either a high or low BMR/kg FFM, may reflect a different degree of sympathetic nervous system activity, and provide a possible explanation for the differences in BMR. Assessment of sympathoadrenal activity is usually based on the measurement of

state. Thyroid hormones have been shown to alter the number and affinity of adrenoreceptors, to modulate receptor coupling to the effector system and to alter the activity of the effector system's regulatory and catalytic components (Tsai *et al.*, 1978; Kunos, 1981; Malbon & Greenberg, 1982; Lefkowitz *et al.*, 1984). Whether or not the differences in thyroid hormone levels between the two groups in this study induced differences in catecholamine responsiveness and if so, whether this contributed to the differences in BMR between the groups cannot be deduced.

Summary

Investigation of the high and low BMR groups allowed us to make some assessment of the factors responsible for variation in BMR in relation to the FFM. The data suggested that the contribution of error in measurement of the fat-free tissue is likely to have been small. In contrast, within-subject differences clearly had a more important effect. When these had been taken into account it was estimated that the genuine inter-individual variance in BMR in relation to the FFM was in the region of 100 kcal/day. The hormonal results strongly suggested that this was at least in part attributable to differences in the levels of thyroid hormones. Thyroid status did not however, provide the complete explanation, leaving a residual variance of around 70 kcal/day. It seems probable that differences in the composition of the FFM are also likely to be involved, although the study provided no adequate data to test this contention. In addition the possibility that differences in a catecholamine process may also have contributed, cannot be ruled out.

CHAPTER 6

GENERAL DISCUSSION.

The studies presented in this thesis have been concerned with variability in in basal metabolic rate, examining the extent of differences between individuals and their cause. In particular the roles of differences in body composition and hormonally mediated metabolic activity were explored. In the majority of people BMR accounts for the largest part of daily energy expenditure, study of the factors that affect BMR is therefore central to our understanding of the causes of variation in daily energy needs.

Measurements in almost 100 healthy women indicated that variability in BMR was large; some 500 kcal/day separated the 15% of the women at the top end of the range from the 15% at the bottom, equivalent to a standard deviation of 159 kcal/day. The differences were initially investigated in terms of differences in body composition. It was found that the between-subject differences could best be explained by differences in FFM, which accounted for 45% of the total variance. In accord with several previous investigations it was found that once differences in the mass of fat-free tissue had been taken into account neither age nor body fatness any any significant effect on BMR (James *et al.*, 1978; Bernstein *et al.*, 1983; Garrow & Webster, 1985; Ravussin *et al.*, 1986; Lawrence *et al.*, 1988). However, the residual standard deviation of the regression equation describing the relationship between BMR and FFM (118 kcal/day) suggested that at a given FFM considerable variation existed in the BMR of individual women; as much as 400 kcal/day could apparently separate the BMR of physically comparable women. Two groups of women characterised by particularly high or low BMRs in relation to their FFM were selected for further investigation with the aim of elucidating the factors responsible for these very large differences.

Repeat measurements of BMR in the two groups suggested that part of the variance in BMR relative to FFM, could be attributed to within-subject differences in BMR rather than to genuine between-subject variation. The data did not allow for an exact quantification of the contribution of intra-individual differences to the

118 kcal/day residual standard deviation, but it was possible to make a reasonable estimate that it lay somewhere between about 60 and 100 kcal/day. This would leave a between-subject variation in the region of 70 to 100 kcal/day. Based on data from other studies it seems likely that the intra-individual variance is probably closer to the lower estimate, 60 kcal/day, and correspondingly inter-individual to the upper estimate, 100 kcal/day.

The magnitude of real inter-individual variation has implications as to the accuracy with which an individual's BMR can be predicted from their FFM. With a variance as large as the residual standard deviation would at first suggest, around 120 kcal/day a great deal of uncertainty would be attached to the prediction. If however, this figure is partly a reflection of wthin-subject differences and inter-individual variation is less, closer to 70 kcal/day one can have much more confidence of obtaining a reasonable estimate of an individual's basal metabolic rate. This is an issue of some practical importance. The most recent FAO/WHO/UNU committee on energy and protein requirements has adopted the principle that total energy expenditure (TEE), and therefore requirements, should be calculated as multiples of the BMR; 1.56 x BMR for women with a light level of activity, 1.82 x BMR for heavy activity and so on (FAO/WHO/UNU, 1985). Although originally envisage for use only in groups, it can often be instructive to make an estimate of an individual's energy expenditure in this way, to assess for example, how much weight a person could reasonably be expected to lose on a given diet. In most cases BMR cannot be conveniently measured and it is necessary to make a prediction. It follows that any error in the estimation of BMR will translate through to an error in estimation of daily energy requirements.

Whilst variation in BMR in relation to the FFM was found to be less than the residual standard deviation of the regression equation at first indicated, it did exist. As pointed out above, it was estimated to be in the region of 100 kcal/day. Differences in the mass of fat-free tissue are not therefore, a complete explanation

for differences in BMR between individuals. The tendency to regard them as such, likely stems from the observations that variation in BMR between groups in relation to sex, race age and so on, is largely eliminated when the weight of the FFM is taken into account. As pointed out above, the studies presented here were able to confirm that the relationship between BMR and FFM was essentially independent of both differences in body fatness and age. Findings such as this do not justify however, the assumption that FFM is the genesis of all differences in BMR - clearly when only 45% of variance in BMR in the 97 women could be explained in these terms it cannot be - nor do they allow the extrapolation that FFM is the 'active' portion of body weight. It certainly includes the most metabolically active tissues and organs, the liver, brain and so on, yet the very fact that approximately half its weight is constituted by relatively inactive muscle precludes its designation as the 'active' fraction of body mass. Indeed, it is probably in the heterogeneity of the fat-free tissue that at least part of the explanation for variation in BMR lies.

Reworking of the cadaver data presented by Greenwood & Brown (1913) suggested that differences in the composition of the FFM, specifically differences in the size of the metabolically active organs, could potentially explain much of the variation observed in BMR in relation to weight. The residual standard deviation of the regression equation relating the estimated metabolic rate of the liver, kidneys, heart and brain to body weight was 100 kcal/day. This compares to the residual standard deviations observed when BMR is correlated with weight which lie in the region of 110 to 150 kcal/day. The results of animal studies reported by Koong & Ferrell (1990) also emphasised the potential importance of variations in organ size. In the studies presented here, it was not possible to determine the contribution of differences in the composition of the FFM to the observed variance in BMR. It had been hoped to make at least some qualitative assessment by measuring organ - liver, kidney, heart and brain - size in the high and low BMR groups. None of the

available techniques proved suitable however. Ultra sound was not judged sufficiently accurate, the ionising radiation involved in computerized axial tomography (CT) precluded its use and nuclear magnetic resonance (NMR), which seemed the most promising, was prohibitively expensive. Similarly, no adequate estimate of muscularity was obtained in the women. Limb circumference measurements proved too crude an index to assess the exact proportion of of muscle occupying the FFM. Other more sophisticated techniques are available, creatinine and 3-methyl histidine excretion for example. Even these however, cannot provide an exact measure of muscle mass (Lukaski, 1987). Accurate assessment of of the composition of the FFM is probably not possible with currently available techniques. This is certainly an area deserving future investigation, because until we are able to quantify the composition of the FFM more precisely I would suggest that our understanding of the nature of variation in BMR cannot be complete.

The other possibility investigated in this thesis was that variation in BMR may be a consequence of differences in the metabolic activity of the tissues. Since both thyroid hormones and catecholamines have been implicated in the control of cellular thermogenesis the studies were particularly concerned with the role of these hormones in determining BMR. The relatively reduced BMR of the patients undergoing ß-blockade therapy compared to the control subjects strongly suggested that BMR has an adrenergically mediated component, supporting the work of Jung et al. (1980). On this basis it seemed reasonable to postulate that differences in sympathoadrenal activity may be responsible for some of the differences observed in BMR in relation to the FFM. However, when the hypothesis was subsequently put to the test by measuring urinary catecholamine levels in the high and low BMR groups no significant differences were evident. It is difficult to interpret this finding with any certainty. Firstly there is some suggestion that urinary catecholamines may not be an adequate index of sympathoadrenal activity between

individuals. However, even accepting that they are, it is possible that the two groups may have differed in their responsiveness to catecholamines. The possibility that differences in adrenergically mediated process may contributed to the differences in BMR cannot therefore be excluded.

Aside from pointing to a role for the catecholamines in determination of BMR, the observation that the ß-blockers depress basal metabolism has implications for the clinical use of these drugs. The weight gain which could potentially result from a lowered BMR would certainly not be desirable in the cardiovascular patients to whom ß-blockers are widely prescribed. The general usefulness of ß-blockade therapy is undisputed but I would suggest that this side effect is sufficiently important to warrant further investigation.

The significantly different levels of thyroid hormones between the high and low BMR groups certainly suggests that differences in thyroid status are involved in explaining variation in BMR in relation to the FFM. It was possible to take this further however, and make an estimate of the extent of their contribution. The data suggested that differences in T_3 levels are probably responsible for around 20% of the total variance in BMR and for about 35% of the variance at any given FFM (see below for derivation of these estimates). In view of the great deal of research effort that has focused on the general area of thyroid status and metabolic rate it is perhaps surprising that this appears to be one of the few studies to specifically explore the relative importance of differences in thyroid levels in explaining variations in BMR in normal, euthyroid individuals. It seems reasonable to assume that under the influence of thyroid hormones, the the metabolic activity of the tissues and organs of the women differed. Quite what form these differences may take is a matter of some conjecture. The precise effects of the thyroid hormones, or indeed the catecholamines, on energy requiring processes at cellular level - protein turnover, substrate cycling, ionic pumping and so on - remains to be fully elucidated. Even more fundamentally, the relative contributions of these processes to basal energy

expenditure are far from clear. For instance, estimates of the contribution of the Na^+/K^+ pump activity to BMR have ranged form 5% (Sestoft, 1980) to 50% (Sims, 1987). This is an area where much work remains. It would certainly aid our understanding of the part played by thyroid hormones and catecholamines in the determination of BMR if we knew the relative importance of the processes they are thought to affect.

Taken together, the studies presented here have allowed us to make an assessment of the respective roles of the various factors investigated in explaining variance in basal metabolic rate. The findings are summarised in the form of a hypothetical analysis of variance table (Table 6.1).

(1) Total variance:

In the original cross-sectional study of the 97 women the total variance in BMR was equal to 25316.

(2) FFM:

It was calculated that 45% of this variance - 11392 - could be explained by differences in the mass of fat-free tissue, leaving a residual variance of 13924.

ie. 25316 - 11392 = 13924.

(3) Within-subject variance:

On the basis of the repeat measurements of BMR in the group of 19 women it was estimated that within-subject variance in BMR lay between 60 kcal and 100 kcal/day. Comparison with other studies in the literature suggests that the lower estimate is perhaps likely to be the more realistic. Taking the 60 kcal/day value therefore,

Table 6.1

Hypothetical analysis of variance table indicating relative importance of the factors investigated in explaining variance in BMR

	Explained.	Residual	$\sqrt{\text{Resid}}$	% total variance	% variance at given FFM
(1) Total		25316	159		
(2) FFM	11392	13924	118	45 %	
(3) Within	3600	10324	104	15 %	25 %
(4) T_3	5088	5236	72	20 %	35%
Other				20 %	40%

$$\text{within-subject variance} = 60^2$$
$$= 3600$$

On this basis approximately 15% of the total variance in BMR in the initial study can be attributed to within-subject differences and some 25% of variance in relation to the FFM. A residual variance of 10324 remains,

$$\text{ie. } 13924 - 3600 = 10324$$

(4) T3:

After adjusting for differences in FFM and T3 in the group of 19 women the residual standard deviation was found to be 94 kcal/day, equal to a residual variance of 8836 (942). Making an allowance for the contribution of intra-individual variance (3600, from above) it is possible to arrive at an estimate of the contribution of differences in T_3 levels to total variance in BMR and of the residual variance which remains:

$$\text{Residual variance} = 8836 - 3600$$
$$= 5236$$

$$T_3 \text{ variance} = 10324 - 5236$$
$$= 5088$$

This estimate suggests that differences in T_3 are able to explain in the region of 20% of the total variance in BMR and some 35% of the variance at any given FFM.

Having then, taken into account variance attributable to FFM, T_3 and within-subject variance we are left with a residual variance of 5236; 20% of the total variance in BMR is left unexplained and about 40% of the variance in relation to the FFM. The residual variance in BMR when FFM alone has been taken into account corresponds to a residual standard deviation of 118 kcal/day. When an estimate of within-subject variance is allowed for, this value falls to about 100

kcal/day, and further to approximately 70 kcal/day when T_3 levels are taken into account. It seems reasonable to assume that the remaining variance is probably attributable to differences in the size of the metabolically active organs - liver, kidney, heart and brain.

It is not suggested that these estimates are by any means the definitive values - they are 'best guesses' with the data available. They are likely however, to be of the right order of magnitude and give some insight into the relative importance of the factors responsible for variation in BMR. At the least , they provide a peg on which to hang future work.

In the course of the investigations - the primary consideration of which was to explain variance in BMR - other related issues were highlighted which are worth comment. One particularly salient point to emerge was the observation that BMR expressed kg FFM is not constant with weight but tends to be higher in lighter compared to heavier individuals. Those with relatively enlarged FFMs - men, the obese - generally have a lower BMR/kg FFM than those with less fat-free tissue - women, lean individuals and so on. It was postulated that these differences may again to be a consequence of differences in the composition of the FFM, to the relative proportions of active and inactive tissues. The observation has important implications for the use of FFM as a metabolic reference standard. Expressing BMR 'per kg FFM' could potentially lead to misinterpretation of data when the groups or individuals concerned are of different body size. This is precisely the situation which the use of a reference standard is intended to avoid. FFM may still be useful in this role however. It was found, in the present studies and those by Miller & Blyth (1953) and Lawrence et al. (1988), that BMR was approximately constant, across or between groups, when divided by a power function of FFM rather than FFM itself; by $FFM^{0.7}$ in our group of 97 women, $FFM^{0.5}$ for Lawrence *et al's* analysis and so on. As a reference standard therefore, it might be

more appropriate to relate BMR to o power function of FFM than than to simply express metabolic rate 'per kg' FFM.

The studies also highlighted a potential problem with the FAO/WHO/UNU (1985) equations for predicting the BMR of 30-60 year olds. It became clear that both the male and female equations had a tendency to over-estimate the BMRs of those individuals at the upper end of the age range. An inspection of the relevant literature revealed that in both cases over half the measurements from which the equations were derived (Schofield, 1985b) were made on individuals between the ages of 30 and 40. The inclusion of a disproportionate number of younger men and women likely explains the over-estimation in our older individuals, The FAO/WHO/UNU (1985) report states that in relation to weight, BMR declines little over the age range 30 to 60, only about 1% per decade. The study presented in Chapter 3 however, suggests that the fall is greater than this, closer to 4% per decade, occasioned by the progressive increase in the proportion of fat making up body mass. It is perhaps not surprising therefore, that equations derived from measurements made on predominantly younger individuals over-estimate the BMRs of those at the upper end of the age range. The age ranges for the WHO equations were not made by inspecting the data, but rather were selected to approximate to the commonly used clinical divisions of human life span (Schofield, 1985a). In view of the age-related changes in body composition which occur over the years 30 to 60 and their effect on the relationship between BMR and weight this particular subdivision may not be the most appropriate.

It is perhaps appropriate to end, as this thesis began, with the quote from Dr. Elsie Widdowson, " Much more research lies ahead before we can begin to understand why one person can live on half the calories of another." I would like to think the work on BMR presented here has taken us way forward and, at the least, highlighted areas deserving further investigation. Fifty years on however,

Dr. Widdowson's remarks still hold true and there is much to occupy the attentions of future investigators.

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