THE USE OF ULTRASOUND IN THE ASSESSMENT OF CANINE OBESITY.

by

MICHAEL J.A. WILKINSON LIC.VET., M.R.C.V.S.

Thesis submitted for the degree of Doctor of Philosophy in the Faculty of Veterinary Medicine, University of Glasgow.

Department of Veterinary Medicine.
University of Glasgow.
December, 1991.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

AUTHOR'S DECLARATION.

Apart from the help acknowledged below, the work described in this thesis was carried out solely by the author under the supervision of Professor Maxwell Murray.

TABLE OF CONTENTS.

ACKNOWLEDGEMENTS.

I am very grateful to the Waltham Centre for Pet Nutrition, for their financial aid during these years but especially for the encouragement I always was offered.

Without the help and support of many people this thesis would not have been possible. I wish to accord my appreciation to all members of staff and technicians of Glasgow University Veterinary School, in particular to: Mairi Austin, Susan Cain, Charlie Cameron, Susan Fitzpatrick, Pamela Gillan, Gary Jackson, Steve Martin, Allan May, Diana McKechnie, Iain McMillan, Jimmy Murphy, David Newham, Alan Reid and Moira Young, for their invaluable assistance in some of the practical aspects of this work. Special thanks are due to Neil McEwan who has taught me so much throughout these 3 years and to Brian Wright who was always most helpful and generous in providing time, materials and advice. Thanks are also due to the small animal clinicians in the Department of Veterinary Medicine: Dr. Andrew Nash, Joan Barry, Peter Graham, Dr. Chris Little, Joanna Dukes and Carmel Mooney. They generously allowed me to scan many of their clinical cases and granted me free access to their clinical records and observations.

Parts of this work were carried out in other Departments and in this respect I owe thanks to: Professors N.G. Wright and J. Boyd for providing the facilities in the Department of Veterinary Anatomy; Professor P.H. Holmes and Dr. J.M. MacLean of Veterinary Physiology, who made the studies on carcass composition

possible and were extremely generous in offering me their time and advice; Professor G. Hemingway and Dr. J. Parkins in the Department of Animal Husbandry, who provided the means for the chemical analysis of carcasses; Professor D. Onions and the staff in the Department of Veterinary Pathology who carried out the processing of histological specimens.

I also wish to express my sincere gratitude to Calum Paterson who extended my limited knowledge on ultrasound and provided graphic materials for this thesis, and Dr. George Gettinby who gave me priceless guidance in the dark forest of statistics. I thankfully acknowledge the advice given by Dr. Sandy Love, Tim Watson, David Irvine and Sean Callanan.

Professor Max Murray had the burdensome task of being my supervisor and I am most indebted to him for all his help and encouragement during these years.

I also wish to name Juan Escala, Angelo Mtambo and Joseph Ndung'u for their invaluable assistance and friendship.

Finally, and most importantly, I want to express my deepest gratitude to my family, for their constant love and support and especially to my father, to whom I owe so much.

LIST OF ACRONYMS.

BCS - Body Condition Score.

BIA - Bioelectrical Impedance Analysis.

BMI - Body Mass Index (Weight/Height^2).

BW - Body Weight.

cm - centimetres.

CT - Computed Tomography.

DPA - Dual-Photon Absorptiometry.

g - gram(s).

G - Girth.

GI - Girth Index (Girth^2/Height).

GWI - Girth-Weight Index (Girth^2 x Weight / Height^2).

H - Height.

Hz - Hertz.

kg - kilogram(s).

L - Length.

LPL - Lipoprotein Lipase.

m - metre(s).

mg - milligram(s).

MHz - Megahertz.

ml - millilitre(s).

mm - millimetre(s).

mmol- millimole(s).

MRI - Magnetic Resonance Imaging.

NAA - Neutron Activation Analysis.

ns - not significant.

p - probability.

r - coefficient of correlation.

R-sq - the square of the coefficient of correlation (r), expressed as a percentage. Also known as coefficient of determination.

R-sq(adj) - the coefficient of determination adjusted for degrees of freedom. It takes into consideration the number of variables in the model.

s - standard error of estimate.

sec - seconds.

sd - standard deviation.

TBF - Total Body Fat.

W - Weight.

$\bar{X}$ - mean.

LIST OF TABLES.

LIST OF FIGURES.

LIST OF APPENDICES.

SUMMARY.

The aim of the studies reported in this thesis was to evaluate the use of ultrasound in the assessment of canine obesity.

Subcutaneous fat thickness was measured in dog carcasses by means of an A-mode ultrasonographic unit, skinfold callipers and a conventional histometric method. Measurements were taken on six different anatomical locations: axilla, flank, sternum, abdomen, thigh and lumbar. There was a high degree of correlation between ultrasonic and histological fat thickness measurements. However, correlation between the calliper technique and histology was low.

Total body chemical analysis was carried out in the carcasses in order to calculate their total fat content. Fat thickness measurements obtained with histology were highly correlated with the percentage of fat content in the carcass. The relationship between ultrasonic fat thickness measurements and total fat content in the carcass was weaker than the one found between total fat content and histology.

A more sophisticated ultrasonographic unit was used to measure subcutaneous fat in the lumbar area of 100 canine hospital in-patients. These measurements were highly correlated to a condition score assessment of

overall adiposity. There was a statistical difference in fat thickness between dogs of different condition score groups.

These results indicate that ultrasound, specially two-dimensional ultrasound, is an accurate method of measuring subcutaneous fat thickness in dogs and that these measurements can be of value in predicting total body fat in certain canine populations.

To my parents.

CHAPTER I.

GENERAL INTRODUCTION.

CHAPTER I. GENERAL INTRODUCTION.

1.1. THE CLINICAL SIGNIFICANCE OF OBESITY.

Advances in Veterinary Medicine have meant that most of the major infectious diseases in dogs and cats can be controlled or prevented by vaccination of the young population. As a result, more and more animals survive to middle and old age, with the attendant problems that ageing brings. Obesity is a major one of these problems.

In man, the disease consequences associated with obesity are becoming increasingly well defined, with the result that developed nations are extremely health conscious with regard to diet and exercise. By contrast, in dogs and cats, the consequences of obesity are less well documented and understood.

The reduced quality of companionship resulting from lethargy and fatigue as well as the increased surgical risk of the obese pet must be considered. Furthermore, there is now preliminary epidemiological evidence that obesity may be related to circulatory, respiratory, articular and locomotor problems, and with a higher incidence of diabetes mellitus (Edney and Smith, 1986; Lewis, Morris and Hand, 1987). Mortality is said to be 50% higher in people who are 20% overweight and 33% greater in those that are 10% overweight (Mayer, 1972). Similar statistics are not available for the obese canine population but it is now clear that obesity poses a serious threat to the well-being of the animal.

1.2. QUANTIFYING BODY FAT IN PETS.

A major constraint in dealing with obesity in dogs and cats is the lack of techniques to precisely quantify body fat. In man, obesity has been defined as a body weight 15% greater than normal. Normal for a given height and body weight is usually determined from actuarial tables based on statistical data from a large number of people. This approach is obviously not possible in the dog bearing in mind that there are over 100 breeds and a large variation in body build within members of the same breed. Other approaches used in man such as skinfold thickness and girth measurements have not yielded promising results in dogs and cats, and the method of total immersion in water to calculate body density in man, is not feasible for use in pets outside laboratory conditions.

It is important to bear in mind that quantifying the body fat content of people is still an arduous exercise. A large amount of research is carried out every year to validate some of the more classical techniques - such as hydrostatic weighing or skinfold callipers - and to explore new methodologies. Nevertheless, considerable progress has been achieved and most of these methods or a combination of them can provide a good estimate of the degree of obesity in people.

At present, the diagnosis of obesity in the dog and cat is somewhat subjective because it is based on the estimation of body fat storage by observation and by palpation of the rib cage and abdomen. Clinical

assessment is liable to personal preferences and although it might be easy to assess obesity in gross animals, it is certainly more difficult in animals where fat accumulation is not obvious.

Until precise quantitative techniques become available, it will not be possible to estimate accurately the real prevalence of obesity in the dog and cat population, to identify the disease problems which develop as a consequence of excess fat accumulation and, finally, to objectively evaluate the efficacy of measures taken to treat obesity such as nutritional management.

1.3. THE USE OF ULTRASOUND IN THE ASSESSMENT OF OBESITY.

With recent advances in non-invasive diagnostic techniques, in particular ultrasound, a range of novel opportunities has been opened up to the veterinary surgeon. Ultrasound has been used both in humans and in farm animals to measure subcutaneous adipose tissue and these measurements have been related to total body fat content. The technique has proved valuable for measuring backfat thickness in pigs (Giles, Murison and Wilson, 1981) and has being carried out in cattle (Kempster and Owen, 1981) and horses (Henneke, Potter, Kreider and Yeates, 1983).

The great attraction of ultrasound for assessing obesity lies in the fact that it is completely safe and harmless for both the operator and the patient. No hazardous radiation is emitted and there is no compression of the skin or

subcutaneous tissues. Furthermore, it can be a rapid and easy procedure minimizing any inconvenience to the patient.

The availability of a rapid non-invasive method for measuring body fat in dogs and cats would mean that:

a) It would be possible to establish objective criteria to what should be considered as obesity in the dog and cat.

b) The real prevalence of obesity in the dog and cat population would be determined.

c) It would be possible to assess the disease consequences of obesity in pets.

d) It would be possible to evaluate the efficacy of various dietary regimens in the management of obesity.

CHAPTER II.

LITERATURE REVIEW.

CHAPTER II. LITERATURE REVIEW.

2.1. DEFINITION OF OBESITY.

Obesity has been defined in both human and veterinary medicine in very similar terms. Davidson and Passmore (1969) defined obesity as the *"excessive accumulation of fat in the storage areas of the body"*, a definition widely adopted in the veterinary literature (Mason, 1970; de Bruijne and Lubberink, 1977; Ward, 1984; Brown, 1987; Wills, 1988).

A more recent definition given by Mayer (1973) emphasizes the detrimental effects of obesity, *"a pathological condition characterized by an accumulation of fat much in excess of that necessary for optimal body function"*. Various clinicians in the veterinary field also adopt this view in their studies on obesity (Edney, 1974; Markwell, 1988a; Clutton, 1988).

It is important to note that the term "overweight" generally refers to a body weight greater than some arbitrary standard for a given height and does not necessarily imply an excess of body fat. A dog can therefore be "overweight" according to its breed weight range simply because it has a marked muscular development rather than excess of fat. In many cases, however, obesity and overweight will be pointing towards the same pathology: an excessive store of body fat.

Though the concept of obesity is fairly straightforward problems arise, however, both in determining the amount of body fat present in a given individual and in defining what is excessive (Mason, 1970; Houpt and Hintz, 1978; Cohen, 1985; Leiter, 1986).

2.2. THE PREVALENCE OF OBESITY.

2.2.1. IN HUMAN BEINGS.

The complex relationship that arises between human beings and their pets makes mandatory that we should study obesity in the latter under the light of our knowledge of this problem in the former. Many authors feel it is not mere coincidence that obesity should be on the increase in both people and household pets.

In 1951 Armstrong and his co-workers estimated that *"one of the subtler and more serious health hazards of our time is obesity"* and *"there are many indications that overweight is becoming increasingly important in medical practice"* (Armstrong, Dublin, Weathley and Marks, 1951). These authors considered that at least one-fifth of the population of the United States over 30 years of age were overweight, i.e., about 15 million. Since that date there have been reports on the increase in prevalence of obesity in both children and adults (Mayer, 1980; Vasselli, Cleary and Van Itallie, 1983; Gortmaker, Dietz, Sobol and Wehler, 1987), and Friedman (1980) considered that there

were 70 million obese Americans (being 20% or more above their ideal weights) at the beginning of our present decade. Quoting a national health survey, Van Itallie (1985) reported a prevalence of obesity of 26% in U.S. adults (people 20 to 75 years old).

In Canada it has been estimated that 14.1% of adult men and 20.6% of women are overweight (Leiter, 1986).

The trend in European countries is not different from the one seen in the United States. In 1959 in a scientific meeting on the subject of obesity the chairman's opening remarks included the following: *"obesity is unquestionably the most common nutritional disease in Britain and gives rise to more ill-health than all the vitamin deficiencies put together"* (Meiklejohn, 1959). Over 20 years later, a survey of the weight and height of a representative sample of 5000 men and 5000 women in the United Kingdom (aged 16-64 years) showed a prevalence of obesity of 40% among men and 32% among women (Rosenbaum, Skinner, Knight and Garrow, 1985). Recently, Weatherall and Shaper (1988) examined data from 7735 middle-aged men in 24 towns in Britain. They found considerable variation in the rate of obesity between towns, from 11% to 28%.

Similar figures are found in other European countries: Austria: 5%-15% ; Bulgaria: 19.1% ; Federal Republic of Germany: 17.4% ; Netherlands: 17% , and although there were differences in the indices used for obesity as well as differences in age and sex in the various surveys,

nevertheless it was clear that obesity occurs in all parts of Europe (Kluthe and Schubert, 1985).

2.2.2. IN DOGS AND CATS.

It is widely held in the veterinary world that obesity is one of the most common afflictions encountered by the small animal practitioner (Morris, 1974; Andersen and Lewis, 1980; Sibley, 1984; Brown, 1987). Possibly because it is easier to recognize this condition in the dog, there are various authors that emphasize that obesity is certainly the most common nutritional disease occurring in the canine species (Edney, 1974; Lewis, 1978; Ward, 1984; Markwell, 1988a; Crane, 1991).

Surprisingly enough, very few surveys have been published on the incidence of obesity. The first one in the literature appears to be that of Krook, Larsson and Rooney (1960). In their survey of 10,993 canine necropsies analyzed at the Royal Veterinary College in Stockholm obesity was found to be present in 11.6% of dogs.

Ten years later Mason (1970) published the first British survey on the prevalence of canine obesity. In a total of 1,000 dogs over 1 year of age attending as outpatients a hospital clinic, 28% were considered to be obese. In 1978 Edney reported that 34% of 1,134 dogs which attended for veterinary consultation were judged to be *"unarguably obese"*. In that same year, a further survey, this time from the German Federal Republic, reported a prevalence of

30% among dogs of several urban districts (Meyer, Drochner and Weidenhaupt, 1978). In dogs attending a small animal clinic in Linz, Austria, the level of obesity was as high as 44% (Steininger, 1981).

The most recent and most intensive clinical study of obesity in dogs was carried out by Edney and Smith (1986). A total of 8268 dogs were surveyed in 11 veterinary practices in the United Kingdom during a period of 6 months in 1983. Results showed that on average, 24.3% of dogs in the survey were judged to be either obese or gross. In all of these surveys, obesity was assessed according to the clinicians criteria or based on a pre-defined clinical scale, such as the one where the animal is given a score from 1 to 5 according to its general demeanour and to the amount of fat overlying its rib cage and abdomen. A score of 1 would mean that the dog is thin, with little or no fat and skeletal structure obvious, whereas a score of 5 would be given to a gross animal, with large amounts of subcutaneous fat palpable and obvious incapacity due to excess adipose tissue.

There are some who consider the majority of dogs in private ownership to be mildly obese when compared with physically fit members of working breeds (Darke, 1978), whereas others conclude that it is very likely that between one quarter and one third of dogs seen in veterinary practices are overweight (Markwell, 1988b).

If a main characteristic of obesity in dogs is the scarcity of published surveys on its incidence, this is especially true in the feline species. There is

little known about feline obesity. Up to date, there is only one survey on the prevalence of obesity in cats recorded in the literature: Anderson (1973) refers to a study carried out by Edney who found an incidence of 9% (range 6%-12.5%) of 429 cats attending three small animal practices.

It has been said that the current incidence of obesity in the cat population is probably much greater, because of the enhanced palatability and increased popularity of dry cat foods and the more common practice of feeding ad-libitum (Lewis and others, 1987; O'Donnell and Hayes, 1987; Branam, 1988). Many authors agree with Darke (1978) in that a proportion of the cat population is undoubtedly overweight and yet nevertheless it is believed that obesity is much less common in this species when compared to the dog (Anderson, 1973; Morris, 1974; Houpt and Smith, 1981; Markwell, 1988a,b).

All evidence appears to suggest that the cat is better able to control its energy balance, even if presented with diets of variable caloric content, than is the dog (Anderson, 1973; Morris, 1974; Kane, Rogers, Morris and Leung, 1981; O'Donnell and Hayes, 1987; Wills, 1988). The desert-type conditions in which the cat's ancestors developed might have influenced the parsimonious eating habits of this species, while the more northern-based primitive dogs would have developed a tendency to large consumption of calories to maintain body temperature and the energy required for capturing prey along vast hunting territories (Wolter, 1988).

This certainly concurs with other authors in their view of the feline species abilities: *"the cat is born with either superior intelligence or self-control to*

avoid overeating. Wisely, it usually consumes a sufficient quantity of food to maintain good health and then graciously moves on to other activities" (Stallings, 1968).

2.3. THE PATHOGENESIS OF OBESITY.

2.3.1. INTRODUCTION.

The problem of obesity is not new and nevertheless there is as yet no simple explanation of its aetiology and effects. No single causative mechanism has been confirmed in all obese people (Truswell, 1985). Many attempts have been made to evaluate the role of genetic and environmental factors in the development of obesity in human beings but there are many questions that remain unanswered (Hirsch, 1972; Vasselli and others, 1983; Cohen, 1985). Indeed it has been said that it is probably not possible to formulate a unifying theory which will explain why some people become fat while others remain lean, just as there is no simple explanation for the economic success or failure of a nation (Garrow, 1983a). Furthermore, some authors have even challenged the notion of a single "ideal weight" (Garn, Hawthorne, Pilkington and Pesick, 1983).

However, much progress has been made towards a comprehensive understanding of all the complexities involved. Research has opened up avenues for exploring dietary, psychological, biochemical and

neurophysiological disturbances associated with obesity and has produced valuable information for both the clinician and the patient. A number of studies on canine obesity include some of the abnormalities which have been reviewed below but it is important to realize that most of these abnormalities have been found in overweight laboratory animals and humans and have yet to be demonstrated in obese pets.

2.3.2. IN SEARCH OF AN EXPLANATION: THE PRESENT STATUS IN OBESITY RESEARCH.

Heritability.

Since the beginning of our century much research work has been carried out to evaluate the role of genetic and environmental factors in the development of all types of obesity (Vasselli and others, 1983).

Obesity in humans is said to have a heritability of 80% (Kronfeld, 1988), and a very definite familial association (reviewed by Mayer, 1980). Studies in the United States have shown that less than 10% of the children of parents of normal weight are obese, but the proportion rises to 50% if one parent is obese and to 80% if both parents are obese (Mayer, 1955b). It has been said that after adjusting phenotypic variance for age and gender, heritability of percent body fat is 25%, whereas resting metabolic rate and thermic response to food could have a heritability as high as 40% (Bouchard, 1991a). Studies involving pairs of monozygotic twins found that there is a

significant genetic transmission of the amount and distribution of subcutaneous adipose tissue (Selby, Newman, Quesenberry, Fabsitz, Carmelli, Meaney and Slemenda, 1990; Bouchard, Tremblay, Després, Nadeau, Lupien, Thériault, Dussualt, Moorjani, Pinault and Fournier, 1990). Moreover, the response to long-term overfeeding within pairs of twins was significantly less variable than between pairs, suggesting that some individuals are at a greater risk of gaining fat than others due to genotypic influences (Bouchard and others, 1990). Based on the observation that most obese people who lose weight tend to regain it within 5 years, it has been suggested that there is a genetically determined set-point weight for each person (Weigle, 1990). The "set-point" theory argues that attempts to modify this weight put into motion physiological and biochemical mechanisms which will try to oppose any change (Weigle, 1990; Katahn and McMinn, 1990).

However, genetic disorders in which obesity follows a clear Mendelian mode of inheritance constitute a very small fraction of the human obese population (Bouchard and Pérusse, 1988). A substantial body of evidence lends support to the idea that obesity is a multifactorial phenotype and its expression can be attenuated or amplified by many non-genetic factors (Bouchard and Pérusse, 1988; Bouchard, 1991b).

It has been repeatedly observed in farm animals that breeds and individuals within breeds show much variation in both amount and anatomical placement of their fat depots (Koch, Dikeman, Allen, May, Crouse, and

Campion, 1976; Talamantes, Long, Smith, Jenkins, Ellis and Cartwright, 1986).

However, most data on the genetic aspects of obesity comes from studies on laboratory animals. Several types of genetic obesity have been identified in mice and rats (reviewed by Bray and York, 1971; and by Trayhurn, 1984) and yet, as is the case in humans, the primary metabolic or regulatory defect(s) which is responsible for the development of excess body fat in these rodents, has not been consistently identified.

Some of the following factors are under intense investigation:

Cellularity of adipose tissue.

An increase in the total amount of adipose tissue can come about by an increase in either adipocyte size (hypertrophy) or cell number (hyperplasia).

In laboratory animals suffering from genetic obesity abnormalities of adipose tissue cellularity have been extensively documented. The yellow obese mouse and the diabetic mouse (db/db) develop only hypertrophic obesity while the obese hyperglycaemic mouse (ob/ob) and the Zucker "fatty" rat show both increased number and size of fat cells. The New Zealand obese mouse (NZO) does not fall easily into either category (Bray and York, 1971; Johnson and Hirsch, 1972; Johnson, Stern, Greenwood and Hirsch, 1978; Cleary, Vasselli and Greenwood, 1980).

Most obese individuals have some increase in adipocyte size and in nearly all obese humans, particularly those with obesity of juvenile onset and those grossly obese, there is a marked increase in adipocyte number (Hirsch, 1972; Salans, Cushman and Weismann, 1973; Knittle, Ginsberg-Fellner and Brown, 1977; Van Itallie, 1980). Larger fat cells have also been found in obese adult mongrel dogs (Berlan, Carpene, Lafontan and Dang-Tran, 1982; Taouis, Valet, Estan, Lafontan, Montastrue and Berlan, 1989). Roncari, Lau and Kindler (1981) found that the adipocytes of 34 massively obese patients showed a significantly greater replication rate when compared to cells from lean subjects and that this excessive replication persisted throughout the first five subcultures. They concluded that this characteristic was inherent in the cells and might have reflected the operation of genetic factors in this subgroup of the obese population.

It has been said that there could be some "critical periods" early in life in which most adipose cells are produced. Nutritional influences in these periods could result in permanent changes in adipose tissue cellularity thus predisposing the individual to a determined body condition (Hirsch, 1972). In this respect, Johnson, Stern, Greenwood, Zucker and Hirsch (1973) carried out an interesting experiment with rats. They exposed genetically obese rats and normal rats to over and underfeeding prior to weaning by placing them in large or small litters, respectively. During the first 30 days of life the nutritional effect and not the genotype was the predominant influence on the animals' growth. However, by 12 weeks of age genotypic differences became

the major determinant of body weight. Furthermore, while early overfeeding significantly increased adipocyte number in both the obese and nonobese rats, early underfeeding reduced adipocyte number only in the non-obese.

Leung and Robson (1990) argue that obesity in childhood is strongly associated with obesity in adulthood although the association with obesity during infancy is less clear. The hyperplastic form of obesity in humans has been said to be much more resistant to weight reduction in a long term basis when compared to the hypertrophic form (Krotkiewski, Sjöström, Björntorp, Carlgren, Garellick and Smith, 1977), but this has not been fully confirmed and requires further investigation (Pi-Sunyer, 1988).

Furthermore, it was postulated that the adipocyte number remained fixed in the adult and that any reduction in body weight in adulthood was really a decrease in fat cell volume, while the number of cells remained constant (Hirsch, 1972; Knittle and others, 1977). Although this had been documented in normal rats (Hirsch and Han, 1969; Greenwood and Hirsch, 1974), it was questioned whether it actually occurred in human adipose tissue (Jung, Gurr, Robinson and James, 1978).

Thus, for years it was thought that any attempt to fatten or thin normal rats after the earliest weeks of life lead only to changes in cell size, with no effect on the number of adipocytes (Hirsch, 1972). It was later shown that adipose tissue of adult rats fed a high-fat diet can experience an increase in the number of lipid-filled cells (Klyde and Hirsch, 1979). Furthermore, in another study, adult rats of various strains became obese when they were fed a

highly palatable diet for several months (Faust, Johnson, Stern and Hirsch, 1978). Analysis of their adipose tissue morphology revealed increases in both adipocyte size and number in most fat depots. Interestingly enough, when the rats were returned to a normal diet, there was a clear weight loss during which only adipocyte size returned to normal, but it was not possible to reduce the number of adipocytes to its previous value.

It has then been suggested that the achievement of some specific mean adipocyte size would trigger fat cell hyperplasia thereby producing a more long-term stimulus to weight gain in the form of excess adipocytes that require lipid filling. This has been termed the "ratchet effect" and it implies that the body's fat content can readily increase, but only under extreme circumstances will it decrease below a minimum level. This minimum level would be set by the total number of adipocytes in combination with their tendency to remain lipid-filled (Vasselli and others, 1983).

In the bovine species, adipose tissue from animals of the leaner Holstein breed was found to contain smaller and fewer cells than the respective tissue from the fatter Hereford x Angus animals (Hood and Allen, 1973a). During growth of the bovine animal, the increase in adipose tissue mass is accompanied by both fat cell hypertrophy and hyperplasia but it would seem that adipocyte proliferation stops in most fat depots at an early age (Hood and Allen, 1973a; Cianzio, Topel, Whitehurst, Beitz and Self, 1985).

Porcine adipose tissue is believed to expand by a combination of adipocyte hyperplasia and hypertrophy up to 5 months of age, after which adipose growth is accomplished by cellular hypertrophy only (Anderson and Kauffman, 1973).

Recently, relevant investigations have been carried out in genetically obese swine. Hausman, Campion and Thomas (1983), studied foetuses of 110 days of gestation from lean and obese sows and found that adipocytes from obese foetuses were larger than cells from lean foetuses. In a further ontogenic study comparing subcutaneous adipose tissue of genetically obese and lean foetuses, it was found that as early as 90 days of gestation the fat cells of obese foetuses were larger than the adipocytes of lean foetuses (Hausman, 1985). Similar results were reported by Mersman (1986) who discovered larger adipocytes in obese than lean pigs at birth and all subsequent post-partal ages.

The mechanisms underlying all of these abnormalities in adipose tissue cellularity and their significance in the development of obesity remain largely unknown.

Lipid metabolism.

A possible explanation for the increased adipocyte size and/or adipocyte number observed in obese individuals, is that they could have a

defective lipid metabolism. Excessive quantities of body fat would be maintained in adipose tissue due to an increased deposition of body fat (defective lipogenic activity) or to a decrease in fat utilization (defective lipolytic rate).

Defects in lipogenesis.

It has been found that the first detectable metabolic abnormality in the genetically obese Zucker rat is an increase in the activity of the enzyme lipoprotein lipase (LPL) (Gruen, Hietanen and Greenwood, 1978; Boulangé, Planche and de Gasquet, 1979; Cleary and others, 1980). At the luminal surface of capillaries LPL catalyzes the breakdown of circulating triglycerides to provide free fatty acids to the fat cell for re-esterification and triglyceride storage. It has also been found that obese Zucker rats show an increased synthesis of hepatic fatty acids which contributes to the maintenance of hyperlipidaemia and obesity in these animals (Nauss-Karol, Triscari and Sullivan, 1982).

A higher LPL activity was demonstrated in the fat pads of an obese line of chickens (Hermier, Quignard-Boulangé, Dugail, Guy, Salichon, Brigant, Ardouin and Leclercq, 1989). These animals showed evidence of an enhanced storage capacity in their adipose tissue at 2 weeks of age when compared to lean chickens.

Genetic variation in anabolic and catabolic processes in adipose tissue of sheep was found to be related to degree of fatness. Low rate of lipolysis and high activity of LPL were thought to result in high rates of fat deposition (Sinnet-Smith and Woolliams, 1988).

Extensive research in pigs demonstrated that adipose cell lipogenic capacity is elevated several fold in the genetically obese pig (Weisenburg and Allen, 1973; Hood and Allen, 1973b; Steele, Frobish and Keeney, 1974; Martin and Herbein, 1976; Scott, Cornelius and Mersman, 1981). Increased fatty acid synthesis, increased incorporation of glucose, acetate, and glycerol for lipid synthesis and elevated LPL activity, were documented in these studies. Furthermore, McNamara and Martin (1982) found that selection for different amounts of backfat in swine caused a threefold increase in adipose tissue LPL activity in a 110 days foetal pigs. Genetically obese foetuses had three times more LPL activity than did foetuses of the same age selected for low backfat. In a similar study, Hausman and others (1983) showed that all adipocytes from obese foetuses (110 days of age) were LPL-positive whereas all cells from lean foetuses were negative for LPL activity. Recently, Campion, Hausman, Stone and Klindt (1988) studied the influence of maternal obesity on the foetal development of pigs. They found that both adipose tissue LPL activity and serum triglyceride levels were higher in foetuses from obese dams than in foetuses from lean dams.

Elevated LPL activity in adipocytes of obese humans has also been documented (Pykälistö, Smith and Brunzell, 1975; Schwartz and Brunzell, 1981). Moreover, the activity of this enzyme has been shown to further increase after weight loss and the increase persists in the stable reduced patient 28 months after initial weight loss. In subjects who regained their lost weight, the LPL activity decreased to the previous obese levels (Schwartz and Brunzell, 1981).

At present, it is not known whether this metabolic alteration could have a key role in the pathogenesis of obesity and in the phenomenon of rapid regain of lost weight seen in many obese humans after dieting (the so-called "yo-yo syndrome").

Defects in lipolysis.

A defective lipid mobilization could lead to excessive fat accretion in the obese animal. Although Standal and Vold (1973) did find lower lipolytic rates in the fat pads of genetically obese pigs, other investigators have concluded that there is no apparent lipolytic defect in obese animals that could explain their excessive accumulation of body fat (Weisenburg and Allen, 1973; Mersmann, 1985; Mersmann, 1986).

However, it has been demonstrated that the lipolytic effect of adrenalin is reduced in adipose tissue of obese mongrel dogs. In two independent studies, it was found that the balance between α-2 and β-adrenoreceptors was shifted in the adipose tissue of the obese, such that there was an increase in

α-2 adrenoreceptors (antilipolytic effect) and a decrease in β-adrenoreceptors (lipolytic effect) (Berlan and others, 1982; Taouis and others, 1989). The authors did not speculate on whether this abnormality in the adrenergic status of obese dogs was a cause or a consequence of obesity.

Energy expenditure.

Great interest has been placed in the possibility that obese or obese-prone individuals could show a reduced energy expenditure when compared to normal subjects. The obese-prone individual would "burn off" less calories and thus store more energy than the lean one (Scott, 1981; Mersmann, Pond and Yen, 1984; Truswell, 1985).

Conflicting results have been published due to the different methodologies employed and the great number of factors that influence energy expenditure. One major area of controversy is the so-called "basal metabolic rate" (BMR) or the daily energy expended for the maintenance of normal physiological processes. Although alterations in the basal metabolic rate of obese individuals have been long suspected (Pawan, 1959; Bray, 1972; Atkinson and Bray, 1978), it has not been possible to consistently identify this defect (Leiter, 1986). Basal metabolism is difficult to measure because it varies among individuals and is influenced by age, sex, racial origin, body composition, environmental temperature, emotions, disease, genetic factors,

overfeeding and underfeeding (Atkinson and Bray, 1978; Björntorp and Yang, 1982; Garrow, 1983a).

In genetically obese rats, it has been shown that a defect in thermoregulatory thermogenesis might exist as early as 7 days after birth (Planche, Joliff, de Gasquet and Le Liepvre, 1983). These authors found that 1-week-old genetically obese Zucker rats showed a reduction in oxygen consumption, respiratory CO_2 production and in-vivo oxidation of injected [$^{1\text{-}14}$ C] palmitic acid at 330^{0}C and 280^{0}C, when compared to their lean counterparts. The authors concluded that this defect in energy expenditure was more than adequate to account for the 50% greater fat content of 7-day-old obese pups.

A decreased thermic response to food could also account for a reduced energy expenditure in the obese-prone. The thermic effect of food is the caloric expenditure occurring after food ingestion. Thus, it has been said that subjects who are overeating and yet are able to maintain low weight gains, could convert their excessive caloric intake simply into heat (Miller, Mumford and Stock, 1967). Obesity-prone rats have been shown to be unable to increase fat oxidation when fed a high-fat diet, thus being predisposed to developing dietary obesity (Chang, Graham, Yakubu, Lin, Peters and Hill, 1990). In humans, it was found that the thermogenic response to a mixed meal was significantly reduced in the obese group when compared to the lean

control group. And this was so even after weight loss in the obese patients (Bessard, Schutz and Jéquier, 1983).

Schutz, Bessard and Jéquier (1984) compared the overall response to food intake during a whole day in 20 young obese women with that in eight non-obese control women. The thermogenic response was blunted in obese women compared with that of controls and there was an inverse correlation between percentage body fat and diet-induced thermogenesis. Swaminathan, King, Holmfield, Siwek, Baker and Wales (1985) found that obese subjects showed very little increase in metabolic rate following ingestion of fat and this was significantly different from that seen in lean subjects. The thermogenic response to a mixed meal was also significantly lower in obese subjects when expressed as a percentage change.

The thermic effect of food at rest, during 30 minutes of exercise, and after exercise was studied in eight lean and eight obese individuals by Segal, Gutin, Nyman and Pi-Sunyer (1985). Their results showed that the thermic effect of food was significantly higher for the lean than the obese men in the rest, after-exercise and exercise conditions. Recently, it was demonstrated that the thermic effect of food in seven obese subjects was significantly lower than in lean individuals, adding further evidence for a defective thermogenesis in obesity (Segal, Edaño and Tomas, 1990).

Nevertheless, it is also possible that the thermogenic defect in obesity is a consequence rather than a cause of obesity (Danforth, 1983), and there are

authors who even question whether dietary-induced thermogenesis is really demonstrable at all (Hervey and Tobin, 1983).

Recently, interest has been drawn to the role that brown adipose tissue might play in the development of obesity (Rothwell and Stock, 1979; Himms-Hagen, 1990). Brown fat cells are involved in generating heat and thus in energy expenditure in many animal species including man. It has been suggested that a defect in thermogenesis in brown fat could be an important factor in the pathogenesis of obesity (Himms-Hagen, 1984; Glick, Bray and Teague, 1984).

Heat production in brown adipose tissue is stimulated by noradrenalin and in one study obese women showed a decreased production of this neuropeptide (Bessard and others, 1983). A decrease in brown adipose tissue response to noradrenalin has been documented in the genetically obese Zucker rat (Marchington, Rothwell, Stock and York, 1983), and in the genetically obese mouse (ob/ob) a reduced noradrenalin turnover has been demonstrated in its brown adipose tissue (Knehans and Romsos, 1982).

The issue is still unclear and it has been said that alterations in dietary induced thermogenesis and brown adipose tissue could hardly explain the wide variations in brown fat activity seen in animals without corresponding differences in obesity (Garrow, 1983a).

Other abnormalities.

Intense study has been undertaken in an effort to learn more about the internal control of food intake and the metabolic abnormalities that could influence the development of obesity (Mayer, 1955a,b; Morrison, 1959; Bray and York, 1979; Royal College of Physicians, 1983; Mrosovsky, 1986; Bray, 1987). The anatomic distribution of adipose tissue is now believed to have an important role on the morbidity that accompanies human obesity and it has been shown that there are site-specific differences in number, size and metabolic behaviour of adipocytes (Leibel, Edens and Fried, 1989).

It has been said that obese or obese-prone individuals could have an abnormal preference for highly palatable foods and an abnormal response to satiety signals (Lewis and others, 1987; Neale, 1988a). A recent study found overeating and impulsive eating behaviour in a sample of 96 obese women, suggesting the presence of an abstinence violation effect (Schlundt, Hill, Sbrocco, Pope-Cordle and Kasser, 1990). Previous studies of the feeding behaviour of obese men and women failed to detect differences in food item preferences or feeding patterns (Spitzer and Rodin, 1981).

Obesity in both human beings and rodents is associated with hyperinsulinaemia and resistance to the cellular effects of insulin (Elahi, Nagulesparan, Hershcopf, Muller, Tobin, Blix, Rubenstein, Unger and Andres, 1982; Golay, Swislocki, Chen, Jaspan and Reaven, 1986). This

abnormality has also been demonstrated in the bovine species (McCann and Reimers, 1986), in sheep (Bergman, Reulein and Corlett, 1989) and in fat ponies (Jeffcoat and Field, 1985).

Insulin can enhance lipogenesis and increase LPL in adipose tissue (Kazdová, Fábry and Vrána, 1974; Gruen and others, 1978; Hausman and Jewell, 1988). Thus, it could well be that the hyperinsulinaemia observed in obese individuals has an important role in the genesis of obesity (Johnson and others, 1973; Johnson and others, 1978; Gruen and others, 1978).

Insulin has also been shown to have a major role in the control of food intake in animals. The infusion of this hormone in some specific areas of the brain causes a reduction on food intake and body weight (Woods, Porte, Bobbioni, Ionescu, Sauter, Rohner-Jeanrenaud and Jeanrenaud, 1985). It has been postulated that a failure to transport sufficient insulin to critical brain sites and/or absent or deficient insulin receptors in the brain, may contribute to the overeating that accompanies some forms of obesity, like the one developed by the Zucker "fatty" rat (Woods and others, 1985).

Other substances have been implicated in the control of food intake. Cholecystokinin, a peptide found both in the brain and gastrointestinal tract, is known to be a satiety signal for the brain. A decreased sensitivity to this substance has been documented in the obese mouse (ob/ob) and in the Zucker rat (McLaughlin, 1982). Both strains of genetically obese animals show hyperphagia although this is not essential for the development of their obesity (Bray, 1987).

Other peptides, the endogenous opioids, are thought to play an important part in food intake regulation, many having an effect in stimulating appetite (Yim and Lowy, 1984). Some have been said to be elevated in the pituitary of the genetically obese mouse (McLaughlin, 1982).

Finally, some workers have studied the possible role of thyroid hormones and the sympathetic nervous system in the regulation of energy metabolism. Danforth (1983) found that obese subjects showed a blunted increase in production of triiodothyronine (T_3) with overfeeding. This hormone plays an important role in thermogenesis and, under normal circumstances, an increase in caloric intake will trigger an increased production of T_3. On the other hand, Young and Landsberg (1982) have shown that the sympathetic nervous system responds to changes in caloric intake. These authors suggest that the association between obesity and hypertension may reflect a continual stimulation of the sympathetic nervous system due to chronic overfeeding. This view is supported by Clark, Rattigan and Colquhoun (1991) who hypothesize that the increase in sympathetic activity could be an attempt by the body to stimulate facultative thermogenesis to burn-off the excess energy. The ability to increase sympathetic nervous activity may be blunted in those who have a tendency to become obese. Recently, Bray (1991) reviewed the role of the sympathetic nervous system in experimental obesity. He showed evidence that in most known models of obesity in rats there is a reduced level of sympathetic activity together with an increased level and/or responsiveness to adrenal steroids.

2.3.3. FEEDING AND EXERCISE.

Introduction.

The concept that overeating accounts for 95% of cases of obesity (Armstrong and others, 1951) has been losing ground in human medicine for 30 years (Mayer, 1980; Kronfeld, 1988). Nevertheless, some believe that calorie overconsumption is the major contributor to body weight gain (Rampone and Reynolds, 1988).

The role of overfeeding in the pathogenesis of obesity in dogs and cats has been given different relevance. The view of some authors is that obesity is very often just the result of overfeeding the pet (Leonard, 1971; Morris, 1974; Kaufman, 1986). But what is generally accepted is that the two most important factors leading to obesity in pets are:

- an excess intake of food and,
- reduced physical activity.

(Staff Report, 1965; Lewis, 1978; Andersen and Lewis, 1980; Ettinger, 1983; Markwell, 1988a; Little, 1990).

Many authors consider that there are two stages in the development of obesity. During the initial growth phase there is a positive energy balance. In other words, the animal is under a dietary intake that exceeds its requirements and the excess energy goes then to increase both lean body mass and body fat. A second, static phase, follows in which dietary intake and energy expenditure

are balanced. This phase is the result of various feed-back mechanisms which reduce dietary intake, but since they are triggered by the excess weight gain, body weight remains constant in the obese state. At this point the animal may consume relatively few calories (Lewis, 1978; Andersen and Lewis, 1980; Sibley, 1984; Ward, 1984; Brown, 1987; Lewis and others, 1987).

However, there are many factors influencing the amount of food and exercise the pet is going to get:

Social and environmental pressures.

The human factor.

Joshua (1970) pointed out that obesity is seldom a problem in working dogs unless there is a frank pathological disturbance. The condition appears to be essentially related to the household pet dog. The author underlines that in an affluent and urbanized society the confinement of dogs to smaller living premises and the very limited time the average working owner can devote to exercising the animal together with the increased financial ability to acquire pet food of high nutritional value, are all factors that favour a high incidence of obesity.

In man, it is now accepted that physical activity involves an increase in energy expenditure and is therefore beneficial in preventing weight gain and fat deposition (Royal College of Physicians, 1983; Cohen, 1985; Tremblay, Després, Leblanc, Craig, Ferris, Stephens and Bouchard, 1990). The dog is a

member of the genus *Canis* which also includes the wolf, the coyote and the four species of jackal. These constitute a group of social carnivores with a body adapted for fast running over long distances and skillful hunting of prey (Clutton-Brock, 1984). It is clear that our canine companions have become more placid than their wild counterparts (such as the Dingo or Australian wild dog). In situations where food is provided and readily available, less energy will be required for locating and ingesting nutrients and competition becomes unnecessary and can even be counterproductive (Craig, 1981).

An interesting investigation into the factors relating to obesity was carried out by Mason (1970) who found that the prevalence of the condition was higher among dogs owned by obese people than among dogs owned by people of normal physique. Again, the prevalence was also higher among dogs of people in middle and elderly age groups than among dogs owned by people under 40 years of age. She concluded that the association between obese pets and obese owners could be related partly to lack of exercise, as such people do not usually find pleasure in taking their pets for walks, and partly to the family eating habits, such that these dogs receive large quantities of hypercaloric food. The higher incidence of overweight dogs among middle-aged and elderly owners is explained by the author as a result of a lack of regular exercise. Among others, this view is supported by Nind (1988) who finds there is a very distinctive relationship between obese animals and obese owners, and by Ward (1984) who thinks that the older, overweight dog owner is likely to eat frequently and exercise infrequently, thus establishing an

environment conducive to obesity in his/her pets. In this respect, it has been shown that daily caloric intake is largely determined by the owner's control over the number of meals per day together with the quantity and composition of the diet (Mugford and Thorne, 1980).

Indeed pet-ownership can be a complicated psychological relationship. Darke (1978) relates that there are owners who feel their pet should eat the same food as themselves, as part of a "humanizing process", while others apparently gain satisfaction and companionship by fulfilling the pet's repeated demands for food not realizing that, given the chance, most small animals will eat more than their basic energy requirements. This is what Kronfield (1988) has called *"an interactive reward system based on feeding"*. Lewis and others, (1987) state that the obese dog or cat is often a helpless victim of its owner's compulsions and they list some of the following reasons why people overfeed their pets:

* Treating pets like people.
* Ignoring the calories that come from food items consumed outside meal time.
* Cultivating the animal's taste for highly palatable foods.
* Feeding the same quantity regardless of the animal's needs.
* Interpreting eating as a sign of health.

Stogdale and Moore (1980) were presented with a chronic skin complaint in a dog that had been overweight for 5 years. The dog had been

allowed to become a very fussy eater and would refuse any food other than prime quality meat. The obese owner fed excessive amounts of food to the animal and still thought that the pet was not eating enough.

Joshua (1970) drew attention to a further reason for overfeeding, subtler but nonetheless important, when she pointed out that *"another feature of affluent society is the tendency of many to regard it as a right to indulge in as many of the pleasures of existence as possible with the minimum of inconvenience"*. She relates this tendency in society to the increasing resort to prepared canned rations fed in large quantities to the pet.

There are also pressures upon the owners themselves. Morris (1974) finds it not surprising that canine obesity is so common when owners are "bombarded" daily by some pet food advertisements stressing the desirability of increased palatability: they come to believe that unless the dog eats substantially more than it needs for normal body weight maintenance, it does not like the food. Figures show that in 1977 dogs in Britain ate 362,000 tonnes of canned food worth £144.3 million. Cats consumed 244,000 tonnes costing £120.5 million (Veterinary Record, 1989). These figures have increased since then, mostly due to the switch to prepared pet foods rather than household meals, and partly due to the increase in the number of pets. Estimates show that after a slight decline some 10 years ago, the number of dogs has increased to 7.4 million while the cat population has grown steadily to 6.8 million. In 1990, around £1000 million was spent in dog and cat foods alone (Veterinary Record, 1991).

Houpt and Smith (1981) are of the opinion that an increase in the palatability of diets is probably one of the most important causes of obesity in pets, and Brown (1987) states that obesity in canine patients is principally attributed to the use of these highly palatable/energy dense diets, which are offered in large amounts on a frequent or easily accessible basis. Yet this would seem to apply principally to home-made diets. In a previously quoted survey, it was found that dogs fed on home-prepared food and table scraps were more inclined to obesity than those fed entirely on tinned meat (Mason, 1970). It is said that when very palatable food is available, the animal will maintain a higher weight (Houpt, 1982; Brown, 1987), and that alimentary behaviour is strongly influenced by the available quantity of food but most of all by its quality (Ballarini, 1990).

Most of the work on diet palatability and diet-induced obesity has been carried out in laboratory animals. It has been shown that normal rats can become obese when fed high fat diets (Schemmel, Mickelsen and Gill, 1970), concentrated sugar solutions (Kanarek and Hirsch, 1977), or a mixture of highly palatable "supermarket" food items (Sclafani and Springer, 1976). In all these studies, the animals became noticeably heavier than the control rats fed a normal, less-palatable chow diet. Carbohydrates have also been incriminated in the development of obesity in humans (Cohen, 1985), although there are some who consider that a high level of sugars can be beneficial when combined with a low-fat diet and regular exercise in a control programme of obesity (Bray, 1987). In one study, dogs fed a high carbohydrate

diet did not develop a greater adipose tissue mass than those fed a carbohydrate-free diet (Romsos, Belo, Bennink, Bergen and Leveille, 1976). Thus, the issue is not as clear as it would seem.

Fat has been shown to increase palatability of diet for most dogs and cats (Houpt and Smith, 1981; Kane, Morris and Rogers, 1981) and in one study both male and female dogs had a significant preference for diets containing sucrose over the bland diet (Houpt, Coren, Hintz and Hilderbrandt, 1979). Cats appear to be more particular about sugar in their diets and although sucrose in milk is accepted they tend to dislike sweetened water (Houpt and Smith, 1981; O'Donnell and Hayes, 1987). Romsos, Hornshuh and Leveille (1978), studied the influence of a high fat diet and a high carbohydrate diet on food intake and on body weight and body fat changes in adult dogs. They found that both groups of dogs increased their body weights during the experiment, 78-80% of which was a result of increase in body fat. But they also discovered that by 12 weeks the increase in body weight in dogs fed the high-fat diet was twice the increase observed when the high-carbohydrate diet was fed. Adult cats have been said to get fat quicker if they are on a diet rich in fat (Scott, 1984).

Higher in caloric density, fat is also more digestible and is used and stored by the body more efficiently than either protein or carbohydrate (Schemmel and others, 1970; Wood and Reid, 1975; Romsos and others, 1978; Lewis and others, 1987; Fraley, 1988).

Therefore, it would appear that highly palatable fatty or sweet foods (high in caloric density) are likely to be consumed more readily and to be utilized more efficiently by the dog and cat, thus increasing the risk of obesity (Scott, 1984; Lewis and others, 1987; Brown, 1987).

Social facilitation.

This refers to the behaviour observed in normal animals when they are fed together. Following a natural instinct, a competition is established which often results in more food intake than if each pet was fed separately (Houpt and Hintz, 1978; Lewis and others, 1987).

Age.

The incidence of obesity increases with age, probably due to the reduction both in metabolic rate and physical activity (Mason, 1970; Ward, 1984; Kaufman, 1986; Branam, 1988). Other problems associated with the geriatric patient and which produce prolonged inactivity (chronic anaemia, arthritis, ocular disease) have also been incriminated in the development of obesity (Staff Report, 1965).

Overfeeding at an early age is thought to be responsible for certain cases of obesity in children and it is widely believed that overweight children are at an increased risk of becoming overweight adults (Royal College of Physicians, 1983). In one study, pups that had been allowed free access to a

commercial puppy food were 22% to 25% heavier at one year of age than those fed 20% less (Hand, 1990). This study stresses the potential influence that early calorie overconsumption can have in the development of obesity.

Sexual status.

Size and location of fat depots vary with sexual status in human beings. Men have more visceral fat than women and they tend to develop a "centripetal" adiposity as opposed to the "peripheral" or gynoid type frequently found in women (Leibel and others, 1989; Campaigne, 1990).

In dogs, Krook and others (1960), discovered that obesity was predominant in females in their investigation of 971 cases of obesity, and Meyer and Stadtfeld (1978) reported an average 16% more fat for females than males in their studies. In a widely quoted survey (Mason, 1970), it was found that the incidence of obesity was higher in females (32%) than in males (23%). But the author very rightly underlines that the survey did not take account as to whether or not the females were entire or spayed. Neutering is believed by many to be a predisposing factor in the development of obesity (see below), and therefore it cannot be concluded that females in this study were more prone to obesity per se.

Edney and Smith (1986) also found an overall higher incidence of obesity in females but their results show that many of the overweight females were spayed.

Climate.

A normal observation in dogs is that they tend to eat more when it is cold and less when it is hot. This would be part of the control of body temperature (Houpt and Hintz, 1978; Ward, 1984).

Other factors.

The control of food intake is a complex system with many variables. It has been clearly established that there are several regions of the brain that have an important role in the control of nutrient balance and dietary intake (Bray and York, 1979). These central areas of control integrate information arising from various internal and external signals. Internal signals include:

- Mechanical and chemical stimulation of the gastrointestinal tract and release of cholecystokinin.
- Plasma glucose levels and changes in the concentrations of various metabolic substrates in body fluids such as fatty acids and aminoacids.
- Hormonal responses to sight, smell and ingestion of food.
- Body stores of glycogen and fat.
- Ovarian and testicular hormones.
- Sympathetic and parasympathetic nervous system activity.
- Adrenal cortex activity and glucocorticoid levels.

- Possibly psychological factors.

(Heinbecker, White and Rolf, 1944; Houpt and Hintz, 1978; Ward, 1984; Lewis and others, 1987; Brown, 1987; Bray, 1987; Ballarini, 1990).

Much investigation is needed to establish the precise role of each of these factors in the development of obesity in dogs and cats (Lewis and others, 1987).

2.3.4. GENETIC FACTORS.

Since Mayer (1955a) studied a family of Shetland Sheepdogs with hereditary obesity appearing as a recessive character, genetic alterations have been long suspected to play a role in some cases of obesity in dogs and cats (Staff Report, 1965). There appears to be a breed predisposition in the tendency to become obese of some dogs. Surveys on the incidence of canine obesity show that Labradors, Cocker Spaniels, Collies and some of the Terriers (the Cairn and the Scottish) have a greater incidence of obesity than other breeds such as the German shepherd dog or the Boxer (Krook and others, 1960; Mason, 1970; Edney and Smith, 1986). However, breed predisposition is at present not well defined (Kronfeld, 1988). Even within one breed, some dogs are said to be "easy keepers" and will gain pathological amounts of weight while their kennel mates maintain optimum body weight on the same dietary regimen (Lewis, 1978; Houpt, 1982).

Cats, on the other hand, do not seem to show any breed predisposition to obesity (Wills, 1988).

Some primary defects related to the pathogenesis of obesity in laboratory animals and humans (see above), such as alterations in adipocyte number and size or alterations in satiety signals, are suspected to play a role in the pathogenesis of canine and feline obesity (Andersen and Lewis, 1980; Sibley, 1984). The finding of larger adipocytes and a deficient lipolytic response to cathecolamines in adipose tissue of obese adult dogs (Berlan and others, 1982; Taouis and others, 1989), although probably a consequence rather than a cause of their obesity, opens new paths into the investigation of the pathogenesis of this problem in dogs.

2.3.5. METABOLIC AND ENDOCRINE ABNORMALITIES.

A number of factors known to predispose or to cause obesity in humans have been considered in reviews of canine obesity. These factors include:

- Emotional trauma.
- Hypopituitarism.
- Cerebral, cortical or hypothalamic lesions.
- Chromophobe adenoma of the pituitary.
- Insulin secreting tumours.
- Diabetes mellitus.
- Hyperadrenocorticism (Cushing's syndrome).
- Hypothyroidism.
- Hypogonadism.

(Lewis, 1978; Andersen and Lewis, 1980; Sibley, 1984).

However, in man these are responsible for less than 5% of obesity cases (Bray, 1972); in dogs and cats the same trend is suspected (Ettinger, 1983; Clutton, 1988). Obesity is certainly seen in hypothyroid and Cushingoid dogs but these cases constitute only a small percentage of the overall obese population.

It is considered that obesity increases the risk and severity of diabetes mellitus in both dogs (Mattheeuws, Rottiers, Kaneko and Vermeulen, 1984) and cats (Panciera, Thomas, Eicker and Atkins, 1990), and it has been known for a long time that hyperadrenocorticism and hypothyroidism can produce obesity in pets.

Meijer (1980), found abdominal enlargement, hepatomegaly and skin atrophy present in 90% or more of cases of hyperadrenocorticism in dogs, with polydipsia/ polyuria/ polyphagia, anoestrus in females and decreased exercise tolerance the next most common clinical findings. Occasionally, generalized obesity was the only obvious clinical sign early in the course of the disease. The high levels of cortisol in the animal suffering from the disease result in gradual wasting of skeletal muscles due to the catabolic effect of cortisol on protein metabolism. Limb muscles atrophy and abdominal muscles are weakened, resulting in a pendulous abdomen. At the same time, accumulation of fat in the abdomen and liver, resulting in hepatomegaly, contribute to the "pot-bellied" appearance of affected animals. Whether naturally occurring or iatrogenic owing to steroid overdose,

hyperadrenocorticism is rare in cats (Scott, 1987; Peterson and Randolph, 1989). The condition has been associated with complicated, non-responsive diabetes mellitus (Fox and Beatty, 1975; Nelson, Feldman and Smith, 1988). Abdominal enlargement is one of the signs present in reported cases. Other signs that may appear are polyuria, polydipsia, polyphagia, lethargy, bilaterally symmetric alopecia, and elevated glucose and cholesterol levels.

The most notable signs of canine hypothyroidism are lethargy, lack of endurance and increased sleeping (Belshaw and Rijnberk, 1980). The decreased metabolic rate in the affected animal together with the lethargy and lack of endurance, result in a greater deposition of fat, especially in the trunk. In addition, myxoedema with the resultant thickening of the skin exaggerates the appearance of obesity.

There are no well documented cases of hypothyroidism in the cat (Wilkinson, 1984). Martin and Capen (1983) reported the case of a cat with reduced levels of serum thyroxine and triiodothyronine. The cat was obese and poorly groomed and there was thinning and bilaterally symmetrical loss of hair. Further work is needed to clearly establish this condition in cats.

Finally, hypogonadism following ovariohysterectomy or castration has long been implicated in the pathogenesis of obesity (Staff Report, 1965). Anderson (1973) reported that 68% of 81 spayed bitches were considered to be obese in one survey and that four out of 10 castrated males showed excessive fat deposition in another survey. The case of a 3 years old

spayed female Sheltie dog presented with signs of lethargy and marked obesity was reported (Forbes and White, 1987). The animal was also shown to be hypothyroid following a minimal response to the administration of thyroid-stimulating-hormone (TSH) but did not respond to thyroid replacement.

Houpt and others (1979) studied the effect of ovariohysterectomy on food intake and weight gain in eight Beagle bitches. Four of them were neutered while the remaining four were sham operated (i.e., the abdomen was opened, the ovaries were located and the abdomen was closed). Their results show that ovariohysterectomized bitches gained more weight that did their sham operated controls by the 90th post-operative day despite being fed on the same diet. Both groups were fed *ad libitum* but spayed females tended to eat more than their entire counterparts.

It is known that neutering decreases physical activity in both males and females (Lewis, 1978). Less roaming decreases the animal's dietary energy needs but usually there is no compensatory decrease in food intake and obesity is then likely to occur (Andersen and Lewis, 1980). On the other hand, oestrogen and testosterone reduce appetite while increasing energy expenditure and therefore, following ovariohysterectomy or castration, food intake and the efficiency of energy utilization often increase (Houpt and others, 1979; Houpt, 1982; Branam, 1988). In one study, it was found that the resting energy expenditure was reduced by 4% in a group of spayed female dogs (Anantharam-Barr, 1990).

Neutering probably predisposes to obesity in cats. It has been shown to reduce roaming and therefore physical activity, and in one study male cats showed an increased efficiency of energy utilization after castration which led to a higher weight gain when compared to the control group (Wills, 1988). Megestrol acetate, commonly used for the control of oestrus in dogs and cats, in high doses was found to be associated with greater fat deposition in a group of kittens when compared to controls (Chen and Bellenger, 1987).

Nevertheless, the relationship between neutering and obesity in pets has not been widely investigated and it is not clear whether an increase in food intake, a decrease in activity, or both are responsible for the gain in weight (Leonard, 1971; Houpt, 1982).

2.4. THE DETRIMENTAL EFFECTS OF OBESITY.

The health risks of obesity have been well established in humans. One of the worst risks is diabetes mellitus. It has been estimated that 90% of cases of diabetes mellitus in people are obesity induced (Friedman, 1980). Coronary heart disease, hypertension, cirrhosis, appendicitis, gallstones, cardiorespiratory dysfunction and osteoarthritis all form part of the long list of health risks that can affect the obese person (Mayer, 1980; Van Itallie, 1980; Cohen, 1985; Pi-Sunyer, 1991).

Recently, attention has been focussed on the regional distribution of fat in obese people as this appears to have a direct bearing on the pathological consequences of obesity (Baumgartner, Roche, Chumlea, Siervogel and Glueck, 1987; Després, Moorjani, Lupien, Tremblay, Nadeau and Bouchard, 1990). The android or centripetal accumulation of body fat, primarily associated with males, has been shown to increase the risk of hyperinsulinaemia, insulin resistance, hyperglycaemia, hypertriglyceridaemia, hypercholesterolaemia and hypertension that can accompany obesity (Campaigne, 1990).

The consequences of obesity in dogs and cats are not so well defined and in any case they seem to differ considerably from those seen in humans. The obese pet is suspected to be at an increased risk of a variety of diseases but confirmation from trials using objective measurements of fat content is lacking (Markwell, 1988b). The obese pet is considered by many people to be less healthy in appearance and there is a decrease in both the length and the quality of the animal's life (Joshua, 1970; Branam, 1988).

The following deleterious effects have been associated with obesity in dogs and cats:

DIABETES MELLITUS.

It has been said that 10% of obese dogs are diabetic and that 60% of dogs suffering from diabetes mellitus are obese (Dumon, 1988). In their study of over 10,000 canine subjects, Krook and others (1960) found that there was

a strong relationship between obesity and diabetes mellitus. Obesity tended to occur at an earlier age than did diabetes mellitus. Mattheeuws and others (1984a,b) have shown that, as is the case with humans, obesity in dogs is associated with hyperinsulinaemia and glucose intolerance. The greater the degree of obesity and the longer its duration, the more severe the hyperinsulinaemia and the glucose intolerance in the affected dogs. In one study of 13 cats suffering from diabetes mellitus, it was found that 10 were obese, and obesity was the most frequent clinical finding accompanying the usual signs of diabetes mellitus (Moise and Reimers, 1983). Recently, Nelson, Himsel, Feldman and Bottoms (1990) have shown that obese cats have an impaired glucose tolerance and altered insulin response to glucose infusion.

The need to secrete an excessive amount of insulin to maintain normal glucose levels in the obese is partly associated to insulin resistance in peripheral tissues. Various possible mechanisms have been proposed to explain this abnormality in humans and laboratory animals. A primary metabolic defect in liver, muscle or adipose tissue and/or β-cell hypersecretion have been implicated in the pathogenesis of insulin-resistant obesity (Nestel and Goldrick, 1976; Elahi and others, 1982). The metabolic defect in peripheral organs might be explained by the reduced numbers of cellular insulin receptors found in the obese (Soll, Kahn, Neville and Roth, 1975). As obesity worsens, a decrease in glucose transport activity would accompany the reduced number of insulin receptors (Ciaraldi, Kolterman and Olefsky, 1981; Garvey, Maianu, Huecksteadt, Birnbaum, Molina and Ciaraldi, 1991). Insulin resistance in obese sheep is associated with a decreased number

of insulin receptors in peripheral tissues with little or no change in insulin post-receptor effects (Bergman and others, 1989).

It has also been postulated that the high levels of circulating free fatty acids associated with a greater adipose mass would play an important role in the hyperinsulinaemia of obesity. The increase in lipid fuel availability/oxidation in obesity would displace glucose for energy utilization leading to hepatic insulin resistance (Bevilacqua, Bonadonna, Buzzigoli, Boni, Ciociaro, Maccari, Giorico and Ferrannini, 1987) or to impaired glucose disposal in skeletal muscle (Sugden and Holness, 1990). In this line, Landsberg (1990) considers that insulin resistance might be a compensatory mechanism to try and limit further weight gain and re-establish energy balance in the obese.

ARTICULAR AND LOCOMOTOR PROBLEMS.

Obesity is often an unappreciated cause of osteoarthritis. The increase in weight imposes an abnormal stress and trauma on otherwise normal joint surfaces, thus creating the perfect microenvironment for the development of degenerative arthritis (Staff Report, 1965; Joshua, 1970). Joint injury is accompanied by pain, leading to immobility and thus reduced energy expenditure, which in turn aggravates the obesity (Edney, 1974).

In their survey of over 8,000 dogs, Edney and Smith (1986) were unable to find an association between obesity and locomotor problems. Only in gross animals was there a tendency towards articular and locomotor troubles.

CIRCULATORY PROBLEMS.

Cardiac functions are impaired and abnormal ECG patterns can be seen in overweight dogs (Staff Report, 1965). Fat is a relatively avascular tissue but it requires perfusion, and this will necessitate an increase in both circulating blood volume and cardiac workload. This increased cardiac workload is imposed on a heart that could already be weakened by fatty infiltration (Edney, 1974; Clutton, 1988).

The cardiorespiratory syndrome of obesity has been well documented in man (Cherniack, Zwillich, Macklem, Kryger and Olson, 1986) and some of the detrimental effects of obesity in the cardiovascular system of humans are suspected to affect the obese dog (Hamlin and Buffington, 1989). Weight gain has been clearly associated with elevations in blood pressure, heart rate, plasma volume and cardiac output in the dog (Rocchini, Moorehead, Wentz and Deremer, 1987; Wehberg, West, Kieswetter and Granger, 1990).

DIGESTIVE DISORDERS.

Flatulence, indigestion or constipation are said to be common in the obese, probably due to fat deposition in the mesentery, omentum, liver, pancreas and intestinal wall (Staff Report, 1965; Andersen and Lewis, 1980).

Hepatic lipidosis has been reported to occur in obese dogs (Lewis, 1978; Brown, 1987) impairing normal liver function. Barsanti, Jones, Spano and Taylor (1977), described this condition in three cats and thought that obesity could have been implicated in the pathogenesis.

RESPIRATORY DISTRESS.

Fat dogs can often show respiratory embarrassment. This is due to the fact that excessive adipose tissue in the intrathoracic and intraperitoneal areas will reduce the lung volume to some degree. Moreover, inspiratory muscles may be weakened by poor physical condition and fatty infiltration. This results in decreased endurance, fatigue and dyspnoea (Joshua, 1970; Lewis, 1978; Stogdale and Moore, 1980; Ward, 1984; Clutton, 1988).

INCREASED SURGICAL RISK.

The obese patient has to be considered at a particular surgical risk. Excessive fat will influence drug disposition in the body. Repeated injections, infusions or continuous administration of anaesthetic drugs (as in inhalation anaesthesia) are more hazardous in the obese patient due to the high lipid solubility of these agents (Clutton, 1988). Depth of anaesthesia may thus be exceedingly difficult to assess and dangerously prolonged. Moreover, the detoxification of other anaesthetic agents may be seriously impaired due to fat-induced changes in the heart, liver, kidneys and pancreas (Staff Report, 1965; Clutton, 1988). The cardiac reserve is reduced predisposing to cardiac arrest during general anaesthesia. Hepatomegaly, resulting from fatty infiltration, may also limit the functional residual capacity of the lungs by limiting diaphragmatic excursion, thus exacerbating hypoxaemia (Clutton, 1988).

Surgical access in fat patients is far more difficult than in animals of normal physique because of large amounts of adipose tissue obscuring the surgical field, and surgery is further complicated by the fact that adipose tissue, being relatively avascular, tends to undergo necrosis as a result of surgical trauma and produces an ideal medium for bacterial multiplication (Joshua, 1970; Edney, 1974).

INTERFERENCE WITH DIAGNOSTIC PROCEDURES.

Diagnosis in the overweight patient is greatly hampered. Abdominal palpation and auscultation are often difficult and are virtually impossible in the grossly obese animal (Joshua, 1970; Lewis and others, 1987). Heart and lung sounds may be obscured by the excess fat overlying the rib cage. Clinical exploration of abdominal organs is also difficult due to deposition of thick layers of fat between muscles of the abdominal wall.

HEAT INTOLERANCE.

The insulating properties of excess subcutaneous fat are said to cause heat intolerance in fat patients. When the hot days of the summer arrive, these animals are more prone to suffer acute distress due to the impossibility to dissipate the excess heat (Edney, 1974; Andersen and Lewis, 1980).

DERMATOLOGICAL PROBLEMS.

The overweight animal is thought to be more prone to various dermatoses. The skin folds resulting from rolls of subcutaneous fat are

potentially a good medium for bacterial growth (Staff Report, 1965), and the heat intolerance produced by the excess of subcutaneous fat could also have a detrimental influence on the skin (Lewis, 1978). There is one report of an 8-years-old Fox Terrier spayed bitch which had a chronic dermatitis that virtually disappeared after weight reduction (Stogdale and Moore, 1980). However, in a previously quoted survey (Edney and Smith, 1986), there was no clear relationship between skin problems and obesity in dogs.

DECREASED RESISTANCE TO INFECTIOUS DISEASES.

It has been demonstrated that genetically obese laboratory animals show a significant derangement in cell-mediated immunity and a higher incidence of infectious diseases and infection-related mortality (Chandra, 1980; Conge, Gouache, Joyeux, Goichot and Fournier, 1988). The mechanism(s) underlying this altered immunity is unknown.

Williams and Newberne (1971), infected three groups of Beagles with a *Salmonella* inoculum. They found that the obese group was the most severely affected when compared to the normal and thin groups. In a previous study, it was shown that obese dogs were more susceptible than their lean countermates to infection with canine Distemper virus (Newberne, 1966). A similar conclusion was obtained by Fiser, Rollins and Beisel (1972), who found that clinical illness after viral infection was most severe and fulminant in dogs fed a high-fat ration than in dogs fed a normal ration.

Decreased immunocompetence could have serious consequences in aged, obese animals that are exposed to virulent organisms during times of stress, boarding or hospitalization (Branam, 1988).

REPRODUCTIVE PROBLEMS.

Disruption of the reproductive ability of women suffering from an excess body fat has been clearly established (Frish, 1987). Some obstetrical conditions have been associated with the overweight dog, such as increased dystocia (Lewis, 1978), pyometra (Krook and others, 1960), and decreased reproductive efficiency (Brown, 1987). Edney and Smith (1986) did not find a clear relationship between obesity rating and reproductive problems.

NEOPLASIA.

Neoplasia has been said to be more common in overweight dogs when compared with those of optimum body weight (Lewis and others, 1987), but this remains to be proven. In one study it was found that being underweight at 1 year of age was strongly protective for subsequently developing mammary tumours in dogs (Sonnenschein, Glickman, McKee and Goldschmidt, 1987) and in a further epidemiological survey obese dogs had a significantly higher risk of developing bladder cancer when exposed to topical insecticidal preparations than did lean animals (Glickman, Schofer, McKee, Reif and Goldschmidt, 1989).

It is possible that nutritional status may modulate hormonal levels in such a way that the subsequent risk of developing mammary tumours is

modified. On the other hand, it is also possible that adipose tissue acts as a storage depot for insecticides, thus prolonging their carcinogenic activity. However, these are only preliminary studies and more research is clearly needed to establish if obesity, or the metabolic changes associated with it, presupposes an increased risk for developing cancer. It would be unsafe to conclude from these two studies that there is a clear link between obesity and neoplasia in dogs.

INCREASED RISK OF FELINE UROLITHIASIS SYNDROME.

Over 400 clinical cases of feline urolithiasis syndrome (FUS) were studied by Walker, Weaver, Anderson, Crighton, Fennell, Gaskell and Wilkinson (1977). They found that the proportion of overweight cats (one-third) was unexpectedly high, and that a history of previous FUS was commoner in overweight and lazier cats than in cats of normal physique.

2.5. QUANTIFYING OBESITY.

2.5.1. IN PEOPLE.

Obesity in man is a well-known concept but it is not readily defined satisfactorily and the measurement of body fat is not an easy task (Cohen, 1985). There have been numerous approaches to define what constitutes obesity and to evaluate the amount of fat present in the body.

Some of these approaches are:

Weight and height tables.

A number of physical indices have been used for the measurement of obesity. In general, these obesity indices represent different combinations of weight and height, such as weight divided by height, height divided by the cube root of weight, weight expressed as a percentage of the mean weight for a given height and sex, etc. (Colliver, Frank and Frank, 1983). For many years obesity has been defined according to "relative weights", which are a percentage of the normal or ideal weight of a person of the same height, sex and possibly frame size. Relative weights are based on data collected by life insurance companies and this represents a biassed sample. People who undergo a life insurance medical examination are self-selected and are probably not representative of the general population (Garrow, 1983b; Pi-Sunyer, 1988).

The validity of these obesity indices has been repeatedly questioned because they do not differentiate between those who are muscular and those who are obese, and are highly dependent on age (Brožek and Keys, 1950; Mayer, 1980; Cohen, 1985; Geissler and Miller, 1985; Fung, Lee, Lau, Chow, Wong and Davis, 1990). Furthermore, it has been said that no weight-height index can perfectly define adiposity since two people of the same weight and height can actually differ in fatness (Garrow, 1983b).

One of the most successful indices is the "Quetelet Index". This was first proposed by a Belgian astronomer (Quetelet) and is also known as the Body Mass Index (BMI). It is based on the observation that among adults of normal build but different height, weight is roughly proportional to height square (Garrow, 1983b). Although this index (wt/ht^2) has been extensively used and is considered to be a reliable indicator of obesity in most individuals (Garrow, 1987, 1990), some limitations on its use have been found (Garn, Leonard and Hawthorne, 1986; MacDonald, 1986). The main disadvantages being that it does not distinguish between overweight due to obesity and due to marked muscular development, and does not take into account differences in body composition and frame size (Garrow, 1983b; McLaren and Meguid, 1988).

Skinfold thickness.

A considerable proportion of the body fat lies in the subcutaneous tissue and is relatively easy to pull it up between the thumb and forefinger

into a fold. The thickness of this fold of skin and subcutaneous tissue can be measured by applying some sort of calliper to either side of it, and has been widely used to give an estimate of body fat content in man. Several sets of tables exist that relate skinfold thickness (or a transform of the sum of multiple measurements at different sites) to body fat measured by another method such as density (Edwards, Hammond, Healy, Tanner and Whitehouse, 1955; Sloan, 1967; Durnin and Womersley, 1974; Womersley and Durnin, 1977; Must, Dallal and Dietz, 1991). Formulas combining skinfold measurements with surface area have been proposed as a valid universal estimate of adiposity (Katch, Benhke and Katch, 1979).

The technique has many attractive features: it is simple, relatively quick and inexpensive and data can be collected on large numbers of subjects. However, a number of limitations have been associated with the calliper method which may result in inaccurate estimates of subcutaneous fat thickness and, consequently, of total body fat (Fanelli and Kuczmarski, 1984; Scherf, Franklin, Lucas, Stevenson and Rubenfire, 1986). These limitations have been reviewed by Kuczmarski, Fanelli and Koch (1987), and include:

* inability to control inter and intrasubject variation in skinfold compressibility.
* the fat-muscle interface can not always be palpated.
* the degree of compression involved with skinfold measurements varies with the location of the body and the calliper design.
* impossibility of obtaining interpretable measurements on very obese subjects.

Chemical analysis.

Total body chemical analysis on human cadavers is both aesthetically and technically very demanding and the very few studies reported in the literature have been reviewed elsewhere (Garrow, 1983b). A fact emerging from these studies was that the fat-free body was of a roughly constant composition, although the amount of fat was remarkably variable. In the analysis of the six adult human cadavers reviewed by Garrow, fat content varied between 4.3% to 27.9%. The fat-free body contained about 73% water and approximately 20% protein.

Body density.

For this technique and for the measurement of body water and body potassium (see below), the assumption is made that the body of a person consists of fat (with a density of 0.90 g/ml) and fat-free tissue (with a density of 1.10 g/ml). Thus, if the average density of the body is measured, the ratio of fat to fat-free mass can be calculated (Garrow, 1987).

The practical difficulty is to obtain an accurate measurement of the volume of the subject in order to calculate his/her body density. This is usually done by underwater weighing. The volume can be calculated from the weight of water displaced by the subject when completely submerged in a water tank, having measured the residual air in the lungs (Garrow, Stalley, Dietholm, Pittet, Hesp and Hallidey, 1979). Another possibility for calculating the subject's volume is to measure the displacement of air by the body in an air-tight chamber of known volume (Tanner, 1959). Both techniques are

prone to a number of errors and are rather complex (Garrow, 1983b). They require a high degree of training and cooperation on the part of the subject, and estimating the amount of air in the intestines, which will also displace water, can prove very difficult. Moreover, errors in the measurement of body density can be introduced if there is no food and beverage restriction prior to carrying out the procedure, because these can affect body weight and increase intestinal gas (Thomas, Crough and Araujo, 1988).

Body water.

For this technique, the assumption is made that the fat-free mass compartment of the body contains approximately an average of 73% of water and the other body compartment, fat, contains no water. If the total body water of the subject is measured, then the proportions of fat and fat-free mass can be calculated (Garrow, 1987).

Total body water may be measured by administration (orally or by injection) of a known dose of water labelled with either deuterium (^{2}H), tritium (^{3}H), or the stable isotope of oxygen (^{18}O). After allowing some 3 to 4 hours for the dose to reach an isotopic equilibrium in all body fluids, a sample is taken for measurement of the dilution (Garrow, 1983b). Tritium is easily measured by scintillation counting but involves radiation to the subject. For the other stable isotopes a very careful preparation of the dose to be administered is mandatory. The sample must be accurately weighed and any contamination from atmospheric water avoided.

However, the adipose tissue of the body is said to contain 15% of water. Therefore the greater the adipose tissue mass a person has, the higher the estimated water content of fat-free tissue (Garrow, 1987). This factor must be taken into consideration in extremely obese people.

Body potassium.

The potassium content of fat-free mass is approximately 60 mmol/kg in women and 66 mmol/kg in men. Fat contains no potassium, so if the total body potassium is measured then the ratio of fat to fat-free mass can be calculated (Garrow, 1987). The potassium content of the body can be measured either by detecting the radiation of the isotope ^{40}K coming from the human body (Forbes, Gallup and Hursch, 1961), or by administering to the subject a dose of the radioactive isotope ^{42}K, which has a radiation similar to that of ^{40}K. Both techniques require expensive equipment.

The assumption is made that the potassium content in fat-free tissue remains constant, but this has not been clearly established yet (Garrow, 1983b).

Electrical conductivity and bioelectrical impedance analysis (BIA).

This method is based on the principle that the electrical conductivity of lean tissue is far greater than that of fat, due to the much higher electrolyte content of lean tissue. The subject is introduced in a selenoidal coil through which a current is passed, thus creating an electromagnetic field. This induces

a current in the subject and a second electromagnetic field. The change in the coil impedance when the coil is empty and when the subject is in it, is proportional to the total electrical conductivity of the body, which in turn, is proportional to fat-free mass (Presta, Wang, Harrison, Björntorp, Harker and Van Itallie, 1983). While the accuracy of this technique has not yet been defined, it could prove a useful and simple method of assessing body condition in human beings (Presta and others, 1983).

Electrical conductivity has also been used to measure the depth of the subcutaneous fat layer. This is based on the different electrical resistance offered by muscle and fat, which permits the detection of the interface between these two layers. Two wires have to be introduced through the skin and subcutaneous tissues under local anaesthesia (Booth, Goddard and Paton, 1966).

Bioelectrical impedance analysis (BIA) is based in the same principle, i.e., the low conductivity of adipose tissue. By attaching four electrodes to the skin of the patient, a current is produced and impedance measurements taken (Kushner and Schoeller, 1986). Since the impedance of the body is mostly determined by the low-impedance lean component and this is mainly composed of water, it is possible to relate these measurements to total body water and hence to the fat-free mass (Kushner and Schoeller, 1986). This method has been found to be highly correlated to the measurement of body composition using deuterium oxide dilution techniques (Kushner and

Schoeller, 1986; Kushner, Kunigk, Alspaugh, Andronis, Leitch and Schoeller, 1990).

Soft-tissue roentgenogram.

The shadow of the skin and the subcutaneous tissue can be readily distinguished from that of the underlying muscle on a suitably exposed X-ray film. This permits the measurement of subcutaneous fat thickness for evaluation of body composition (Tanner, 1959).

The great problem with this technique is that it can only be used over a limited number of relatively safe sites because it involves undesirable radiation exposure to the subject (Haymes, Lundegren, Loomis and Buskirk, 1976). High cost is also a drawback.

Computed tomography (CT).

Computed tomography scans are thin cross-sectional, radiographic images of the body. The technique is very sensitive to slight differences in attenuation and therefore depicts the soft tissues with great clarity. The scans are computed reconstructions of thousands of separate determinations of attenuation and the data can be analyzed using special software (Borkan, Gerzof, Robbins, Hults, Silbert and Silbert, 1982).

The technique has been successfully utilized to measure both internal and subcutaneous fat in men and women (Borkan and others, 1982a; Ashwell, Cole and Dixon, 1985) and therefore has potential for evaluating body

condition. However, the cost of the equipment and the risk of radiation exposure constitute limitations on its use.

Neutron Activation Analysis (NAA).

This technique relies on the ability to change stable isotopes of body elements into radioactive ones (Foster and Fowler, 1988). This is achieved by directing neutrons at the body, some of which will be captured by nuclei. These nuclei become unstable and will need to release energy and/or particles in order to regain their stable state. These emissions will vary depending on the type of nucleus affected by the neutron, and can be determined by adjusting the energy of the incident neutrons. Hence, the type and amount of radioactivity emitted will give information on the elemental composition of the absorbing body.

Although the technique has successfully been used in humans (Cohn, Vaswani, Vartsky, Yasumura, Sawitsky, Gartenhaus and Ellis, 1982), sophisticated and expensive equipment is needed for its use (Foster and Fowler, 1988).

Dual-Photon Absorptiometry (DPA).

This method is based on the differential attenuation by body tissues of transmitted photons at two energy levels (Heymsfield, Wang, Heshka, Kehayias and Pierson, 1989). By measuring the attenuated photon intensities and applying two algebraic equations, it is possible to calculate the bone mineral and soft-tissue mass of the body (Peppler and Mazess, 1981). In turn,

the soft-tissue compartment can be divided into fat and fat-free mass by applying a phantom-derived calibration equation (Peppler and Mazess, 1981; Heymsfield and others, 1989). The technique has proved useful in determining fat content in 13 healthy adults (Heymsfield and others, 1989) and in three obese women undergoing semistarvation (Koyama, Nishizawa, Yamashita, Furumitsu, Hagiwara, Ochi and Morii, 1990).

Magnetic Resonance Imaging (MRI).

MRI has been recently developed in medical imaging primarily for the detection of abnormalities such as cancer and various myopathies (Egerter, 1989). This technique works by observing the property of spin exhibited by certain nuclei (usually those of H). Spin gives the nucleus a magnetic moment. When an external magnetic field is applied, the nuclei will align with it at a frequency proportional to the magnetic field. Both parallel and anti-parallel alignment is possible, with an energy difference between them; it is precisely this energy difference that is used for producing the MRI signal.

MRI imagers are expensive and are unlikely to be acquired for body composition studies alone. However, they are giving encouraging results. It has been possible to accurately measure subcutaneous and total abdominal fat in people and these measurements were well correlated with percentage body fat obtained by hydrostatic weighing (Staten, Totty and Kohrt, 1989). Recently, Fuller, Fowler, McNeill and Foster (1990), reported a very high

correlation between MRI and the average of six other methods used simultaneously for the estimation of total body fat in human subjects.

Fat soluble gases.

Some gases such as xenon, krypton and cyclopropane are much more soluble in fat than in water. If the subject is placed in an atmosphere containing a known concentration of one of these gases, it should be possible to calculate his/her body fat content by observing the amount of gas taken up (Lesser, Deutsch and Marfofsky, 1971).

Besides other problems, this method has a very limited use for routine data collection, owing to the fact that the equilibration time is too long (Garrow, 1983b).

Metabolic parameters.

By measuring some metabolic variables one can calculate the total mass of the active body tissues. Among these markers are included maximal oxygen consumption and 24-hour urinary excretion of 3-methylhistidine and creatinine.

Oxygen consumption is related to the muscle mass of the subject but its use as an index of body composition is probably limited by the large variability in aerobic fitness among the population (Mendez, Lukaski and Buskirk, 1984).

Creatinine and 3-methylhistidine are both derived from the catabolic processes of muscle and are excreted quantitatively by the kidney. From these two, 3-methylhistidine secretion is believed to be a better predictor of fat-free mass and thus, of body composition (Mendez and others, 1984). A major practical difficulty in all of these techniques is the need of collecting a 24-hour urine sample and of the patient abstaining from eating any meat for the previous 48 hours.

Ultrasound.

The principle behind this technique is the same as the one used for the skinfold calliper method, i.e., body condition is assessed by actually measuring subcutaneous fat thickness at multiple anatomical sites and then relating these measurements to total fatness as estimated with some other technique.

Although some authors have not found ultrasound superior to the skinfold calliper technique (Borkan, Hults, Cardarelli and Burrows, 1982; Chumlea and Roche, 1986), the fact is that thickness of the subcutaneous fat layer has been successfully measured in humans by means of both A-mode ultrasound (Bullen, Quaade, Olesen and Lund, 1965; Booth and others, 1966; Haymes and others, 1976; Balta, Ward and Tomkins, 1981; Volz and Ostrove, 1984) and B-mode ultrasound (Fanelli and Kuczmarski, 1984; Weits, Van der Beek and Wedel, 1986; Kuczmarski and others, 1987).

This method offers various advantages over other techniques for the assessment of obesity:

a) the technique is completely safe and non-invasive, it does not involve any undesirable radiation and the skin is not damaged in the process (Haymes and others, 1976; Payne, 1985).
b) it overcomes some of the limitations of the skinfold calliper, such as variability in compression and positioning of the calliper tips, impossibility of palpating the fat-muscle interface in very obese individuals, and changes in skin and fat tissue elasticity (Kuczmarski and others, 1987).
c) it is a very sensitive technique. Ultrasound scans can reliably detect density interfaces with an accuracy of 1mm (Fanelli and Kuczmarski, 1984).
d) the technique can be relatively simple and quick and there are now units in the market sufficiently portable for use in survey and clinical settings (Kuczmarski and others, 1987).

However, ultrasound is not free from drawbacks. A certain degree of experience is necessary for interpretation of the data and of the anatomical topography under study especially when using A-mode units. Standardization of methodologies is needed and the cost of equipment is high and is a further consideration.

2.5.2. IN LARGE ANIMALS.

Except in horses, interest in the conformation of farm animals has been based upon the belief that the appearance of the exterior of the animal is closely correlated with the carcass and its quality. Conformation gives an external indication of the desirability of the carcass and its meat (Barton, 1967). Body condition is also of interest because of its relationship with reproductive performance, both in livestock animals and in horses (Henneke and others, 1983).

Various methods have been used for assessing body condition in large animals:

Condition scoring.

This is the most simple and widely adopted way of determining the animal's conformation. It involves giving a score based on visual appraisal, usually on a scale from 1 to 10, which takes into account the whole animal (Barton, 1967). The technique has been developed for use in horses. A condition score system using a scale of 1 to 9 (with 1 being extremely emaciated and 9 being extremely fat), was developed by Henneke and others (1983) for comparison of horses with differing amounts of body fat. Their scoring system was based on visual appraisal and palpable fat cover at six areas of the horse's body. The system was applied to 32 mares of different body condition and although the emphasis on each of the six body areas

varied according to the individual and the stage in its reproductive cycle, it was found that the condition score correlated well with percentage body fat as estimated with ultrasound.

Carroll and Huntington (1988), utilized a body condition scoring system in horses based on a scale from 0 to 5, taking into account the deposition of fat in different areas by examining the neck, back, ribs, pelvis and rump of the animal. They found that body weight correlated with condition score in 372 horses and ponies of different body size and fat content. However, correlation between body weight and girth^2 x length was much better than between the former and condition score.

Physical measurements.

Measuring rods and tapes of various kinds have been used to calculate appropriate dimensions which would allow prediction of body condition (Barton, 1967).

Using different combinations of weight, height, girth and length both in cattle (Gresham, Holloway, Butts and McCurley, 1986) and in horses (Henneke and others, 1983; Carroll and Huntington, 1988), estimates of carcass composition, body weight and body fat percentage have been made in the live animal. The accuracy of these measurements is probably greater if the measuring points can be precisely determined than when the points are vaguely located at a site overlaid with soft tissues. Thus, height and circumference measurements will yield more accurate results than most width

measurements in which prominent bony reference points are more difficult to find (Barton, 1967).

Direct probing.

Previous methods of determining backfat thickness on live pigs involved introducing a steel ruler or a "lean-meter" (which measures the change in resistance as the probe goes from fat to muscle) through the skin and subcutaneous tissue of the animal (Hazel and Kline, 1952; East, Taylor, Miller and Widdowson, 1959). These methods were not satisfactory because the pig is hurt and is difficult to manage, and therefore only a limited number of measurements could be obtained from each pig (East and others, 1959; Giles and others, 1981).

Skinfold callipers.

The determination of skinfold thickness at various body sites in cattle, has been attempted as an indicator of body condition with varying degrees of success. While in two studies it was found that skinfold thickness over the 11th and 13th ribs respectively was a poor predictor of total fatness, a further study found a strong relationship between anal fold thickness and fat percentage in the carcass (reviewed by Simm, 1983).

Body water.

The technique is very similar to the one used in humans. It involves injecting a known amount of a substance, which meets the qualifications of a

biological tracer, into the body. Total body water is estimated from dilution of the tracer after it has equilibrated with body water. Water labelled with the isotopes deuterium or tritium has proven to be a good biological tracer (Arnold, Hentges and Trenkle, 1985). Consequently, deuterium oxide and tritiated water have been used to estimate body water and thus body composition in pigs (Ferrell and Cornelius, 1984; Rudolph, Stahly and Cromwell, 1988), sheep (Panaretto, 1963; Searle, 1970), goats (Panaretto, 1963; Dunshea, Bell, Chandler and Trigg, 1988) and cattle (Arnold and others, 1985). The technique was successfully used for determining body water in racing pigeons (Mulligan, MacLean and Preston, 1990).

Body water has also been estimated using urea as a biological marker. Bartle, Kock, Preston, Wheeler and Davis (1987) showed that the urea dilution technique was a valid estimator of in vivo composition in growing finishing cattle.

Body potassium.

Total body potassium has been used to estimate fat-free mass and other body components in the live animal. By injecting a very small amount of radioactive isotopes of potassium, it has been possible to predict body composition in sheep (Domingo, Trigg and Topps, 1973) and pigs (Domermuth, Veum, Alexander, Hedrick, Clarl and Eklund, 1976).

Electrical conductivity.

As is the case with humans, the various body constituents of livestock, primarily muscle and fat, show differences in electrical conductivity. Fat has a much lower electrical conductivity than muscle due to the different electrolyte content. By introducing the animal in an electromagnetic field, the change in current induced by the animal's body can be measured, and this measurement can be used to calculate the ratio between fat and muscle content.

This technique has been successfully employed to predict body composition in live pigs (Domermuth and others, 1976).

Radiographic technique.

X-rays have been used in live animals to estimate the weight and proportion of bone, but with little success (Barton, 1967).

Somewhat more fruitful has been the use of this method for determining backfat thickness in pigs. Observations are usually taken on the animal held in a sling with feet protruding, and the X-rays are emitted perpendicular to the dorsal line of the swine. The technique is somewhat difficult and not infrequently there is poor definition of the lower limit of fat (Stouffer, 1963). Furthermore, it is considered to be an expensive and slow method for determination of fat thickness (East and others, 1959).

Ultrasound.

Since the 1950s, ultrasonic techniques have been used to evaluate the carcass composition of live cattle (Simm, 1983).

A continuous scanning technique producing a cross-sectional picture, was utilized by Stouffer (1963) to measure the fat thickness of cattle between the 12th and 13th ribs, over the *longissimus dorsi* muscle. Although repeatability of these studies was found to be very good, the comparison of ultrasonic estimates on the live animal with similar measurements obtained directly from the carcass, was somewhat disappointing. The author suggested these differences could be due to the major changes introduced during the slaughtering operation.

The rate of fat deposition and *longissimus* muscle growth was evaluated in cattle, using an A-mode ultrasonographic unit, by McReynolds and Arthaud (1970). The ultrasonic estimates were in general highly correlated with actual carcass measurements. Kempster and Owen (1981) examined the relationship between the ultrasonically measured fat areas and actual subcutaneous fat percentage obtained by dissection in a group of 313 cattle. Using a modified linear scanner (Scanogram, Ithaca, New York), fat areas were measured over the muscle *longissimus thoracis* at the 10th and 13th ribs. Within the context of the trial, the accuracy of the technique was considered to be satisfactory by the authors. In a later study, Kempster, Cuthbertson, Jones and Owen (1981) carried out a series of trials to determine the precision of two different ultrasonic machines for predicting the body composition of live cattle. Fat

thickness measurements and the areas of fat and *longissimus thoracis* and *lumborum* were taken at the 10th and 13th ribs and at the position of the third lumbar vertebra. A better prediction was achieved by the Scanogram (modified linear scanner) when compared to the Sonatest (simple A-mode ultrasonic machine). Both techniques were able to identify differences in carcass composition among cattle of the same breed, sex and live weight. However, even within these well defined circumstances, there was considerable unexplained variation in the prediction of carcass composition.

Alliston and Hinks (1981) used the same three body sites to scan 45 crossbred cattle, using a B-mode ultrasonographic unit (Danscanner, Medico Technical Institute, Copenhagen). Before slaughter and subsequent carcass dissection, the animals were "condition scored" according to external fat development. The authors found that ultrasonic measurements gave useful predictions of carcass fat and were more accurate than the condition scoring system.

Ultrasound has also been used for the measurement of backfat thickness in live pigs. An early report of this technique came from East and others (1959), who described the use of a simple A-mode device on 77 live pigs. Although backfat thickness as assessed by ultrasound showed certain variability when compared to the direct measurement of fat on the carcass, the authors thought that the accuracy of the technique would be greatly enhanced if repeated measurements on a large number of days were taken. More encouraging results were obtained by Price, Pearson, Pfost and Deans

(1960). They measured backfat thickness and depth of lean tissues on a total of 158 hogs using a similar ultrasonographic unit. Fat thickness measured ultrasonically was a good predictor of carcass composition and was highly correlated to both the live probe technique and actual carcass measurement. More recently, Giles and others (1981) compared the use of three different ultrasonic machines for estimating backfat thickness. The three devices (Sonatest, Scanoprobe, and Scanogram) all proved to be highly correlated to the measurement of backfat thickness as assessed by the ruler probe technique and calliper measurement of the carcass fat.

The technique has also been tried in sheep. Although an ultrasound machine was successfully utilized by Bennet, Meyer and Kirton (1988) to select rams with high and low fat depth, other reports have questioned the use of ultrasound for predicting the body composition of live sheep (Kempster, Arnall, Alliston and Barker, 1982; Leymaster, Mersmann and Jenkins, 1985).

Finally, the usefulness of ultrasound for measuring fat depth has been documented in horses. Westervelt, Stouffer, Hintz and Schryver (1976) carried out a series of experiments in horses and ponies and showed that there was a high correlation of ultrasonic measurements to actual fat cover (as measured in the fresh carcass). Furthermore, their ultrasonic data were found to be useful in the prediction of total body fat as determined by total chemical analysis. More recently Henneke and others (1983) also used the ultrasound technique to calculate percent body fat in 32 mares of variable body

condition. Ultrasonic scans of subcutaneous fat thickness were taken at a point half-way between the first sacral vertebra and the tailhead, 5 cm lateral to the spinous processes. These measurements were subsequently used to calculate per cent body fat using the equation developed by Westervelt and others (1976). A good correlation was found between percent body fat calculated in this way and a condition scoring system devised by the authors.

2.5.3. IN DOGS AND CATS.

The diagnosis of gross obesity in pets requires little clinical skill, but when the deposition of body fat is not so obvious, it can prove difficult to determine what constitutes obesity and what does not. Some of the criteria used in man to define obesity, such as standard weights and combinations of weight and height, are not possible to apply in dogs bearing in mind that there are over 100 breeds and a 35-fold weight variation across this range (Kirkwood, 1985). Standard weight tables for different breeds of dogs have been produced (Lewis and others, 1987; Rainbird, 1988) but they are not generally very helpful because body builds vary widely, even among dogs of the same breed and sex, and because they cannot be applied to mongrels which are just as prone to obesity as pure breeds (Joshua, 1970; Andersen and Lewis, 1980; Lewis and others, 1987; Hand, 1990). Similar tables do not exist for cats and the only available criteria for comparison is that a cat of common domestic breed weighing more than 5.5 kg is likely to be overweight (Lewis and others, 1987; Wills, 1988). Other parameters such as girth circumference

have been found to be of value for predicting body weight in a limited group of dogs (Pendergrass, Bartley, Nagy, Ream and Stuhlman, 1983) but this is not an easy method to apply due to the wide range in frame size, variability in coat thickness and difficulty in obtaining repeatable measurements (Anderson, 1973).

Skinfold thickness, a technique readily used in man, is thought to be of little value in dogs and cats because the skin in these animals is very easily lifted from the subcutaneous tissue (Anderson, 1973; Lewis and others, 1987).

Total body water as an estimator of body condition has been measured using tritiated water in growing Beagles (Sheng and Huggins, 1971; Sheng, Kamonsakpithak, Naiborhu, Chuntananukoon and Huggins, 1977). These authors found that there was generally poor agreement between calculated and measured water using this technique. However, it is possible that the differences might be due to errors being introduced in the complicated process of whole body desiccation that was undertaken to calculate total body water. On the other hand, the technique was successfully utilized by Romsos and others (1978) to estimate body water, body fat-free mass and body fat in 14 growing dogs.

In one study, ultrasound was used to measure subcutaneous fat thickness along the dorsum of 24 female Beagle dogs (Anderson and Corbin, 1982). The authors found that ultrasonic measurements taken on the live

animal were 30% to 40% smaller than measurements of subcutaneous fat thickness made directly on the carcass using a measuring tape. Carcass measurements of subcutaneous fat thickness were positively correlated with percent body fat calculated by total chemical analysis.

Other parameters such as body density or creatinine excretion could only be used in laboratory conditions and are somewhat difficult to apply in pets (Anderson, 1973).

Therefore, at present, the assessment of obesity in dogs and cats is based on the observation by eye and by palpation of the amount of adipose tissue overlying the thorax and abdomen. By this method a dog will be considered too thin if the ribs are easily seen, normal if they are more difficult to see but readily palpable, and fat if the ribs are not seen and a considerable layer of fat is palpable. A gross animal will be the one were the rib cage is impossible to be palpated because of the thick layer of adipose tissue overlying it. Although this is certainly a simple and practical way of diagnosing obesity, it must be considered to be largely subjective (Joshua, 1970; Mason, 1970; Anderson, 1973; Lewis, 1978; Andersen and Lewis, 1980; Markwell, 1988b; Clutton, 1988; Markwell, van Erk, Parkin, Sloth and Shantz-Christienson, 1990).

CHAPTER III.

OBJECTIVES.

CHAPTER III. OBJECTIVES.

In the preceding pages the problem of obesity has been reviewed under many different aspects: those concerning its prevalence in both people and pets, its aetiology and development, and the deleterious effects that accompany obesity or that obesity can cause.

However, the major concern of this thesis is related to the diagnosis of obesity in pets. The quantification of total body fat is important for an objective treatment and prevention of obesity in our companion animals. Moreover, such quantification would allow monitoring of their growth and a better understanding of the physiological changes that accompany it. Quantifying body fat in pets has been the object of scarce research and is presently based on subjective assessment. The work herewith presented was undertaken in an effort to investigate the possible use of a diagnostic tool, namely ultrasound, in the assessment of body fat in relation to obesity in pets.

The objectives were:

1. To determine if ultrasound was capable of accurately measuring subcutaneous fat thickness in dogs.

To achieve this objective, ultrasonic measurements of fat thickness at various anatomical locations were compared to measurements taken with a conventional histometric method at the same locations. Furthermore, the

skinfold calliper technique, widely used in humans, was also compared against the histometric method.

The results of this investigation are described in Chapter 5.

2. To determine if the ultrasonic technique could be used to predict total body fat in dogs.

Measurements of subcutaneous fat thickness obtained with ultrasound, skinfold callipers and the histometric method were used to predict fat content calculated by total body chemical analysis. Hence, it was possible to compare the relative merit of each of these three techniques for estimating fat content.

Results are presented in Chapter 6.

3. To develop a database of ultrasonic measurements of subcutaneous fat from a field situation: a referred population of dogs. This database would allow a comparison between the ultrasonic technique, a clinical scoring of obesity, some physical measurements such as weight, height, length and girth, and individual characteristics such as age, sex and breed.

Results are presented in Chapter 7.

CHAPTER IV.

PRINCIPLES OF ULTRASONOGRAPHIC IMAGING.

CHAPTER IV. PRINCIPLES OF ULTRASONOGRAPHIC IMAGING.

4.1. GENERAL PRINCIPLES.

Ultrasound is a form of acoustical (mechanical) energy where the frequency at which the vibrations occur is higher to the one detectable by the human ear (Ziskin, 1975).

Most audible sounds are in the frequency range of about 20 to 20,000 Hertz (Hz) although some animals can detect sounds of considerably higher frequencies (Ziskin, 1975). Bats, for example, have the ability of producing ultrasonic blips during their flights which help them navigate by listening to the returning echoes. The same physical principle was used for developing SONAR (Sound Navigation and Ranging) during the Second World War, for locating underwater objects such as submarines. After the war, techniques were developed for using ultrasound in medical diagnosis (Cameron and Skofronick, 1978).

Diagnostic ultrasound originates from crystals with the "piezoelectric" effect, discovered by Jacques and Marie Curie in about 1880 (Ziskin, 1975). The term "piezo" is derived from a Greek word meaning "pressure". When a high frequency electrical pulse is passed through the crystal, it will contract and expand at the pulse frequency, generating sound waves or vibration. Each crystal has a natural resonant frequency of vibration: the thinner the crystal,

the higher the frequency at which it will oscillate (Cameron and Skofronick, 1978). The vibrating crystal is placed in close contact with the skin and pulses of ultrasound are transmitted into the body. Water or a jelly paste are normally used as coupling agents because they enhance the transmission of the ultrasonic beam and the detection of returning echoes (Cameron and Skofronick, 1978).

Ultrasonic waves behave very much in the same way as audible sound: the emitted vibrations travel through the medium, be it gas, liquid or solid, at a constant speed until they encounter a reflecting surface. This causes some of the sound beam to be reflected back towards the source. The vibrations that the returning echoes produce on the crystal generate a voltage across it which can be amplified and stored. Thus the name "transducer" is given to ultrasound generators, because these are devices capable of converting electrical energy to mechanical energy and vice versa (Wells, 1981). Most transducers contain piezoelectric crystals such as quartz, lead zirconate titanate, lead metaniobate and barium titanate. Some polymer materials such as polyvinylidine fluoride (PVDF) have also been used (Payne, 1985).

In the case of diagnostic ultrasound, the vibrations produced by the transducer pass into the tissue under study and when a change in density is encountered, some of the energy is reflected back to the transducer. The reflected energy is reconverted into an electrical impulse and fed to a receiving device for amplification and recording. The delay between the formation of the wave and the reception of the echo, allows for the calculation

of the distance between the transducer and the reflecting surface (Cameron and Skofronick, 1978). In ultrasound scanners, this distance is recorded as a pixel or spike on a television screen or oscilloscope; the spike will be proportional to the distance travelled by the echo and will enable not only the measurement of distance but a visual picture of it as well.

To understand the medical applications of ultrasound, we need to examine some of the properties of this form of energy.

All sound, be it ultrasound or audible sound, is a mechanical disturbance in a gas, liquid or solid that travels outward from the source at a certain speed. The vibrations cause local increases (compressions) and decreases (rarefactions) in pressure, relative to atmospheric pressure (Cameron and Skofronick, 1978). These changes spread in the same direction the sound wave travels (Figure 4.1). Line A shows a single wave or cycle. As we move along the horizontal axis, representing time, the pressure starts at zero, rises to a peak and continues to a negative before returning to zero. Line B shows a continuous wave, made up of single waves strung together.

The characteristics of a sound wave are defined by a number of parameters (Figure 4.2). These are:

The *Amplitude* (A) is the height of the wave or peak pressure. It gives a measure of the strength (loudness) of the sound wave.

The *Period* (T) is the time it takes for one cycle of the wave to be completed.

The *Frequency* (f) is the number of times the wave is repeated per second. It can be calculated by dividing the period (T) into 1.

The *Velocity* (v) is the speed of the wave. It will vary according to the type of medium in which the wave is travelling. For example, sound travels at 330 m/sec in air but it is much faster in water (1480 m/sec) and bone (2700-4100 m/sec) (Wells, 1977).

The *Wavelength* (λ) is the distance travelled by the wave in one single cycle.

The relationship between the frequency of vibration (f) of the sound wave, its wavelength (λ) and velocity (v) is defined by the equation:

$$v = \lambda \times f$$

As mentioned above, most audible sound is in the frequency range of 20 to 20,000 Hz. A Hertz is equivalent to one cycle per second and a MegaHertz (MHz) is a million cycles per second. Hence, the frequency range above 20,000 Hz is called ultrasound. Typical frequencies used for visualizing internal organs in medicine are in the 1 to 10 MHz range (Taylor and Kremkau, 1985).

A further difference between audible and ultrasound is the wavelength. The wavelengths of audible sounds are proportionally far greater than those of ultrasound. This is also true for the amplitude or power of the wave, which is considerably greater in audible sound when compared to ultrasound. The common unit of sound pressure or amplitude is the *decibel* (db). This unit is not an absolute measurement of the intensity of sound but it allows

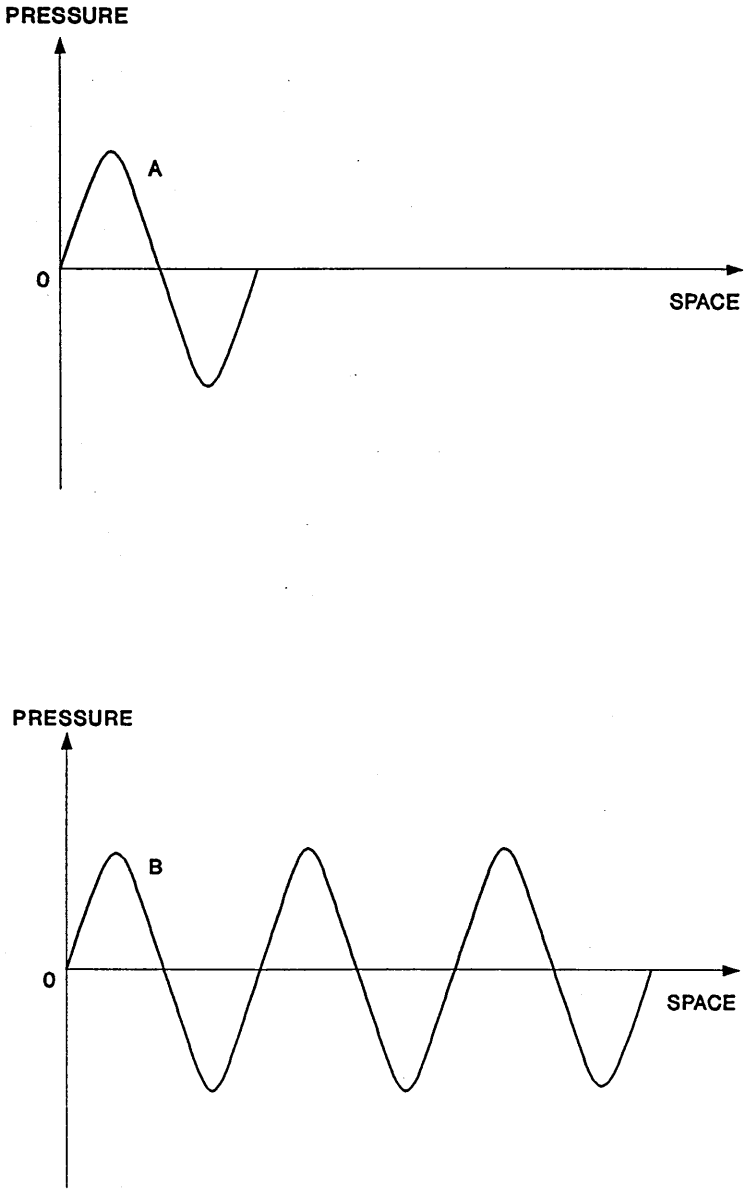


FIGURE 4.1. The propagation of a sound wave.

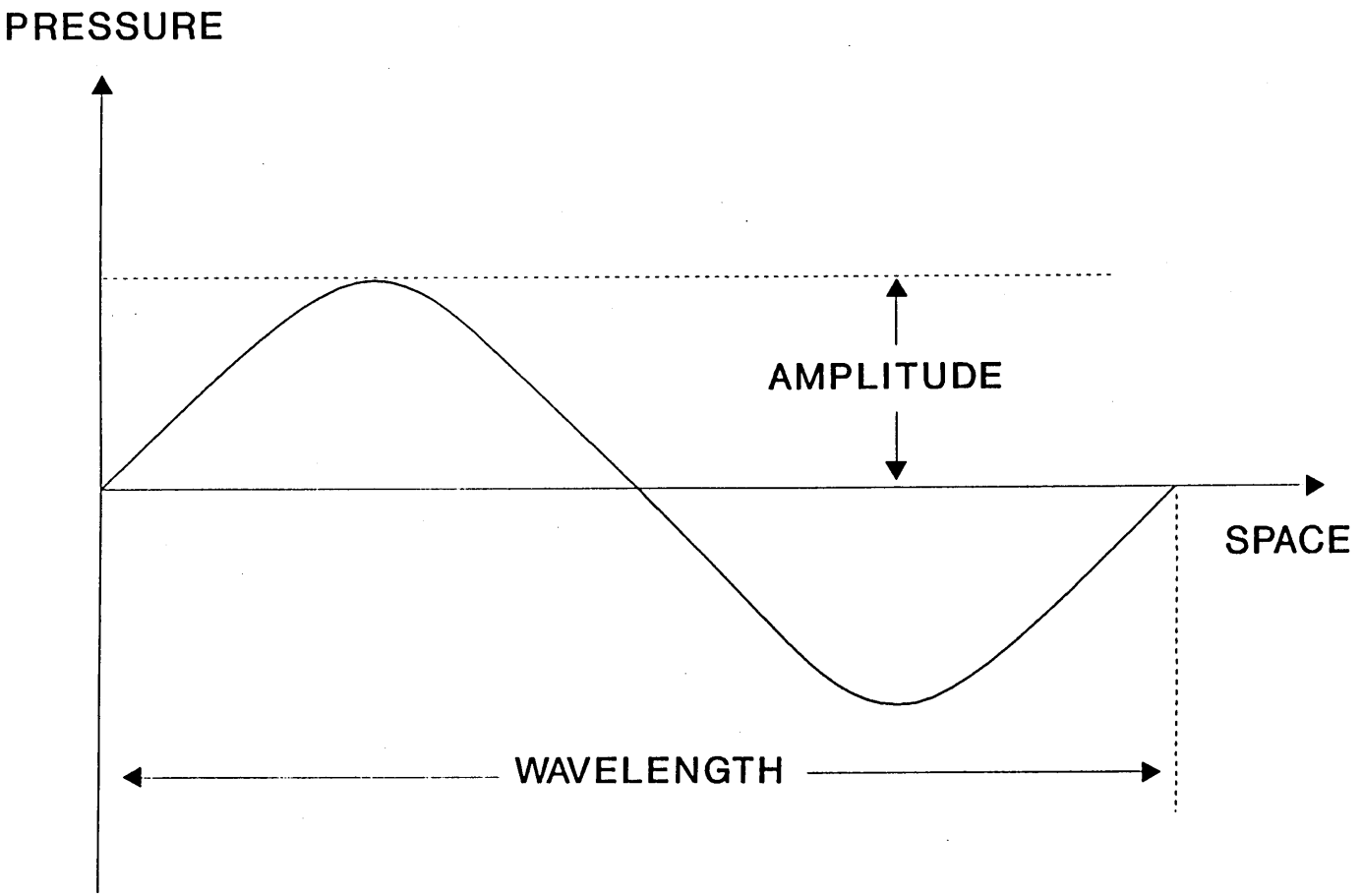


FIGURE 4.2. Some parameters defining a sound wave.

comparison between different sound waves (Wells, 1977). For instance, many countries have regulations on permissible noise levels, typically less than 55 db (Cameron and Skofronick, 1978). At times, the amplitude of echoes in ultrasound is referred to as X number of decibels: the greater the amplitude, the greater the echo.

4.2. ULTRASOUND KINETICS IN BODY TISSUES.

ATTENUATION.

The most important change ultrasound undergoes while travelling through the soft tissues of the body is known as *attenuation.* This phenomenon is the progressive weakening of the sound beam as it travels, and is influenced by factors such as the wavelength of the sound wave, the density of the tissue, and the number and type of interfaces in the tissue (Ziskin, 1975). The attenuation of an ultrasound beam occurs basically through three processes: Absorption, Reflection and Scattering (Ziskin, 1975; Taylor and Kremkau, 1985). As the sound wave travels, a certain amount of its energy is absorbed by the tissue (*absorption*). This form of attenuation is precisely the principle behind ultrasound used in therapeutic diathermia. In the case of diagnostic ultrasound this effect is practically negligible due to the low energy levels utilized (Wells, 1981).

Whenever the ultrasound beam abandons a tissue and continues through another tissue of differing density, a portion of the beam will be redirected back to the transducer (*reflection*). This phenomenon constitutes

the basis of diagnostic ultrasound and it occurs every time the sound beam hits an acoustic interface. An interface occurs whenever two tissues of different acoustic impedance are in contact with each other. The acoustic impedance of a tissue is primarily determined by its density (Ziskin, 1975).

The difference between the acoustic impedances of the two tissues will determine the amount of sound reflected back to its source: the greater this difference, the greater the percentage of sound reflected and the smaller the amount of sound that will continue travelling through the tissues. This is why most ultrasound scanners cannot "see" through gas or bone: the difference in acoustic impedance between these and soft tissue is so large that most of the sound beam is reflected (Wells, 1977).

Finally, when the sound wave encounters an interface which is irregular and smaller than the sound beam, part of it is scattered in all directions (*scattering*). A portion of the beam will be reflected directly back to the transducer, producing an echo known as non-specular reflection. However, most reflected echoes in diagnostic ultrasound are specular, that is, the angle of reflection is equal to the angle of incidence. These type of reflections occur whenever the acoustic interface is larger than the sound beam (Ziskin, 1975).

A common and troublesome artifact appearing on ultrasound images are those reflected echoes which bounce back and forth between two interfaces or between the transducer and a tissue interface. These are called "reverberation echoes" and can give misleading information on the screen

(Taylor and Kremkau, 1985). A sound beam reverberating between the transducer and a tissue interface will produce an initial echo which will indicate the true position of the interface, and multiple low-amplitude echoes at greater depths which indicate the time-lapse between the second, third or fourth returning echoes (Taylor and Kremkau, 1985). On the other hand, if the sound wave is bouncing back and forth between two internal interfaces, the returning echoes will be delayed and the recorded distance will be deeper in the tissue than the original reflecting surface.

RESOLUTION.

Resolution is the ability to differentiate two closely spaced interfaces and is usually expressed as a distance. Resolution in ultrasound scanners has two components: axial and lateral. Axial resolution is the ability to separate two adjacent interfaces that lie along the path of the sound beam whereas lateral resolution refers to the resolution perpendicular to the axis of the sound beam. In general terms, as the frequency of the sound wave increases, so also does resolution (Ziskin, 1975).

FOCUSING.

The ultrasound beam that comes out of the transducer is approximately of the same diameter as the transducer. However, when the beam travels beyond the field nearest to its source it becomes divergent, hence decreasing the ability to differentiate two structures at right angles to the sound beam. Thus, the width of the beam at the near field can be narrowed to improve

lateral resolution and this is known as focusing (Ziskin, 1975; Taylor and Kremkau, 1985).

In general, transducers emitting high frequency ultrasound will have a focused region closer to them than those transducers operating on lower frequencies (Wells, 1977). In other words, because penetration is reduced with increasing frequency, it is important to select a transducer that is focused closest to the depth of interest.

4.3. DIFFERENT MODALITIES.

The echoes produced by the different interfaces present in a tissue are detected by the transducer and converted into electrical energy. After modification, these electrical signals can be displayed on a screen in a number of ways (display formats or "modes"). These are:

AMPLITUDE MODE (A-MODE).

This is the simplest (one-dimensional) modality of ultrasonographic imaging. The so-called A-mode units display echo amplitude (A) against time. Echoes appear as spikes on the screen, the distance between different spikes being related to the distance between successive tissue interfaces (Ziskin, 1975).

A-mode machines have been used to measure skin thickness in people. Alexander and Miller (1979) were able to measure the thickness of the skin

on the forearm of 10 normal male and female adults using a 15 MHz A-mode unit. They found ultrasonic measurements of skin thickness to be almost equivalent to measurements of skin thickness obtained with a conventional radiological method. Utilizing very similar equipment, Tan, Statham, Marks and Payne (1982) found a slight difference, not statistically significant, between two independent observers measuring skin thickness in normal adults. The authors concluded that the pulse echo technique has a high reproducibility and is a reliable method for determining the thickness of human skin.

The skin thickness of 22 females with systemic sclerosis was measured using a 15 MHz A-scan (Serup, 1984a). The author was able to demonstrate an increase in the thickness of the skin of these patients, at both the extensor and flexor aspects of their forearms, when compared to healthy females matched for age. The same ultrasonographic unit was successfully used for measuring the full thickness of sclerotic plaques in the skin of patients suffering from localized scleroderma (Serup, 1984b). Corticosteroid-induced thinning of the dermis has been monitored using an A-mode device (Tan, Marks and Payne, 1981), and the technique has been used for quantifying the degree of cutaneous oedema following patch test reactions in contact dermatitis patients (Serup, Staberg and Klemp, 1984).

Other pathological conditions affecting the skin in which A-mode ultrasound has been used as an exploratory tool, include: squamous cell carcinoma, basal cell carcinoma, epidermoid cyst, lipoma, lymphoedema,

keratoacanthoma and malignant melanoma (Tan, Marks, Roberts and Guibarra, 1981; Hughes, Black, Srivastava, Dalziel and Marks, 1987). Moreover, the technique has proved useful for measuring the thickness of nails (Finlay, Moseley and Duggan, 1987) and of the oral mucosa of humans (Daly and Wheeler, 1971).

As it was seen above (Chapter 2), A-mode ultrasound has been widely used in humans to measure the thickness of subcutaneous fat. Many authors have found a high degree of correlation for the measurement of this fat layer between ultrasound and other techniques such as direct needle puncture (Bullen and others, 1965), skinfold callipers (Booth and others, 1966; Haymes and others, 1976; Volz and Ostrove, 1984), computed tomography (Black, Vora, Hayward and Marks, 1986), electrical conductivity (Booth and others, 1966), infrared interactance (Conway, Norris and Bodwell, 1984) and surgical incision (Balta and others, 1981). However, it was not in all cases that A-mode ultrasound was highly correlated with other techniques (Borkan and others, 1982b; Chumlea and Roche, 1986).

Various pulsed echo scans have been used in body composition studies of farm animals. Determinations of backfat thickness on live pigs have been carried out using this technique (East and others, 1959; Price and others, 1960; Giles and others, 1981). The thickness of the subcutaneous fat layer running along the dorsum has been measured with A-mode ultrasound in cattle (McReynolds and Arthaud, 1970; Kempster and others, 1981; Gresham

and others, 1986) and sheep (Bennett and others, 1988). These measurements were used to predict body composition.

BRIGHTNESS MODE (B-MODE).

B-mode ultrasonography is the most versatile of the various modes available. It is a two-dimensional display of dots. A wide beam of ultrasound is directed at the area of interest and a cross-section of its anatomy is depicted (Ziskin, 1975). The position of the dot on the screen is determined by its depth in the tissue and therefore by the time it takes for the echo to return to the transducer. The image is usually composed of dots with varying shades of grey, their brightness being proportional to the amplitude of the returning echo (Wells, 1981). Different organs each have a characteristic appearance (different acoustic impedance) and so can be identified.

This modality of ultrasonic imaging has been used both in human and veterinary medicine to visualize the female reproductive tract and the abdominal organs such as the liver, spleen and kidneys (Freiherr, 1989). Using higher frequencies than those utilized for visualizing internal organs, it has been possible to obtain cross-sectional pictures of normal human skin by means of B-scans (Dines, Sheets, Brink, Hanke, Condra, Clendenon, Goss, Smith and Franklin, 1984; Fornage and Deshayes, 1986). It has also been possible to evaluate changes in skin thickness in patients suffering from systemic sclerosis (Myers, Cohen, Sheets and Bies, 1986) and to assess burn depth in thermal injury (Kalus, Aindow and Caulfield, 1979). Nodular lesions

of the skin were visualized with a B-mode unit (Miyauchi, Tada and Miki, 1983) and the extension and local invasiveness of various skin tumours have been evaluated using this technique (Rukavina and Mohar, 1979; Shafir, Itzchak, Heyman, Azizi, Haggai and Hiss, 1984; Murakami and Miki, 1989).

The merit of this form of ultrasound imaging in measuring subcutaneous fat thickness in humans has been well established (Fanelli and Kuczmarski, 1984; Fried, Coughlin and Griffen, 1986; Weits and others, 1986; Kuczmarski and others, 1987; Hansen and Kehrer, 1987). Furthermore, since the early reports on the use of real time scanners in farm animals (Stouffer, 1963), numerous studies have shown the efficacy of B-scans in measuring the various components of body composition (Chapter 2). Fat areas measured over the back of cattle have been found to be highly correlated with actual subcutaneous fat percentage obtained by dissection (Alliston and Hinks, 1981; Kempster and Owen, 1981). Similar results were obtained by comparing subcutaneous adipose tissue thickness measured with a B-scan to direct in situ ruler measurements in pigs (Weingand, Hartke, Noordsy and Ledeboer, 1989). In horses, rump fat thickness measured with B-mode ultrasound has been used to estimate body fat percentage (Westervelt and others, 1976; Henneke and others, 1983).

The differences in display format between B-mode and A-mode ultrasound have been depicted in Figure 4.3.

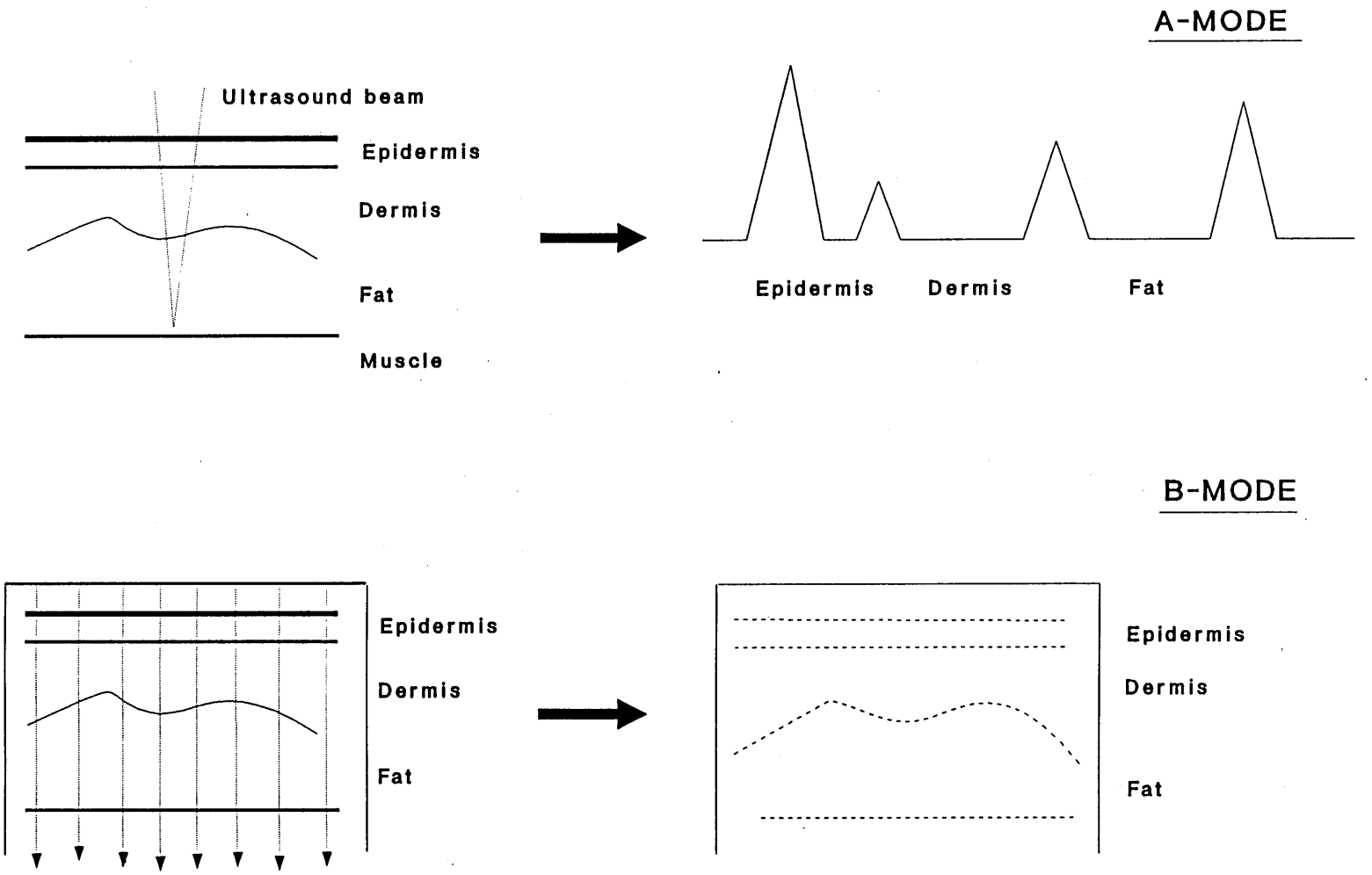


FIGURE 4.3. Differences in display format between A and B-mode ultrasound.

TIME-MOTION MODE (M-MODE) AND DOPPLER ULTRA-SONOGRAPHY.

These two modalities are used to measure motion in the body. M-mode displays dots exactly in the same way as B-mode, but in a one-dimensional format. Basically, the transducer is held stationary as in the A-scan and the echoes appear as grey dots as in the B-scan (Cameron and Skofronick, 1978). M-mode machines are used to monitor the movement of the heart walls and heart valves and to measure cardiac chamber dimensions (Cameron and Skofronick, 1978).

Doppler ultrasonography is based on the "Doppler effect": when a source of sound of known frequency is moving towards a listener, it has a higher pitch than when it is moving away from him. The same happens when the listener is moving towards the source (Cameron and Skofronick, 1978). This effect is exploited in ultrasonic imaging for measuring the speed of blood flow across valves or within vessels (Taylor and Kremkau, 1985).

CHAPTER V.

ULTRASONOGRAPHIC MEASUREMENT OF SUBCUTANEOUS FAT THICKNESS IN DOGS AND ITS CORRELATION WITH TWO OTHER TECHNIQUES.

CHAPTER V. ULTRASONOGRAPHIC MEASUREMENT OF SUBCUTANEOUS FAT THICKNESS IN DOGS AND ITS CORRELATION WITH TWO OTHER TECHNIQUES.

5.1. INTRODUCTION.

In humans, a considerable amount of body fat is deposited immediately beneath the skin. For many years, the measurement of this layer of fat has been taken to be a good estimator of total adiposity. The studies of Durnin and Womersley (1974) showed that skinfold measurements at four body sites (biceps, triceps, subscapular and suprailiac) could be used to predict body fat, measured by hydrostatic weighing, over a wide range of ages and body weights.

Picking up a skin fold and applying a gentle pressure with the tips of a calliper is a very simple, inexpensive method for determining degree of obesity. However, the skinfold calliper technique has a number of disadvantages (reviewed by Kuczmarski and others, 1987), the most important of these being: the difficulty in controlling inter and intra-subject variation in skinfold compressibility, the impossibility of obtaining reliable measurements in very obese individuals and the inability to palpate the fat-muscle interface in a number of subjects. Edwards and others (1955) showed that the pressure exerted by different callipers had a significant effect on the observed thickness

of the fold and the consistency with which measurements could be repeated. They found significant inter-observer variation and that the accuracy of this technique would vary according to the anatomical site under study. Similar conclusions were reached by Ruiz, Colley and Hamilton (1971) when they tested for inter-observer variation taking skinfold measurements at one body site.

Skinfold callipers have seldom been used in domestic animals to estimate body condition. Their value for use in dogs and cats is reportedly low (Anderson, 1973; Lewis and others, 1987). These authors point out that the skin of most dogs and cats is easily detached from the underlying adipose tissue, hence making the measurement of the thickness of subcutaneous fat an unreliable exercise.

Ultrasound has been used to overcome some of the limitations of the skinfold technique. The ultrasonic probe does not compress the skin, can be used on very obese subjects and does not affect skin or fat tissue elasticity after repeated measurements. Besides, it is not necessary to palpate the fat-muscle interface and the probe can usually be applied to almost any body site.

Various ultrasonographic devices have been used for a number of years on livestock to predict carcass composition. In particular, A-mode units have been successfully used for measuring backfat thickness on live pigs (Price and

others, 1960; Giles and others, 1981) and cattle (McReynolds and Arthaud, 1970). Consequently, this technique has also been developed for use in humans and the subcutaneous layer of fat has successfully been measured in people using A-mode ultrasound (Balta and others, 1971; Haymes and others, 1976; Volz and Ostrove, 1984).

An A-mode ultrasound machine, designed for use in human dermatology, was purchased by the Department of Veterinary Medicine of Glasgow University Veterinary School. Our preliminary investigations using this equipment on live dogs showed that the layer of fat being measured varied among different body sites and depending on the positioning of the probe. Furthermore, although there was usually consistency in the interpretation of readings done by each observer, there were inter-observer discrepancies as to what was being measured. Thus, an experiment was designed to validate this technique for measuring subcutaneous fat in dogs.

5.2. OBJECTIVES.

The purposes of this experiment were the following:

1. To determine if A-mode ultrasound could reliably measure subcutaneous fat thickness in dogs.

2. To develop a skill in the interpretation of the echograms obtained with the ultrasonographic unit.

3. To assess differences in the thickness of the subcutaneous layer of fat according to anatomical location.

4. To compare three techniques (histology, ultrasound and skinfold callipers) for the measurement of subcutaneous fat.

5.3. MATERIALS AND METHODS.

POPULATION UNDER STUDY.

Twenty eight stray dogs, obtained from a charitable organization shortly after they had been euthanased, were studied (Table 5.1). Euthanasia was carried out due to the impossibility of finding a home for these animals, and was performed using intravenous administration of pentobarbitone sodium. Most were mongrel dogs of various sizes. They were subjectively classified into one of three age groups according to the wear and tear of dentition (Appendix 5.1.):

* Young: from 6 months to 3 years of age.
* Adult: from 3 to 6 years of age.
* Old: over 6 years.

Assessment of their body condition was performed based on the 5-point clinical scale proposed by Edney and Smith (Table 5.2). According to this scale, dogs can be classified into one of five body condition groups mainly by observing and palpating the amount of fat present in the animal's body and noting its general demeanour and body weight. The terms "emaciated" and "thin" were used in this study to signify the groups described as "thin" and "lean" by Edney and Smith.

BODY SITES SELECTED FOR INVESTIGATION.

Dogs were placed in right lateral recumbency and the hair coat over the left side was gently clipped using an electrical razor (John Oster Manufacturing Company, Milwaukee, Wisconsin, U.S.A.).

Six anatomical sites were carefully defined, and marked using an ink circle of about 30 mm in diameter. These sites were chosen because they were spread throughout those parts of the canine body where fat is thought to accumulate and which are used in the clinical assessment of obesity, namely the trunk and abdomen. Indeed fat deposition in dogs tends to be over the trunk, abdomen and inguinal area (Joshua, 1970; Evans and Christensen, 1979; Habermehl, 1981) and large amounts of adipose tissue may be found in the lumbar region (Joshua, 1970). The latter region has been used for measuring subcutaneous fat thickness in cattle (Kempster and others, 1981; Alliston and Hinks, 1981), pigs (East and others, 1959; Price and others, 1960) and dogs (Anderson and Corbin, 1982).

TABLE 5.1.

The population of dogs under study.

	BREED	AGE GROUP[1]	SEX[+]
Dog 1	Cross	Young	Male
Dog 2	Cross	Young	Female
Dog 3	Cross	Young	Female
Dog 4	Cross	Young	Female
Dog 5	Cross	Young	Male
Dog 6	Cross	Young	Male
Dog 7	Pitbull Terrier	Adult	Male
Dog 8	Cross	Young	Female
Dog 9	Cross	Young*	Male
Dog 10	Cross	Young	Female
Dog 11	Cross	Young	Female
Dog 12	Cross	Adult	Male
Dog 13	Cross	Young	Female
Dog 14	Cross	Young	Female
Dog 15	Cross	Young	Male
Dog 16	Cross	Old	Female
Dog 17	Cross	Old	Male
Dog 18	Welsh Corgi	Old	Male
Dog 19	Cross	Old	Female
Dog 20	Cross	Old	Female
Dog 21	WHWT	Old	Female
Dog 22	WHWT	Old	Female
Dog 23	Cross	Old	Male
Dog 24	Cross	Old	Female
Dog 25	Cross	Old	Male
Dog 26	Cross	Young	Female
Dog 27	Cross	Young	Female
Dog 28	Cross	Young	Female

[1] Estimated by the wear and tear of dentition.

Young: 6 months to 3 years / Adult: 3 to 6 years / Old: >6 years.

[+] All males were entire. Sexual status of females was not determined.

* This dog was a puppy, approximately 8-10 months old.

WHWT: West Highland White Terrier.

TABLE 5.2.

Five-point clinical scale for body condition scoring in dogs*.

EMACIATED	Pronounced underweight, skeletal structure obvious.
THIN	Little body fat evident, rib cage visible.
NORMAL	Moderate amount of body fat, rib cage easily palpable but not too obvious.
FAT	Rib cage not visible, difficulty in palpating bones of chest, fat deposition in abdomen and trunk.
GROSS	Unable to feel ribs, large amounts of fat can be grasped by hand.

* Based on Edney, A. T. B. and Smith, P. M. (1986).
The Veterinary Record. 118: 391-396.

Taking the above into consideration, the anatomical sites were defined as follows:

Axilla: over the deep pectoral muscle, caudal to the *triceps brachii* muscle.

Flank: over the 9th intercostal space, just above the costochondral junction.

Sternum: 1 cm caudal to the xiphoid cartilage and lateral to the linea alba.

Abdomen: on the left lateral wall of the abdomen, over the *obliquus externus* muscle.

Thigh: on the right inner thigh, midway on the diagonal line traced from the ischiatic tuberosity to the lateral condyle of the femur.

Lumbar: between the 3rd and 5th lumbar vertebrae, 2 to 3 cm lateral from the spine.

MEASUREMENTS.

Skinfold calliper and ultrasonic measurements were carried out within 1 to 4 hours post-mortem, at room temperature. The body was still warm and the skin retained its elasticity and suppleness.

Skinfold calliper.

All skinfolds were measured with a Holtain skinfold calliper (Holtain Ltd., Crosswell, Crymych, Dyfed SA41 3UF, U.K.). This instrument is designed to give a constant pressure of 10 g/sq mm over its entire operating

range. The dial follows 0.2 mm divisions but readings of 0.1 mm are easily estimated.

Measurements were performed following the manufacturers recommendations as far as it was possible. The thumb and forefinger of the left hand are used to pick up a fold of skin and subcutaneous tissue while the callipers are applied to the fold, below the pinch point, using the right hand. The grip on the trigger is then released so that the jaws of the calliper can exert their full pressure. In some cases, the registered measurement decreased as the operator watched the dial, particularly in obese dogs. The greater thickness of the fat fold in these animals made it more difficult to maintain a constant pressure with the calliper tips. However, this was usually solved by holding a firmer pinch with the left hand and taking a reading immediately after the calliper had exerted its full pressure.

Two readings, separated by an interval of half a minute, were recorded at each anatomical location.

Ultrasound.

Immediately following skinfold measurements, ultrasound was used for measuring subcutaneous fat thickness at the same anatomical locations.

An A-mode ultrasonographic unit (Dermascan-A, Cortex Technology Aps, Aalborguej 53E, 9560 Hadsund, Denmark) was utilized for the experiment (Figure 5.1). This lightweight device operates with a 20 MHz focused transducer and has a tissue resolution of 0.075 mm. The reflected

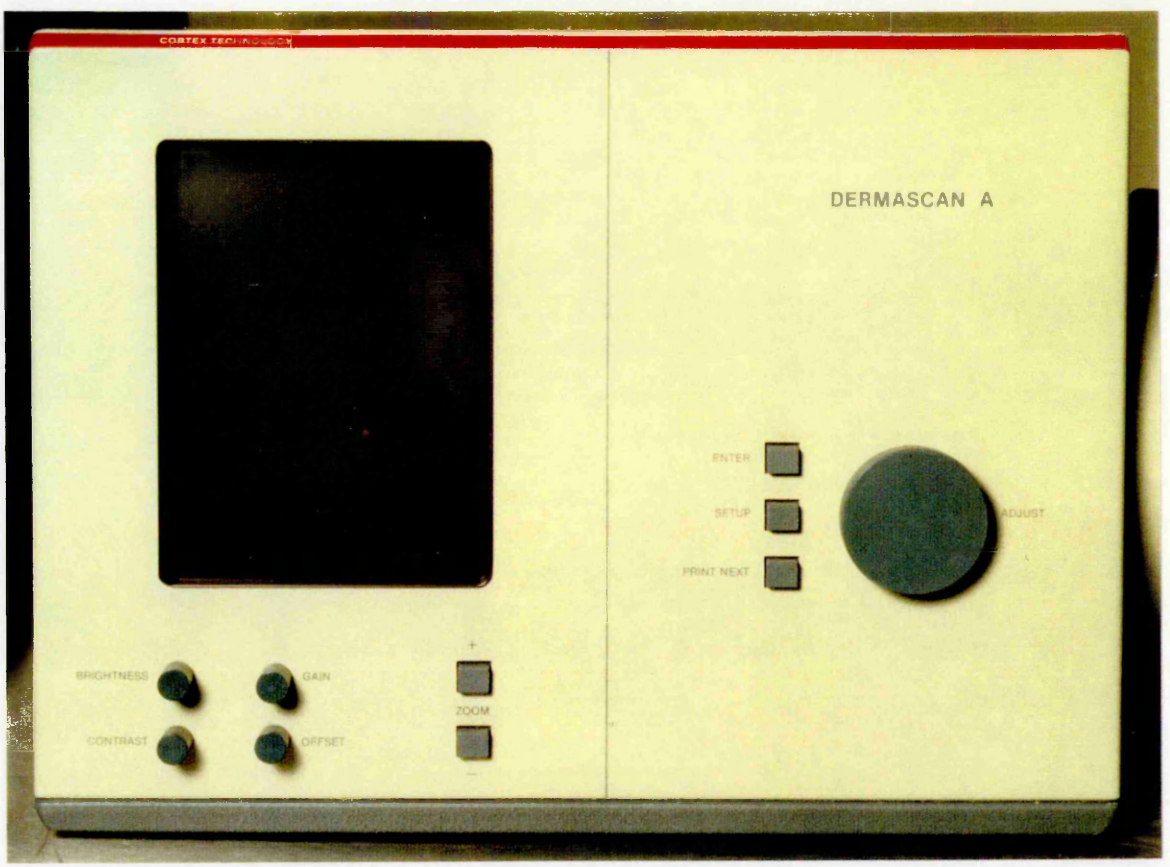


FIGURE 5.1. The Dermascan-A.

echoes are shown as peaks on the screen allowing measurements in millimetre intervals up to a depth of 13.1 mm. The velocity of sound was set to 1470 m/sec, which is the speed of ultrasound in fat and has been used for measuring subcutaneous fat in both humans and animals (Escoffier, Querleux, de Rigal and Leveque, 1986; Hamlet, Rezvani and Hopewell, 1986).

The skin was moisturized using a drop of water to allow a better coupling with the sound beam. The probe was held manually against the skin surface, taking great care not to compress the subcutaneous tissues. A beam of ultrasound is then sent from the transducer into the skin and subcutaneous tissues. Whenever the sound beam goes through a change in tissue density, a fraction is reflected and picked up by the transducer (Chapter 4). On the data screen, the reflected echoes are shown as peaks indicating the precise location, with reference to the skin surface, of the various structures in and underneath the skin.

The probe was placed on five different positions within each of the inked circles defining the anatomical locations. Starting at the 12 o'clock position, the probe was moved clockwise to the 3, 6, 9 o'clock positions, and finally to the centre of the circle.

After carefully altering the probe's orientation at the 12 o'clock position, the echo pattern was obtained on the display screen and the picture was frozen and stored. The probe would then be moved to the next position within the circle and so on, until five pictures (echograms) were obtained at

each anatomical site. This procedure was done adjusting the viewing field range on the data screen to 6.5 mm. The maximum viewing field range (13.1 mm) was also used for taking a further five echograms. However, the latter were not used for measuring the subcutaneous fat layer because in these echograms the fat/muscle interface was difficult to distinguish from echoes arising from other subcutaneous structures.

The time needed for the operator to be satisfied with the picture on the screen was variable. Not only were there differences in echo pattern depending on the body site under study, but the positioning of the probe and, to a certain extent, the body condition of the animal, had an influence on the type of echogram. In some cases, 10 minutes had elapsed before an acceptable picture could be frozen on the data screen.

All echograms were subsequently stored on a portable computer (SupersPort 2, Zenith Data Systems) for later analysis.

Histology.

Following ultrasonic investigation, the same anatomical sites were biopsied. A scalpel blade was used to make an incision down to the muscle fascia and the whole anatomical area defined by the inked circle was removed. The biopsy specimen was placed on a piece of cardboard and fixed in 10% buffered neutral formalin for 24 to 48 hours. Tissues were then trimmed to 5 mm thickness, processed and embedded in paraffin wax using a

V.I.P.-1000 Tissue Processor and Tissue Tek 2 Embedding System (Miles Laboratories, Stoke Poges, Buckinghamshire, England). Sections 4 μm thick were cut on a Lietz 1512 rotary microtome. After incubation at 60^0C for 30 minutes, the sections were stained by the hematoxylin and eosin method using D.P.X. mounting medium.

All sections were examined under the microscope in order to measure the thickness of the immediate subcutaneous fat, that is, the layer of fat comprised between the dermis and the cutaneous muscle. A total of six measurements were performed in each section using a graded lens, moving from left to right across the slide. The mean thickness of the fat layer for each anatomical site was calculated from these measurements.

STATISTICAL METHODS.

The mean, standard deviation and range of measurements of subcutaneous fat thickness obtained with the calliper, ultrasound and histological techniques, were calculated.

To measure the association between any two of these techniques, the coefficient of correlation (r) was determined (Snedecor and Cochran, 1967a). The level of significance was chosen to be 5% ($p < 0.05$).

The relationship between techniques was graphically represented by plotting the best fitted straight line between two sets of measurements (Wardlaw, 1985).

One way analysis of variance (Snedecor and Cochran, 1967b) was used to test differences in subcutaneous fat thickness between groups of dogs with different body condition score. To further identify which groups were different from one another, the Newman-Keuls Range Test was utilized (Snedecor and Cochran, 1967b).

5.4. RESULTS.

CLINICAL SCALE.

The various condition-scored groups of dogs showed differences in the thickness of subcutaneous fat being measured (Appendix 5.2.). Histological and calliper measurements of subcutaneous fat were significantly different between both the "thin" and "normal" groups of dogs when compared to the "fat" group. Fat measurements obtained with ultrasound were significantly greater in "fat" dogs when compared to "thin" dogs, but the difference between "fat" and "normal" dogs was not statistically significant.

No significant difference was found with ultrasound, histology or the calliper technique between "normal" and "thin" dogs.

The insufficient number of dogs classified as "emaciated" and "gross" did not allow comparison with other condition score groups.

SKINFOLD CALLIPERS.

It was normally easy to pick up a skin fold between thumb and forefinger in these group of animals. In the case of obese dogs, the operator had the impression of not being able to lift the full thickness of the fat fold along with the skin. In any case, there was good repeatability of the two calliper measurements taken 30 seconds apart: the difference was usually less than 0.5 mm.

For each of the six body sites, the mean of the two calliper measurements was calculated. This value was halved since it theoretically represents a double fold of fat (Appendix 5.3). This practice has been recommended for allowing comparison with ultrasound measurements, which represent a single fat thickness (Hicks, Hope, Turnbull and Verel, 1956; Borkan and others, 1982b; Volz and Ostrove, 1984; Black, Vora, Hayward and Marks, 1988).

Although there was a certain uniformity in the thickness of the fat layer measured by the callipers in each animal, nevertheless differences of up to 6.8 mm could be found between two anatomical locations in the same dog.

The thickness of the subcutaneous fat fold recorded with the callipers, was generally greater in the lumbar area (mean= 3.34 mm), independent of body condition. On the other hand, the anatomical location defined as "thigh" usually showed the least fat thickness (mean= 1.66 mm). Table 5.3 shows the

mean, standard deviation, and range values for the calliper measurements at each body site.

ULTRASOUND.

The echograms obtained by the ultrasound machine were analysed 12 to 24 hours after collection. Fat thickness measurements were carried out directly on the screen, using electronic callipers.

Typically, a good A-mode echogram would show a few high-amplitude echoes, representing the epidermis, followed by a series of echoes of similar amplitude given by the dermal structures (Figure 5.2). Peaks representing the dermis would be followed by an echolucent area (anechoic gap) at the end of which a high amplitude echo, indicating the position of the fat/muscle interface, would appear. The distance between the last dermal peak and the fat/muscle interface (anechoic gap), gave the thickness of the subcutaneous fat layer.

For each anatomical location, the calculated thickness of subcutaneous fat (Appendix 5.4) was the mean of five measurements (five echograms). Skin thickness, represented by the epidermis and dermis, was not included in these measurements.

In a number of echograms, additional or spurious echoes were found within the echolucent fat gap (Figure 5.3). This was the case even when the

"gain" control, which allows adjustment of echo amplitude, was reduced to a minimum in an attempt to remain with a single peak on the screen corresponding to the fat/muscle interface. These additional echoes were probably caused by the connective tissue mesh present in the fat layer (see below). Moreover, the echo-pattern arising from the fat/muscle interface was variable. Most times it consisted of two peaks separated by a short echolucent gap, probably representing the thickness of the cutaneous muscle; but in other occasions, only a single peak could be distinguished on the screen. Whether the latter pattern was due to the cutaneous muscle being too thin for the ultrasound beam to resolve its thickness or to some other reason, is unknown.

Subcutaneous fat thickness varied depending on anatomical location. Fat deposition was greatest at the lumbar area (mean= 2.09 mm). The echograms obtained from this site usually showed a thicker layer of subcutaneous fat when compared to other anatomical locations. This increased thickness of the subcutaneous fat layer probably accounts for the greater ease with which lumbar echograms were interpreted. A clearly defined echolucent gap was generally distinguished between the dermis and the fat/muscle interface. By contrast, the thigh area yielded poorly defined echograms. Abundant spurious echoes appeared on the screen when this body site was interrogated with the A-scan. The inner thigh can be a lean part of the body, where the subcutaneous fat layer is thin and important blood vessels (e.g., the femoral trunk) run superficially across it. These factors, together

with difficult accessibility, might explain the low quality of ultrasonic data obtained from this site.

With regards to the anatomical location defined as "sternum", and especially in the case of females, the abundant vasculature of the zone made interpretation of the echograms difficult at times. Additional echoes produced by small blood vessels within the fat layer were misleading. Thus, there were some occasions when it was not possible to define the subcutaneous fat layer on the data screen (see Appendix 5.4).

The mean, standard deviation and range of fat thicknesses measured with the ultrasound technique at six body sites are shown in Table 5.4.

HISTOLOGY.

On gross examination, the average histological specimen showed a layer of fat situated between the skin (epidermis and dermis) and the cutaneous muscle (panniculus carnosus). Underneath the cutaneous muscle, more adipose tissue was usually present (Figure 5.4). Specimens were examined under a microscope incorporating an eyepiece measuring graticule. The full thickness of the fat layer was measured at six different points, moving the slide from left to right (Appendix 5.5). Care was taken to avoid measuring areas where histological processing had introduced tissue distortions or artifacts. As far as possible, subcutaneous fat was measured where there was a

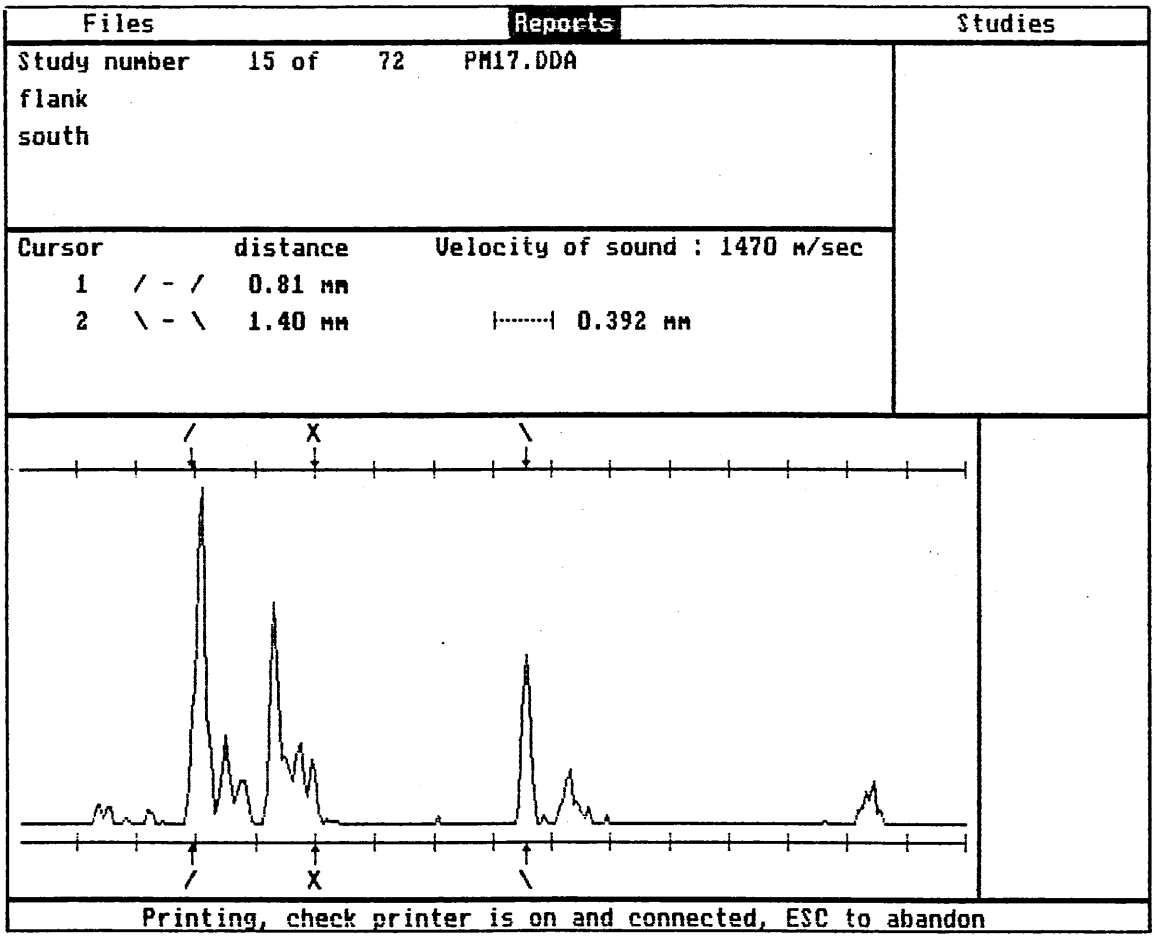


FIGURE 5.2. A-mode echogram showing skin (/ /) and subcutaneous fat (\ \) thickness.

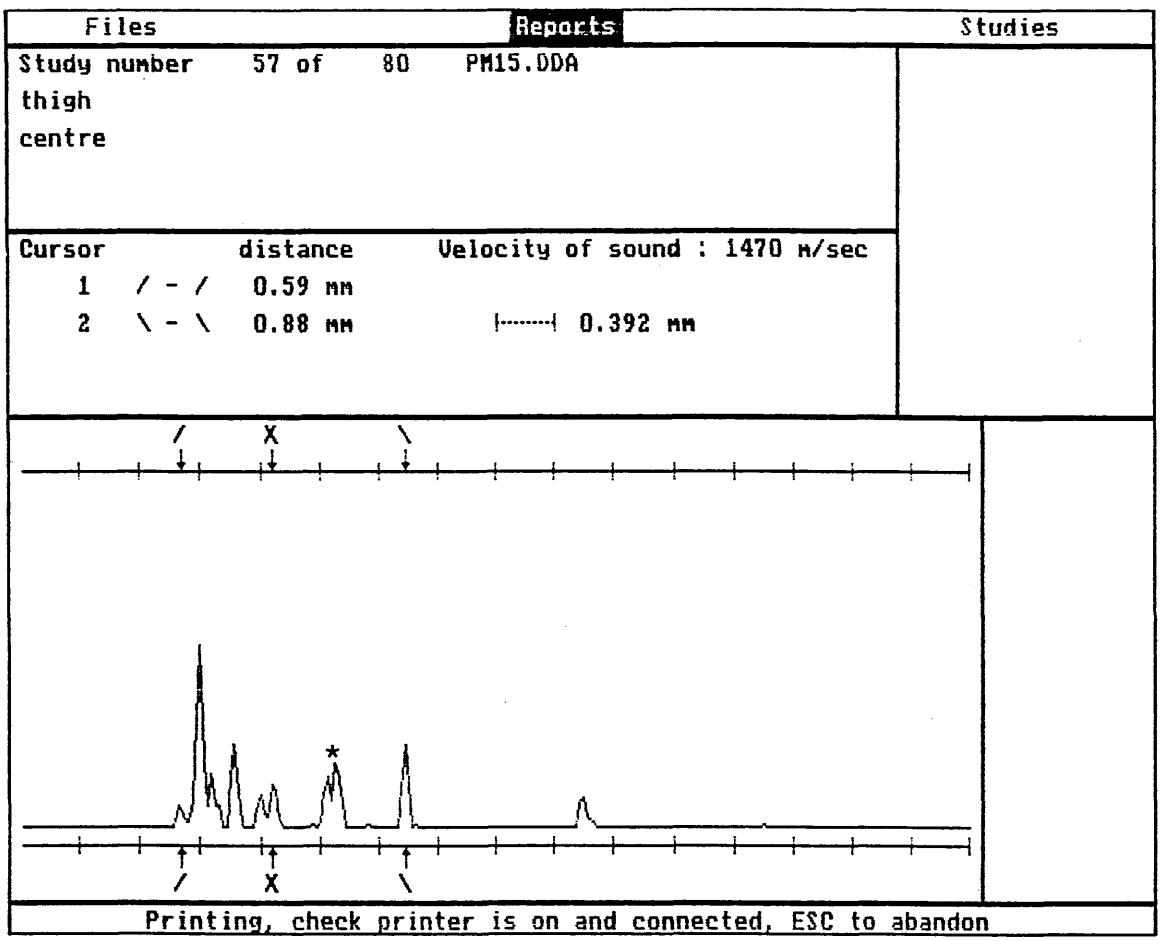


FIGURE 5.3. A-mode echogram showing an spurious echo (*) within the fat layer.

TABLE 5.3.

Subcutaneous fat thickness (mm) measured with skinfold callipers at six anatomical locations in 25 dogs.

BODY SITE	MEAN	SD	RANGE
Axilla	2.77	2.04	0.95-9.00
Flank	2.11	0.93	0.90-4.90
Sternum	2.64	1.26	0.80-5.75
Abdomen	2.21	1.18	0.95-5.90
Thigh	1.66	1.49	0.65-8.35
Lumbar	3.24	1.47	1.40-8.10

TABLE 5.4.

Subcutaneous fat thickness (mm) measured with ultrasound at six anatomical locations in 28 dogs.

BODY SITE	MEAN	SD	RANGE
Axilla	1.11	0.43	0.60-2.28
Flank	0.93	0.38	0.43-2.39
Sternum	1.06	0.42	0.35-1.90
Abdomen	1.06	0.49	0.42-2.40
Thigh	0.98	0.53	0.42-2.88
Lumbar	2.09	1.03	0.41-4.66

compact mass of adipocytes limited by the dermis above and the cutaneous muscle beneath. Nevertheless, at times it was not possible to differentiate a clear-cut fat layer and no measurements were taken. On these occasions, histological processing was repeated on another piece of sample in an attempt to obtain a subcutaneous fat measurement. However, in a reduced number of specimens, adipocytes were "washed-away" during processing, or there was severe distortion of the dermis, or no subcutaneous structure was visible underneath the fat. It was primarily in specimens with a finer layer of adipose tissue that these histological alterations were found.

Histological examination was very revealing and aided with the interpretation of the Dermascan data. The subcutaneous fat layer was not consistently homogeneous. Connective tissue bundles were present between the adipocytes, running in stripes across the fat (Figure 5.5). Other structures, such as small blood vessels and hair follicles were also seen within the adipose tissue layer.

The density of all these subcutaneous structures, and hence their acoustic impedance, differ from that of adipose tissue. It is therefore reasonable to suggest that many of the spurious echoes seen on the ultrasound screen were caused by these structures. Numerous blood vessels and connective tissue bundles were seen in histological preparations obtained from the thigh and sternum areas. These two anatomical locations had yielded the poorest echographic data (see above). Connective tissue was also seen in other body sites but not so abundantly.

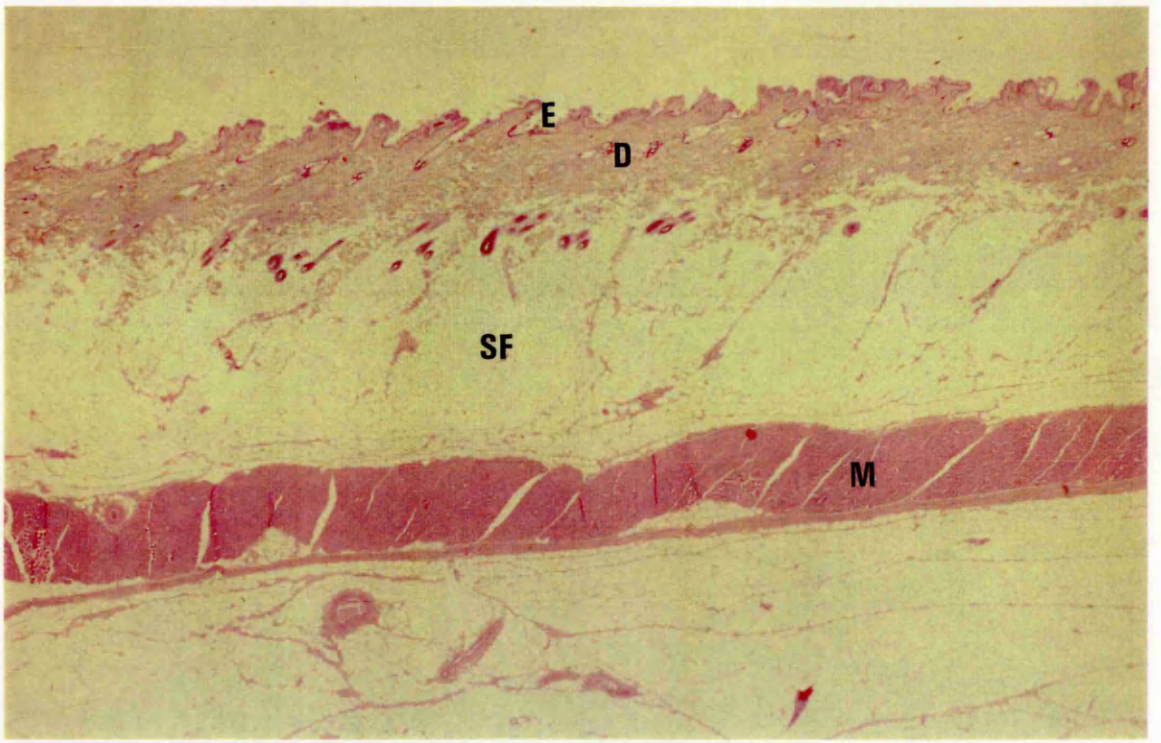


FIGURE 5.4. Gross appearance of histological specimen showing the epidermis (E), dermis (D), subcutaneous fat (SF) and cutaneous muscle (M).

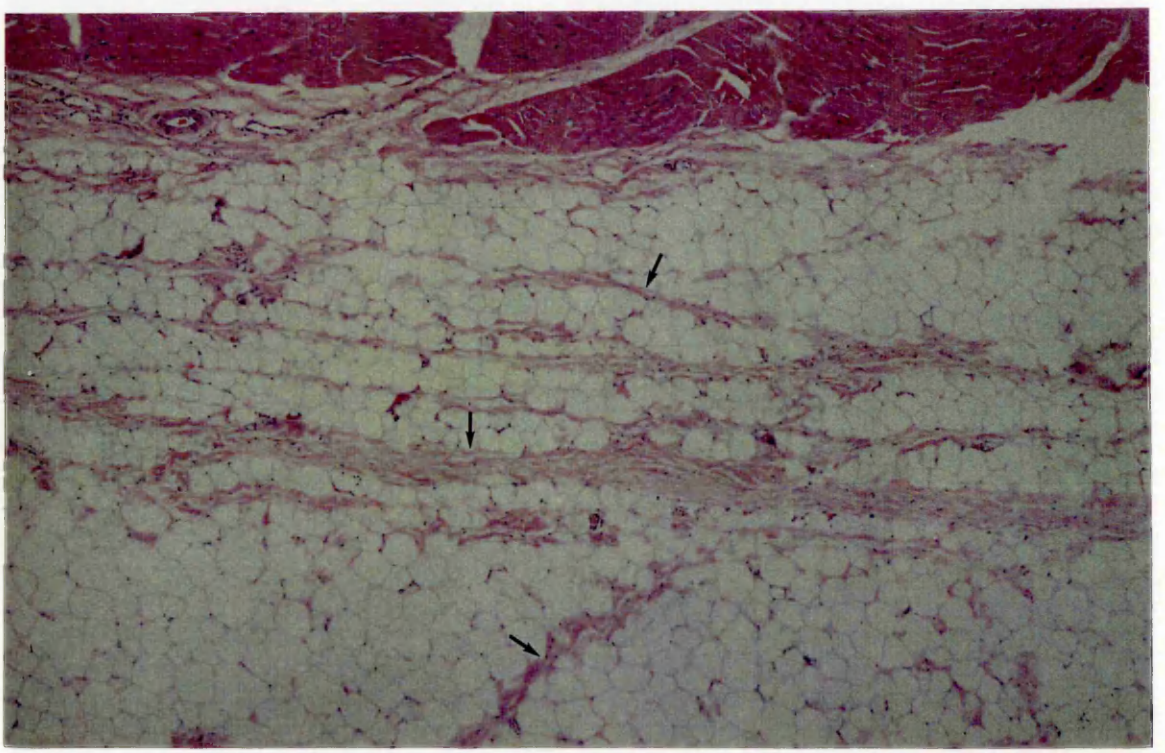


FIGURE 5.5. Bundles of connective tissue (arrows) within the subcutaneous fat layer.

The best histological specimens for subcutaneous fat measurement were obtained from the lumbar area. A thick, well-defined layer of fat was easily distinguished between dermis and cutaneous muscle.

A further interesting finding was that the subcutaneous fat layer varied in thickness along the slide. Although this variation was normally less than a millimetre, it was sufficient to render taking multiple measurements advisable. Changes in fat thickness were partly due to the irregular, winding shape of the dermis/fat interface. Thus, care was taken to include the minimum and maximum depth of fat visible on the histological specimen, together with other four thickness measurements, in order to calculate an average.

Differences of up to 4.2 mm were found between two body sites within the same animal, indicating the variable thickness of the subcutaneous layer of fat. The general trend found with the calliper and ultrasound techniques with regards to a greater fat deposition in the lumbar area, was confirmed with histological examination. The mean subcutaneous fat thickness at this anatomical site (2.18 mm) was the highest when compared to other sites (Table 5.5). The thigh area, however, was not found to be the leanest after histological examination, as opposed to findings with the calliper and ultrasound techniques.

CORRELATION BETWEEN TECHNIQUES.

As illustrated in Figure 5.6, there was a high degree of correlation between the ultrasound technique and histology for the measurement of subcutaneous fat ($r = 0.82$; $p < 0.001$). This correlation was not the same for all anatomical sites (Table 5.6). Some areas, such as the axilla ($r = 0.83$) and especially the lumbar ($r = 0.96$) showed a higher degree of agreement between ultrasound and the histometric technique in measuring subcutaneous fat. The correlation at the axilla and lumbar sites is illustrated in Figure 5.7 and 5.8, respectively.

The calliper method was not so highly correlated with histology (Figure 5.9). The correlation coefficient ($r = 0.63$) between the techniques was somewhat low. The various anatomical areas each showed a different degree of correlation (Table 5.7). Illustrated in Figures 5.10 and 5.11 is the correlation between histology and the calliper technique at the axilla ($r = 0.70$) and lumbar ($r = 0.76$) areas, respectively.

Finally, a comparison between ultrasound and the calliper technique gave a low, although significant, degree of correlation ($r = 0.51$; $p < 0.001$). As can be seen in Table 5.8, correlation coefficients between the two techniques were low at most body sites.

TABLE 5.5.

Subcutaneous fat thickness (mm) measured with histology at six anatomical locations in 28 dogs.

BODY SITE	MEAN	SD	RANGE
Axilla	1.55	0.88	0.46-3.53
Flank	1.18	0.58	0.27-2.38
Sternum	1.55	0.84	0.44-3.23
Abdomen	1.30	0.76	0.33-3.63
Thigh	1.20	1.12	0.17-4.90
Lumbar	2.18	1.22	0.46-5.13

TABLE 5.6.

Correlation coefficients between ultrasound and histology for the measurement of subcutaneous fat at six body sites.

BODY SITE	r	p
Axilla	0.83	< 0.001
Flank	0.72	< 0.001
Sternum	0.79	< 0.001
Abdomen	0.71	< 0.001
Thigh	0.77	< 0.001
Lumbar	0.96	< 0.001

TABLE 5.7.

Correlation coefficients between the skinfold calliper technique and histology for the measurement of subcutaneous fat at six body sites.

BODY SITE	r	p
Axilla	0.70	< 0.001
Flank	0.57	< 0.005
Sternum	0.49	< 0.05
Abdomen	0.44	< 0.05
Thigh	0.53	< 0.01
Lumbar	0.76	< 0.001

TABLE 5.8.

Correlation coefficients between the skinfold calliper technique and ultrasound for the measurement of subcutaneous fat at six body sites.

BODY SITE	r	p
Axilla	0.47	< 0.05
Flank	0.55	< 0.005
Sternum	0.39	< 0.05
Abdomen	0.27	n.s.
Thigh	0.33	n.s.
Lumbar	0.69	< 0.001

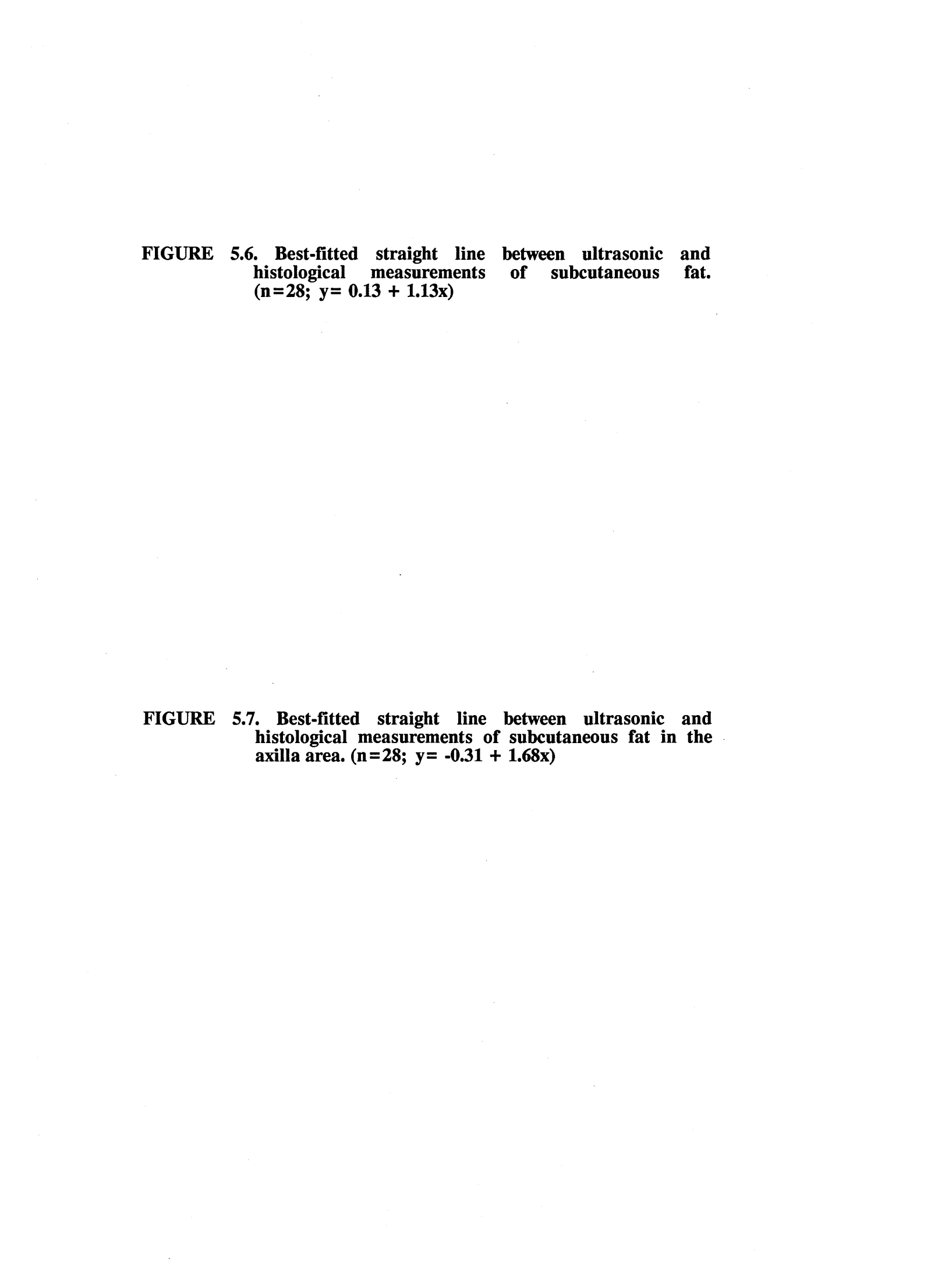


FIGURE 5.6. Best-fitted straight line between ultrasonic and histological measurements of subcutaneous fat. (n=28; y= 0.13 + 1.13x)

FIGURE 5.7. Best-fitted straight line between ultrasonic and histological measurements of subcutaneous fat in the axilla area. (n=28; y= -0.31 + 1.68x)

FIGURE 5.6.

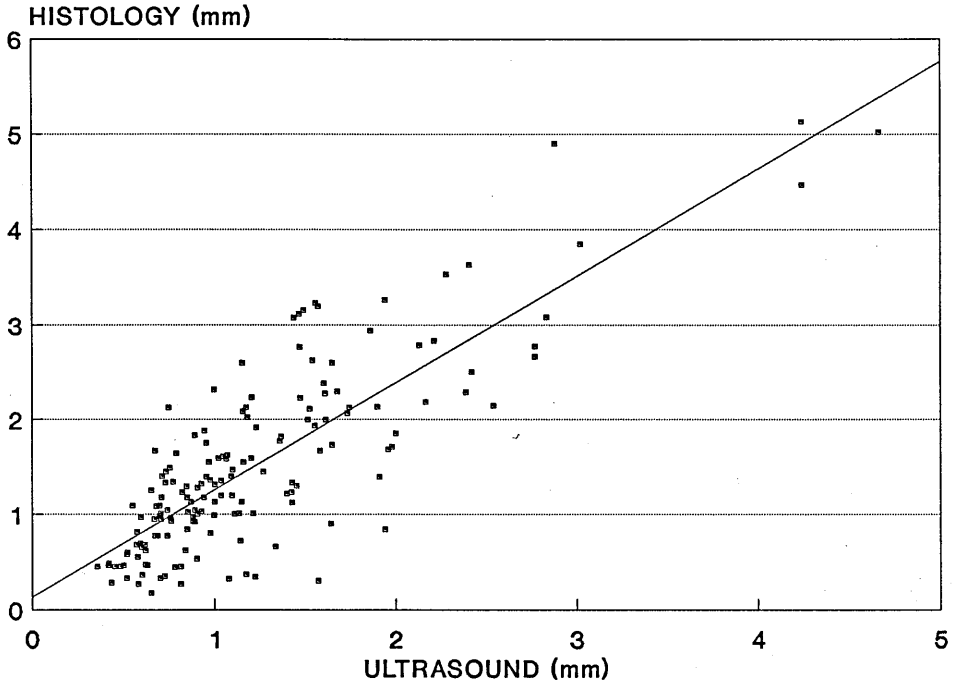


FIGURE 5.7.

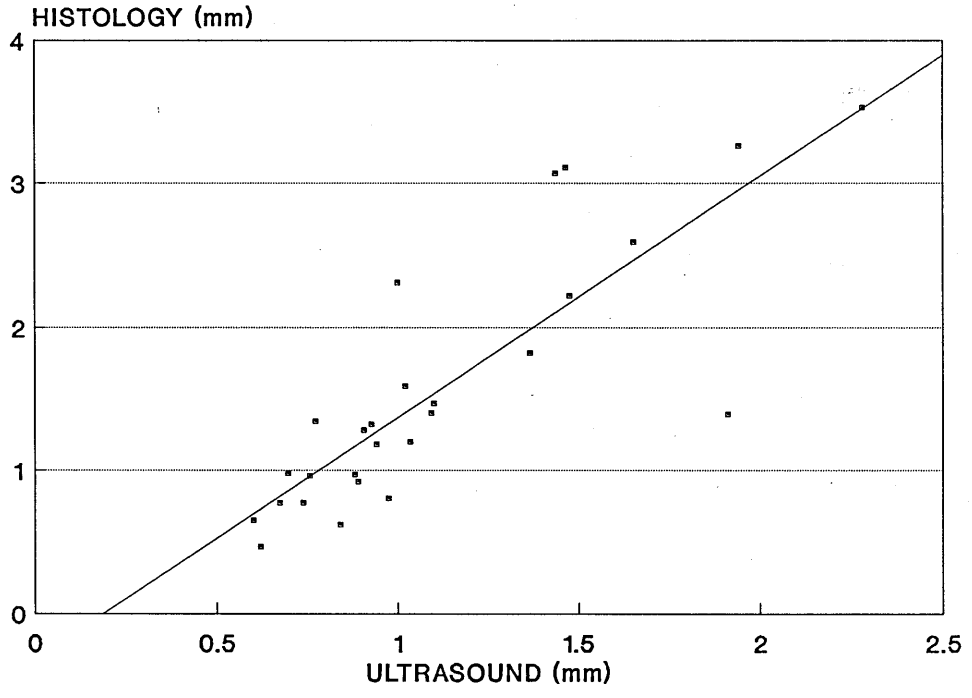


FIGURE 5.8. Best-fitted straight line between ultrasonic and histological measurements of subcutaneous fat in the lumbar area. (n=28; y= -0.21 + 1.14x)

FIGURE 5.9. Best-fitted straight line between histological and calliper measurements of subcutaneous fat. (n=25; y= 0.48 + 0.41x)

FIGURE 5.8.

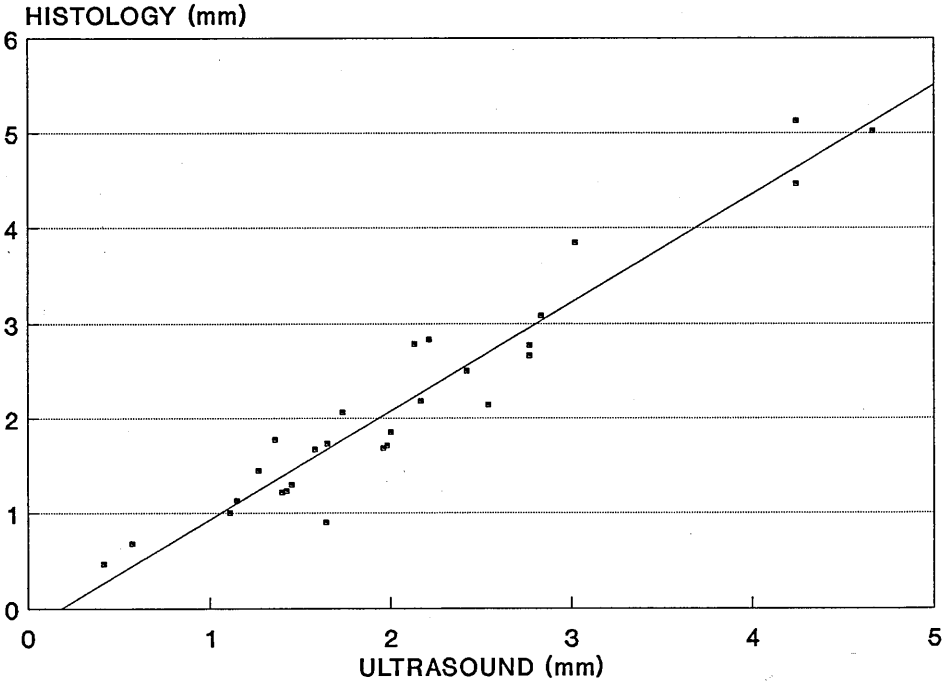


FIGURE 5.9.

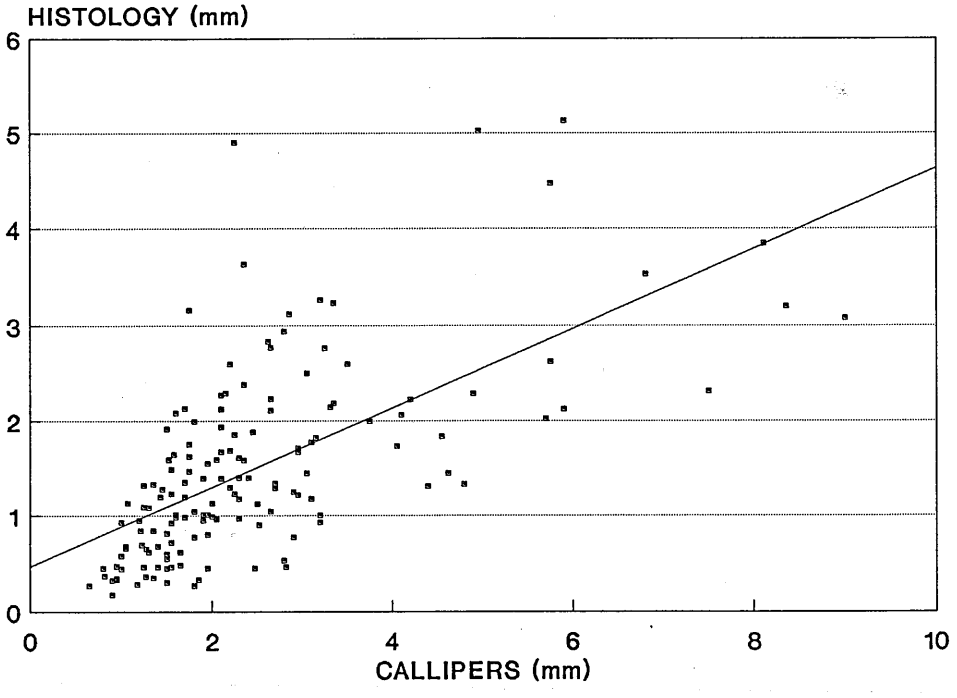


FIGURE 5.10. Best-fitted straight line between histological and calliper measurements of subcutaneous fat in the axilla area. (n=25; y= 0.74 + 0.3x)

FIGURE 5.11. Best-fitted straight line between histological and calliper measurements of subcutaneous fat in the lumbar area. (n=25; y= -0.12 + 0.66x)

FIGURE 5.10.

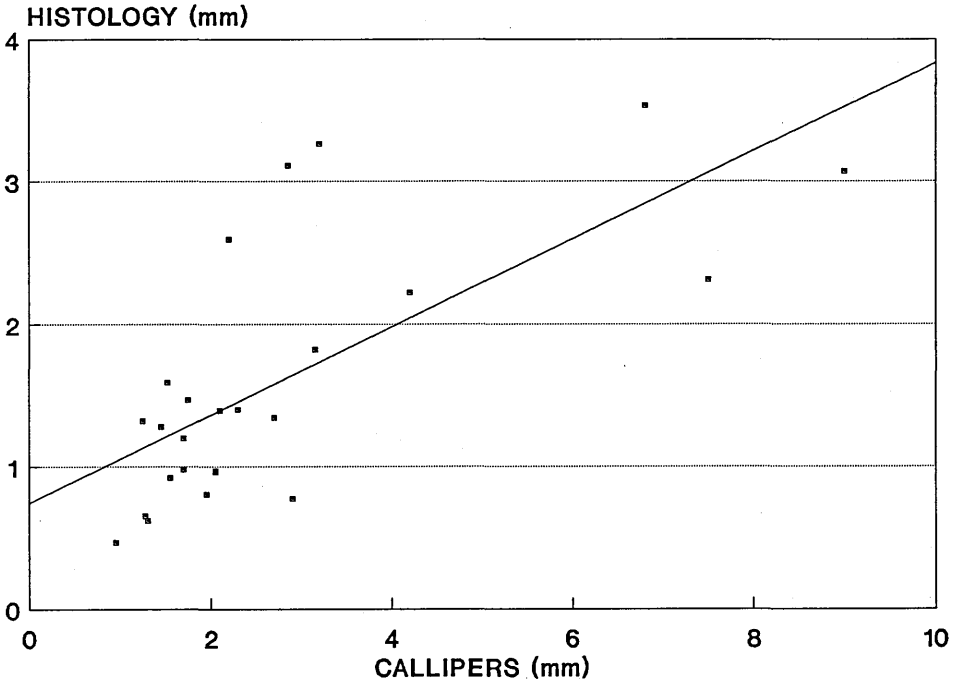
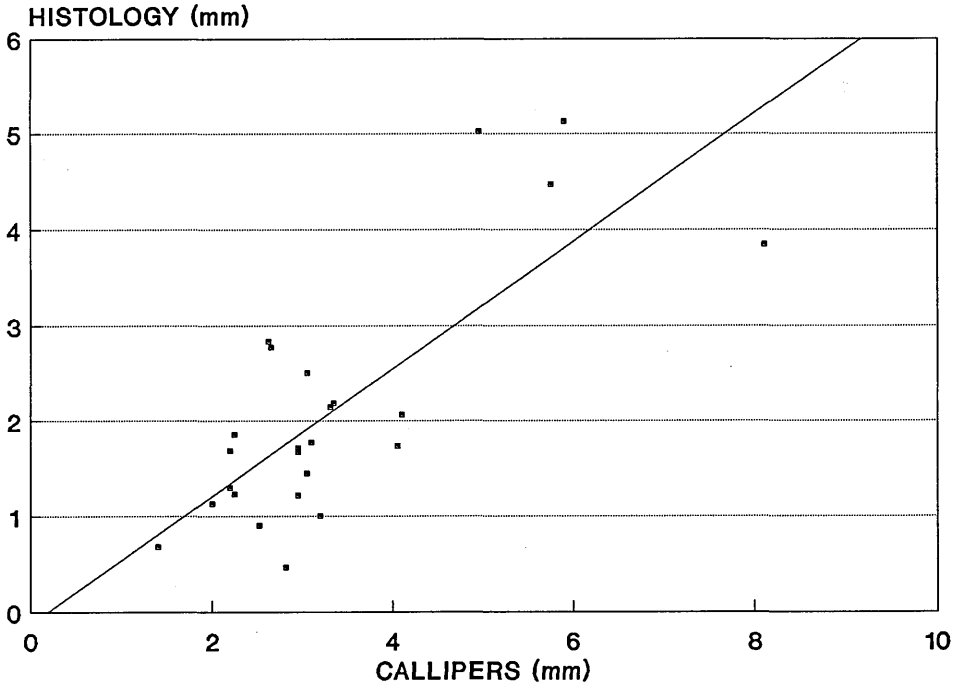


FIGURE 5.11.



5.5. DISCUSSION

The interest in measuring subcutaneous fat arises from the relationship between the peripheral fat depot and total adiposity. Since the 1950s, the measurement of the thickness of subcutaneous fat has been used in multiple studies of human body composition and livestock carcass composition (Edwards, 1950; Edwards and others, 1955; Tanner, 1959; Simm, 1983). By contrast, in dogs, very little attention has been paid to the distribution of body fat and its bearing on the animal's health. There are a two reports in the literature where subcutaneous fat has been measured by means of B-mode ultrasound for assessing body condition in dogs (Houpt and Hintz, 1978; Anderson and Corbin, 1982). The skinfold calliper technique is believed to be of no value (Anderson, 1973; Lewis and others, 1987).

In this experiment, three techniques were used for measuring the thickness of the superficial fat layer of dogs: histology, ultrasound and skinfold callipers.

A conventional histometric method was chosen in order to obtain a set of in vitro measurements of fat thickness against which the ultrasound and calliper techniques could be validated. Since each of the three methods chosen to measure subcutaneous fat relies on a totally different means of obtaining this measurement, it was anticipated that the values found with each technique would not be identical with one another, but the aim was to establish if a trend existed.

Several workers have validated A-mode ultrasonographic units by comparing fat thicknesses obtained with this technique to the same measurements taken by other methods. In livestock industry, both the surgical introduction of a ruler probe along the back of the live animal (Price and others, 1960; Giles and others, 1981) and direct carcass measurements (East and others, 1959; McReynolds and Arthaud, 1970; Bennett and others, 1988) have been used to assess the validity of A-mode ultrasound in predicting backfat thickness and carcass composition. In humans, a high degree of correlation has been established between A-mode ultrasound and measurements of subcutaneous fat thickness obtained with soft-tissue roentgenograms (Haymes and others, 1976), needle puncture (Bullen and others, 1965), and direct visualization following surgical incision (Balta and others, 1981).

These correlations have been taken as indicators of the validity of the ultrasonic technique as a method for measuring subcutaneous adipose tissue.

It is important to note here that most of these workers validated A-mode scans operating at lower frequencies than the one used in this study. Thus, the depth of subcutaneous fat interrogated with our equipment was lower than the one presented in other studies. Using a higher ultrasonic frequency means higher resolution but lower penetration. With our equipment, it was possible to accurately measure the superficial layer of subcutaneous fat, that is, the one comprised between the dermis and the cutaneous muscle.

In the dogs used in this study, a high degree of correlation was found between the ultrasound technique and the histometric method ($r = 0.82$). In general, subcutaneous fat thicknesses measured with histology were slightly larger than the corresponding ones obtained with ultrasound.

Dykes and Marks (1977) found some degree of dermal shrinkage following histological processing of human skin specimens. A similar finding for pig dermal thickness was obtained by Hamlet and others (1986). They found ultrasonic measurements of pig skin thickness slightly larger than the ones obtained with histology. By contrast, Tan and others (1982) found large differences in human skin thickness measured by an A-mode device when compared to histological measurements. They partly attributed these differences to a loss of resting dermal tension when the skin was excised and placed in vitro, such that histological measurements were always greater than the ultrasonic ones. A loss of tissue tension might explain the larger values for fat thickness obtained with histology in this study. However, it is more likely that the slight differences between ultrasound and histology were due to different techniques being employed. Using a B-scan, Anderson and Corbin (1982) found ultrasonic measurements of subcutaneous fat thickness to be 30% to 40% smaller than the corresponding ones taken directly on the carcass of 24 Beagle dogs. However, this difference was probably due to the positioning of the dog at the time of ultrasonic measurement. The hind legs were placed beneath the body and thus, the skin over the top of the back was stretched and resulted in a thinner layer of fat being measured.

Ultrasound and histology correlated better in some anatomical locations than in others. The lumbar area showed the best agreement between the two techniques (r= 0.96), followed by the axilla (r= 0.83), sternum (r= 0.79), thigh (r= 0.77), flank (r= 0.72) and abdomen (r= 0.71).

In people, different body sites have yielded different degrees of correlation between ultrasound and alternative methods of measuring subcutaneous fat. Haymes and others (1976), comparing ultrasound to soft-tissue roentgenograms, found a higher correlation at the mid-triceps site (r= 0.88) than at the suprailiac site (r= 0.78). Volz and Ostrove (1984) found A-mode ultrasound to be more highly correlated with the calliper technique at the suprailiac site than at six other anatomical locations. In cattle and sheep, certain body sites along the back of the animal have shown higher correlations than others when ultrasound and carcass measurements have been compared (Alliston and Hinks, 1981; Leymaster and others, 1985).

The disparity between reports regarding the anatomical locations which give better results in ultrasound scanning are probably due to differences in the type of subjects or animals being examined, differences between machines, differences in methodology and operator's experience.

Nevertheless, a comparison between ultrasonic fat measurements and corresponding histologic measurements in this study, provided a useful evaluation of the A-mode machine. The higher correlation obtained at the lumbar site is attributed to the particular characteristics of the fat layer in this

region. Greater in thickness and relatively free of vasculature when compared to other sites, it was easier to measure with the A-mode. Most echograms obtained from this area showed a clear-cut echolucent gap between the last dermal echo and the muscle fascia, representing the subcutaneous fat layer.

In other anatomical areas, interpretation of the echograms was more difficult due to numerous spurious echoes caused by connective tissue bundles and blood vessels situated among the adipocytes. The greater difficulty in the interpretation of the echograms might explain the lower correlation with histology at these sites.

Interpretation of the A-scan data can be difficult. This modality of ultrasound provides only a one-dimensional linear array representing depths between different tissue interfaces. Actual images of tissues are not available and hence, differentiation of tissue boundaries becomes harder. Using an A-mode device of similar frequency to the one utilized in this study, Alexander and Miller (1979) found that the dermis/subcutaneous fat echo was not easily distinguished from other dermal and fat structures in human skin. In a previously quoted survey, Tan and others (1982), comparing ultrasonic and histologic measurements of human skin thickness, showed that the dermis/subcutaneous fat junction is not uniformly straight. A similar finding was obtained in this study. Invaginations of fat into the dermis in a particular area could make the subcutaneous fat layer appear thicker than in the surrounding area. The multiple measurements taken in this experiment

both with the ultrasonic and histometric techniques helped to overcome this problem.

In the present study, it was found that additional or spurious echoes frequently appeared on the display screen, at the position of the adipose tissue layer. Histology showed abundant connective tissue bundles running through the fat and the spurious echoes were hence attributed to these. The connective tissue present in the subcutis of sheep and carnivores has been shown to be particularly plentiful (Habermehl, 1981). Booth and co-workers (1966) reported finding two distinct discontinuities in the subcutaneous fat of three subjects and intermediate discontinuities on a number of other subjects. They attributed these additional echoes to a membrane running through the subcutaneous tissue. Subsequently, Haymes and others (1976) found more than one discontinuity on the echograms of several subject's subcutaneous fat, and Borkan and others (1982b) found it difficult to remain with a single echo representing the fat/muscle interface in a series of 39 adult men.

It is thought that these additional echoes arise from extraneous membranes dispersed throughout the adipose tissue layer (Volz and Ostrove, 1984). Pigs' backfat comprises two layers of fat separated by a membrane which gives an additional signal on the display screen (East and others, 1959; Giles and others, 1981).

Skinfold calliper measurements of subcutaneous fat did not correlate with histology as well as ultrasonic measurements. The overall correlation coefficient between histology and the calliper technique was r= 0.63.

No study comparing these two techniques was found in the literature. Haymes and others (1976) found a high correlation between calliper and roentgenogram measurements of subcutaneous adipose tissue at the triceps and suprailiac sites of 22 women and 20 men. Besides, the calliper technique has been found to be well correlated with A-mode ultrasound in numerous studies where both methods were utilized to measure subcutaneous fat in humans (Bullen and others, 1965; Booth and others, 1966; Haymes and others, 1976; Volz and Ostrove, 1984; Black and others, 1986).

In this study of 28 dogs, measurements of subcutaneous fat thickness obtained with the Holtain skinfold calliper were poorly correlated with corresponding ultrasonic measurements (r= 0.50). Only in the lumbar area was there a relatively good correlation between the two methods (r= 0.69).

Skinfold callipers are believed to be of little value for measuring subcutaneous fat in dogs because the skin fold appears to lift off the subcutaneous tissue resulting in a false thickness of fat being measured (Anderson, 1973; Lewis and others, 1987). However, no data have been published to confirm this belief.

The disagreement between ultrasound and skinfold callipers in this study, was probably due to intrinsic differences between the two methods. The depth of fat being measured by the A-scan was probably lower than the one detected by the calliper technique. As mentioned above, the high ultrasonic frequency used in this study, allowed the measurement of the layer of fat comprised between the dermis and the cutaneous muscle. This was also the fat layer measured with histology. By contrast, the Holtain skinfold calliper must have measured not only the most superficial subcutaneous fat, but also a certain amount of adipose tissue situated beneath the cutaneous muscle. Moreover, a considerable amount of adipose tissue was found beneath the cutaneous muscle when biopsy specimens were taken for histology. Fat was deposited in successive layers between muscle fasciae and was a few centimetres thick in obese dogs. It is reasonable to suggest that the Holtain calliper was able to measure part, but not all, of this fat depot.

In conclusion, subcutaneous fat thickness in dogs was accurately measured by A-mode ultrasound. There was a high degree of correlation between ultrasonic measurements of subcutaneous fat and the corresponding ones obtained by histological processing. The skinfold calliper technique, on the other hand, was not highly correlated with either ultrasound or histology. This was probably due to inherent differences between techniques, such that the calliper method was including fat situated beneath the cutaneous muscle and hence was measuring a greater thickness of fat.

CHAPTER VI.

PREDICTING TOTAL BODY FAT IN DOGS BY MEANS OF ULTRASOUND, HISTOLOGY AND SKINFOLD CALLIPERS.

CHAPTER VI. PREDICTING TOTAL BODY FAT IN DOGS BY MEANS OF ULTRASOUND, HISTOLOGY AND SKINFOLD CALLIPERS.

6.1. INTRODUCTION.

Obesity in dogs has been shown to be as common a nutritional disease as it is in humans, affecting the animal's general health by contributing to various pathological problems (Hand, 1990). It has been clearly established that obese dogs are at a higher risk of developing diabetes mellitus (Mattheeuws and others, 1984a,b), cardiovascular abnormalities (Rocchini and others, 1987; Wehberg and others, 1990), and decreased resistance to infectious diseases (Newberne, 1966; Williams and Newberne, 1971; Fiser and others, 1972). Furthermore, the fat dog often shows respiratory embarrassment (Lewis, 1978; Stogdale and Moore, 1980) and has to be considered a particular surgical risk (Clutton, 1988). Epidemiological studies have found an association between obesity in dogs and a higher incidence of articulomotor problems (Edney and Smith, 1986) and certain neoplastic conditions (Sonnenschein and others, 1987; Glickman and others, 1989).

In people, the deleterious effects of obesity, have spurred researchers into the task of trying to establish how much fat a person contains and define what is an excessive accumulation of body fat. The management and, more

importantly, the prevention of obesity makes this scientific effort one that is worth while.

Although in Veterinary Medicine obesity is also considered a nutritional health problem, its definition remains largely subjective (Joshua, 1970; Mason, 1970; Anderson, 1973; Lewis, 1978; Andersen and Lewis, 1980; Markwell, 1988b; Clutton, 1988; Markwell and others, 1990).

Various methods of quantifying the amount of fat present in the body, have been devised in humans to assess degree of obesity (reviewed in Chapter 2). In particular, the measurement of the subcutaneous fat depot has been thought to give a good estimate of total adiposity (Edwards and others, 1955; Fletcher, 1962; Mayer, 1980; Garrow, 1982).

Subcutaneous fat has been quantified by methods such as the skinfold technique (Durnin and Womersley, 1974), soft-tissue roentgenogram (Haymes and others, 1976), ultrasound (Volz and Ostrove, 1984), computed tomography (Borkan and others, 1982a), direct needle puncture (Bullen and others, 1965) and infrared interactance (Conway and others, 1984). Fat thickness measurements are subsequently used to predict total adiposity calculated by another method such as density or body potassium. Hence, methods based on the anatomical distribution of tissues (e.g., skinfold callipers and ultrasound) are validated against techniques which describe the chemical composition of the body. The latter are techniques such as body

density, body potassium or body water, based on the assumption that the body can be divided into fat and a fat-free compartment. By estimating one component of the lipid-free body, these techniques are able to estimate the lipid-free mass as a whole and, by subtraction, the fat component (Fuller and others, 1990).

A more reliable validation for a technique estimating body fat is direct chemical analysis of the whole body (Fuller and others, 1990). This method provides an absolute and direct measure of total fat content against which other measurements can be tested for their predictive value. However, whole body chemical analysis is a major undertaking and Garrow (1983b), could only find six such analysis of adult human cadavers in the literature.

Chemical analysis of the whole or part of the carcass has been widely carried out in farm animals to establish the value of other techniques, such as ultrasound, in predicting body composition (Hankins and Ellis, 1934; Barton, 1967; Westervelt and others, 1976; Simm, 1983; Leymaster and others, 1985; Gresham and others, 1986).

The same principle has been used in Beagle dogs, where carcass chemical analysis was carried out to validate an ultrasonic technique (Anderson and Corbin, 1982) and total body water analysis (Sheng and Huggins, 1971; Sheng and others, 1977).

6.2. OBJECTIVES.

Having established the validity of an A-mode ultrasound scan for measuring subcutaneous fat thickness in dogs (Chapter 5), the work presented in this chapter was carried out with the following purposes:

1. To determine if ultrasonic measurements of subcutaneous fat thickness in dogs could be used to predict total body fat calculated by chemical analysis in the same dogs.

2. To evaluate, in like manner, histologic and calliper measurements of fat thickness.

3. To determine which anatomical sites yielded a better prediction of total adiposity.

6.3. MATERIALS AND METHODS.

The same population of stray dogs described in the previous chapter was used in this study.

Subsequent to obtaining calliper and ultrasonic readings of subcutaneous fat thickness, a biopsy specimen had been taken from each of six anatomical sites. The whole carcass was then placed in sterile plastic bags

and kept frozen at -20^{0}C. The carcasses remained in the deep freezer for a period of no more than 16 weeks, until it was possible to perform total chemical analysis. The latter procedure was not carried out in the first three dogs. Their bodies had been disposed of immediately after ultrasonic measurements and biopsy samples for histology were completed.

CARCASS PROCESSING.

Prior to grinding, carcasses were weighed and subsequently cut into pieces of approximately 15 x 6 x 4 cm, using an electrical meat-processing band saw. The carcass was left to defrost at room temperature for approximately 12 hours.

The entire carcass (including viscera) was then ground on a carcass grinder (Karl Schnell GmBH & Co, 7065 Winterbach, Germany), processed once through a 15 mm plate and once through a 5 mm plate. In two or three cases a second pass through the 5 mm plate was carried out in order to obtain a finer sample. The grinder was thoroughly cleaned using hot, soapy water before the next dog carcass was processed. After grinding, the whole carcass was collected in clean, dry, plastic bins and was thoroughly mixed using a shovel.

From each carcass, two samples of approximately 2 kg each were placed in plastic containers and the remaining carcass material was discarded.

The material in the containers was thoroughly mixed and a 150 g sample was obtained from each one, accurately measured to the nearest milligram on an electronic scale and placed on pre-weighed plastic cups.

The material inside the plastic cups was freeze dried (Edwards High Vacuum, Crawley, West Sussex, England) and its water content was extracted for a period of no less than 48 hours. Subsequently, aliquots of the dried samples had their fat content determined by ether extraction.

Ash and nitrogen content were also calculated from aliquots of the dried samples.

DETERMINATION OF FAT CONTENT.

Fat extraction was carried out following two different procedures. Procedure 1 was based on a standard Soxhlet extraction and procedure 2 was based on a hydrolysed fat extraction. The second procedure is recommended for samples with bound fat or with a high fat content, and it was suggested as a "back-up" methodology to the standard Soxhlet extraction (Dr. Wills, J. M., personal communication). An adaptation of the amended regulation 3(2)(b) of the EEC's Feeding Stuffs Regulations 1982 (Her Majesty's Stationery Office, McCorquodale Printers Ltd., p.3) was carried out in this study. It entailed a pre-treatment of the sample by heating with hydrochloric acid 3N during 1 hour. The acid helps release the bound fat in the sample. After heating, the mixture was cooled and 1 or 2 g of filtration aid (Celite Analytical

Filter Aid, BDH Ltd., Poole, England) were added to prevent any loss of fat during filtration. The mixture was then filtered through a fat-free, double filter paper (Whatman 541, Whatman International Ltd., Maidstone, England). The residue on the filter paper was washed with cold water until a neutral filtrate could be obtained. Subsequently, the residue was placed on a watch glass and dried for 90 minutes at 100^0C. The dried residue was then processed following the standard Soxhlet extraction method (Procedure 1). The latter was carried out in the following way: 1 g of the dried sample was weighed to the nearest milligram transferred into an extraction thimble and covered with fat-free cotton wool. The thimble was placed on a Soxhlet extraction apparatus and fat was extracted with ether (boiling range 40-60^0C) for 6 to 8 hours. The ether extract was then collected on a dry, pre-weighed flask and the solvent was removed by distillation. The resulting residue was dried in an oven at 102^0C for 2 hours, cooled and weighed.

DETERMINATION OF PROTEIN CONTENT.

Approximately 0.5 g of dried sample were placed on a semi-automated Kjeldahl apparatus (Kjel-Foss Automatic 16210, A/S N. Foss Electric, Denmark). This apparatus measures the nitrogen content of the sample by digestion with sulphuric acid and potassium sulphate, with a mercury catalyst. Crude protein was then calculated by multiplying the nitrogen content by 6.25.

DETERMINATION OF ASH CONTENT.

A silica crucible basin was heated at 600^0C for 4 to 6 hours, allowed to cool in a desiccator and weighed. Approximately 4 g of the dried carcass sample were placed in the basin and into a muffle furnace. The temperature of the furnace was maintained at 500^0C until a whithish-grey ash was left in the basin.

The basin and its content were allowed to cool in a desiccator and weighed. The weight of the residue was then multiplied by 1000 and divided by the weight of the original aliquot to obtain the number of g/kg of total ash in the sample (Ministry of Agriculture, Food and Fisheries, 1981).

STATISTICAL METHODS.

A two-sample T-test (Eason, Coles and Gettinby, 1980) was used to assess differences between two sets of data such as fat content measured by the Soxhlet and fat content measured by the modified ether extraction methods.

One way analysis of variance and the Newman-Keuls range test (Snedecor and Cochran, 1967b) were used to detect differences in fat, protein and ash content between groups of dogs with differing body condition score.

Simple linear regression (Wardlaw, 1985) was used to explain the variation in total body fat by means of fat thickness measurements at any one anatomical area. All anatomical areas were combined to provide a predictive

value for total adiposity based on multiple regression analysis (Snedecor and Cochran, 1967c).

Finally, the relationship between variables was graphically represented by plotting best fitted straight lines (Wardlaw, 1985).

The level of significance was chosen to be 5% ($p < 0.05$).

6.4. RESULTS.

FAT CONTENT.

The two methodologies employed to calculate total body fat yielded very similar results (Appendix 6.1). Statistical analysis showed no significant difference between the two methods. Consequently, only one set of results (the Soxhlet fat) was used for comparison with histology, ultrasound and skinfold callipers.

The amount of fat in this group of dogs varied widely. Values of fat content, expressed as a percentage of whole body tissue, ranged from 7.7% to 45.2%. On average, bitches were found to have 6% more fat than dogs, but the difference was not statistically significant. Water content was obtained by subtracting calculated dry matter from the original tissue. The higher the fat content in the body, the lower was the calculated water content (Appendix 6.2; Figure 6.1).

In general, dogs which had been classified as "obese" using the 5-point clinical scale, showed higher fat contents than animals classified as "normal" or "thin" (Table 6.1). However, this was not always the case and a significant variation was found in the fat content of dogs clinically classified in the same group. For instance, dog no.7 , a pit bull terrier classified as "normal" had a fat content of 9.2%. By contrast, dog no.18, a Welsh Corgi, also classified as "normal", had 37.8%, a fat content in line with dogs classified as "obese".

Statistical analysis showed a significant difference in fat content between both the "thin" (mean= 11.8% ; SD= 5.05) and "normal" (mean= 20.1% ; SD= 9.63) groups of dogs when compared to the "fat" group (mean= 36.5% ; SD= 5.83). There was no significant difference in fat content between dogs classified as "normal" and those classified as "thin". The insufficient number of dogs evaluated as "gross" or "emaciated" did not allow statistical comparison with the other condition score groups. However, it is interesting to note that the greatest fat content was obtained from the one dog (no.17) classified as "gross" in this series (Table 6.1). Similarly, the carcass containing less amount of fat was that of dog no.12, clinically assessed as "emaciated".

The differences in total fat percentage between the various body condition score groups are represented in Figure 6.2.

FIGURE 6.1.

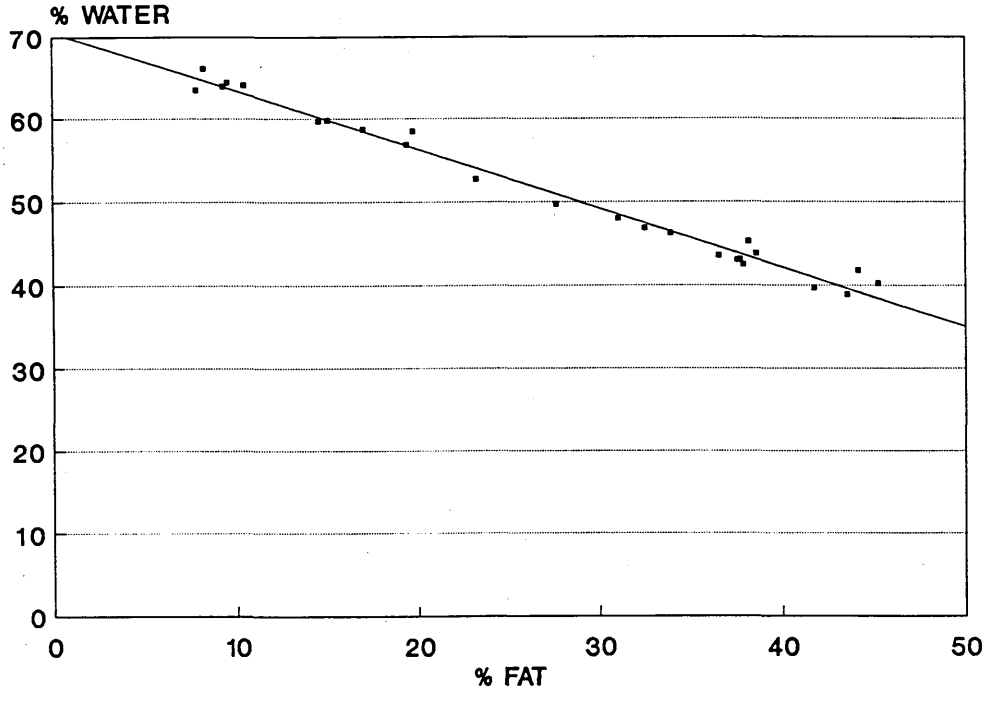


FIGURE 6.1. The relationship between water and fat content in 25 dog carcasses.

TABLE 6.1.

Comparison between body condition score, some physical parameters and total body fat in 25 dogs.

	B.C.S.	AGE GROUP[1]	SEX*	B.W.	T.B.F.
Dog 4	Thin	Young	Female	16	19.3
Dog 5	Normal	Young	Male	18.2	14.5
Dog 6	Normal	Young	Male	21	16.9
Dog 7	Normal	Adult	Male	26	9.2
Dog 8	Thin	Young	Female	18	10.4
Dog 9	Normal	Young	Male	10.2	19.6
Dog 10	Thin	Young	Female	18	9.5
Dog 11	Fat	Young	Female	20.8	37.7
Dog 12	Emaciated	Adult	Male	20.8	7.7
Dog 13	Fat	Young	Female	19.6	31.0
Dog 14	Thin	Young	Female	20.2	8.2
Dog 15	Normal	Young	Male	18.8	15.0
Dog 16	Fat	Old	Female	19.8	36.5
Dog 17	Gross	Old	Male	44	45.2
Dog 18	Normal	Old	Male	10.2	37.8
Dog 19	Fat	Old	Female	20	44.1
Dog 20	Fat	Old	Female	13.8	38.1
Dog 21	Fat	Old	Female	11.2	38.6
Dog 22	Fat	Old	Female	10.2	33.9
Dog 23	Normal	Old	Male	22	27.6
Dog 24	Fat	Old	Female	20.6	43.5
Dog 25	Fat	Old	Male	29.6	37.5
Dog 26	Fat	Young	Female	12.3	32.5
Dog 27	Fat	Young	Female	24.2	41.7
Dog 28	Fat	Young	Female	14.4	23.2

[1] Estimated by the wear and tear of dentition.

* All males were entire. Sexual status of females was not determined.

Young: 6 months to 3 years / Adult: 3 to 6 years / Old: >6 years.

Body weight is in kg.

Total body fat is expressed as a percentage of whole body tissue.

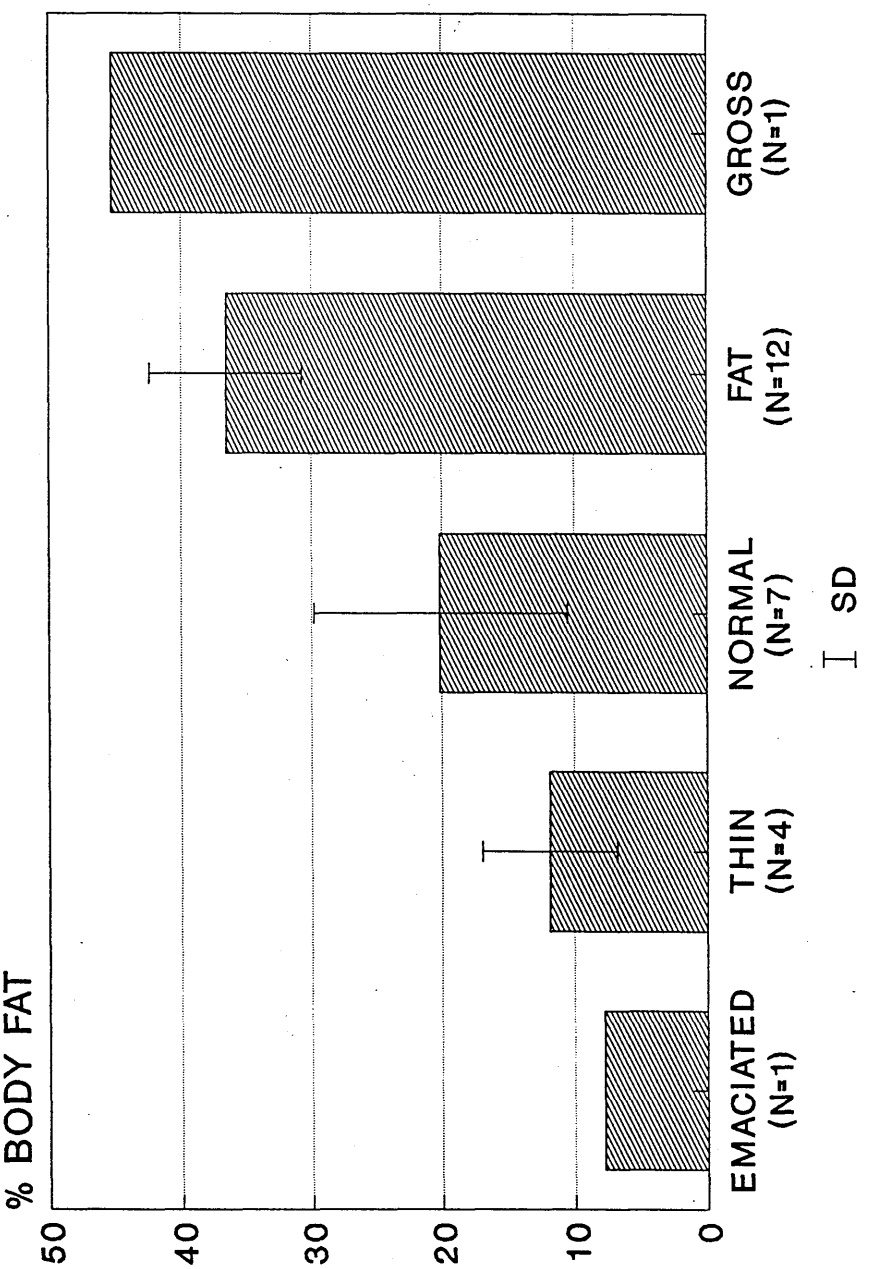


FIGURE 6.2. Body fat percentage of dogs in the different condition score groups.

PROTEIN AND ASH CONTENT.

The protein and ash content of the 25 dog carcasses, expressed as a percentage of whole body tissue, is presented in Appendix 6.3. The protein content was found to range from 12.3% to 21.4% (mean= 17.3%). There was a strong negative correlation (r= -0.89) between the protein and fat percentages; the higher the fat content, the lower the protein. Statistical analysis showed a significant difference in protein content between groups of dogs with different body condition scores. Both the "thin" and "normal" groups had a significantly greater protein content when compared to the "fat" group.

The percentage of ash in the dried carcass material ranged from 2.1% to 4.7% (mean= 3.3%). Dogs clinically classified as "fat" had a significantly lower ash content than dogs in the "normal" group.

The body composition of these 25 dogs is graphically represented in Figure 6.3. Carbohydrates were not included because the technical error of chemical analysis usually exceedes the figure of 1% to 2% sugar content normally found in dogs (Meyer and Stadtfeld, 1978).

PREDICTION OF TOTAL BODY FAT.

Measurements of subcutaneous fat thickness obtained with histology at six anatomical areas were used to predict total adiposity (Table 6.2). Although there was a statistically significant association between percentage body fat

and fat thickness measurements in all body sites, correlation coefficients were not very high. Only in the anatomical area defined as flank was there a high coefficient of correlation ($r = 0.71$; $p < 0.001$) between the two sets of data.

Ultrasonic fat thickness measurements did not predict percentage body fat more accurately than the corresponding histological ones (Table 6.3). The association between total adiposity and subcutaneous fat measured with ultrasound, was not statistically significant at the sternum and thigh areas. At other body sites, a significant association was found but coefficients of correlation were somewhat low.

Similar results to the ones obtained with histology were found when calliper measurements of subcutaneous fat were used to predict total body fat. A statistically significant association was found in all body sites (Table 6.4). Correlation coefficients were generally lower than the ones obtained between histology and total body fat. The highest association between calliper measurements and percentage body fat was found at the sternum site ($r = 0.71$; $p < 0.001$).

When all body sites were used as predictor variables in the regression equation, it was found that histology was able to account for 66% of the variation in body fat percentage. On the other hand, ultrasound explained 43% of the variation in total body fat, and the calliper technique explained 58% (Table 6.5).

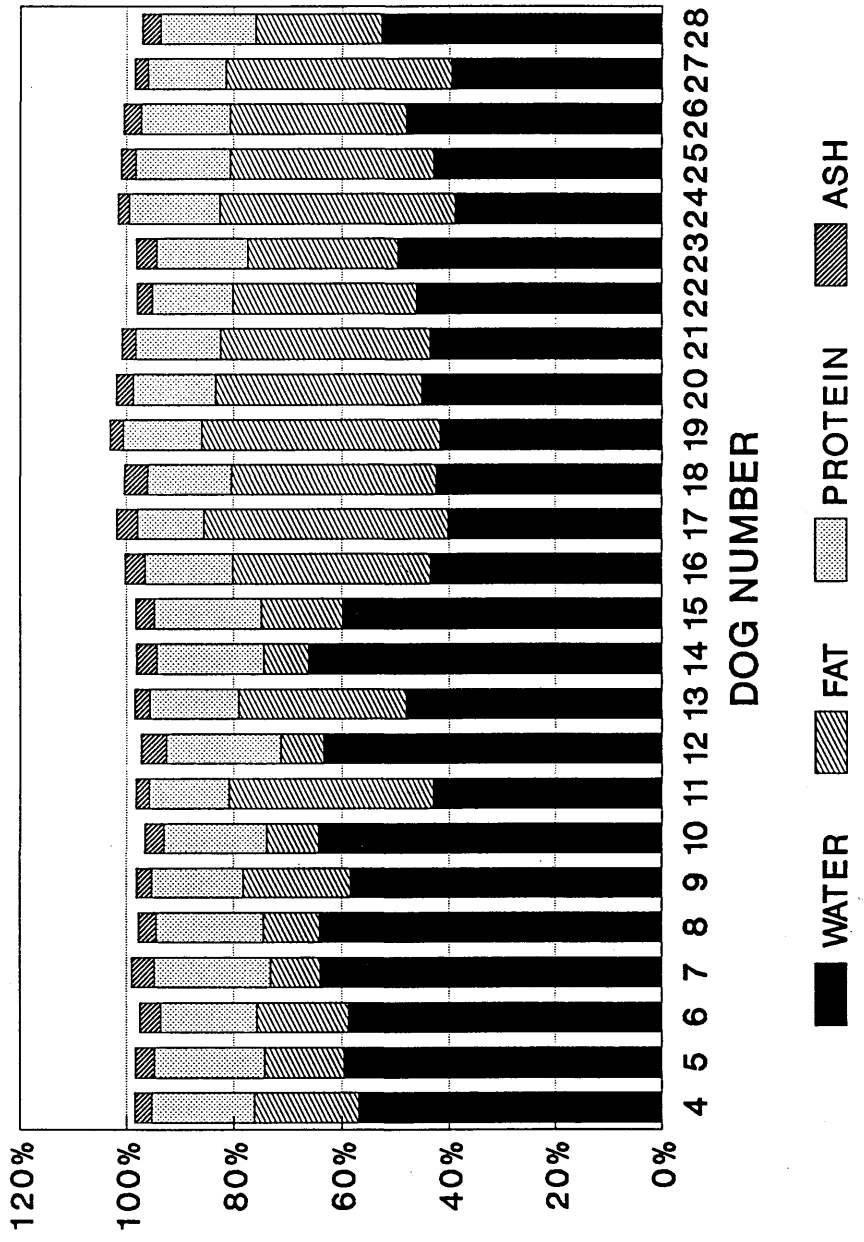


FIGURE 6.3. Body composition in 25 dogs.

TABLE 6.2.

Regression line parameters for the estimation of percentage body fat using histological measurements of subcutaneous fat thickness in 25 dogs.

BODY SITE	INTERCEPT	SLOPE	r	p
Axilla	13.0	8.88	0.61	< 0.001
Flank	8.99	15.4	0.71	< 0.001
Sternum	16.5	7.60	0.52	< 0.01
Abdomen	13.4	10.4	0.64	< 0.001
Thigh	18.0	7.60	0.65	< 0.001
Lumbar	18.1	4.31	0.42	< 0.05

TABLE 6.3.

Regression line parameters for the estimation of percentage body fat using ultrasonic measurements of subcutaneous fat thickness in 25 dogs.

BODY SITE	INTERCEPT	SLOPE	r	p
Axilla	11.3	14.1	0.48	< 0.05
Flank	13.6	14.6	0.45	< 0.05
Sternum	15.9	10.2	0.33	n.s.
Abdomen	12.6	13.4	0.53	< 0.01
Thigh	20.3	7.01	0.28	n.s.
Lumbar	17.3	4.88	0.40	< 0.05

TABLE 6.4.

Regression line parameters for the estimation of percentage body fat using calliper measurements of subcutaneous fat thickness in 25 dogs.

BODY SITE	INTERCEPT	SLOPE	r	p
Axilla	16.6	3.84	0.60	< 0.001
Flank	7.91	9.14	0.66	< 0.001
Sternum	7.83	7.33	0.71	< 0.001
Abdomen	14.0	6.0	0.54	< 0.005
Thigh	20.6	3.94	0.45	< 0.05
Lumbar	13.1	4.20	0.47	< 0.05

TABLE 6.5.

Multiple regression parameters for the estimation of percentage body fat in 25 dogs.

PARAMETER	HISTOLOGY	ULTRASOUND	CALLIPERS
R-sq	66.7%	43.2%	58.1%
R-sq (adj)	54.2%	24.2%	44.1%
s	8.71	11.31	9.71

If body weight was added as one more predictor variable in the equation, there was only a slight improvement in the above percentages (Table 6.6).

Thus, multiple regression analysis showed that there was considerable unexplained variation in the percentage of body fat.

To investigate differences in body fat distribution, which might have accounted for the unexplained variation in percentage body fat, the set of 25 dog carcasses was divided into two groups: one group composed of those animals classified as young or adult (15 carcasses), and another group composed of dogs over 6 years of age (10 carcasses). The latter group included animals which showed signs of endocrine disease (see discussion).

Prediction of total body fat in young and adult dogs.

Histological measurements of subcutaneous fat in young and adult dogs were used to predict their percentage of body fat (Table 6.7). A statistically significant association between total adiposity and subcutaneous fat was found in all anatomical areas with the exception of the axilla. Correlation coefficients were particularly high at the flank ($r = 0.83$) and lumbar ($r = 0.75$) areas. Figures 6.4 and 6.5 represent the association between total body fat and histological fat thickness measurements at the flank and lumbar areas, respectively.

By contrast, linear regression analysis showed that ultrasonic fat thicknesses did not accurately predict percentage body fat in young and adult dogs (Table 6.8). Only in the lumbar area (r= 0.66) was there a significant association between percentage body fat and ultrasonic fat (Figure 6.6).

On the other hand, the calliper technique predicted total fat with a similar degree of accuracy as that of histology in young and adult dogs. The association between subcutaneous fat thickness and percentage body fat was statistically significant in all body sites with the exception of the abdomen (Table 6.9). A high degree of correlation was found at the sternum (r= 0.89), lumbar (r= 0.82) and flank (r= 0.75) areas. Figures 6.7 and 6.8 illustrate the association between total body fat and fat thickness measurements obtained with the callipers at the sternum and lumbar areas.

When all anatomical areas were used as predictor variables in the regression equation, it was found that histological fat thickness measurements were able to account for 90% of the variation in total body fat (Table 6.10). Ultrasonic fat measurements explained 60% of the variation in total body fat, while calliper measurements explained 88%.

With the exception of the calliper technique, these percentages were not significantly improved by using body weight as another predictor variable in the regression equation. Calliper measurements, in combination with body weight, were able to account for 96% of the variation in fat content.

TABLE 6.6.

Multiple regression parameters for the estimation of percentage body fat in 25 dogs, when body weight was included in the equation.

PARAMETER	HISTOLOGY	ULTRASOUND	CALLIPERS
R-sq	71.9%	44.8%	65.2%
R-sq (adj)	58.8%	22.0%	50.8%
s	8.72	11.47	9.11

TABLE 6.7.

Regression line parameters for the estimation of percentage body fat using histological measurements of subcutaneous fat thickness in young and adult dogs.

BODY SITE	INTERCEPT	SLOPE	r	p
Axilla	12.4	6.11	0.34	n.s.
Flank	5.36	13.8	0.83	< 0.001
Sternum	10.8	7.20	0.56	< 0.05
Abdomen	11.9	7.70	0.56	< 0.05
Thigh	7.53	17.3	0.66	< 0.01
Lumbar	6.18	6.21	0.75	< 0.001

TABLE 6.8.

Regression line parameters for the estimation of percentage body fat using ultrasonic measurements of subcutaneous fat thickness in young and adult dogs.

BODY SITE	INTERCEPT	SLOPE	r	p
Axilla	9.71	10.1	0.29	n.s.
Flank	6.86	15.3	0.43	n.s.
Sternum	12.9	6.73	0.27	n.s.
Abdomen	11.0	9.29	0.47	n.s.
Thigh	29.8	-11.9	-0.34	n.s.
Lumbar	6.94	6.17	0.66	< 0.01

TABLE 6.9.

Regression line parameters for the estimation of percentage body fat using calliper measurements of subcutaneous fat thickness in young and adult dogs.

BODY SITE	INTERCEPT	SLOPE	r	p
Axilla	-0.62	11.8	0.51	< 0.05
Flank	-11.0	18.1	0.75	< 0.001
Sternum	-0.05	8.73	0.89	< 0.001
Abdomen	3.06	9.42	0.44	n.s.
Thigh	-3.8	21.1	0.51	< 0.05
Lumbar	1.11	5.47	0.82	< 0.001

TABLE 6.10.

Multiple regression parameters for the estimation of percentage body fat in young and adult dogs.

PARAMETER	HISTOLOGY	ULTRASOUND	CALLIPERS
R-sq	90.7%	60.6%	88.5%
R-sq (adj)	81.4%	31.1%	79.9%
s	5.03	9.26	5.01

FIGURE 6.4. Best-fitted straight line between histological measurements of subcutaneous fat in the flank area of young and adult dogs and percentage body fat. (n=15; y= 5.36 + 13.8x)

FIGURE 6.5. Best-fitted straight line between histological measurements of subcutaneous fat in the lumbar area of young and adult dogs and percentage body fat. (n=15; y= 6.18 + 6.21x)

FIGURE 6.4.

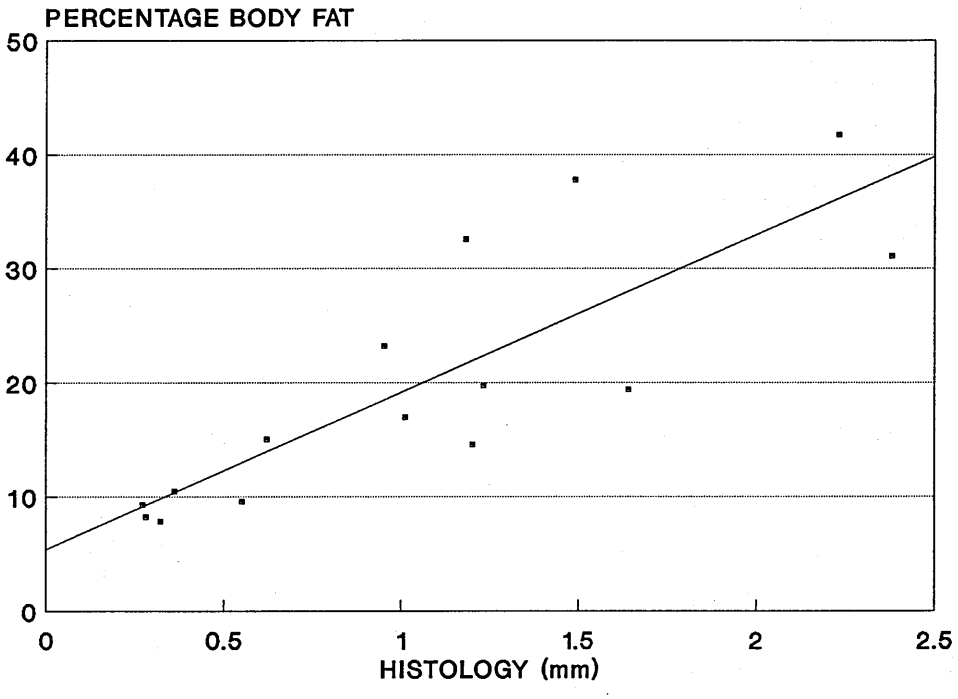


FIGURE 6.5.

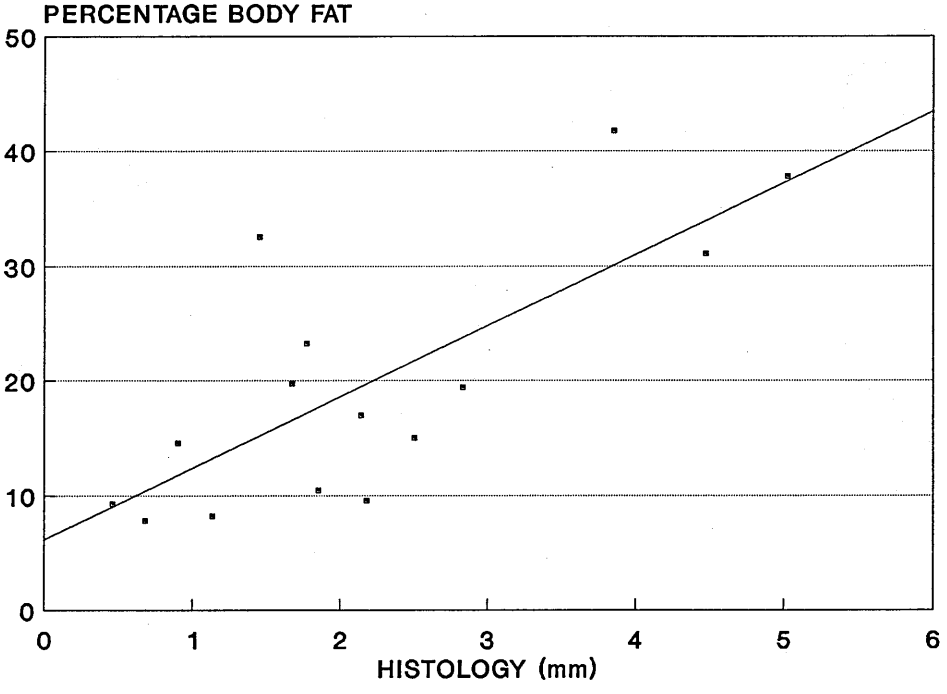


FIGURE 6.6. Best-fitted straight line between ultrasonic measurements of subcutaneous fat in the lumbar area of young and adult dogs and percentage body fat. (n=15; y= 6.94 + 6.17x)

FIGURE 6.7. Best-fitted straight line between calliper measurements of subcutaneous fat in the sternum area of young and adult dogs and percentage body fat. (n=15; y= -0.05 + 8.73x)

FIGURE 6.6.

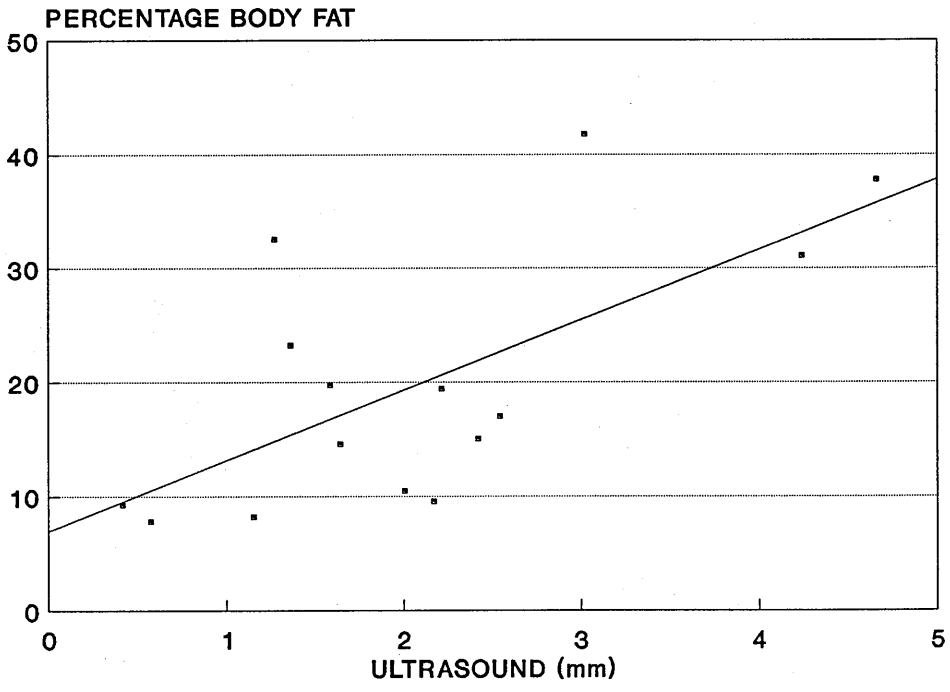


FIGURE 6.7.

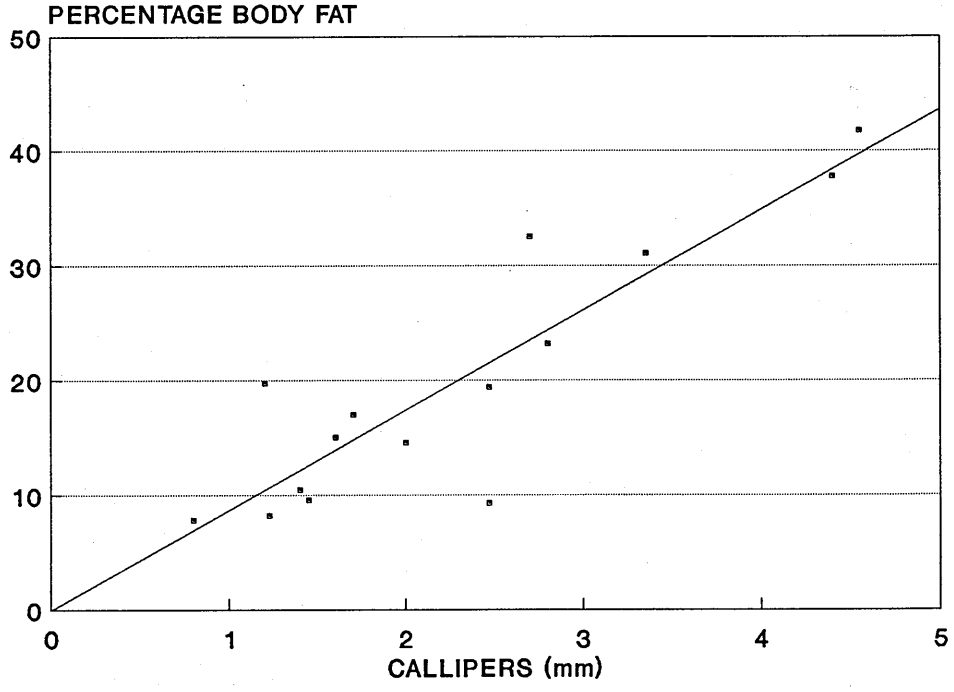


FIGURE 6.8.

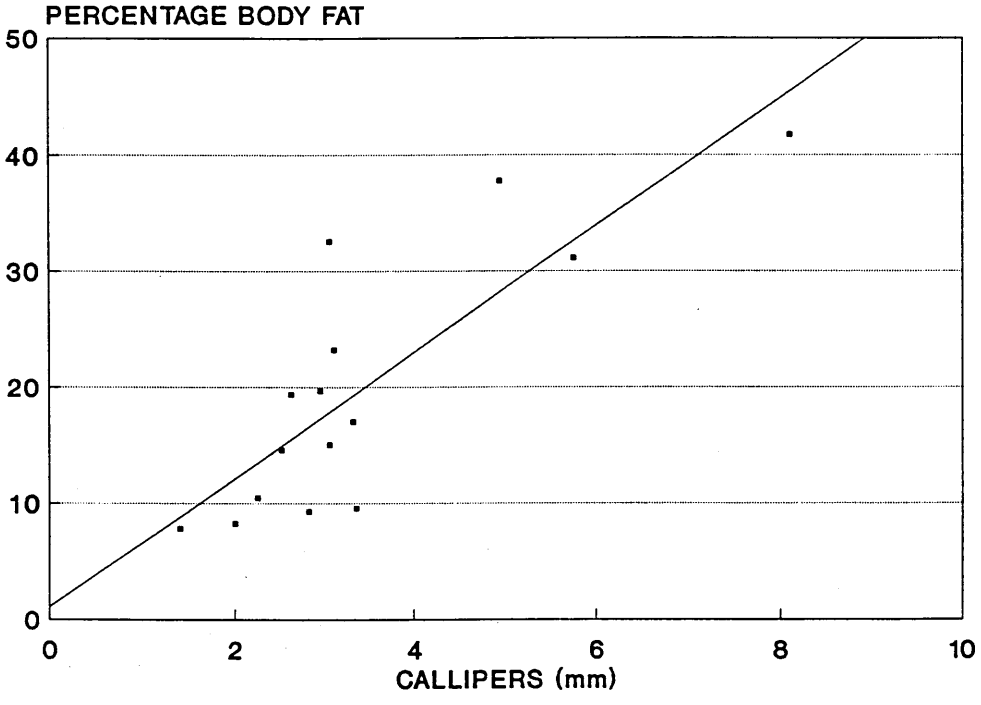


FIGURE 6.8. Best-fitted straight line between calliper measurements of subcutaneous fat in the lumbar area of young and adult dogs and percentage body fat. (n=15; y= 1.11 + 5.47x)

Prediction of total body fat in old dogs.

Measurements of subcutaneous fat thickness obtained by histology in those animals over 6 years of age, were used to estimate their total fat content (Table 6.11). The association between percentage body fat and histological measurements was statistically significant at only two body sites: the axilla ($r = 0.67$) and thigh ($r = 0.69$). Coefficients of correlation were generally low.

A similar trend was obtained with ultrasound (Table 6.12). Fat thickness measurements obtained with this technique were only significantly associated with overall fat content at the thigh area ($r = 0.68$).

Calliper fat measurements were not significantly associated with percentage body fat at any of the six anatomical locations defined on the carcass (Table 6.13).

When multiple regression analysis was carried out using all anatomical areas as predictor variables, it was found that almost 80% of the variation in total body fat was explained by histology, 61% by ultrasound and 63% by the calliper technique (Table 6.14).

As was the case in young and adult dogs, when body weight was added to the regression equation, little change was found in the predictive value of histological and ultrasonic measurements. However, the predictive value of

TABLE 6.11.

Regression line parameters for the estimation of percentage body fat using histological measurements of subcutaneous fat thickness in old dogs.

BODY SITE	INTERCEPT	SLOPE	r	p
Axilla	30.2	3.70	0.67	< 0.05
Flank	31.3	5.08	0.40	n.s.
Sternum	34.0	2.42	0.38	n.s.
Abdomen	37.6	0.39	0.03	n.s.
Thigh	33.3	2.54	0.69	< 0.05
Lumbar	33.4	2.51	0.58	n.s.

TABLE 6.12.

Regression line parameters for the estimation of percentage body fat using ultrasonic measurements of subcutaneous fat thickness in old dogs.

BODY SITE	INTERCEPT	SLOPE	r	p
Axilla	30.3	6.05	0.62	n.s.
Flank	32.9	5.15	0.49	n.s.
Sternum	34.2	3.37	0.24	n.s.
Abdomen	34.4	2.96	0.19	n.s.
Thigh	32.2	5.08	0.68	< 0.05
Lumbar	32.1	3.15	0.55	n.s.

TABLE 6.13.

Regression line parameters for the estimation of percentage body fat using calliper measurements of subcutaneous fat thickness in old dogs.

BODY SITE	INTERCEPT	SLOPE	r	p
Axilla	33.9	1.02	0.48	n.s.
Flank	32.6	2.09	0.45	n.s.
Sternum	34.0	1.35	0.33	n.s.
Abdomen	32.6	1.99	0.60	n.s.
Thigh	36.2	0.85	0.34	n.s.
Lumbar	32.9	1.68	0.37	n.s.

TABLE 6.14.

Multiple regression parameters for the estimation of percentage body fat in old dogs.

PARAMETER	HISTOLOGY	ULTRASOUND	CALLIPERS
R-sq	79.9%	61.6%	63.8%
R-sq (adj)	39.6%	0.0%	0.0%
s	4.07	5.62	5.46

calliper measurements was clearly improved when body weight was included in the equation: 80% of variation in fat content could then be explained.

6.5. DISCUSSION.

The two methodologies employed to calculate total fat content, namely a Soxhlet fat extraction and a hydrolysed fat extraction, yielded similar results. Even though two different aliquots were used, the calculated fat content normally differed by less than 1.5%. In general, the hydrolysed fat extraction was able to obtain a few more grams of fat but the difference with the Soxhlet extraction was statistically negligible.

For evaluation with subcutaneous fat thickness measurements, the Soxhlet fat was chosen because it is a widely used, standardized technique. However, in future studies involving dog carcass material, especially when obese dogs are included, it is probably recommendable to use the hydrolysed fat extraction, since this method achieves a more complete extraction of lipid content.

A remarkable variability in fat content was found in the 25 dog carcasses analysed in this study. Total fat, expressed as a percentage of whole body tissue, ranged from 7.7% to 45.2%. This range of fat content was very similar to that found by Anderson and Corbin (1982). They analysed the body composition of 24 Beagle bitches after discarding their viscera and obtained body fat contents ranging from 4.5% to 45.2%.

Fat content was negatively correlated to water content (r= -0.99). The greater the accumulation of fat, the lower the percentage of water in the body. This linear negative relationship between the water and fat content of animals has been known for a long time (Moulton, 1923). The total body water of man and domestic animals is variable and depends mainly on the amount of fat present in the body. Thus, a lean animal might have 70% of its weight in water whilst an obese one will only have 45% (Reece, 1991).

In their analysis of 60 dogs, Meyer and Stadtfeld (1978) showed that the water content of the body was mainly determined by the proportion of fat. They found a strong negative correlation (r= -0.95) between the fat and water content of dogs. The range of water contents found in the 25 dogs analysed in this study, were similar to those found by Meyer and Stadtfeld. However, the mean water content obtained by these authors (56%) was slightly greater than the one found in the present study (51%). The large proportion of fat dogs included in the present work (almost half of the total) probably accounts for the difference.

The lowest fat content found by Meyer and Stadtfeld was 10%. Some dogs analysed in the present study were in poor nutritional condition and yields of less than 10% body fat were obtained. Dog no.12, assessed as "emaciated", had only 7.7% of body fat. Gunn (1978) could find no dissectible fat in the carcass of some athletic greyhounds.

Widdowson, McCance and Spray (1951) analysed the body composition of an adult human male who had died of infective endocarditis

and found a fat content of 1.1%. The authors concluded that almost non-existent body fat stores seem to be compatible with life, if not with health.

Dogs classified as "thin" in this study had, nevertheless, a significant amount of fat located in the lumbar area, underneath the skin and between successive muscle fasciae. This area seems able to sustain considerable amount of fat in dogs, even after substantial weight loss.

Three dogs in this series had a fat content over the maximum of 41% obtained by Meyer and Stadtfeld (1978). One of them had been clinically classified as "gross" and yielded a fat content of 45.2%.

Meyer and Stadtfeld (1978) found a greater amount of fat in bitches when compared to dogs. In the present study, bitches had on average 6% more fat than males but the difference was not statistically significant. In any case, the 25 dog carcasses analysed in this study did not constitute a representative sample of the general dog population and more females (15 out of 25) were represented in the series.

Mean protein content was 17.3% of the original carcass material and, in general, a lower protein content was accompanied by a greater percentage of body fat. Incidentally, the largest amount of protein was obtained from dog no.7, an adult male pit bull terrier, probably reflecting the great muscle development in this breed.

Both the protein and ash contents found in the 25 dog carcasses analysed in this study, were very similar to the ones obtained by other authors

(MacFarlane, 1976; Meyer and Stadtfeld, 1978). No figures for protein or ash content were given by Anderson and Corbin (1982) in their analysis of 24 Beagle dogs. These authors had clipped all the hair coat of the animals and it would have been interesting to compare the protein content of their dogs with the ones found in this study.

The 5-point scale used in this study for assessing body condition (Chapter 5) was able to detect broad differences in fat content. Based on a clinical evaluation of fat storage over the body, dog no.17 had been classified as "gross" and actually yielded the largest fat content in this series of 25 dog carcasses. The clinical scale also detected the leanest dog in the series. Classified as "emaciated", dog no.12, an adult Alsatian cross, had a fat content of 7.7%.

Statistical analysis showed that there was a significant difference in body fat content between dogs classified as "fat" and those classified as either "normal" or "thin". However, no difference was found between the "normal" and "thin" groups. Moreover, the variation in fat content among dogs of the same condition score was noteworthy. Dog no.4, for example, classified as "thin", had much more fat than other dogs in the same group. Similarly, a Welsh Corgi classified as "normal" (dog no.18) yielded a percentage of body fat (37.8%) more in line with the values obtained for fat dogs; and dog no.25, a Collie-cross thought to be obese, had a fat content (23.2%) which could well have been within the "normal" score.

The foregoing observations would seem to indicate the limitation of the clinical scoring system in assessing fat content in dogs. It is a valuable method for detecting broad differences in body condition but provided little information about the actual amount of fat present in the body.

In humans, a visual assessment of the patient's body condition is thought to provide a qualitative estimate of obesity (Mayer, 1980). However, little information is gained about the quantity of fat that person is carrying. For example, Widdowson and co-workers (1951) analysed the body composition of four human cadavers. They found a greater percentage of fat in the body of a "thin" woman when compared to the fat content of an adult male thought to be "overweight".

The predictive value of subcutaneous fat thickness measurements to estimate total body fat content was analysed. When all 25 dog carcasses were included, it was found that prediction of total body fat varied depending on technique and anatomical location.

Histological measurements of fat thickness obtained from all six anatomical areas, were significantly correlated with the percentage of body fat in the carcass. The best site for prediction of fat content appeared to be the flank ($r = 0.71$; $p < 0.001$). A similar trend was obtained when calliper measurements of subcutaneous fat were used in the prediction. All anatomical areas showed a significant correlation with percentage body fat but with this technique the sternum appeared to be the best site for prediction ($r = 0.71$; $p < 0.001$).

Ultrasonic fat thickness measurements, on the other hand, were not significantly correlated with fat percentage in the carcass at the sternum and at the thigh. The A-scan data obtained from these two sites had been more difficult to interpret than in other anatomical sites (Chapter 5). Errors introduced in measuring the subcutaneous fat layer on the echograms, might explain the lack of correlation with total body fat at these two locations.

Although a significant correlation was found between fat percentage in the carcass and subcutaneous fat thickness at most anatomical locations, nevertheless correlation coefficients were generally low. Moreover, when fat measurements from all anatomical areas were used in the prediction equation, statistical analysis showed that a considerable percentage of variation in body fat content remained unexplained. Over 30% of variation was unexplained by histological measurements whilst ultrasound failed to account for more than half of the variation.

A possible explanation for the unaccounted differences in fat content, were the 10 old dogs included in this study. These animals were all over 6 years of age and many showed signs of having suffered some pathological condition. Dog no.22 had a large testicular mass whilst dogs no.23, 24, 25 and 29, all presented signs suggestive of Cushing's syndrome, such as an obvious pendulous abdomen (pot-bellied appearance), bilaterally symmetrical alopecia and generalized thinning of the skin.

It is well known that in some endocrine disorders such as hyperadrenocorticism (Cushing's syndrome), a redistribution of body fat stores ensues, such that a greater amount of adipose tissue is located intra-abdominally (Heinbecker and Pfeiffenberger, 1950; Dumon, 1988; Feldman, 1989). Furthermore, a sharp increase in fat deposition after 5 years of age has been found in dogs (Mason, 1970) and in obese, older dogs, fat can accumulate around internal structures such as the iliac crests (Brown, 1987).

In humans, advancing age and increasing fatness can bring about changes in body fat distribution, and more fat will tend to be deposited internally (Brožek and Keys, 1950; Leibel and others, 1989; Schwartz, Shuman, Bradbury, Cain, Fellingham, Beard, Kahn, Stratton, Cerqueira and Abrass, 1990). Scherf and co-workers (1986) found that skinfold calliper measurements of subcutaneous fat did not accurately predict total body fat in formerly obese adults. These patients had undergone rapid and pronounced weight reduction. It was thought that a disproportion between the subcutaneous and internal fat depots had ensued such that subcutaneous fat measurements did not reflect overall fatness.

In the present study, when subcutaneous fat measurements obtained from 10 old dogs were used to predict total body fat, a low degree of correlation was found at most anatomical sites. Fat thickness measurements obtained with histology were significantly associated with total body fat only at the axilla ($r = 0.67$; $p < 0.05$) and thigh ($r = 0.69$; $p < 0.05$) areas. Similarly,

ultrasonic measurements of subcutaneous fat were significantly associated with total fat only in one anatomical site: the thigh ($r = 0.68$; $p < 0.05$). A poorer correlation still, was found between the calliper technique and the percentage of fat in the carcass. These two sets of measurements were not significantly correlated at any of the six anatomical areas.

In these old dogs, there was considerable variation in body fat percentage which remained unexplained by either histology, ultrasound or the calliper technique.

However, when subcutaneous fat thickness measurements obtained from young and adult dogs were used for predicting their total fat content, a good correlation was generally found. Thus, histological measurements of subcutaneous fat were able to explain 90% of the variation in total fat. The best sites for predicting fat content with histology, were the flank ($r = 0.83$; $p < 0.001$) and the lumbar ($r = 0.75$; $p < 0.001$). A similar trend was obtained with the calliper technique, which was able to explain 88% of the variation in body fat content. The sternum ($r = 0.89$; $p < 0.001$), lumbar ($r = 0.82$; $p < 0.001$) and the flank ($r = 0.75$; $p < 0.001$), were the best sites for prediction.

Ultrasound, however, could only explain 60% of the variation in the percentage of body fat, and only at the lumbar area was there a significant correlation between subcutaneous fat and total fat ($r = 0.66$; $p < 0.01$).

Thus, it would appear that a different distribution of fat stores in the 10 old dogs included in this study, had a negative influence in the predictive

value of fat thickness measurements. A greater deposition of fat internally, due to age and/or disease, may have caused a disproportion between the peripheral and internal fat depots rendering subcutaneous fat measurements of little value in predicting total fat.

By contrast, in young and adult healthy dogs, subcutaneous fat had a value in predicting total body fat. Histological measurements of subcutaneous fat were the most reliable estimators of body fat content in these dogs, followed by the calliper technique and ultrasound.

Body weight slightly improved the predictive value of histological and ultrasonic measurements and notably increased the predictive value of calliper measurements, when included as another variable in the prediction equation. It might be that calliper measurements were generally more associated with body weight than either histological or ultrasonic measurements. In fact, calliper measurements were better predictors of body weight than either histology or ultrasound (Appendix 6.4). However, the improvement in predicting total body fat when body weight was included in the equation is difficult to explain, on the basis of the very low correlation found between body weight and total fat in these 25 dogs ($r = 0.14$; $p > 0.1$).

The predictive value of the ultrasonic technique was somewhat disappointing. Although fat thickness measurements obtained with ultrasound at the lumbar area were significantly correlated with total body fat,

ultrasound was inferior to histology and the calliper technique for predicting fat percentage.

The discrepancy in the accuracy of predicting total body fat between histology and ultrasound was unexpected, as the two techniques had been shown to be highly correlated in measuring subcutaneous fat thickness (Chapter 5). Where this correlation had been higher, i.e., the lumbar area, the agreement between histology and ultrasound for predicting total fat was also higher. The coefficient of correlation of these two techniques with total fat in 25 dogs, was very similar (Tables 6.2 and 6.3). Nevertheless, ultrasound was clearly inferior for predicting total fat in both the old and young/adult groups of dogs.

Interpretation of the A-scan data had not been easy (Chapter 5). Errors in the thickness of fat being measured with ultrasound must have made histological measurements of subcutaneous fat more accurate than corresponding ultrasonic ones. This difference is the most likely explanation for the considerable discrepancy in their respective value as predictors of overall fatness.

The skinfold calliper technique was of use in estimating total body fat in these dogs. Measurements of subcutaneous fat obtained by this method were able to explain 88% of the variation in fat content of young and adult dogs. The anatomical sites where prediction of fat content was best (sternum,

flank and lumbar) were similar to the ones obtained with histology (flank and lumbar) and ultrasound (lumbar). In old dogs, however, skinfold calliper measurements were poorly correlated with total body fat.

The calliper technique is believed to be of little value for use in dogs or cats because the skin is easily lifted away from the subcutaneous tissues (Lewis and others, 1987). In the present study, an effort was made to pick up subcutaneous tissue together with the skin fold, pinching it away from underlying muscle. As discussed above, it was felt that the callipers were measuring a greater depth of fat than either histology or ultrasound. However, there was more adipose tissue situated between muscle fasciae which was probably not detected by the callipers.

The elasticity of the skin and subcutaneous tissues was probably unaltered when measurements were taken, a few hours after death. Lee and Ng (1965) found little change in skin elasticity in their study with human cadavers. They were studying bodies which had been in the cold chamber for an average of 2 days before measurements with skinfold callipers were taken.

In conclusion, the fat content of dogs in this study was remarkably variable. The clinical scoring system provided a valuable estimate of body condition but little information on the actual amount of fat present in the body. In some dogs classified with one condition score, actual fat content was in fact more similar to the one found in dogs of a different condition score.

Subcutaneous fat thickness measurements were of value in predicting the total body fat of young and adult dogs. Prediction of total fat was best when histological measurements of subcutaneous fat were used in the regression equation, in particular those obtained at the flank and lumbar areas. Calliper fat measurements were highly correlated with overall fatness at the sternum, flank and lumbar areas, whereas ultrasonic measurements of subcutaneous fat were correlated with total fat content only at the lumbar site.

In old dogs, subcutaneous fat measurements were not good estimators of total body fat. These animals were all over 6 years of age and many had signs of past endocrine disease. It was thought that a redistribution of fat stores due to age, increasing fatness and/or systemic disease, had introduced a disproportion between the peripheral and internal fat depots, which made subcutaneous fat measurements unrelated to overall fatness.

CHAPTER VII.

MEASURING SUBCUTANEOUS FAT IN A HOSPITALIZED POPULATION OF DOGS.

CHAPTER VII. MEASURING SUBCUTANEOUS FAT IN A HOSPITALIZED POPULATION OF DOGS.

7.1. INTRODUCTION.

Advances in medical technology have produced major improvements in the quality of ultrasonic imaging. In particular, the introduction of two-dimensional ultrasound scanners opened-up a range of novel opportunities for non-invasive medical diagnosis, both in humans and animals (Simm, 1983; Taylor and Kremkau, 1985; Freiherr, 1989). Brightness-mode (B-mode) scanners provide a two-dimensional picture of the tissue(s) under investigation (Wells, 1977). This offers an important advantage over amplitude-mode (A-mode) units, which provide only a one-dimensional array representing the depth of various tissue interfaces (Cameron and Skofronick, 1988). Subcutaneous structures such as fasciae or connective tissue bundles are more easily identified using a B-mode scan (Fanelli and Kuczmarski, 1984). The two-dimensional picture displays anatomical features and tissue boundaries which cannot be found in those units that do not produce a cross-sectional view of the structures being interrogated. Since Booth and co-workers (1966) described the difficulties in discerning the fat/muscle interface from other additional echoes, various authors have reported on the pitfalls of A-mode data interpretation (Haymes and others, 1976; Volz and Ostrove, 1984). Some studies found A-scan measurements of human

subcutaneous fat to be poorly correlated to corresponding ones obtained with the skinfold calliper technique (Borkan and others, 1982b; Chumlea and Roche, 1986). Furthermore, it has been said that the development of more sophisticated ultrasonic devices, incorporating two-dimensional pictures, has provided greater accuracy in the measurement of subcutaneous fat in humans (Fanelli and Kuczmarski, 1984; Weits and others, 1986; Kuczmarski and others, 1987).

In an effort to make interpretation of ultrasonic data easier, a simple B-mode scan was developed by Stouffer (1963) for use in cattle. This device was able to measure fat thickness and rib eye areas on live animals with great accuracy. Since the studies of Stouffer, two-dimensional scanners have been proved to be generally more accurate than A-mode machines for predicting carcass traits (Kempster and others, 1981; Simm, 1983). B-mode scans have also been used to predict body composition of horses (Westervelt and others, 1976) and pigs (Weingand and others, 1989). Moreover, Anderson and Corbin (1982) were able to measure subcutaneous fat thickness in a group of 24 Beagle dogs, using a simple B-mode device. Their measurements were used to predict total body fat calculated by chemical analysis.

Recent developments in technology have allowed the incorporation of ultrasound devices in the field of clinical dermatology. Using higher frequencies than those utilized for visualizing internal organs, it has been possible to obtain cross-sectional pictures of normal (Dines and others, 1984; Fornage and Deshayes, 1986) and diseased skin (Myers and others, 1986).

Two-dimensional scanners have provided information on the extent of skin burn depth (Kalus and others, 1979), nodular lesions of the skin (Miyauchi and others, 1983) and the local invasiveness of skin tumours (Shafir and others, 1984; Murakami and Miki, 1989). A further development in ultrasonic imaging has been the incorporation of the C-scope into some ultrasonic devices dedicated to skin applications. The C-scope provides information on the angular position of the echo-producing structure and thus, an image is composed which resembles a conventional radiographic tomogram (Wells, 1977). Such a machine, incorporating A, B and C-modes, was acquired by the Department of Veterinary Medicine of Glasgow University Veterinary School, to monitor the local invasiveness of equine skin tumours. This provided an opportunity to include a B-mode ultrasonic scan into the studies on canine obesity.

7.2. OBJECTIVES.

1. To measure subcutaneous fat thickness in live dogs by means of a two-dimensional ultrasonic scanner and skinfold callipers.

2. To assess differences in the thickness of this fat layer according to the clinical body condition of the animal.

3. To study the relationship between some physical parameters such as weight, length, girth and height to body condition.

4. To assess differences in the clinical body condition of a sample of hospitalized dogs, according to sex and age.

7.3. MATERIALS AND METHODS.

POPULATION.

Over 1000 dogs are referred annually to the Small Animal Clinic of Glasgow University Veterinary School with a variety of medical conditions. These animals may suffer from endocrine, gastrointestinal, cardiovascular, renal, respiratory, infectious and dermatological problems, as well as neoplastic conditions. A total of 100 dogs from this population were utilized in the present study. Animals with endocrine diseases such as diabetes mellitus and hyperadrenocorticism, which are known to affect body fat distribution, were not included. Hypothyroid dogs were also excluded.

The owner's consent as well as that of the clinician in charge of the case, was sought before carrying out any investigation. Once this consent was obtained, the animal's breed, sex and age were recorded. The initial complaint, which had caused the referral, was obtained from the clinical history and by discussing each individual case with the clinician responsible for it. Hence, it was possible to exclude any confirmed or suspected cases of diabetes, hyperadrenocorticism or hypothyroidism.

BODY CONDITION ASSESSMENT.

By palpating the amount of fat overlying the rib cage and abdomen, and by a general assessment of nutritional status, a body condition score was given to each animal. This scoring system was based on Edney and Smith's (1986) 5-point clinical scale (Chapter 5). In brief, dogs with an obvious skeletal structure were classified as "emaciated" whilst those with little body fat present and visible rib cage were assessed as "thin". When the ribs were easily palpable under a moderate layer of fat covering the thorax, the animal was classified as "normal". Dogs with obvious fat deposition over the trunk and abdomen, whose ribs were not visible and were difficult to palpate, were considered to be "fat". Finally, when large amounts of adipose tissue could be grasped by hand and the ribs were not palpable, the dog was classified as "gross".

ULTRASOUND.

Following condition scoring, subcutaneous fat thickness was measured using a three-dimensional scanner: the Dermascan-C (Cortex Technology Aps, Aalborgvej 53E, DK-9560 Hadsund, Denmark). This unit was specifically developed for skin applications and combines A/B and C-mode scanning facilities (Figure 7.1). The third dimension is added by fast computer controlled rectilinear scanning, which continually displays multiple B-scans on the screen. The 20 MHz transducer automatically moves through the scanning

FIGURE 7.1. The Dermascan-C.

field interrogating and depicting successive slices of skin. It is contained in a probe which, prior to scanning, is filled with distiled water and sealed using a plastic ring covered with cling-film. The transparent cling-film lies across the probe's opening of 30 x 30 mm, while the transducer moves in different planes inside the water-filled probe (Figure 7.2). Water enhances the transmission of the ultrasonic wave from the transducer into the skin, through a layer of ultrasound gel.

The velocity of sound was adjusted to be 1470 m/sec which is the speed of ultrasound in fat (Escoffier and others, 1986; Hamlet and others, 1986). A small circular area, approximately 30 mm in diameter, was defined in the lumbar region of the animal, between the 3rd and 5th lumbar vertebrae, 2 to 3 cm lateral from the spine (Chapter 5). This site was chosen following observations made in previous work (Chapter 6) which showed that measurements of subcutaneous fat obtained with ultrasound in the lumbar area were significantly correlated to total body fat. The hair covering this area was gently clipped with scissors and a thin layer of salt-free ultrasound gel (2-5 mm thick) was applied. The hair was clipped to avoid unnecessary reflections before ultrasound reached the outer layers of the skin.

Dogs were made to lie on an examining table with chest and abdomen touching its surface. The articulated arm holding the transducer probe was brought towards the animal and the probe was gently positioned on the lumbar area, slightly touching the skin surface. If the animal moved, care was taken while repositioning the probe, so that it would not compress the skin

FIGURE 7.2. Water-filled probe containing the transducer.

and subcutaneous tissues. The transducer inside the water-filled probe was automatically moved through the scanning field until the operator was satisfied with a picture. The latter was frozen on the screen and stored on a computer for later analysis.

Pictures obtained with the ultrasound unit were analysed 20 to 60 minutes after collection. The A-mode function was used to measure distances on the screen. By selecting this mode from the control options, electronic callipers appeared on the picture together with the A-mode representation of the cross-section of tissue selected by the operator. The electronic callipers were suitably placed to measure the distance from the lower limit of the dermis to the cutaneous muscle, i.e., the subcutaneous fat layer. After the second calliper was positioned on the screen, the machine automatically displayed the distance comprised between the two. Subcutaneous fat thickness was measured in this way at four different sites across the entire section of skin displayed on screen. Mean fat thickness was calculated from these four measurements.

CALLIPERS.

Ultrasound gel was removed from the lumbar site and two readings of subcutaneous fat thickness were obtained with a Holtain skinfold calliper (Holtain Ltd, Crosswell, Crymych, Dyfed SA41 3UF, U.K.) on the same site. This procedure was carried out following the manufacturer's recommendations as described previously (Chapter 5). A fold of skin and

subcutaneous tissue was firmly lifted and held between thumb and forefinger. The calliper tips were then applied at 1 to 2 cm distance from the fingers. Readings were taken as soon as the needle had stopped, usually 3 to 4 seconds after releasing the grip on the trigger. In some obese dogs it was difficult to lift up a skin fold and maintain the grip on it. In these cases, the reading was taken as soon as the calliper tips were left to exert their full pressure on the fold. The mean of the two calliper measurements was halved since it theoretically represents a double fold of fat (Hicks and others, 1956). This mean allowed comparison with ultrasonic measurements, which represent a single fat thickness (Borkan and others, 1982b; Volz and Ostrove, 1984).

PHYSICAL PARAMETERS.

Following calliper measurements, the animal was brought down from the examining table and placed on the floor. Height, girth and length in centimetres were then obtained with the dog standing erect, using a measuring tape. Height (H) was defined as the distance from the tip of the acromion to the floor, whilst length (L) was the distance from the tip of the acromion to the base of the tail. Girth (G) was defined as the thoracic circumference measured immediately posterior to the forelimbs. Finally, weight in kilograms (W) was recorded after a 12 to 24 hours fast, with the dog standing quietly on an electronic scale.

When all measurements were completed the animal was returned to its hospital accommodation.

STATISTICAL METHODS.

The chi-square test (Bishop, 1966) was used to find evidence, if any, to suggest that a particular age group or sexual status was more prone to obesity than another. The mean, standard deviation and range of measurements of subcutaneous fat thickness obtained with ultrasound and the calliper technique were calculated.

To assess the degree of association between any two sets of measurements, the coefficient of correlation was determined (Snedecor and Cochran, 1967a). One-way analysis of variance and the Newman-Keuls range test (Snedecor and Cochran, 1967b) were utilized to detect differences in fat thickness measured by ultrasound and the calliper technique in the various body condition score groups. Simple linear regression (Wardlaw, 1985) was used in order to assess the value of weight, girth, height, length or a combination of these, as predictors of body condition.

The level of significance was chosen to be 5% ($p < 0.05$).

7.4. RESULTS.

The hospitalized dogs included in this study suffered from a variety of medical conditions. Dermatological and gastrointestinal problems constituted

almost half of the total number of cases (42%). Gastrointestinal complaints (21%) ranged from chronic diarrhoeas to gastric ulcers. Cardiovascular problems (16%) and neoplastic conditions (11%) were the two next largest groups on the list. Care was taken not to include animals with oedema, scars or subcutaneous lumps at the site of measurements. Eight per cent of dogs were referred for a respiratory complaint, 8% for urogenital problems, two dogs were referred with pyrexia of unknown origin and two dogs had a neurological problem. The total number was completed with a group of "other conditions" (11%). This group included animals with musculoskeletal and reproductive problems as well as some nonspecific conditions such as lymphadenopathy and intermittent anorexia. Most dogs were purebred (89%) and the two sexes were equally represented (50% females, 50% males). There were a total of 21 spayed bitches whereas only one neutered male was included in the study. Age of dogs ranged from 5 months to 14 years, the largest group being that composed of middle-age animals (44% between 4 and 8 years). Thirty two per cent were between 5 months and 3 years of age, and 24% were over 8 years old.

Clinical scoring for body condition was quick and easy to perform. The largest group was that composed of dogs classified as "fat" (38%), followed by "normal" (30%), "thin" (19%), "emaciated" (8%) and "gross" (5%). The chi-square test showed that there was a difference in the prevalence of obesity among sexes in this population (Table 7.1). A significantly greater number of females were classified as "fat" or "gross" than were males. Neutered females

were almost half of the total number of bitches (21 out of 50) but the chi-square test did not find any evidence to suggest that spaying and obesity were associated (Table 7.2). However, it is interesting to note that among the five animals classified as "gross" in this study, four were neutered females; the fifth was an entire bitch.

Obesity was found in all age groups, with no evidence to suggest that a particular group contained a significantly larger number of obese dogs (Table 7.3). In the group including animals over 8 years of age, a greater proportion of dogs were obese than were non-obese (Table 7.3).

Due to insufficient numbers, it was not possible to analyse differences in the prevalence of obesity among the various breeds of dogs. Certain breeds which are thought to be more prone to obesity than others, like Labradors and Cairn Terriers, had a large proportion of their members scored as "fat" in this study (Appendix 7.1).

ULTRASOUND.

The majority of animals tolerated the scanning process well. Some dogs, of a more excitable nature, needed the presence of a nurse to keep them quiet and immobile while the measurement was being taken. In these cases, it was usually necessary to reposition the ultrasound probe a number of times because the animal would move and displace the probe from the target site of

TABLE 7.1.

Chi-square table showing the actual and expected numbers of obese ("fat" & "gross") and non-obese dogs according to their sex.

	MALES	FEMALES	TOTAL
OBESE	14	29	43
	(21.5)	(21.5)	
NON-OBESE	36	21	57
	(28.5)	(28.5)	
TOTAL	50	50	100

In parenthesis are expected numbers.

TABLE 7.2.

Chi-square table showing the actual and expected numbers of obese ("fat" & "gross") and non-obese bitches according to their sexual status.

	ENTIRE	NEUTERED	TOTAL
OBESE	15	14	29
	(16.8)	(12.1)	
NON-OBESE	14	7	21
	(12.1)	(8.8)	
TOTAL	29	29	50

In parenthesis are expected numbers.

TABLE 7.3.

Chi-square table showing the actual and expected numbers of obese ("fat" & "gross") and non-obese dogs according to age group.

	5mo-3yrs	4-8yrs	>8yrs	TOTAL
OBESE	10	20	13	43
	(13.7)	(18.9)	(10.3)	
NON-OBESE	22	24	11	57
	(18.2)	(25)	(3.6)	
TOTAL	32	44	24	100

In parenthesis are expected numbers.

measurement. No sedation was used and the only restraining, when needed, was manual.

Scanning the subcutaneous tissues of the lumbar area and finding a suitable picture of subcutaneous fat generally took between 2 and 6 minutes. It was usually more difficult in dogs with a short hair coat, such as Dobermans and Boxers. The skin surface of these animals is very densely covered with hair follicles (Muller, Kirk and Scott, 1989), which reflected part of the sound wave back to the transducer. Scanning these animals took anything between 10 to 20 minutes. The problem would have been solved by using an electrical razor, instead of scissors, to clip the hair coat short. This was not done because a bald patch ensues which is very unaesthetic and which owners dislike.

Pictures obtained with the ultrasound unit were analysed 20 to 60 minutes after collection. Interpretation was usually simple. The epidermis appeared as a strong echogenic thin layer followed by a wider, but less echogenic, layer of dermis (Figure 7.3). Immediately beneath the dermis was an echolucent layer of variable thickness, the subcutaneous fat, limited by the cutaneous muscle, which usually appeared as a thin band of intermediate echogenicity (Figure 7.3). Beneath the cutaneous muscle a wide echolucent area, probably representing more fat, could be seen. However, it was generally not possible to distinguish the lower limit of this layer with the 20 MHz transducer. In many dogs, thin bands of intermediate echogenicity could

be found running across this second layer of fat, probably caused by connective tissue bundles or fasciae (Figure 7.4).

The layer of fat comprised between the lower limit of the dermis and the cutaneous muscle was measured at four different points using the A-mode function (Appendix 7.2). Table 7.4 lists the mean, standard deviation and range of subcutaneous fat thickness measured with ultrasound in the various condition score groups. Mean thickness of subcutaneous fat was greatest in the group of dogs classified as "gross" (mean= 4.07 mm). Fat dogs had a thicker layer of fat (mean= 2.75 mm) than either "normal" (mean= 1.65 mm), "thin" (mean= 0.96 mm) or "emaciated" dogs (mean= 0.45 mm).

One way analysis of variance and the Newman-Keuls range test showed that there was a significant difference between the "gross", "fat", "normal" and "thin" groups of animals. No statistical difference was found between ultrasonic measurements of subcutaneous fat taken on dogs classified as "emaciated", when compared to those measurements taken on "thin" animals. Hence, ultrasound detected differences in the thickness of subcutaneous fat in animals of diverse body condition. These differences are graphically represented in Figure 7.5. The overall correlation between ultrasonic fat thickness measurements and body condition score was $r= 0.79$ ($p< 0.001$). Ultrasonic measurements were then corrected for height by grouping them into one of four categories, according to the stature of the animal in which

they were taken. These categories, adapted from Lewis and others (1987), were the following:

* Toy dogs: 13-29 cm high.
* Small dogs: 30-41 cm high.
* Medium dogs: 42-56 cm high.
* Large dogs: 57-65 cm high.
* Giant dogs: >66 cm high.

Ultrasonic measurements of subcutaneous fat were significantly correlated to body condition score in all categories except in toy dogs (Table 7.5). The best correlation was achieved in large (r= 0.83) and small dogs (r= 0.82).

CALLIPERS.

The calliper technique was usually easy to perform. However, in some obese dogs it was difficult to lift up a fold of skin and subcutaneous tissue. This was more noticeable in gross animals, where the fat underneath the skin easily slipped away from the calliper tips, and in dogs with encapsulated fat stores in the lumbar area. The fascia covering these localized fat stores made it difficult to pinch a skin fold and take a measurement. In both gross animals and in those with encapsulated fat deposits, the reading was taken immediately after the calliper had exerted its full spring pressure.

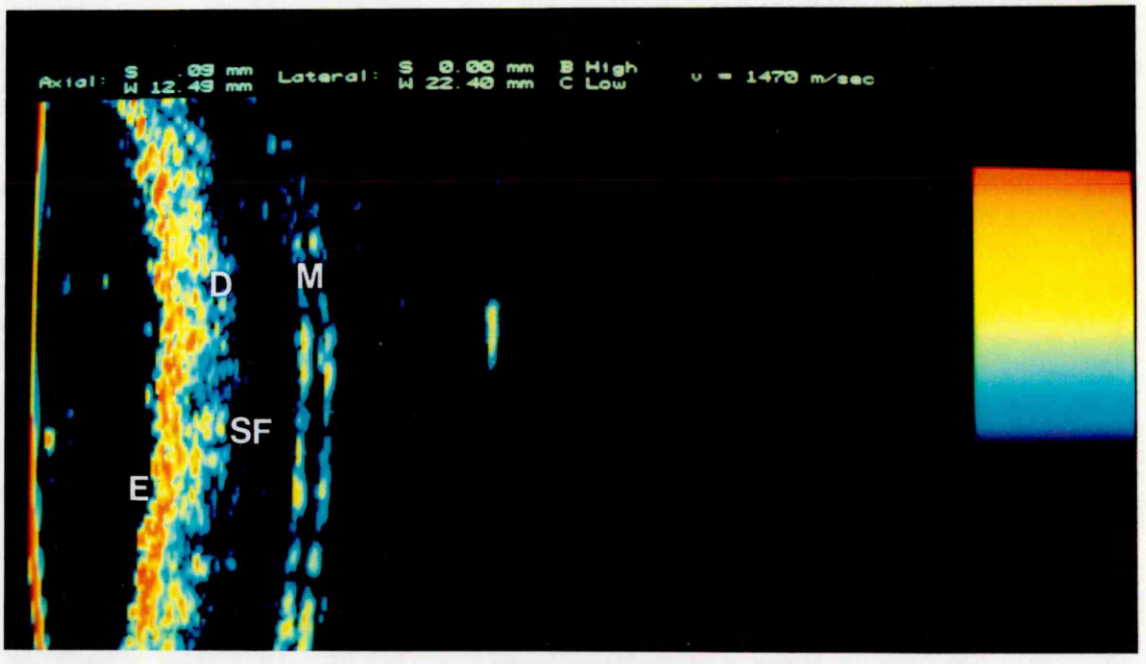


FIGURE 7.3. Dermascan-C picture showing the epidermis (E), dermis (D), subcutaneous fat (SF) and cutaneous muscle (M).

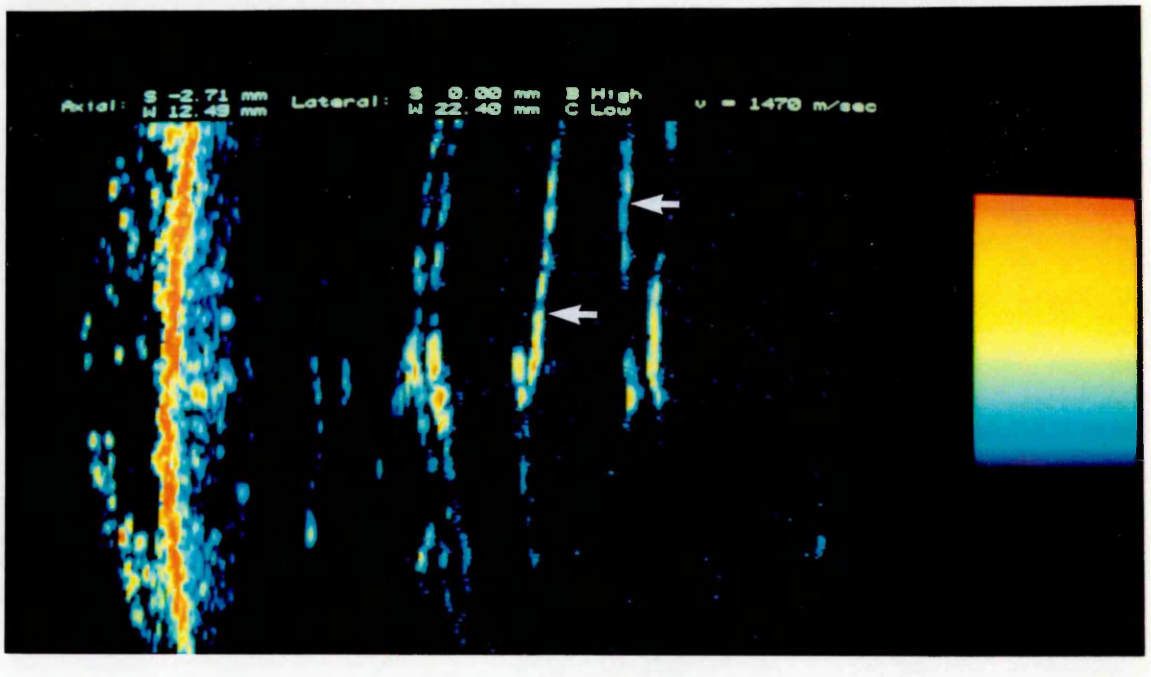


FIGURE 7.4. Fine echogenic bands beneath the cutaneous muscle, probably caused by bundles of connective tissue or fasciae.

TABLE 7.4.

Mean, standard deviation and range of subcutaneous fat thickness (mm) measured with ultrasound in 100 dogs.

B.C.S.	MEAN	SD	RANGE
Emaciated	0.45	0.08	0.31-0.58
Thin	0.96	0.53	0.43-2.86
Normal	1.65	0.69	0.48-3.45
Fat	2.75	0.79	1.43-4.82
Gross	4.07	0.88	2.78-5.14

TABLE 7.5.

Correlation coefficients between ultrasonic measurements of subcutaneous fat, corrected for height, and body condition score in 100 dogs.

B.C.S.	r	p
Giant	0.78	< 0.05
Large	0.82	< 0.001
Medium	0.76	< 0.001
Small	0.82	< 0.001
Toy	0.33	n.s.

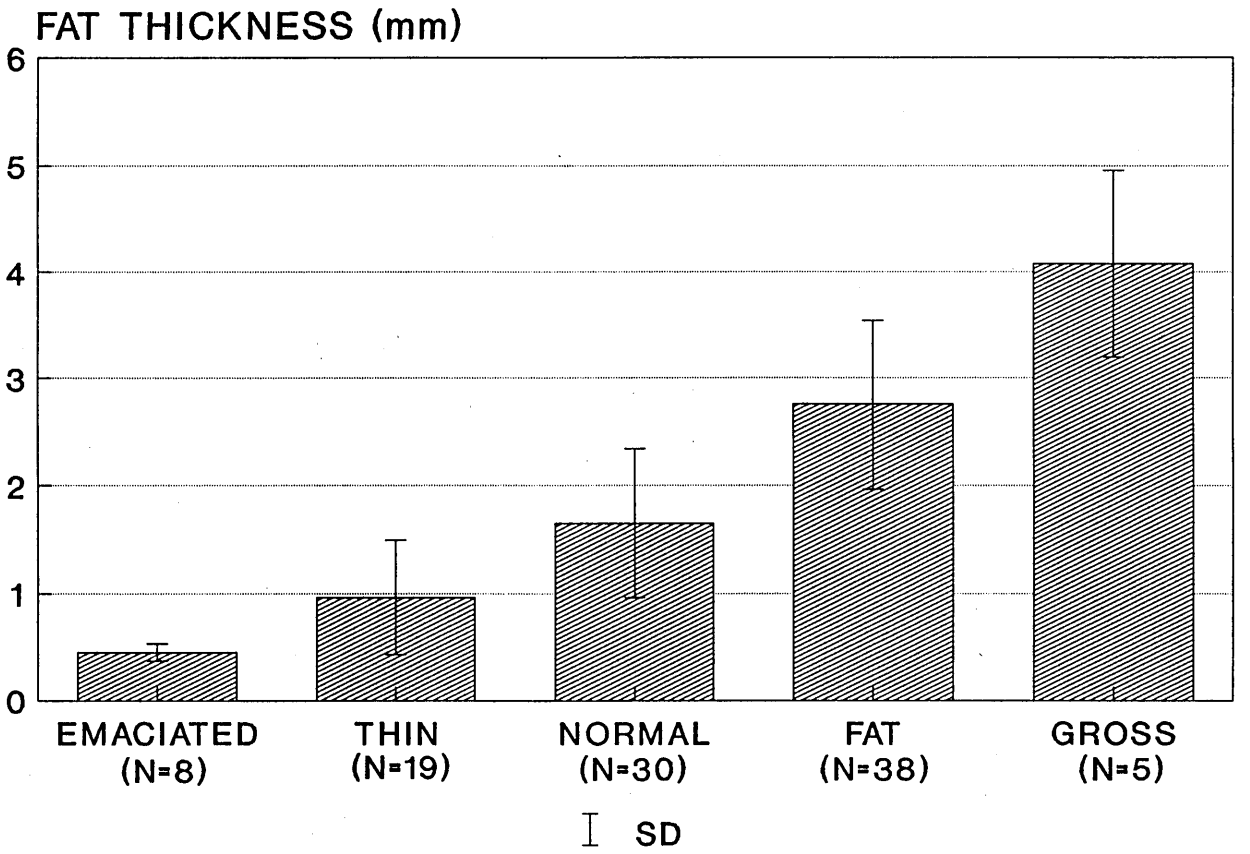


FIGURE 7.5. Subcutaneous fat thickness measured with ultrasound in dogs of differing body condition.

The mean of the two calliper readings taken from the lumbar area was halved in order to obtain a single fat measurement (Appendix 7.3). Table 7.6 shows the mean, standard deviation and range of subcutaneous fat thickness obtained with the calliper technique in the various condition score groups. Animals clinically assessed as "gross" had the deepest layer of fat measured with the callipers (mean= 9.76 mm). Fat thickness decreased together with a lower condition score so that the thinnest fat layer was found in the "emaciated" group of dogs (mean= 2.07 mm). One way analysis of variance and the Newman-Keuls range test showed a significant difference in calliper measurements between "normal", "fat" and "gross" dogs. No statistical difference was found between the "emaciated" and "thin" groups, nor between "thin" and "normal" dogs.

When calliper measurements were corrected for height, according to the stature of the animal (see above), the best correlation between them and body condition score was obtained in large and giant dogs (Table 7.7). As it had been the case with ultrasound, correlation with condition score was significant in all groups except in toy dogs. The overall agreement between calliper measurements and body condition score was statistically significant ($r= 0.66$; $p< 0.001$).

Ultrasonic fat thicknesses were then compared to the corresponding ones obtained with the skinfold calliper. There was a high degree of correlation between the two techniques for the measurement of subcutaneous

TABLE 7.6.

Mean, standard deviation and range of subcutaneous fat thickness (mm) measured with the calliper technique in 100 dogs.

B.C.S.	MEAN	SD	RANGE
Emaciated	2.07	0.33	1.35-2.40
Thin	2.66	0.77	1.55-5.15
Normal	3.72	1.47	1.95-9.00
Fat	4.94	1.28	2.45-8.85
Gross	9.77	3.78	6.75-15.7

TABLE 7.7.

Correlation coefficients between calliper measurements of subcutaneous fat, corrected for height, and body condition score in 100 dogs.

B.C.S.	r	p
Giant	0.79	< 0.05
Large	0.79	< 0.001
Medium	0.62	< 0.001
Small	0.62	< 0.005
Toy	0.42	n.s.

fat ($r = 0.79$; $p < 0.001$). However, the level of agreement varied depending on the body condition of the animal. Correlation between ultrasound and skinfold callipers was best in dogs classified as "normal" (Table 7.8). When both sets of measurements were corrected for height, it was found that correlation coefficients were generally good in all five categories (Table 7.9). Agreement between ultrasound and the calliper technique was best in large ($r = 0.91$), medium ($r = 0.81$) and small ($r = 0.71$) dogs.

PHYSICAL PARAMETERS.

There was a wide variety of sizes and conformations represented in the 100 dogs seen in this study. From a Jack Russell terrier weighing 4.3 kg to a male Rottweiler weighing 72 kg after a few months on a weight-reducing diet (its original weight had been 101 kg). This dog had the widest girth measurement (108 cm). Height varied from 85 cm in a Great Dane to 22 cm in a Jack Russell and a Cairn terrier. The longest dog was a Great Dane 79 cm long, and the shortest was the above mentioned Jack Russell, measuring 25 cm in length.

A high degree of correlation was found between the weight of the animal and its girth, length and height in the different condition score groups (Table 7.10). Linear regression analysis showed that girth measurements could account for more than 85% of the variation in body weight. The mean, standard deviation and range of physical parameters measured in the various condition score groups are listed in Table 7.11. One way analysis of variance

TABLE 7.8.

Correlation coefficients between ultrasonic and calliper measurements of subcutaneous fat in 100 dogs.

B.C.S.	r	p
Emaciated	-0.32	n.s.
Thin	0.69	< 0.001
Normal	0.72	< 0.001
Fat	0.43	< 0.01
Gross	0.81	n.s.

TABLE 7.9.

Correlation coefficients between ultrasonic and calliper measurements of subcutaneous fat, corrected for height, in 100 dogs.

B.C.S.	r	p
Giant	0.68	n.s.
Large	0.91	< 0.001
Medium	0.81	< 0.001
Small	0.71	< 0.001
Toy	0.70	< 0.05

TABLE 7.10.

Correlation between weight and three other physical parameters in the various body condition score groups.

	WEIGHT&GIRTH		WEIGHT&LENGTH		WEIGHT&HEIGHT	
B.C.S.	r	p	r	p	r	p
Emaciated	0.94	< 0.001	0.95	< 0.001	0.86	< 0.005
Thin	0.92	< 0.001	0.88	< 0.001	0.88	< 0.001
Normal	0.92	< 0.001	0.91	< 0.001	0.87	< 0.001
Fat	0.98	< 0.001	0.88	< 0.001	0.89	< 0.001
Gross	0.96	< 0.05	0.92	< 0.05	0.68	n.s.

TABLE 7.11.

Mean, standard deviation and range of physical parameters (cm) measured in 100 dogs.

	WEIGHT			GIRTH		
B.C.S.	X	SD	RANGE	X	SD	RANGE
Emaciated	25.5	9.5	10-41	71.3	10.7	49-84
Thin	21.2	9.4	6-38	66.0	11.2	43-87
Normal	24.0	8.4	4-45	69.4	9.0	40-84
Fat	24.9	12.4	7-51	70.1	13.3	49-93
Gross	38.4	21.0	16-72	85.2	15.7	64-108
	LENGTH			**HEIGHT**		
Emaciated	60.6	8.8	48-76	61.6	11.2	45-85
Thin	55.8	10.8	34-73	54.7	13.7	30-82
Normal	55.2	10.1	25-79	51.3	11.8	22-81
Fat	50.6	10.2	34-71	44.6	10.2	22-68
Gross	52.2	9.0	39-64	48.8	8.3	35-57

and the Newman-Keuls range test showed a significant difference in the weight and girth of "gross" animals when compared to those of the other condition score groups. Emaciated animals were significantly taller than those classified as "gross". No other difference in morphological characteristics was detected among condition score groups.

Weight, girth and length measurements were corrected for height to allow comparison with body condition score (Table 7.12). Weight and girth were significantly correlated with the clinical assessment of body condition in all groups except in giant dogs. However, correlation coefficients, save in the case of toy animals, were low and the overall correlation between these physical parameters and condition score was not significant. Multiple linear regression showed that when girth, height, length and weight were used as independent variables in the prediction equation, they could only account for 55% of the variation in condition score. Various combinations of weight, height, girth and length were formulated. Among these, the Body Mass Index (weight/height^2), a girth index (girth^2/height) and a girth-weight index (girth^2 x weight/height^2) gave the best correlation with condition score (Table 7.13). However, simple linear regression showed that these physical parameters could account for less than half of the variation in condition score.

The prediction of condition score improved significantly when ultrasonic measurements of subcutaneous fat were combined with physical parameters into one prediction equation (Table 7.14). Ultrasound, in

TABLE 7.12.

Correlation between weight, girth and length with body condition score in 100 dogs.

	WEIGHT		GIRTH		LENGTH	
STATURE	r	p	r	p	r	p
Giant	0.23	n.s.	0.36	n.s.	-0.19	n.s.
Large	0.67	< 0.001	0.62	< 0.001	-0.09	n.s.
Medium	0.57	< 0.001	0.65	< 0.001	0.01	n.s.
Small	0.50	< 0.05	0.55	< 0.05	0.21	n.s.
Toy	0.88	< 0.001	0.92	< 0.001	0.94	< 0.001

TABLE 7.13.

Correlation between the Body Mass Index (BMI), a Girth Index (GI), and a Girth-Weight Index (GWI), with body condition score in 100 dogs.

	BMI		GI		GWI	
STATURE	r	p	r	p	r	p
Giant	0.79	< 0.05	0.77	< 0.05	0.72	< 0.05
Large	0.73	< 0.001	0.67	< 0.001	0.70	< 0.001
Medium	0.65	< 0.001	0.68	< 0.001	0.55	< 0.001
Small	0.50	< 0.05	0.52	< 0.05	0.49	< 0.05
Toy	0.84	< 0.005	0.90	< 0.001	0.90	< 0.001

TABLE 7.14.

Multiple regression parameters for the estimation of body condition score using ultrasonic fat thickness measurements in combination with physical parameters.

	U+W+H+L+G			U+BMI		
STATURE	S	R-SQ	R-SQ(adj)	S	R-SQ	R-SQ(adj)
Giant	0.33	97.2%	90.2%	0.43	88%	83.2%
Large	0.56	79.9%	74.6%	0.61	72%	69.5%
Medium	0.55	69.9%	65.5%	0.58	63.9%	62%
Small	0.56	71.6%	59.8%	0.52	69.5%	65.4%
Toy	0.09	97.1%	92.2%	0.20	71.4%	61.9%
	U+GI			U+GWI		
Giant	0.43	87.8%	83%	0.46	86.1%	80.5%
Large	0.65	69.2%	66.4%	0.64	70%	67.3%
Medium	0.58	64.5%	62.6%	0.61	60.8%	58.7%
Small	0.51	70.7%	66.8%	0.52	69.6%	65.6%
Toy	0.15	84%	78.6%	0.15	83.2%	77.6%

combination with the weight, height, length and girth of the animal could account for at least 70% of the variation in condition score.

7.5. DISCUSSION.

The population of dogs seen in this study did not constitute an epidemiological survey of obesity. It was a sample of hospitalized animals suffering from a variety of medical conditions but free from endocrine disorders or local abnormalities at the site of measurements. The latter were the main criteria for inclusion into the study, together with owner's and clinician's consent.

Although a certain effort was made to have obese animals in the population, it was the high number of fat dogs among the available in-patients that made obesity so prevalent (43%). "Emaciated" and "gross" animals were hard to find, and those classified as "thin" were not too common. These findings are in agreement with general surveys on canine obesity, which have shown that the majority of household dogs are in the "normal" and "fat" groups (Mason, 1970; Edney and Smith, 1986). Undernourished or excessively obese animals are much more difficult to find. Steininger (1981) found as many as 44% of dogs visiting a small animal clinic in Austria to be obese, and Edney (1978) reported a prevalence of obesity of 34% among a population of 1134 dogs.

In the present study, a significantly larger number of females were included in the "fat" and "gross" categories than were males. Other authors have found bitches to be more prone to obesity than male dogs (Mason, 1970; Meyer and Stadtfeld, 1978), and Edney and Smith (1986) reported neutering to double the likelihood of obesity in females. Although there was no evidence for the latter in the present study, it is interesting to note that 66% of all neutered females were classified as "fat" or "gross".

Following ovariohysterectomy or castration, food intake and the efficiency of energy utilization often increase (Houpt and others, 1979; Houpt, 1982; Branam, 1988). Neutering is thought to decrease physical activity without a compensatory change in dietary intake, enhancing the development of obesity in dogs (Andersen and Lewis, 1980). In the present study, four out of five animals with massive fat accumulation were spayed bitches.

Obesity was found in all age groups with no evidence to suggest that older animals were at a particular risk. Mason (1970) found a sharp increase in the incidence of canine obesity after 4 years of age, but no association between obesity and age was reported by Edney and Smith (1986).

Surveys on the incidence of canine obesity have found certain breeds such as Labradors, Cocker Spaniels and some of the Terriers, to be more prone to obesity than others (Krook and others, 1960; Mason, 1970; Edney and Smith, 1986). No conclusion on breed predisposition to obesity can be drawn from the present population of hospitalized dogs. However, it was

noted that six out of eight Labradors and three out of three Cairn Terriers included in this study, were classified as "fat". From a total of six Cocker Spaniels, two were assessed as "fat" and one was thought to be "gross".

The ultrasonic technique was easily carried out in most dogs. Only a few animals, of a more excitable nature, posed problems when lifted on to the examining table or when the ultrasound probe was placed on their lumbar area. These dogs moved and wriggled, slowing the process because the probe had to be repositioned a number of times. The lumbar area had been chosen because previous work (Chapter 6) showed that ultrasonic fat thickness measurements in this site were significantly correlated to total body fat. It is also easily accessible and known to be a characteristic location for fat deposition in dogs (Evans and Christensen, 1979; Habermehl, 1981).

Ultrasonic pictures of subcutaneous fat were readily obtained. The three-dimensional display allowed direct visualization of structures which had been previously unidentified using a one-dimensional unit (Chapter 5). Other authors have commented on the greater ease of interpretation in B-mode scans as opposed to A-mode units (Simm, 1983; Fanelli and Kuczmarski, 1984; Weits and others, 1986).

The high-frequency transducer utilized in the present study (20 MHz) provided a fine degree of resolution but a low penetration. With this equipment, specifically designed for dermatological applications, it was

possible to accurately measure the superficial layer of subcutaneous fat. This layer is located between the dermis and the cutaneous muscle.

In humans, subcutaneous fat has been successfully measured with B-mode units operating at low frequencies. Hansen and Kehrer (1987) were able to measure the thickness of subcutaneous fat in 100 subjects using a 5 MHz transducer. They recorded fat depths ranging from 5 to 69 mm. Similar values for obese people were obtained by Kuczmarski and co-workers (1987) and by other authors operating at slightly higher frequencies (Fanelli and Kuczmarski, 1984; Weits and others, 1986).

Low-frequency B-scans have been employed for measuring subcutaneous fat in farm animals (Miles, Pomeroy and Harris, 1972; Kempster and Owen, 1981; Leymaster and others, 1985; Weingand and others, 1989). Moreover, a 2 MHz B-mode scan was utilized in the only documented study of ultrasound and body composition of dogs found in the literature (Anderson and Corbin, 1982). The authors reported fat thickness measurements ranging from 1 to 44.5 mm along the back of 24 Beagle bitches. In the present study, fat depth varied within much finer limits (0.31 to 5.14 mm). Nevertheless, statistical analysis showed that there was a significant difference in the thickness of this fat layer between most condition score groups. Thus, ultrasound detected a deeper layer of adipose tissue in animals classified as "fat" (mean= 2.75 mm) when compared to those classified as "normal" (mean= 1.65 mm). Subcutaneous fat was thickest in animals assessed as "gross" (mean= 4.07 mm) and thinnest in those classified as

"emaciated" (mean= 0.45 mm). Only between "thin" and "emaciated" animals was there no statistical difference in ultrasonic fat thickness measurements.

The skinfold calliper detected a similar trend. Measurements of subcutaneous fat obtained with this technique were significantly different between "normal", "fat" and "gross" animals. No difference was found between the "normal" and "thin" groups nor between "thin" and "emaciated" dogs.

Ultrasound and the calliper technique correlated well when all measurements of subcutaneous fat were compared (r= 0.79). However, skinfold callipers were measuring a greater thickness of subcutaneous fat. After being halved in order to obtain a single fat measurement, calliper values ranged from 1.35 to 15.75 mm, considerably more than the range of ultrasonic values. This discrepancy in what was actually being measured might explain the different degree of correlation between the two techniques in the various condition score groups. After being corrected for height, ultrasonic and calliper measurements of subcutaneous fat were in better agreement. Neither of the two techniques, however, was significantly correlated to condition score in toy dogs. Only nine animals fell within this size category and all but one had been classified as "fat". It was therefore a biassed sample and this might explain the low correlation between fat thickness measurements and condition score.

In any case, ultrasound proved to be superior to the calliper method for predicting condition score. Ultrasonic fat thickness measurements could

account for 63% of the variation in condition score whereas the calliper technique accounted for only 44%.

In very obese dogs and in those with encapsulated fat stores around the lumbar area, the calliper technique was difficult to apply. The large amounts of subcutaneous adipose tissue in overtly fat animals could not be firmly pinched between the calliper tips. On the other hand, fasciae covering encapsulated fat deposits made it hard to separate the latter from other subcutaneous tissues.

In humans, skinfold callipers are of limited use in some overweight individuals and almost impossible to apply in very obese ones (Garrow, 1983b; Fanelli and Kuczmarski, 1984). The inability to palpate the fat/muscle interface, the sliding of the calliper tips on large skin folds and the impossibility to contain great amounts of subcutaneous fat within the calliper, are some of the problems encountered in gross subjects (Weits and others, 1986; Kuczmarski and others, 1987). No study documenting the use of the skinfold technique in dogs could be found in the literature. Anderson (1973), reporting on his experience with this technique, indicates that the skin fold is readily lifted from the subcutaneous tissues and hence, does not correlate with degree of obesity. This view is supported by other authors (Lewis and others, 1987; Crane, 1991) and by the present study, which found a low degree of association between skin fold thickness and body condition.

In carnivores and sheep, subcutaneous connective tissue is loosely structured and plentiful, allowing the skin to be easily moved over its supporting tissue (Habermehl, 1981). The skin of the dog, in particular, is characteristically very loose and endowed with ample subcutaneous adipose tissue, especially on the dorsal aspect of neck and trunk (Sisson, 1975). In the present study, an effort was made to lift up the subcutaneous adipose tissue together with the skin. However, the operator was under the impression that a large proportion of it could not be properly included into the skin fold. This was especially noticeable in very fat dogs. Some obese animals presented encapsulated fat deposits in the lumbar area, which have been reported elsewhere (Joshua, 1970; Sibley, 1984). A common occurrence in these and some of the other fat dogs, was the apparent loss of cutaneous elasticity. In fact, the great mass of underlying adipose tissue covered by strong fascia and firmly held in place, was probably over-stretching the skin and making the formation of a skin fold almost impossible.

The great variety of sizes and conformations characteristic of the canine species was represented in this study. The smallest dog was a Jack Russell Terrier 25 cm long, 22 cm high and weighing 4.3 kg. The tallest was a Great Dane (85 cm) and the heaviest was a Rottweiler. When this animal was originally presented with a skin complaint its weight was 101 kg. At the time when measurements were taken, the owners had managed to reduce it to 72 kg. A high degree of correlation was found between the weight of the animal and its girth, length and height in the various condition score groups. The one

exception was the lack of significant relationship between weight and height in dogs classified as "gross". Weight and girth^2 x length were highly correlated in a group of 372 horses studied by Carroll and Huntington (1988). Pendergrass and co-workers (1983) found a good degree of association between the length and girth of 63 mongrel dogs and their body weight. Verryn and Geerthsen (1988) were able to predict body weight and other conformation values of mature German shepherd dogs from physical parameters obtained in young animals.

It is known that many morphological parameters and linear dimensions such as height, increase with weight in dogs (Kirkwood, 1985). However, the way in which these morphological parameters change with weight can differ considerably among breeds and sizes due to the fact that there is a 35-fold weight range of dog breeds (Kirkwood, 1985). Moreover, body weight is thought to be a poor indicator of obesity in dogs, due to numerous differences in conformation between breeds and the great variety of types within each breed (Joshua, 1970; Lewis, 1978). Hence, comparing the weight of an individual to breed weight standards is of little value, and inappropriate for use in mongrel dogs (Anderson, 1973; Hand, 1990; Little, 1990).

Dogs classified as "gross" in this study were heavier and had a wider thoracic circumference than animals in other condition score groups. No other statistical difference in physical parameters was found. To analyse the possible relationship between the body condition of the animal and its morphological

characteristics, weight, length and girth measurements were corrected for height. This was done by grouping dogs into one of five statures: toy, small, medium, large and giant. Various other combinations of the above morphological parameters, including the Body Mass Index (weight/height^2), were also compared to body condition score. In general, coefficients of correlation between all of these physical parameters and condition score were low, except in toy dogs. Weight, girth and length were highly and significantly associated to body condition score in animals 13 to 29 cm tall. This was probably due to minor variability within each of the two parameters being compared at any one time. All of these animals except one had been classified as "fat" (condition score 4) and their weights, girths and lengths differed very slightly. Hence, correlation was high. At best, physical parameters could only explain 55% of the variation in the condition score of all animals.

Various physical measurements describing size and conformation of cattle have often proved of little value in predicting actual body composition (reviewed by Barton, 1963). Henneke and others (1983) developed a 9-point clinical scale for body condition assessment in horses. They found this condition score system more closely related to ultrasonic measurement of body fat than any other physical measurement including weight, height and girth. In the present study, the overall correlation with condition score improved significantly when ultrasound was combined with physical parameters into one prediction equation.

In conclusion, subcutaneous fat thickness in the lumbar area of 100 dogs was readily visualized and accurately measured with a three-dimensional ultrasonic scan. There was a significant difference in the thickness of this fat layer among animals clinically classified as "thin", "normal", "fat" and "gross". No statistical difference was found between "thin" and "emaciated" animals.

There was in general a good agreement between ultrasound and skinfold callipers for the measurement of subcutaneous fat. However, ultrasound was found to be more related to body condition score than the calliper technique.

Various physical parameters, including weight, girth and length, were correlated to body condition score. Coefficients of correlation were usually low, and considerable variation in condition score remained unexplained by these parameters. A combination of ultrasonic fat thickness measurements and physical parameters yielded a high degree of association with condition score.

CHAPTER VIII.

GENERAL DISCUSSION AND CONCLUSIONS.

CHAPTER VIII. GENERAL DISCUSSION AND CONCLUSIONS.

Over the last 15 years, a vast amount of research has been carried out on the subject of obesity. This scientific effort has sprung from the conviction that a substantial increase in body fat content frequently results in the impairment of human health, a conviction found in the early works of Hippocrates and over the ensuing centuries (Bray, 1990). Obesity has been associated with a higher risk of developing cardiovascular disease, diabetes mellitus, hypertension, gallbladder disease, insulin resistance and osteoarthritis (Armstrong and others, 1951; Mayer, 1980; Van Itallie, 1980; Royal College of Physicians, 1983; Pi-Sunyer, 1991). However, despite the many years during which this condition has been known and studied, its definition remains undetermined. Most textbooks on human obesity point to the fact that it is not clear what the criterion for "normal" weight or "desirable" fat content should be (Cohen, 1985; Leiter, 1986; Stordy, 1988; Pi-Sunyer, 1988). Moreover, some authors have challenged the concept that there is a single ideal weight (Garn and others, 1983) or that there are objective definitions of obesity at all (Rothblum, 1990). Recent studies have shifted the emphasis towards the regional distribution of body fat, since it appears that central adiposity is more commonly associated with the health risks that accompany obesity (Baumgartner and others, 1987; Leibel and others, 1989; Weigle, 1990; Campaigne, 1990; Després and others, 1990). Hence, a new definition of obesity has recently been proposed by Björntorp (1990) in which

only abdominal obesity is distinguished as obesity. The problem now will be to define what constitutes excessive abdominal fat...

If this is the situation in human medicine, it is hardly surprising that objective definitions of obesity are lacking in veterinary medicine. Increased body fat content in dogs has been associated with diabetes mellitus (Krook and others, 1960; Dumon, 1988), hyperinsulinaemia and glucose intolerance (Mattheeuws and others, 1984a,b), hypertension (Rocchini and others, 1987; Wehberg and others, 1990), respiratory distress (Stogdale and Moore, 1980), increased surgical risk (Clutton, 1988) and decreased resistance to infectious diseases (Newberne, 1966; Williams and Newberne, 1971; Fiser and others, 1972). Other health hazards such as dermatological problems, heat intolerance, digestive disorders and osteoarthritis are suspected to be aggravated by excess body fat (Staff Report, 1965; Joshua, 1970; Edney, 1974; Lewis, 1978; Andersen and Lewis, 1980; Brown, 1987). However, objective measures of obesity are needed to confirm these health risks and to clarify what constitutes an excessive body fat content.

In humans, a considerable amount of body fat is located immediately beneath the skin. The proportion of fat contained in this depot has been a subject of controversy (Lohman, 1981); in a recent study it was estimated to be 53.7% for males and 62.6% for females (Hattori, Numata, Ikoma, Matsuzaka and Danielson, 1991). For a number of years this layer of fat has been taken to be a good estimator of total adiposity in both humans (Edwards, 1950; Edwards and others, 1955; Mayer, 1980; Mueller and

Wohlleb, 1981; Garrow, 1982) and farm animals (Hankins and Ellis, 1934; Price and others, 1960; Barton, 1967; Westervelt and others, 1976). Subcutaneous fat has been measured by methods such as soft-tissue roentgenograms (Barton, 1967; Haymes and others, 1976), computed tomography (Borkan and others, 1982a, 1983; Ashwell and others, 1985; Weingand and others, 1989), electrical conductivity (Booth and others, 1966), direct needle puncture (East and others, 1959; Bullen and others, 1965; Giles and others, 1981) and infrared interactance (Conway and others, 1984). The method most widely used for measuring subcutaneous fat thickness in humans is the skinfold calliper. Since the studies of Edwards (1950), Durnin and Womersley (1974) and Womersley and Durnin (1977), the technique has been extensively employed in anthropometric and nutrition studies. However, due to some limitations associated with the calliper technique such as the impossibility of obtaining reliable measurements in the very obese and inter-subject variation in skinfold compressibility, ultrasound has been proposed as an alternative method for measuring subcutaneous fat in humans (Balta and others, 1981; Volz and Ostrove, 1984; Weits and others, 1986; Hansen and Kehrer, 1987; Kuczmarski and others, 1987). The technique has been used in livestock industry since the 1950s and both A-mode and B-mode machines have been tested (reviewed in Chapter 2 of this thesis). The main advantage of the ultrasound technique is that it is completely safe and non-invasive, it does not involve any undesirable radiation or discomfort to the patient. Hence, it is an attractive alternative to the rather subjective

assessment of body fat stores (Mason, 1970; Anderson, 1973; Markwell, 1988b; Clutton, 1988) presently available for quantifying obesity in dogs.

Little is known about the applicability of the ultrasound technique in canine obesity. Houpt and Hintz (1978) refer to its use for measuring body fat in a clinical setting but only Anderson and Corbin's (1982) paper gives a detailed account on the value of this technique for measuring subcutaneous fat in dogs. The objective of the experiment described in Chapter 5 was to determine the usefulness of an ultrasound technique for measuring subcutaneous fat thickness in dogs. An A-mode (one-dimensional) ultrasound scan was utilized to measure fat thickness at six different locations in 28 dog carcasses. Ultrasonic measurements were validated against a conventional histometric method. A high degree of correlation was found between ultrasonic and histologic measurements of subcutaneous fat, indicating the validity of the ultrasonic technique for measuring fat cover in dogs.

Other workers have validated A-mode ultrasonographic units by comparing fat thicknesses obtained with this technique to the same measurements taken with other methods. The surgical introduction of a ruler probe along the back of live hogs (Price and others, 1960; Giles and others, 1981), and direct carcass measurements in cattle (McReynolds and Arthaud, 1970), sheep (Bennett and others, 1988) and pigs (East and others, 1959), have been used to assess the validity of A-mode ultrasound in predicting backfat thickness and carcass composition. In humans, a high degree of correlation has been established between A-mode ultrasound and fat

measurements obtained with needle puncture (Bullen and others, 1965), soft-tissue roentgenograms (Haymes and others, 1976) and direct visualization following surgical incision (Balta and others, 1981). Histology, in particular, has been widely used in dermatological studies to validate skin thickness measurements obtained with ultrasound (Dykes and Marks, 1977; Tan and others, 1982; Dines and others, 1984; Shafir and others, 1984; Hamlet and others, 1986; Hughes and others, 1987; Murakami and Miki, 1989). However, various authors have reported difficulties in the interpretation of the echoes obtained with A-mode units (Booth and others, 1966; Haymes and others, 1976; Volz and Ostrove, 1984). This modality of ultrasound provides only a one-dimensional linear array, with echoes and gaps representing depths between different tissue interfaces. Actual images of tissues are not available. Hence, differentiation of boundaries can prove difficult (Booth and others, 1966; Borkan and others, 1982b). This was certainly the experience found in the present thesis. In some anatomical areas such as the thigh and the sternum, numerous spurious echoes obscured interpretation of the echograms and the subcutaneous fat layer was not easily defined. Booth and co-workers (1966) reported finding distinct discontinuities in the subcutaneous fat of obese men which they attributed to a membrane running through the subcutaneous tissue. Similar findings were obtained by Haymes and others (1976) and Volz and Ostrove (1984). In one study, computed tomography allowed visualization of septae in the adipose tissue of obese subjects which had hampered A-scan assessment of subcutaneous fat thickness (Black and others, 1988). These discontinuities are attributable to connective tissue

bundles which run among the adipocytes and which are particularly plentiful in the subcutis of carnivores (Habermehl, 1981). Small blood vessels and dermal invaginations, frequently found in histologic specimens examined in this thesis, probably caused additional spurious echoes.

These difficulties in the interpretation of ultrasonic data were not found when a three-dimensional scanner was used for measuring subcutaneous fat in live dogs (Chapter 7). Pictures obtained with this unit allowed direct visualization of structures which were previously indistinguishable with the A-mode machine.

The fact that certain anatomical areas showed better agreement than others between histology and ultrasound, was in line with other studies carried out in humans (Bullen and others, 1965; Haymes and others, 1976; Volz and Ostrove, 1984) and farm animals (Alliston and Hinks, 1981; Leymaster and others, 1985). The best agreement in the present thesis was found in the lumbar area, which usually showed a well delineated subcutaneous fat layer. Its thickness was, on average, larger than in the other anatomical sites and the fat/muscle interface was readily identified.

Having established the validity of ultrasound for measuring subcutaneous fat thickness in dogs, the next objective was to determine if these measurements could be used to predict total body fat. The results described in Chapter 6 show that ultrasound was inferior to histology and the calliper technique for predicting body fat content in 25 dog carcasses. This

could be partly due to the difficulties encountered in the interpretation of the A-mode echograms, which probably introduced errors in the thickness of the fat layer being measured. Borkan and co-workers (1982b) found skinfold callipers to be a more accurate means of measuring subcutaneous fat and predicting total body fat in humans than was ultrasound. They were using an A-mode device and encountered difficulties in the interpretation of the echograms. Similar conclusions were reached by Chumlea and Roche (1986) after using an A-mode device on elderly men and women.

In the 25 dogs analysed in this thesis, a considerable proportion of the variance in fat content remained unexplained by either histology, ultrasound or the calliper technique.

It is not clear whether measurements of subcutaneous fat thickness are a reliable indicator of total adiposity (Borkan and others, 1982b; Davies, Jones and Norgan, 1986; Weits and others, 1986; Stordy, 1988; Heymsfield and Williams, 1988). Considerable debate exists at present surrounding this question, which is of central importance for the assessment of obesity. Since Edwards (1950) postulated that subcutaneous fat thickness changes in proportion with body weight, numerous studies have relied on the measurement of subcutaneous fat to predict total adiposity (reviewed in Chapter 2). However, there is now a substantial body of evidence to suggest that the relationship between subcutaneous and total body fat may not be constant (Young, Blondin, Tensuan and Fryer, 1963; Katch and others, 1979;

Borkan and others, 1982b; Scherf and others, 1986; Hattori and others, 1991). In 1956, Allen and co-workers concluded that the relationship between external and internal adiposity in humans is not linear but it is best described by a parabola (Allen, Peng, Chen, Huang, Chang and Fang, 1956). Durnin and Womersley (1974) found considerable changes in the position of regression lines predicting body density from skinfold measurements in an ageing population. They attributed these changes to a greater proportion of body fat being deposited internally rather than subcutaneously. Since that study, other authors have reported changes in the proportion of fat situated subcutaneously depending on age, sex and total fat content (Brožek and Keys, 1950; Pi-Sunyer, 1988; Leibel and others, 1989). Scherf and others (1986) showed that most regression equations derived from skinfold measurements presently being used for predicting total body fat, significantly overestimated percent body fatness, determined by hydrostatic weighing, in formerly obese adults. They argued that a redistribution of fat stores following rapid and pronounced weight loss had ensued such that much of the loss had affected the internal fat depot, with practically no change in the amount of subcutaneous fat.

Young and others (1963) found that skinfold thicknesses did not reflect changes in body fat content of women older than sixty. They inferred from this result that a greater proportion of body fat in older women is central rather than subcutaneous. This view has been confirmed by studies on fat distribution using computed tomography. It has been shown that

intra-abdominal fat increases with age with practically no change in the amount of subcutaneous fat (Tokunaga, Matsuzawa, Ishikawa and Tarvi, 1983; Borkan and others, 1983; Seidell, Oosterlee, Deurenberg, Hautvast and Ruijs, 1988; Schwartz and others, 1990). Moreover, inherent differences in the proportion of fat situated underneath the skin have been documented between sexes and between different levels of fatness (Grauer, Moss, Cann and Goldberg, 1984; Hattori and others, 1991).

Wright and Russel (1984a) found large diversity in the partition of body fat among various depots in different breeds of cattle and with varying degrees of fatness. They were able to show that these differences in fat partitioning resulted in animals being classified with the same condition score or ultrasonic fat depth, when they actually differed considerably in total fat content (Wright and Russel, 1984b). Recently, body fat was calculated in 105 healthy human adults using a variety of methods (Gray, Bray, Bauer, Kaplan, Gemayel, Wood, Greenway and Kirk, 1990). These authors found that skinfold measurements tended to underestimate percentage body fat in obese subjects. They hypothesized that there was a greater increase in intra-abdominal fat than in subcutaneous fat with increasing body fatness. This view has been confirmed by Hattori and others (1991) who found that females deposited a greater proportion of fat internally with increasing fatness. Comparable results have been obtained in cattle (Belk, Tatum and Williams, 1991).

Furthermore, some authors have reported circumference measurements to be more accurately related to total body fat than either skinfold (Bray, Greenway, Molitch, Dohms, Atkinson and Hamilton, 1978) or ultrasonic measurements (Fanelli, Kuczmarski and Hirsch, 1988). Fanelli and her co-workers concluded that circumference measurements might reflect both internal and subcutaneous adipose tissue while only subcutaneous adipose tissue is measured with ultrasound (Fanelli and others, 1988). Davies and others (1986), using A-mode ultrasound in 25 women and 21 men, documented large individual variations in the proportion of fat situated subcutaneously. They found no relationship between subcutaneous and internal fat masses. Their conclusion was similar to the one reached by other authors; namely, that the variability in quantity and distribution of internal fat renders a measurement of subcutaneous fat inaccurate for predicting total body fat (Borkan and others, 1982b; Tokunaga and others, 1983; Heymsfield and Williams, 1988).

In the present thesis, subcutaneous fat thickness measurements were not highly correlated to body fatness in the total of 25 dog carcasses. Further analysis of the data showed that this correlation was particularly poor in dogs classified as old. These were animals over 6 years of age and most had been classified as fat. Many showed signs of having suffered endocrine disease, in particular hyperadrenocorticism. It was thought that age and/or disease had resulted in a redistribution of fat stores such that more fat was deposited

internally. The disproportion between the internal and external fat depots made subcutaneous fat measurements unrelated to total adiposity.

However, fat thickness measurements obtained in young and adult dogs were in better agreement with total body fat. Histology, in particular, was found to explain more than 90% of the variation in the fat content of these dogs. Hence, it would appear that measurements of subcutaneous fat can be of value in assessing body fatness at least in certain populations of dogs. The work of Anderson and Corbin (1982) would support this view. They found a good degree of correlation between measurements of subcutaneous fat in the carcass and total body fat, in a controlled population of Beagle dogs (females 2 to 4 years old).

The experiment described in Chapter 7, lends further support to the possible value of subcutaneous fat measurements for predicting fatness in dogs. Using more sophisticated ultrasound equipment (three-dimensional scanner), it was possible to overcome some of the limitations found with the previous A-mode device. Subcutaneous fat was readily measured in the lumbar area of 100 canine hospital in-patients. Results showed that ultrasonic fat thickness measurements were in close agreement with a condition score assessment of overall fatness. There was a statistically significant difference in the amount of subcutaneous fat measured in the various condition score groups: a greater score was generally associated with a thicker layer of subcutaneous fat.

Other authors have reported two-dimensional scanners to be more accurate in measuring subcutaneous fat than simpler A-mode units (Kempster

and others, 1981; Simm, 1983; Fanelli and Kuczmarski, 1984; Weits and others, 1986; Kuczmarski and others, 1987). Two-dimensional pictures allow visualization of tissue boundaries, making interpretation of ultrasonic data easier.

The chemical analysis of the whole carcass revealed large differences in the amount of body fat among dogs. These findings were in agreement with the work of Mayer and Stadtfeld (1978) who analysed the body composition of 60 dogs, and of Anderson and Corbin (1982) who determined the fat content of 24 Beagle bitches. Chemically-determined fat content of human cadavers has been shown to vary considerably (reviewed by Garrow, 1983b), and it has long been recognized that fat stores in mammals are extremely variable (Moulton, 1923; Pace and Rathbun, 1945; Maynard, Loosli, Hintz and Warner, 1979).

The large differences in body fat content among dogs analysed in this work roughly reflected the condition scoring system (adapted from Edney and Smith, 1986). In general, animals subjectively classified as "fat" had a greater fat content than those classified as "normal" or "thin".

Clinical condition-score systems have been widely used in cattle to assess conformation and predict carcass composition (reviewed by Barton, 1967). They are thought to give an acceptable estimate of changes in the fat content of dairy cows (Ruegg, 1991). Similar scoring systems have been developed for use in horses (Henneke and others, 1983; Carroll and

Huntington, 1988). In humans, visual rating of obesity is considered to give a reasonable assessment of fat content (Mayer, 1980; Marshall, Hazlett, Spady and Quinney, 1990). However, as shown by Wright and Russel (1984a,b), condition scoring can only assess the subcutaneous fat depot. Hence, differences in partition of fat among the other depots will result in animals of different total fat content being classified with the same condition score. This view is in agreement with results found in the present thesis. The condition scoring system was able to detect major differences in the fat content of 25 dog carcasses, but animals classified with the same condition score actually differed widely in their fat content. Some dogs classified as "normal" had a fat content more in line with those classified as "fat" and vice versa. Thus, it would appear that the scoring system is a valuable method for detecting crude differences in the body composition of dogs and can be of considerable value in a clinical setting. However, it provides little information on actual fat content.

The value of skinfold callipers in the assessment of obesity in dogs remains unclear. As shown in Chapter 5, the correlation between this technique and either histology or ultrasound was generally low. This could be due to differences in the fat depth being measured. Both histology and ultrasound were measuring the layer of fat situated between dermis and cutaneous muscle, whereas a greater fat thickness was detected with the calliper technique.

This method was superior to ultrasound and almost as good as histology for predicting total fat content in the 25 dogs investigated. Although a poor correlation with total body fat was found in old dogs, skinfold measurements of subcutaneous fat were able to explain 88% of the variation in the fat content of young and adult animals. However, in the study described in Chapter 7, the calliper technique was less well correlated to a clinical assessment of adiposity than was ultrasound. Skinfold measurements could only account for 44% of the variation in condition score. The improvement in equipment probably made ultrasonic fat measurements more accurate than when the calliper technique was compared to an A-mode device (Chapters 5 and 6). Moreover, the difficulties in obtaining reliable calliper measurements in some of the fat and gross dogs described in Chapter 7, were not found in the 15 young dogs analysed for total fat content. A high degree of correlation had been found between calliper measurements and fat content in these 15 carcasses. By contrast, correlation between total adiposity and calliper measurements was very low in the 10 dogs classified as old. In general, these animals were considerably fatter than most of the young and adult dogs, and it is possible that calliper measurements taken from them were unreliable.

Skinfold callipers are difficult to apply in obese people (Garrow, 1982; Fanelli and Kuczmarski, 1984; Kuczmarski and others, 1987) and they have been shown to be inaccurate for predicting total adiposity in the very obese (Bray and others, 1978; Wang, Segal, Van Itallie, Kral, Gutin, Wadden and Pierson, 1986; Neale, 1988b; Gray and others, 1990) and in old individuals

(Young and others, 1963). Large fat folds tend to slip between the calliper tips and in obese people not all subcutaneous fat can be grasped into one skin fold (Bray and others, 1978; Kuczmarski and others, 1987; Gray and others, 1990). In some of the obese dogs studied in this thesis it was difficult to pinch a consistent fold of adipose tissue. Moreover, the author had the impression of not being able to lift all of the subcutaneous fat together with the skin, in most of the dogs classified as "fat" or "gross" in this study. This finding is in agreement with the experience of Anderson (1973).

Circumference measurements in humans have proved to be of value in assessing body fat (Seidell and others, 1988; Campaigne, 1990; Schwartz and others, 1990). Selby and others (1990) reported that circumference measurements actually measure a different dimension of fat distribution than skinfold callipers. Furthermore, Fanelli and co-workers (1988) found circumference measurements to be better predictors of body fat in women than were ultrasonic fat thickness measurements. They concluded that the latter were only measuring subcutaneous adipose tissue while circumferences were reflecting both the internal and external fat depots.

Various physical measurements such as length, height and girth, have been used to predict body composition in cattle (Barton, 1967; Gresham and others, 1986) and horses (Henneke and others, 1983), and a high degree of correlation has been generally found between some of these measurements and liveweight (Carroll and Huntington, 1988; Jones, Lawrence, Veevers,

Cleave and Hall, 1989). In the same way, Pendergrass and co-workers (1983) demonstrated a high degree of correlation between length, girth and body weight in 63 mongrel dogs, and similar results were obtained in the hospital population studied in this thesis.

Certain physical parameters are known to change with body weight in dogs, but the way in which these changes take place can differ considerably among the 35-fold weight range of dog breeds (Kirkwood, 1985). Body weight is thought to be a poor indicator of overall adiposity in dogs due to numerous differences in conformation and size within and between breeds (Joshua, 1970; Anderson, 1973; Lewis, 1978; Hand, 1990; Little, 1990). This view was confirmed in the present thesis, where dogs of similar body weight (and size) showed large differences in total fat content. In Anderson and Corbin's (1982) study, live weight was the best single predictor of fat content over a range of body fatness. However, they were using an otherwise homogeneous population of dogs: same breed, size, sex and similar age.

Live weight, but also girth and length were poorly correlated to a condition score assessment of body fat in the 100 dogs studied in this thesis (Chapter 7). However, these physical parameters slightly improved the predictive value of ultrasonic fat thickness measurements for estimating body condition score.

In conclusion, ultrasound is a reliable tool for measuring subcutaneous fat thickness in dogs. Units offering a two-dimensional picture of the structures under study make interpretation of tissue boundaries easier and are to be preferred over simpler one-dimensional units. A transducer operating at a lower frequency than the one used in this thesis would probably allow visualization of deeper fat layers. The technique is quick, simple to use and completely non-invasive, and it would appear to be of value in assessing body fat content in some populations of dogs. However, much work is needed to establish whether subcutaneous fat thickness measurements in dogs truly reflect overall adiposity in both sexes, at different levels of fatness and over a wide range of breeds, sizes and ages. The advantages over ultrasound of techniques such as computed tomography or magnetic resonance imaging, which can measure internal and external adipose tissue, must be considered when choosing a technique for body condition assessment.

Skinfold callipers are probably of limited use in dogs as their skin is easily detached from the underlying adipose tissue and a large proportion of the subcutaneous fat cannot be included into one fold particularly in obese cases.

Body fat content in dogs can be strikingly variable and some animals can sustain large quantities of adipose tissue in their bodies. Thick layers of fat can be found between successive muscle fasciae in obese dogs and the

lumbar area can contain a considerable amount of adipose tissue even in non-obese animals.

A well defined condition-scoring system is of major value in detecting broad differences in total fat content. This method should be used in clinical settings to diagnose and treat canine obesity until more objective measures of total adiposity are well established.

APPENDICES.

APPENDIX 5.1.

Approximate age-groups according to the wear and tear of dentition*.

YOUNG (6months-3years)	All permanent teeth present, little amount of tartar visible, cusps of upper middle incisor still intact but those of lower incisors I (middle) and II (intermediate) may be worn off.
ADULT (3-6years)	Cusps of upper incisors worn off, slight wear of canines, cusps of lower incisor III (corner) may be worn slightly.
OLD (> 6years)	Cusps of lower incisors III (corners) worn off, canines are becoming blunt, considerable amount of tartar may be present.

* From *Current Veterinary Therapy IV.* Editor R.W. Kirk, WB Saunders Company, Philadelphia, 1971. p.786.

APPENDIX 5.2.

Subcutaneous fat thickness (mm) measured with ultrasound, histology and skinfold callipers in dogs of differing body condition score.

ULTRASOUND

	EMACIATED (n=1)	THIN (n=4)	NORMAL (n=10)	FAT (n=12)	GROSS (n=1)
MEAN	0.85	0.95	1.08	1.32	2.44
SD	-	0.26	0.20	0.29	-
RANGE	-	(0.6-1.2)	(0.7-1.4)	(0.9-2.0)	-

HISTOLOGY

	EMACIATED (n=1)	THIN (n=4)	NORMAL (n=10)	FAT (n=12)	GROSS (n=1)
MEAN	0.42	0.95	1.28	1.79	3.42
SD	-	0.45	0.34	0.55	-
RANGE	-	(0.5-1.6)	(0.5-1.7)	(1.1-3.0)	-

CALLIPERS

	EMACIATED (n=1)	THIN (n=4)	NORMAL (n=7)	FAT (n=12)	GROSS (n=1)
MEAN	0.94	1.57	1.94	2.97	4.87
SD	-	0.23	0.25	1.16	-
RANGE	-	(1.3-1.8)	(1.6-2.2)	(1.8-6.0)	-

n= number of dogs in each condition-score group.

APPENDIX 5.3.

Subcutaneous fat thickness (mm) measured with the calliper technique in 25 dogs.

	AXILLA		FLANK		STERNUM	
	Mean	**sd**	**Mean**	**sd**	**Mean**	**sd**
Dog 4	1.45	0.0	1.58	0.01	2.47	0.1
Dog 5	1.52	0.03	1.43	0.01	2.00	0.0
Dog 6	1.70	0.0	1.95	0.07	1.70	0.0
Dog 7	2.30	0.0	1.80	0.0	2.47	0.03
Dog 8	1.30	0.14	1.27	0.03	1.40	0.14
Dog 9	1.25	0.07	1.55	0.07	1.20	0.0
Dog 10	1.55	0.07	1.50	0.0	1.45	0.07
Dog 11	1.95	0.07	1.55	0.07	4.40	0.14
Dog 12	0.95	0.07	0.90	0.0	0.80	0.0
Dog 13	2.85	0.07	2.35	0.07	3.35	0.07
Dog 14	1.27	0.03	1.17	0.03	1.22	0.03
Dog 15	2.05	0.21	1.65	0.07	1.60	0.14
Dog 16	7.50	0.0	4.62	1.59	4.80	0.28
Dog 17	6.80	0.28	4.90	0.28	3.50	0.14
Dog 18	2.90	0.42	1.80	0.0	2.30	0.28
Dog 19	9.00	0.7	3.10	0.14	5.75	0.35
Dog 20	2.70	0.7	1.95	0.63	1.95	0.07
Dog 21	3.20	0.0	2.05	0.07	3.25	0.07
Dog 22	4.20	0.42	1.90	0.0	2.80	0.42
Dog 23	1.70	0.14	1.90	0.0	2.10	0.0
Dog 24	2.20	0.0	2.35	0.07	1.80	0.0
Dog 25	3.15	0.21	2.65	0.07	3.75	0.07
Dog 26	1.75	0.07	2.30	0.28	2.70	0.0
Dog 27	2.05	0.07	2.65	0.07	4.55	0.07
Dog 28	2.10	0.0	1.90	0.0	2.80	0.28

APPENDIX 5.3.(Continued)

	ABDOMEN		THIGH		LUMBAR	
	Mean	**sd**	**Mean**	**sd**	**Mean**	**sd**
Dog 4	1.75	0.28	1.05	0.0	2.62	0.0
Dog 5	1.80	0.28	1.07	0.03	2.52	0.1
Dog 6	1.60	0.0	1.21	0.01	3.31	0.01
Dog 7	1.85	0.07	1.50	0.0	2.82	0.03
Dog 8	1.50	0.0	0.82	0.03	2.25	0.07
Dog 9	1.65	0.07	1.00	0.0	2.95	0.07
Dog 10	1.55	0.07	1.00	0.0	3.35	0.07
Dog 11	1.70	0.0	1.25	0.07	4.95	0.07
Dog 12	0.90	0.07	0.65	0.07	1.40	0.0
Dog 13	2.35	0.07	1.75	0.07	5.75	0.07
Dog 14	1.50	0.1	0.90	0.0	2.00	0.0
Dog 15	1.50	0.0	0.95	0.07	3.05	0.07
Dog 16	2.45	0.07	2.90	0.0	2.95	0.07
Dog 17	5.90	0.21	2.25	0.07	5.90	0.42
Dog 18	2.50	0.14	1.75	0.07	2.20	0.21
Dog 19	5.70	0.42	8.35	0.21	4.10	0.14
Dog 20	1.50	0.0	1.00	0.14	2.20	0.14
Dog 21	1.55	0.07	1.35	0.21	2.65	0.07
Dog 22	2.10	0.14	1.35	0.07	2.95	0.07
Dog 23	2.15	0.07	2.30	0.28	3.20	0.84
Dog 24	2.10	0.0	1.60	0.0	2.25	0.07
Dog 25	2.65	0.07	2.10	0.0	4.05	0.07
Dog 26	1.35	0.07	1.05	0.07	3.05	0.07
Dog 27	2.40	0.0	1.30	0.0	8.10	0.14
Dog 28	3.20	0.21	1.25	0.07	3.10	0.14

Values are means of two measurements after being halved to allow comparison with the ultrasound technique.

The calliper technique was not carried out in dogs no. 1, 2 and 3.

APPENDIX 5.4.

Subcutaneous fat thickness (mm) measured with ultrasound in 28 dogs.

	AXILLA		FLANK		STERNUM	
	Mean	**sd**	**Mean**	**sd**	**Mean**	**sd**
Dog 1	0.73	0.2	0.70	0.1	0.87	0.1
Dog 2	0.94	0.07	0.97	0.14	0.74	0.14
Dog 3	-	-	0.92	0.29	0.97	0.15
Dog 4	0.90	0.23	0.79	0.12	1.41	0.44
Dog 5	1.02	0.18	1.09	0.23	0.99	0.13
Dog 6	1.03	0.15	1.13	0.08	1.90	0.17
Dog 7	1.09	0.34	0.58	0.1	0.48	0.26
Dog 8	0.84	0.36	0.60	0.18	0.63	0.09
Dog 9	0.92	0.16	0.82	0.04	0.70	0.15
Dog 10	0.89	0.09	0.57	0.14	1.03	0.15
Dog 11	0.97	0.23	0.75	0.03	1.00	0.17
Dog 12	0.62	0.16	1.07	0.49	0.81	0.11
Dog 13	1.46	0.06	1.60	0.06	1.55	0.15
Dog 14	0.60	0.23	0.43	0.15	0.59	0.17
Dog 15	0.88	0.18	0.62	0.18	0.70	0.26
Dog 16	0.99	0.22	0.73	0.05	1.42	0.14
Dog 17	2.28	0.14	2.39	0.35	1.15	0.11
Dog 18	0.67	0.12	0.68	0.03	1.04	0.09
Dog 19	1.43	0.15	0.84	0.04	1.53	0.1
Dog 20	0.77	0.08	1.16	0.07	0.35	0.03
Dog 21	1.94	0.17	1.20	0.1	1.47	0.04
Dog 22	1.47	0.19	0.70	0.05	0.90	0.04
Dog 23	0.69	0.09	0.95	0.27	1.17	0.16
Dog 24	1.65	0.12	1.06	0.04	1.51	0.16
Dog 25	1.36	0.13	0.89	0.05	1.61	0.29
Dog 26	1.10	0.2	0.70	0.08	0.84	0.08
Dog 27	0.75	0.1	1.20	0.09	0.89	0.09
Dog 28	1.91	0.19	0.67	0.15	1.86	0.1

APPENDIX 5.4.(Continued)

	ABDOMEN		THIGH		LUMBAR	
	Mean	**sd**	**Mean**	**sd**	**Mean**	**sd**
Dog 1	0.85	0.04	-	-	2.77	0.12
Dog 2	0.69	0.12	-	-	2.13	0.23
Dog 3	0.90	0.17	1.09	0.19	2.83	0.14
Dog 4	1.07	0.22	1.33	0.25	2.21	0.24
Dog 5	0.73	0.11	1.00	0.27	1.64	0.34
Dog 6	1.21	0.28	0.85	0.07	2.54	0.51
Dog 7	0.51	0.19	1.57	0.13	0.41	0.04
Dog 8	0.52	0.13	1.17	0.48	2.00	0.18
Dog 9	0.42	0.09	0.76	0.1	1.58	0.14
Dog 10	0.50	0.1	0.51	0.09	2.16	0.42
Dog 11	1.03	0.09	0.55	0.15	4.66	0.76
Dog 12	1.22	0.33	0.81	0.22	0.57	0.15
Dog 13	2.40	0.14	0.95	0.33	4.24	0.21
Dog 14	0.45	0.19	0.65	0.15	1.15	0.1
Dog 15	0.57	0.03	0.70	0.14	2.42	0.26
Dog 16	0.94	0.25	0.65	0.08	1.98	0.33
Dog 17	1.74	0.22	2.88	0.34	4.24	0.46
Dog 18	1.42	0.08	1.49	0.12	1.45	0.06
Dog 19	1.18	0.1	1.57	0.27	1.73	0.02
Dog 20	1.22	0.15	0.78	0.2	1.96	0.19
Dog 21	1.14	0.37	0.73	0.05	2.77	0.8
Dog 22	0.67	0.08	0.72	0.15	1.40	0.14
Dog 23	1.67	0.1	0.59	0.09	1.10	0.26
Dog 24	1.61	0.26	1.15	0.16	1.42	0.11
Dog 25	1.52	0.03	1.55	0.11	1.65	0.14
Dog 26	1.94	0.38	0.61	0.31	1.26	0.12
Dog 27	0.71	0.02	0.68	0.1	3.01	0.05
Dog 28	0.88	0.23	0.42	0.06	1.36	0.1

Values are means of five measurements.

APPENDIX 5.5.

Subcutaneous fat thickness (mm) measured histologically in 28 dogs.

	AXILLA		FLANK		STERNUM	
	Mean	**sd**	**Mean**	**sd**	**Mean**	**sd**
Dog 1	0.77	0.17	-	-	1.13	0.19
Dog 2	1.18	0.35	1.36	0.33	2.12	0.31
Dog 3	-	-	1.03	0.24	1.55	0.14
Dog 4	1.28	0.12	1.64	0.2	-	-
Dog 5	1.59	0.21	1.20	0.2	0.99	0.32
Dog 6	1.20	0.25	1.01	0.25	2.13	0.06
Dog 7	1.40	0.36	0.27	0.07	0.45	0.37
Dog 8	0.62	0.13	0.36	0.07	0.46	0.09
Dog 9	1.32	0.16	1.23	0.18	0.95	0.07
Dog 10	0.92	0.26	0.55	0.07	-	-
Dog 11	0.80	0.08	1.49	0.13	1.31	0.32
Dog 12	0.46	0.11	0.32	0.07	0.44	0.11
Dog 13	3.11	0.44	2.38	0.13	3.23	0.14
Dog 14	0.65	0.14	0.28	0.03	0.69	0.23
Dog 15	0.97	0.12	0.62	0.1	0.98	0.5
Dog 16	2.31	0.14	1.45	0.23	1.33	0.2
Dog 17	3.53	0.5	2.28	0.31	2.59	0.59
Dog 18	0.77	0.11	0.77	0.09	1.61	0.09
Dog 19	3.07	0.2	1.18	0.13	2.62	0.27
Dog 20	1.34	0.08	1.55	0.27	0.45	0.07
Dog 21	3.26	0.33	1.59	0.07	2.76	0.33
Dog 22	2.22	0.19	1.01	0.05	0.53	0.09
Dog 23	0.98	0.07	1.39	0.05	2.12	0.16
Dog 24	2.59	0.33	1.58	0.08	1.99	0.22
Dog 25	1.82	0.42	1.04	0.05	1.99	0.12
Dog 26	1.47	0.12	1.18	0.14	1.29	0.1
Dog 27	0.96	0.14	2.23	0.21	1.83	0.26
Dog 28	1.39	0.11	0.95	0.12	2.94	0.25

APPENDIX 5.5.(Continued)

	ABDOMEN		THIGH		LUMBAR	
	Mean	**sd**	**Mean**	**sd**	**Mean**	**sd**
Dog 1	1.02	0.2	-	-	2.66	0.31
Dog 2	1.09	0.27	-	-	2.78	0.45
Dog 3	1.00	0.22	-	-	3.08	0.35
Dog 4	1.62	0.19	0.66	0.24	2.83	0.63
Dog 5	1.04	0.18	1.13	0.33	0.90	0.26
Dog 6	1.01	0.23	0.84	0.19	2.14	0.28
Dog 7	0.33	0.13	0.30	0.25	0.46	0.24
Dog 8	0.59	0.14	0.37	0.17	1.85	0.44
Dog 9	0.48	0.1	0.93	0.14	1.67	0.16
Dog 10	0.46	0.1	0.58	0.04	2.18	0.28
Dog 11	1.35	0.27	1.09	0.54	5.02	0.32
Dog 12	0.34	0.07	0.26	0.06	0.68	0.11
Dog 13	3.63	0.21	1.75	0.13	4.47	0.1
Dog 14	0.45	0.12	0.17	0.07	1.13	0.11
Dog 15	0.81	0.14	0.33	0.16	2.50	0.43
Dog 16	1.88	0.37	1.25	0.14	1.71	0.57
Dog 17	2.12	0.17	4.90	0.63	5.13	0.34
Dog 18	1.12	0.2	3.15	0.23	1.30	0.22
Dog 19	2.02	0.09	3.19	0.25	2.06	0.06
Dog 20	1.91	0.27	0.44	0.16	1.68	0.21
Dog 21	0.72	0.34	1.33	0.15	2.77	0.21
Dog 22	1.67	0.05	0.35	0.11	1.22	0.04
Dog 23	2.29	0.17	0.97	0.42	1.00	0.15
Dog 24	2.27	0.85	2.08	0.28	1.23	0.16
Dog 25	2.11	0.18	1.93	0.39	1.73	0.21
Dog 26	0.84	0.07	0.68	0.26	1.45	0.22
Dog 27	1.40	0.1	1.08	0.27	3.85	0.28
Dog 28	0.93	0.14	0.46	0.09	1.77	0.15

Values are means of six measurements.

APPENDIX 6.1.

Percentage of fat over dry matter calculated by the Soxhlet and Hydrolysed fat extraction methods in 25 dogs.

	SOXHLET FAT	HYDROLYSED FAT
Dog 4	44.88	46.40
Dog 5	36.07	38.31
Dog 6	41.11	43.29
Dog 7	25.76	24.89
Dog 8	29.10	29.97
Dog 9	47.44	47.90
Dog 10	26.77	28.22
Dog 11	66.24	66.55
Dog 12	21.33	22.64
Dog 13	59.77	59.48
Dog 14	24.27	25.67
Dog 15	37.37	39.17
Dog 16	64.76	64.41
Dog 17	75.74	74.17
Dog 18	65.86	64.61
Dog 19	75.87	74.21
Dog 20	69.64	65.11
Dog 21	68.60	69.58
Dog 22	63.04	63.76
Dog 23	54.95	53.74
Dog 24	71.33	71.16
Dog 25	65.92	65.80
Dog 26	61.19	61.96
Dog 27	69.15	69.72
Dog 28	49.11	50.36

Values are means of two fat determinations obtained from two different aliquots.

APPENDIX 6.2.

Total body water and fat expressed as percentages of whole body tissue in 25 dog carcasses.

	BODY WATER	BODY FAT
Dog 4	56.89	19.34
Dog 5	59.69	14.53
Dog 6	58.76	16.95
Dog 7	63.99	9.27
Dog 8	64.13	10.43
Dog 9	58.49	19.69
Dog 10	64.41	9.52
Dog 11	43.04	37.73
Dog 12	63.50	7.78
Dog 13	48.02	31.06
Dog 14	66.16	8.21
Dog 15	59.82	15.01
Dog 16	43.54	36.56
Dog 17	40.23	45.26
Dog 18	42.48	37.88
Dog 19	41.78	44.17
Dog 20	45.20	38.16
Dog 21	43.73	38.60
Dog 22	46.21	33.40
Dog 23	49.68	27.65
Dog 24	38.90	43.58
Dog 25	43.02	37.56
Dog 26	46.86	32.51
Dog 27	39.63	41.74
Dog 28	52.73	23.21

Values are means of two chemical determinations obtained from two different aliquots.

APPENDIX 6.3.

Total protein and ash content expressed as percentages of whole body tissue in 25 dog carcasses.

	BODY PROTEIN	BODY ASH
Dog 4	19.05	3.19
Dog 5	20.52	3.54
Dog 6	17.84	3.91
Dog 7	21.49	4.28
Dog 8	19.82	3.40
Dog 9	17.03	2.86
Dog 10	19.02	3.55
Dog 11	14.96	2.44
Dog 12	21.22	4.78
Dog 13	16.46	2.96
Dog 14	19.85	3.82
Dog 15	19.91	3.41
Dog 16	16.37	3.72
Dog 17	12.36	3.88
Dog 18	15.57	4.37
Dog 19	14.72	2.50
Dog 20	15.32	3.17
Dog 21	15.91	2.64
Dog 22	15.01	2.90
Dog 23	16.99	3.77
Dog 24	16.92	2.13
Dog 25	17.67	2.73
Dog 26	16.55	3.40
Dog 27	14.53	2.53
Dog 28	17.57	3.49

Values are means of two chemical determinations obtained from two different aliquots.

APPENDIX 6.4.

Multiple regression parameters for the estimation of body weight.

		YOUNG AND ADULT DOGS	
PARAMETER	HISTOLOGY	ULTRASOUND	CALLIPERS
R-sq	23.5%	26.8%	38.1%
R-sq (adj)	0.0%	0.0%	0.0%
s	5.45	4.69	4.31
		OLD DOGS	
R-sq	94.1%	90.2%	98.1%
R-sq (adj)	82.4%	70.5%	94.3%
s	4.38	5.66	2.49

APPENDIX 7.1.

Number of obese ("fat" & "gross") and non-obese dogs within each breed.

BREED	OBESE	NON-OBESE	TOTAL
German Shepherd	3	6	9
Golden Retriever	3	5	8
Labrador Retriever	6	2	8
Doberman	1	6	7
Cocker Spaniel	3	3	6
Weimaraner	0	4	4
Border Collie	2	2	4
English Bull Terrier	3	1	4
Jack Russell Terrier	2	2	4
Boxer	1	2	3
Cairn Terrier	3	0	3
Great Dane	0	2	2
Rough Collie	0	2	2
Cavalier King Charles	1	1	2
Springer Spaniel	1	1	2
West Highland White Terrier	2	0	2
Rottweiler	2	0	2
Korean Gindo	0	1	1
Irish Setter	0	1	1
Dalmatian	0	1	1
Setter Gordon	0	1	1
Deerhound	0	1	1
Standard Poodle	0	1	1
Old English Sheepdog	0	1	1
Staffordshire Bull Terrier	0	1	1
Greyhound	0	1	1
Chow-Chow	0	1	1
Pointer	0	1	1
Trailhound	0	1	1
Soft-coated Wheaten Terrier	0	1	1
Rhodesian Ridgeback	1	0	1
Bearded Collie	1	0	1
Scottish Terrier	1	0	1
Samoyed	1	0	1

APPENDIX 7.2.

Subcutaneous fat thickness (mm) measured with ultrasound in the lumbar area of 100 dogs.

BODY CONDITION SCORE

EMACIATED		THIN		NORMAL		FAT		GROSS	
Mean	**sd**	**Mean**	**sd**	**Mean**	**sd**	**Mean**	**sd**	**Mean**	**sd**
0.31	0.05	1.15	0.1	3.45	0.1	1.69	0.17	4.61	0.06
0.44	0.06	0.52	0.04	2.17	0.25	2.54	0.19	5.14	0.02
0.37	0.13	1.25	0.19	2.34	0.16	3.45	0.32	2.78	0.65
0.53	0.07	0.76	0.03	1.87	0.02	3.57	0.27	3.89	0.08
0.53	0.04	0.43	0.1	2.10	0.17	3.59	0.09	3.94	0.23
0.41	0.08	0.83	0.13	0.48	0.11	2.65	0.16		
0.43	0.03	0.47	0.02	0.83	0.04	2.15	0.1		
0.58	0.05	0.62	0.03	1.43	0.17	3.96	0.04		
		0.84	0.06	1.63	0.06	2.54	0.27		
		0.90	0.1	1.33	0.05	4.16	0.21		
		2.86	0.09	1.94	0.16	2.43	0.09		
		1.52	0.18	1.60	0.07	2.82	0.23		
		0.78	0.13	2.86	0.07	4.32	0.15		
		0.81	0.09	1.91	0.1	1.64	0.12		
		1.13	0.18	1.02	0.06	2.77	0.18		
		0.89	0.1	0.86	0.05	3.41	0.11		
		0.69	0.16	0.86	0.12	3.47	0.04		
		1.08	0.19	0.92	0.18	2.21	0.21		
		0.68	0.18	3.12	0.07	3.10	0.14		
				1.61	0.15	2.72	0.21		
				1.11	0.12	2.60	0.24		
				1.58	0.13	3.08	0.15		
				1.52	0.24	1.93	0.12		
				2.22	0.24	2.66	0.25		
				2.07	0.33	4.28	0.12		
				1.69	0.09	2.08	0.24		
				1.19	0.08	2.45	0.31		
				0.97	0.11	2.81	0.34		
				1.49	0.1	1.74	0.14		
				1.23	0.02	1.43	0.04		
						2.32	0.06		
						3.02	0.09		
						1.76	0.36		
						2.59	0.37		
						2.40	0.12		
						2.16	0.12		
						3.45	0.14		
						2.06	0.15		

Values are means of 4 measurements.

APPENDIX 7.3.

Subcutaneous fat thickness (mm) measured with the calliper technique in the lumbar area of 100 dogs.

BODY CONDITION SCORE									
EMACIATED		THIN		NORMAL		FAT		GROSS	
Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
2.15	0.21	2.60	0.0	5.75	0.07	4.20	0.14	11.25	1.06
2.05	0.07	2.50	0.0	3.65	0.07	4.55	0.07	15.75	3.18
2.27	0.17	2.45	0.07	4.05	0.07	5.10	0.0	7.47	0.74
1.35	0.07	1.55	0.07	3.80	0.28	5.05	0.07	7.60	0.7
1.95	0.07	3.05	0.07	5.25	0.07	5.85	0.21	6.75	0.07
2.10	0.0	2.55	0.07	1.95	0.07	4.15	0.07		
2.40	0.28	2.10	0.14	2.45	0.07	8.25	0.35		
2.35	0.07	3.20	0.14	5.20	0.14	5.75	0.35		
		1.95	0.07	3.80	0.14	4.05	0.07		
		3.20	0.14	2.50	0.0	8.85	0.21		
		5.15	0.07	3.80	0.28	4.65	0.07		
		2.37	0.03	3.65	0.07	3.85	0.21		
		2.05	0.07	5.25	0.35	5.65	0.21		
		2.10	0.28	3.50	0.0	5.70	0.14		
		2.90	0.14	3.75	0.35	5.35	0.21		
		2.90	0.14	2.75	0.21	4.50	0.0		
		2.00	0.14	2.10	0.0	6.50	0.0		
		3.15	0.21	2.70	0.14	3.70	0.0		
		2.85	0.07	9.00	0.7	3.20	0.28		
				2.85	0.07	5.75	0.07		
				2.30	0.14	2.45	0.07		
				2.07	0.03	4.70	0.28		
				4.37	0.17	3.35	0.07		
				3.30	0.14	5.95	0.07		
				3.30	0.14	5.95	0.07		
				4.55	0.07	3.42	0.1		
				3.02	0.03	6.45	0.07		
				2.45	0.07	5.35	0.07		
				5.60	0.0	4.75	0.07		
				2.95	0.07	3.65	0.07		
						4.85	0.35		
						5.80	0.14		
						3.95	0.07		
						4.05	0.07		
						4.50	0.0		
						4.75	0.07		
						5.30	0.28		
						4.05	0.07		

Values are means of 2 measurements.

REFERENCES.

REFERENCES.

Alexander, H. and Miller, D.L. (1979). Determining skin thickness with pulsed ultrasound. *Journal of Investigative Dermatology.* **72**: 17-19.

Allen, T.H., Peng, M.T., Chen, K.P., Huang, T.F., Chang, C. and Fang, H.S. (1956). Prediction of total adiposity from skinfolds and the curvilinear relationship between external and internal adiposity. *Metabolism.* **5**: 346-352.

Alliston, J.C. and Hinks, C.E. (1981). A note in the use of the "Danscanner" for prediction of the composition of a sample joint from beef cattle. *Animal Production.* **32**: 345-347.

Anantharaman-Barr, G. (1990). The effect of ovarohysterectomy on energy metabolism in dogs. *Veterinary International.* **2** (2): 19-20.

Andersen, G.L. and Lewis, L.D. (1980). Obesity. In: *Current Veterinary Therapy VII.* Editor R.W. Kirk, WB Saunders Company, Philadelphia, 1034-1039.

Anderson, D.B. and Kauffman, R.G. (1973). Cellular and enzymatic changes in porcine adipose tissue during growth. *Journal of Lipid Research.* **14**: 160-168.

Anderson, D.B. and Corbin, J.E. (1982). Estimating body fat in mature Beagle bitches. *Laboratory Animal Science.* **32**: 367-370.

Anderson, R.S. (1973). Obesity in the dog and cat. *The Veterinary Annual.* **13**: 182-186.

Armstrong, D.B., Dublin, L.I., Weathley, G.H. and Marks, H.H. (1951). Obesity and its relation to health and disease. *Journal of the American Medical Association.* **147**: 1007-1114.

Arnold, R.N., Hentges, E.J. and Trenkle, A. (1985). Evaluation of the use of deuterium oxide dilution techniques for determination of body composition of beef steers. *Journal of Animal Science.* **60** (5): 1188-1200.

Ashwell, M., Cole, T.J. and Dixon, A.K. (1985). Obesity: new insight into the anthropometric classification of fat distribution shown by computed tomography. *British Medical Journal.* **290**: 1692-1694.

Atkinson, R.L. and Bray, G.A. (1978). Energy balance in obesity and its relationship to diabetes mellitus. In: *Advances in Modern Nutrition. Vol.II.* Editors H.M. Katzen and R.J. Mahler, John Wiley & Sons, New York, 373-393.

Ballarini, G. (1990). Animal psychodietetics. *Journal of Small Animal Practice.* **31**: 523-532.

Balta, P.J., Ward, M.W.M. and Tomkins, A.M. (1981). Ultrasound for measurement of subcutaneous fat. *Lancet.* **i**: 504-505.

Barsanti, J.A., Jones, B.D., Spano, J.S. and Taylor, H.W. (1977). Prolonged anorexia associated with hepatic lipidosis in three cats. *Feline Practice.* **7**: 52-57.

Bartle, S.J., Kock, S.W., Preston, R.L., Wheeler, T.L. and Davis, G.W. (1987). Validation of urea dilution to estimate in vivo body composition in cattle. *Journal of Animal Science.* **64**: 1024-1030.

Barton, R.A. (1967). The relation between live animal conformation and the carcass of cattle. *Animal Breeding Abstracts.* **35** (1): 1-22.

Baumgartner, R.N., Roche, A.F., Chumlea, Wm.C., Siervogel, R.M. and Glueck, C.J. (1987). Fatness and fat patterns: associations with plasma lipids and blood pressures in adults, 18 to 57 years of age. *American Journal of Epidemiology.* **126** (4): 614-628.

Belk, K.E., Tatum, J.D. and Williams, F.L. (1991). Deposition and distribution of carcass fat for steers differing in frame size and muscle thickness. *Journal of Animal Science.* **69** (2): 609-616.

Belshaw, B.E. and Rijnberk, A.D. (1980). Hypothyroidism. In: *Current Veterinary Therapy VII.* Editor R.W. Kirk, WB Saunders Company, Philadelphia, 994-998.

Bennett, G.L., Meyer, H.H. and Kirton, A.H. (1988). Effects of selection for divergent ultrasonic fat depth in rams on progeny fatness. *Animal Production.* **47**: 379-386.

Bergman, E.N., Reulein, S.S. and Corlett, R.E. (1989). Effects of obesity on insulin sensitivity and responsiveness in sheep. *American Journal of Physiology.* **257** (5): E772-E781.

Berlan, M., Carpene, C., Lafontan, M. and Dang-Tran, L. (1982). Alpha-2 adrenergic antilipolytic effect in dog fat cells: incidence of obesity and adipose tissue localization. *Hormone and Metabolic Research.* **14** (5): 257-260.

Bessard, T., Schutz, Y. and Jéquier, E. (1983). Energy expenditure and postprandial thermogenesis in obese women before and after weight loss. *American Journal of Clinical Nutrition.* **38**: 680-693.

Bevilacqua, S., Bonadonna, R., Buzzigoli, G., Boni, C., Ciociaro, D., Maccari, F., Giorico, M.A. and Ferrannini, E. (1987). Acute elevation of free fatty acid levels leads to hepatic insulin resistance in obese subjects. *Metabolism.* **36** (5): 502-506.

Bishop, O.N. (1966). The chi-square test. In: *Statistics for Biology.* Longmans, Green & Co. Ltd., London, 72-79.

Björntorp, P. and Yang, M.V. (1982). Refeeding after fasting in the rat: effects on body composition and food efficiency. *American Journal of Clinical Nutrition.* **36**: 444-449.

Björntorp, P. (1990). How should obesity be defined?. *Journal of Internal Medicine.* **227**: 147-149.

Black, D.R., Vora, J., Hayward, M. and Marks, R. (1986). Ultrasonic measurement of subcutaneous fat and its correlation with two other techniques. *Bioengineering and the Skin.* **12** (1): 139 (Abstract).

Black, D.R., Vora, J., Hayward, M. and Marks, R. (1988). Measurement of subcutaneous fat thickness with high frequency pulsed ultrasound: comparisons with a calliper and a radiographic technique. *Clinical Physics and Physiological Measurement.* **9** (1): 57-64.

Booth, R.A.D., Goddard, B.A. and Paton, A. (1966). Measurement of fat thickness in man: a comparison of ultrasound, Harpenden callipers and electrical conductivity. *British Journal of Nutrition.* **20**: 719-725.

Borkan, G.A., Gerzof, S.G., Robbins, A.H., Hults, D.E., Silbert, C.K. and Silbert, J.E. (1982a). Assessment of abdominal fat content by computed tomography. *American Journal of Clinical Nutrition.* **36**: 172-177.

Borkan, G.A., Hults, D.E., Cardarelli, J. and Burrows, B.A. (1982b). Comparison of ultrasound and skinfold measurements in assessment of subcutaneous and total fatness. *American Journal of Physical Anthropology.* **58**: 307-313.

Borkan, G.A., Hults, D.E., Gerzof, S.G., Burrows, B.A. and Robbins, A.H. (1983). Relationship between computed tomography tissue areas, thicknesses and total body composition. *Annals of Human Biology.* **10**: 537-546.

Bouchard, C. and Pérusse, L. (1988). Heredity and body fat. *Annual Review of Nutrition.* **8**: 259-277.

Bouchard, C., Tremblay, A., Després, J.P., Nadeau, A., Lupien, P.J., Thériault, G., Dussualt, J., Moorjani, S., Pinault, S. and Fournier, G. (1990). The response to long-term overfeeding in identical twins. *New England Journal of Medicine.* **322** (21): 1477-1482.

Bouchard, C. (1991a). Heredity and the path to overweight and obesity. *Medicine and Science in Sports and Exercise.* **23** (3): 285-291.

Bouchard, C. (1991b). Current understanding of the etiology of obesity: genetic and nongenetic factors. *American Journal of Clinical Nutrition.* **53** (6 Suppl.): 1561S-1565S.

Boulangé, A., Planche, E. and de Gasquet, P. (1979). Onset of genetic obesity in the absence of hyperphagia during the first week of life in the Zucker rat (fa/fa). *Journal of Lipid Research.* **20**: 857-864.

Branam, J.E. (1988). Dietary management of obese dogs and cats. *Veterinary Technician.* **9** (9): 490-493.

Bray, G.A. and York, D.A. (1971). Genetically transmitted obesity in rodents. *Physiological Reviews.* **51** (3): 598-646.

Bray, G.A. (1972). Clinical management of the obese adult. *Postgraduate Medicine.* **51**: 125-130.

Bray, G.A., Greenway, F.L., Molitch, M.E., Dohms, W.T., Atkinson, R.L. and Hamilton, K. (1978). Use of anthropometric measures to assess weight loss. *American Journal of Clinical Nutrition.* **31**: 769-773.

Bray, G.A. and York, D.A. (1979). Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. *Physiological Reviews.* **59**: 719-743.

Bray, G.A. (1987). Obesity - a disease of nutrient or energy balance?. *Nutrition Reviews.* **45** (2): 33-43.

Bray, G.A. (1990). Obesity: historical development of scientific and cultural ideas. *International Journal of Obesity.* **14** (11): 909-926.

Bray, G.A. (1991). Obesity, a disorder of nutrient partitioning: the MONA LISA hypothesis. *Journal of Nutrition.* **121**: 1146-1162.

Brown, S.A. (1987). Obesity. In: *Small Animal Medical Diagnosis.* Editors M.D. Lorenz and L.M. Cornelius, Lippincott Company, Philadelphia, 98-106.

Brožek, J. and Keys, A. (1950). Evaluation of leanness-fatness in man: a survey of methods. *Nutrition Abstracts and Reviews.* **20**: 247-256.

Bullen, B.A., Quaade, F., Olesen, E. and Lund, S.A. (1965). Ultrasonic reflections used for measuring subcutaneous fat in humans. *Human Biology.* **28**: 375-384.

Cameron, J.R. and Skofronick, J.G. (1978). Sound in Medicine. In: *Medical Physics.* John Wiley & Sons, New York, 253-293.

Campaigne, B.N. (1990). Body fat distribution in females: metabolic consequences and implications for weight loss. *Medicine and Science in Sports and Exercise.* **22** (3): 291-297.

Campion, D.R., Hausman, G.J., Stone, R.T. and Klindt, J. (1988). Influence of maternal obesity on foetal development in pigs. *Journal of Animal Science.* **66**: 28-33.

Carroll, C.L. and Huntington, P.J. (1988). Body condition scoring and weight estimation of horses. *Equine Veterinary Journal.* **20** (1): 41-45.

Chandra, R.K. (1980). Cell-mediated immunity in genetically obese mice. *American Journal of Clinical Nutrition.* **33**: 13-16.

Chang, S., Graham, B., Yakubu, F., Lin, D., Peters, J.C. and Hill, J.O. (1990). Metabolic differences between obesity-prone and obesity-resistant rats. *American Journal of Physiology.* **259** (6 Pt 2): R1103-R1110.

Chen, J.C. and Bellenger, C.R. (1987). Obese appearance, mammary development and retardation of hair growth following megestrol acetate administration to cats. *Journal of Small Animal Practice.* **28**: 1161-1167.

Cherniack, R.M., Zwillich, C.W., Macklem, P.T., Kryger, M.H. and Olson, G.H. (1986). Obesity. *American Review of Respiratory Disease.* **134**: 827-828.

Chumlea, W.C. and Roche, A.F. (1986). Ultrasonic and skinfold calliper measures of subcutaneous adipose tissue thickness in elderly men and women. *American Journal of Physical Anthropology.* **71**: 351-357.

Cianzio, D.S., Topel, D.G., Whitehurst, G.B., Beitz, D.C. and Self, H.L. (1985). Adipose tissue growth and cellularity: changes in bovine adipocyte size and number. *Journal of Animal Science.* **60** (4): 970-976.

Ciaraldi, T.P., Kolterman, O.G. and Olefsky, J.M. (1981). Mechanism of the postreceptor defect in insulin action in human obesity. *Journal of Clinical Investigation.* **68**: 875-880.

Clark, M.G., Rattigan, S. and Colquhoun, E.Q. (1991). Hypertension in obesity may reflect a homeostatic thermogenic response. *Life Sciences.* **48** (10): 939-947.

Cleary, M.P., Vasselli, J.R. and Greenwood, M.R.C. (1980). Development of obesity in Zucker obese (fa/fa) rat in absence of hyperphagia. *American Journal of Physiology.* **238**: E284-E292.

Clutton, R.E. (1988). The medical implications of canine obesity and their relevance to anaesthesia. *British Veterinary Journal.* **144**: 21-28.

Clutton-Brock, J. (1984). Dog. In: *Evolution of Domesticated Animals.* Editor I.L. Mason, Longman Group Ltd, Essex, 198-217.

Cohen, J. (1985). Obesity: a review. *Journal of the Royal College of General Practitioners.* **35** (278): 435-441.

Cohn, S., Vaswani, A.N., Vartsky, D., Yasumura, S., Sawitsky, A., Gartenhaus, W. and Ellis, K.J. (1982). In vivo quantification of body nitrogen for nutritional assessment. *American Journal of Clinical Nutrition.* **35**: 1186-1191.

Colliver, J.A., Frank, S. and Frank, A. (1983). Similarity of obesity indices in clinical studies of obese adults: a factor analysis study. *American Journal of Clinical Nutrition.* **38**: 640-647.

Conge, G.A., Gouache, P., Joyeux, Y., Goichot, J. and Fournier, J.M. (1988). Effects of different kinds of experimental obesity in the resistance of mice to infection with *Salmonella typhimurium* and *Klebsiella pneumoniae. Annals of Nutrition and Metabolism.* **32** (3): 113-120.

Conway, J.M., Norris, K.H. and Bodwell, C.E. (1984). A new approach for the estimation of body composition: infrared interactance. *American Journal of Clinical Nutrition.* **40**: 1123-1130.

Craig, J.V. (1981). Domestication. In: *Domestic Animal Behavior.* Prentice-Hall Inc., Englewood Cliffs, New Jersey, 21-31.

Crane, S.W. (1991). Occurrance and management of obesity in companion animals. *Journal of Small Animal Practice.* **32**: 275-282.

Daly, C.H. and Wheeler, J.B. (1971). The use of ultrasonic thickness measurement in the clinical evaluation of the oral soft tissues. *International Dental Journal.* **21** (4): 418-429.

Danforth, E., Jr. (1983). The role of thyroid hormones and insulin in the regulation of energy metabolism. *American Journal of Clinical Nutrition.* **38**: 1006-1017.

Darke, P.G.G. (1978). Obesity in small animals. *The Veterinary Record.* **102**: 545-546.

Davidson, S. and Passmore, R. (1969). Obesity. In: *Human Nutrition and Dietetics.* 4th Edition. E. & S. Livingstone Ltd., Edinburgh, 367-385.

Davies, P.S.W., Jones, P.R.M. and Norgan, N.G. (1986). The distribution of subcutaneous and internal fat in man. *Annals of Human Biology.* **13** (2): 189-192.

de Bruijne, J.J. and Lubberink, A.M.M.E. (1977). Obesity. In: *Current Veterinary Therapy VI.* Editor R.W. Kirk, WB Saunders Company, Philadelphia, 1068-1070.

Després, J.P., Moorjani, S., Lupien, P.J., Tremblay, A., Nadeau, A. and Bouchard, C. (1990). Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. *Arteriosclerosis.* **10** (4): 497-511.

Dines, K.A., Sheets, P.W., Brink, J.A., Hanke, C.W., Condra, K.A., Clendenon, J.L., Goss, S.A., Smith, D.J. and Franklin, T.D. (1984). High frequency ultrasonic imaging of skin: experimental results. *Ultrasonic Imaging.* **6**: 408-434.

Domermuth, W., Veum, T.L., Alexander, M.A., Hedrick, H.B., Clark, J. and Eklund, D. (1976). Prediction of lean body composition of live market swine by indirect methods. *Journal of Animal Science.* **43**: 966-976.

Domingo, E.A., Trigg, T.E. and Topps, J.H. (1973). Estimation of body composition of sheep by isotopic dilution techniques. 1. Exchangeable potassium. *Proceedings of the Nutrition Society.* **32** (1): 20 (Abstract).

Dumon, C. (1988). L'obésité chez le chien. (Obesity in dogs). *Bulletin des Groupements Techniques Vétérinaires.* **2**: 47-59.

Dunshea, F.R., Bell, A.W., Chandler, K.D. and Trigg, T.E. (1988). A two-pool model of tritiated water kinetics to predict body composition in unfasted lactating goats. *Animal Production.* **47**: 435-445.

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

Dykes, P.J. and Marks, R. (1977). Measurement of skin thickness: a comparison of two in vivo techniques with a conventional histometric method. *Journal of Investigative Dermatology.* **69**: 275-278.

Eason, G., Coles, C.W. and Gettinby, G. (1980). Parametric tests. In: *Mathematics and Statistics for the Bio-sciences.* Ellis Horwood Ltd., Chichester, West Sussex, 465-486.

East, E., Taylor, J., Miller, I.T. and Widdowson, R.W. (1959). Measurement of backfat thickness on live pigs by ultrasonics. *Animal Production.* **1**: 129-134.

Edney, A.T.B. (1974). Management of obesity in the dog. *Veterinary Medicine/Small Animal Clinician.* **69**: 46-49.

Edney, A.T.B. (1978). Dietary management in small animal practice. *The Veterinary Record.* **102**: 543-545.

Edney, A.T.B. and Smith, P.M. (1986). Study of obesity in dogs visiting veterinary practices in the U.K. *The Veterinary Record.* **118**: 391-396.

Edwards, D.A.W. (1950). Observation on the distribution of subcutaneous fat. *Clinical Science.* **9**: 259-270.

Edwards, D.A.W., Hammond, W.H., Healy, M.J.R., Tanner, J.M. and Whitehouse, R.H. (1955). Design and accuracy of callipers for measuring subcutaneous tissue thickness. *British Journal of Nutrition.* **9**: 133-143.

Egerter, D.E. (1989). Is MR spectroscopy ready for prime time?. *Diagnostic Imaging.* **11** (2): 127-146.

Elahi, D., Nagulesparan, M., Hershcopf, R.J., Muller, D.C., Tobin, J.D., Blix, P.M., Rubenstein, A.H., Unger, R.H. and Andres, R. (1982). Feedback inhibition of insulin secretion by insulin: relation to the hyperinsulinaemia of obesity. *New England Journal of Medicine.* **306**: 1196-1202.

Escoffier, C., Querleux, B., de Rigal, J. and Leveque, J.L. (1986). In vitro study of the velocity of ultrasound in the skin. *Bioengineering and the Skin.* **2**: 87-94.

Ettinger, S.J. (1983). Body weight. In: *Textbook of Veterinary Internal Medicine.* 2nd Edition. Editor S.J. Ettinger, WB Saunders Company, Philadelphia, 100-102.

Evans, H.E. and Christensen, G.C. (1979). Muscles. In: *Miller's Anatomy of the Dog.* 2nd Edition. WB Saunders Company, Philadelphia, 269-410.

Fanelli, M.T. and Kuczmarski, R.J. (1984). Ultrasound as an approach to assessing body composition. *American Journal of Clinical Nutrition.* **39**: 703-709.

Fanelli, M.T., Kuczmarski, R.J. and Hirsch, M. (1988). Estimation of body fat from ultrasound measures of subcutaneous fat and circumferences in obese women. *International Journal of Obesity.* **12** (2): 125-132.

Faust, I.M., Johnson, P.R., Stern, J.S. and Hirsch, J. (1978). Diet-induced adipocyte number increase in adult rats: a new model of obesity. *American Journal of Physiology.* **235**: E279-E286.

Feldman, E.C. (1989). Adrenal gland disease. In: *Textbook of Veterinary Internal Medicine.* 3rd Edition. Editor S.J. Ettinger, WB Saunders Company, Philadelphia, 1721-1774.

Ferrell, C.L. and Cornelius, S.G. (1984). Estimation of body composition in pigs. *Journal of Animal Science.* **58** (4): 903-912.

Finlay, A.Y., Moseley, H. and Duggan, T.C. (1987). Ultrasound transmission time: an in vivo guide to nail thickness. *British Journal of Dermatology.* **117**: 765-770.

Fiser, R.H., Rollins, J.B. and Beisel, W.R. (1972). Decreased resistance against infectious canine hepatitis in dogs fed a high fat diet. *American Journal of Veterinary Research.* **33**: 713-719.

Fletcher, R.F. (1962). The measurement of total body fat with skinfold callipers. *Clinical Science.* **22**: 333-346.

Forbes, D.C. and White, D.E. (1987). A case of marked and unresponsive obesity. *Canadian Veterinary Journal.* **28**: 187.

Forbes, G.B., Gallup, J. and Hursch, J.B. (1961). Estimation of total body fat from ^{40}K content. *Science.* **133**: 101-102.

Fornage, B.D. and Deshayes, J.L. (1986). Ultrasound of normal skin. *Journal of Clinical Ultrasound.* **14**: 619-622.

Foster, M.A. and Fowler, P.A. (1988). Non-invasive methods for assessment of body composition. *Proceedings of the Nutrition Society.* **47**: 375-385.

Fox, J.G. and Beatty, J.O. (1975). A case report of complicated diabetes mellitus in a cat. *Journal of the American Animal Hospital Association.* **11**: 129-134.

Fraley, J.R. (1988). An evaluation of a dry-fat product as a source of supplemental energy in pig diets. *Journal of Animal Science.* **66**: 1697-1702.

Freiherr, G. (1989). Diagnostic ultrasound: where is it headed?. *Diagnostic Imaging.* **11** (4): 94-103.

Fried, A.M., Coughlin, K. and Griffen, W.O. (1986). The sonographic fat/muscle ratio. *Investigative Radiology.* **21**: 71-75.

Friedman, G.J. (1980). Diet in the treatment of diabetes mellitus. In: *Modern Nutrition in Health and Disease.* 6th Edition. Editors R.S. Goodhart and M.E. Shils, Lea & Febiger, Philadelphia, 977-997.

Frisch, R.E. (1987). Body fat, menarche, fitness and fertility. *Human Reproduction.* **2** (6): 521-533.

Fuller, M.F., Fowler, P.A., McNeill, G. and Foster, M.A. (1990). Body composition: the precison and accuracy of new methods and their suitability for longitudinal studies. *Proceedings of the Nutrition Society.* **49**: 423-436.

Fung, K.P., Lee, J., Lau, S.P., Chow, O.K., Wong, T.W. and Davis, D.P. (1990). Properties and clinical implications of body mass indices. *Archives of Diseases of Childhood.* **65** (5): 516-519.

Garn, S.M., Hawthorne, V.M., Pilkington, J.J. and Pesick, S.D. (1983). Fatness and mortality in the west of Scotland. *American Journal of Clinical Nutrition.* **38**: 313-319.

Garn, S.M., Leonard, W.R. and Hawthorne, V.M. (1986). Three limitations of the body mass index. *American Journal of Clinical Nutrition.* **44** (6): 996-997.

Garrow, J.S., Stalley, S., Dietholm, R., Pittet, P.H., Hesp, R. and Hallidey, D. (1979). A new method for measuring the body density of obese adults. *British Journal of Nutrition.* **42**: 173-183.

Garrow, J.S. (1982). New approaches to body composition. *American Journal of Clinical Nutrition.* **35**: 1152-1158.

Garrow, J.S. (1983a). Luxuskonsumption, brown fat and human obesity. *British Medical Journal.* **286**: 1684-1686.

Garrow, J.S. (1983b). Indices of obesity. *Nutrition Reviews and Abstracts.* **53**: 697-708.

Garrow, J.S. (1987). Energy balance in man - an overview. *American Journal of Clinical Nutrition.* **45** (5): 1114-1119.

Garrow, J.S. (1990). Body composition for the investigation of obesity. *Basic Life Sciences.* **55**: 183-190.

Garvey, W.T., Maianu, L., Hueckstead, T.P., Birnbaum, M.J., Molina, J.M. and Ciaraldi, T.P. (1991). Pretranslational suppression of a glucose transporter protein causes insulin resitance in adipocytes from patients with non-insulin-dependent diabetes mellitus and obesity. *Journal of Clinical Investigation.* **87** (3): 1072-1081.

Geissler, C.A. and Miller, D.S. (1985). Problems with the use of "weight for height" tables. *Journal of Nutrition.* **115** (12): 1546-1549.

Giles, L.R., Murison, R.D. and Wilson, B.R. (1981). A comparison of ultrasound and ruler probe predictors of backfat and eye-muscle measurements in live pigs. *Animal Production.* **32**: 47-50.

Glick, Z., Bray, A. and Teague, R.J. (1984). Effect of prandial glucose on brown fat thermogenesis in rats: possible implications for dietary obesity. *Journal of Nutrition.* **14**: 286-291.

Glickman, L.T., Schofer, F.S., McKee, L.J., Reif, J.S. and Goldschmidt, M.H. (1989). Epidemiologic study of insecticide exposures, obesity and risk of bladder cancer in household dogs. *Journal of Toxicology and Environmental Health.* **28** (4): 407-414.

Golay, A., Swislocki, A.L.M., Chen, Y.D.I., Jaspan, J.B. and Reaven, G.M. (1986). Effect of obesity on ambient plasma glucose, free fatty acids, insulin, growth hormone and glucagon concentrations. *Journal of Clinical Endocrinology and Metabolism.* **63** (2): 481-484.

Gortmaker, S.L., Dietz, W.H.,Jr., Sobol, A.M. and Wehler, C.A. (1987). Increasing pediatric obesity in the United States. *American Journal of Diseases of Children.* **141** (5): 535-540.

Grauer, W.O., Moss, A.A., Cann, C.E. and Goldberg, H.I. (1984). Quantification of body fat distribution in the abdomen using computed tomography. *American Journal of Clinical Nutrition.* **39**: 631-637.

Gray, D.S., Bray, G.A., Bauer, M., Kaplan, K., Gemayel, N., Wood, R., Greenway, F. and Kirk, S. (1990). Skinfold thickness measurements in obese subjects. *American Journal of Clinical Nutrition.* **51** (4): 571-577.

Greenwood, M.R.C. and Hirsch, J. (1974). Postnatal development of adipocyte cellularity in the normal rat. *Journal of Lipid Research.* **15**: 474-483.

Gresham, J.D., Holloway, J.W., Butts, W.T. Jr. and McCurley, J.R. (1986). Prediction of mature cow carcass composition from live animal measurements. *Journal of Animal Science.* **63**: 1041-1048.

Gruen, R., Hietanen, E. and Greenwood, M.R.C. (1978). Increased adipose tissue lipoprotein lipase activity during the development of the genetically obese rat (fa/fa). *Metabolism.* **27**: 1955-1966.

Gunn, H.M. (1978). The proportions of muscle, bone and fat in two different types of dog. *Research in Veterinary Medicine.* **24**: 277-282.

Habermehl, K. (1981). Skin and cutaneous organs. In: *The Anatomy of the Domestic Animals. The Circulatory System, the Skin, and the Cutaneous Organs of the Domestic Mammals.* Editors R. Nickel, A. Schummer, and E. Seiferle, Verlag Paul Parey, Berlin, 441-558.

Hamlet, R., Rezvani, M. and Hopewell, J.W. (1986). Ultrasound measurement of atrophy in pig skin following X- or β- irradiation. *Bioengineering and the Skin.* **2**: 49-57.

Hamlin, R.L. and Buffington, C.A. (1989). Nutrition and the heart. *Veterinary Clinics of North America/Small Animal Practice.* **19** (3): 527-538.

Hand, M.S. (1990). Treating and preventing obesity in small animals. *Veterinary Times.* **20** (9): 14.

Hankins, O.G. and Ellis, N.R. (1934). Physical characteristics of hog carcasses. *Journal of Agricultural Research.* **48**: 257-264.

Hansen, W.E. and Kehrer, H. (1987). Assessment of cutaneous fat and body fat by ultrasound. *Klinische Wochenschrift.* **65**: 407-410.

Hattori, K., Numata, N., Ikoma, M., Matsuzaka, A. and Danielson, R.R. (1991). Sex differences in the distribution of subcutaneous and internal fat. *Human Biology.* **63** (1): 53-63.

Hausman, G.J., Campion, D.R. and Thomas, G.B. (1983). Adipose tissue cellularity and histochemistry in foetal swine as affected by genetic selection for high or low backfat. *Journal of Lipid Research.* **24**: 223-228.

Hausman, G.J. (1985). Cellular and enzyme-histochemical aspects of adipose tissue development in obese (ossabaw) and lean (crossbred) pig foetuses: an ontogeny study. *Journal of Animal Science.* **60**: 1539-1552.

Hausman, G.J. and Jewell, D.E. (1988). The effect of insulin on primary cultures of rat preadipocytes grown in foetal or postnatal pig serum. *Journal of Animal Science.* **66**: 3267-3278.

Haymes, E.M., Lundegren, H.M., Loomis, J.L. and Buskirk, E.R. (1976). Validity of the ultrasonic technique as a method of measuring subcutaneous adipose tissue. *Annals of Human Biology.* **3** (3): 245-251.

Hazel, L.N. and Kline, E.A. (1952). Mechanical measurement of fatness and carcass value on live hogs. *Journal of Animal Science.* **11**: 313-318.

Heinbecker, P., White, H.L. and Rolf, D. (1944). Experimental obesity in the dog. *American Journal of Physiology.* **141**: 549-561.

Heinbecker, P. and Pfeiffenberger, M., Jr. (1950). Further clinical and experimental studies on the pathogenesis of Cushing's syndrome. *American Journal of Medicine.* **9**: 3-23.

Henneke, D.R., Potter, G.D., Kreider, J.L. and Yeates, B.F. (1983). Relationship between condition score, physical measurements, and body fat percentage in mares. *Equine Veterinary Journal.* **15** (4): 371-372.

Hermier, D., Quignard-Boulangé, A., Dugail, I., Guy, G., Salichon, M.R., Brigant, L., Ardouin, B. and Leclercq, B. (1989). Evidence of enhanced storage capacity in adipose tissue of genetically fat chickens. *Journal of Nutrition.* **119**: 1369-1375.

Hervey, G.R. and Tobin, G. (1983). Luxuskonsumption, diet-induced thermogenesis and brown fat: a critical review. *Clinical Science.* **64**: 7-18.

Heymsfield, S.B. and Williams, P.J. (1988). Nutritional assessment by clinical and biochemical methods. In: *Modern Nutrition in Health and Disease.* 7th Edition. Editors M.E. Shiels and V.R. Young, Lea & Febiger, Philadelphia, 817-860.

Heymsfield, S.B., Wang, J., Heshka, S., Kehayias, J.J. and Pierson, R.N. (1989). Dual-photon absorptiometry: comparison of bone mineral and soft tissue mass measurements in vivo with established methods. *American Journal of Clinical Nutrition.* **49**: 1283-1289.

Hicks, D.A., Hope, A., Turnbull, A.L. and Verel, D. (1956). The estimation and prediction of normal blood volume. *Clinical Science.* **15**: 557-565.

Himms-Hagen, J. (1984). Thermogenesis in brown adipose tissue as an energy buffer. Implications for obesity. *New England Journal of Medicine.* **311** (24): 1549-1558.

Himms-Hagen, J. (1990). Brown adipose tissue thermogenesis: interdisciplinary studies. *FASEB Journal.* **4** (11): 2890-2898.

Hirsch, J. and Han, P.W. (1969). Cellularity of rat adipose tissue: effects of growth, starvation and obesity. *Journal of Lipid Research.* **10**: 77-82.

Hirsch, J. (1972). Can we modify the number of adipose cells?. *Postgraduate Medicine.* **51**: 83-86.

Hood, R.L. and Allen, C.E. (1973a). Cellularity of bovine adipose tissue. *Journal of Lipid Research.* **14**: 605-610.

Hood, R.L. and Allen, C.E. (1973b). Lipogenic enzyme activity in adipose tissue during the growth of swine with different propensities to fatten. *Journal of Nutrition.* **103**: 353-362.

Houpt, K.A. and Hintz, H.F. (1978). Obesity in dogs. *Canine Practice.* **5** (2): 54-58.

Houpt, K.A., Coren, B., Hintz, H.F. and Hilderbrandt, J.E. (1979). Effect of sex and reproductive status on sucrose preference, food intake and body weight of dogs. *Journal of the American Veterinary Medical Association.* **174**: 1083-1085.

Houpt, K.A. and Smith, S.L. (1981). Taste preferences and their relation to obesity in dogs and cats. *Canadian Veterinary Journal.* **22**: 77-81.

Houpt, K. (1982). Ingestive behaviour problems of dogs and cats. *Veterinary Clinics of North America/Small Animal Practice.* **12** (4): 683-692.

Hughes, B.R., Black, D., Srivastava, A., Dalziel, K. and Marks, R. (1987). Comparison of techniques for the non-invasive assessment of skin tumours. *Clinical and Experimental Dermatology.* **12**: 108-111.

Jeffcott, L.B. and Field, J.R. (1985). Current concepts of hyperlipaemia in horses and ponies. *The Veterinary Record.* **116**: 461-466.

Johnson, P.R. and Hirsch, J. (1972). Cellularity of adipose depots in six strains of genetically obese mice. *Journal of Lipid Research.* **13**: 2-11.

Johnson, P.R., Stern, J.S., Greenwood, M.R.C., Zucker, L.M. and Hirsch, J. (1973). Effect of early nutrition on adipose cellularity and pancreatic insulin release in the Zucker rat. *Journal of Nutrition.* **103**: 738-743.

Johnson, P.R., Stern, J.S., Greenwood, M.R.C. and Hirsch, J. (1978). Adipose tissue hyperplasia and hyperinsulinaemia in Zucker obese female rats: a developmental study. *Metabolism.* **27**: 1941-1954.

Jones, R.S., Lawrence, T.L.J., Veevers, A., Cleave, N. and Hall, J. (1989). Accuracy of prediction of the liveweight of horses from body measurements. *The Veterinary Record.* **125**: 549-553.

Joshua, J.O. (1970). The obese dog and some clinical repercussions. *Journal of Small Animal Practice.* **11**: 601-606.

Jung, R.T., Gurr, M.I., Robinson, M.P. and James, W.P.T. (1978). Does adipocyte hypercellularity in obesity exist?. *British Medical Journal.* **2**: 319-321.

Kalus, A.M., Aindow, J. and Caulfield, M.R. (1979). Application of ultrasound in assessing burn depth. *Lancet.* **i**: 188-189.

Kanarek, R.B. and Hirsch, J. (1977). Dietary-induced overeating in experimental animals. *Federation Proceedings.* **36**: 154-158.

Kane, E., Rogers, Q.R., Morris, J.G. and Leung, P.M. (1981). Feeding behaviour of the cat fed laboratory and commercial diets. *Nutrition Research.* **1**: 499-507.

Kane, E., Morris, J.G. and Rogers, Q.R. (1981). Acceptability and digestibility by adult cats of diets made with various sources and levels of fat. *Journal of Animal Science.* **53**: 1516-1523.

Katahn, M. and McMinn, M.R. (1990). Obesity. A biobehavioral point of view. *Annals of the New York Academy of Sciences.* **602**: 189-204.

Katch, F.I., Benhke, A. and Katch, V.L. (1979). Estimation of body fat from skinfolds and surface area. *Human Biology.* **51**: 411-424.

Kaufman, E. (1986). Obesity in dogs. *Veterinary Technician.* **7** (2): 75-80.

Kazdová, L., Fábry, P. and Vrána, A. (1974). Effect of small doses of insulin in vivo on the proliferation and cellularity of adipose tissue. *Diabetologia.* **10**: 77-83.

Kempster, A.J. and Owen, M.G. (1981). A note on the accuracy of an ultrasonic technique for selecting cattle of different breeds for slaughter at equal fatness. *Animal Production.* **32**: 113-115.

Kempster, A.J., Cuthbertson, A., Jones, D.W. and Owen, M.G. (1981). Prediction of body composition of live cattle using two ultrasonic machines of differing complexity: a report of four separate trials. *Journal of Agricultural Science, Cambridge.* **96**: 301-307.

Kempster, A.J., Arnall, D., Alliston, J.C. and Barker, J.D. (1982). An evaluation of two ultrasonic machines (Scanogram and Danscanner) for predicting the living composition of live sheep. *Animal Production.* **34**: 249-255.

Kirk, R.W. (1971). Table of dentition. In: *Current Veterinary Therapy IV.* Editor R.W. Kirk, WB Saunders Company, Philadelphia, 786.

Kirkwood, J.K. (1985). The influence of size on the biology of the dog. *Journal of Small Animal Practice.* **26**: 97-110.

Kluthe, R. and Schubert, A. (1985). Obesity in Europe. *Annals of Internal Medicine.* **103**: 1037-1042.

Klyde, B.J. and Hirsch, J. (1979). Increased cellular proliferation in adipose tissue of adult rats fed a high-fat diet. *Journal of Lipid Research.* **20**: 705-715.

Knehans, A.W. and Romsos, D.R. (1982). Reduced norepinephrine turnover in brown adipose tissue of ob/ob mice. *American Journal of Physiology.* **242**: E253-E261.

Knittle, J.L., Ginsberg-Fellner, F. and Brown, R.E. (1977). Adipose tissue development in man. *American Journal of Clinical Nutrition.* **30**: 762-766.

Koch, R.M., Dikeman, M.E., Allen, D.M., May, M., Crouse, J.D. and Campion, D.R. (1976). Characterization of biological types of cattle. III. Carcass composition, quality and palatability. *Journal of Animal Science.* **43**: 48-62.

Koyama, H., Nishizawa, Y., Yamashita, N., Furumitsu, Y., Hagiwara, S., Ochi, H. and Morii, H. (1990). Measurement of composition changes using dual-photon absorptiometry in obese patients undergoing semistarvation. *Metabolism.* **39** (3): 302-306.

Kronfeld, D.S. (1988). Cutting down on canine obesity. *Veterinary Practice.* **20** (19): 9-10.

Krook, L., Larsson, S. and Rooney, J.R. (1960). The interrelationship of diabetes mellitus, obesity and pyometra in the dog. *American Journal of Veterinary Research.* **21**: 120-124.

Krotkiewski, M., Sjöström, L., Björntorp, P., Carlgren, G., Garellick, C. and Smith, V. (1977). Adipose tissue cellularity in relation to prognosis for weight reduction. *International Journal of Obesity.* **1**: 395-416.

Kuczmarski, R.J., Fanelli, M.T. and Koch, G.G. (1987). Ultrasonic assessment of body composition in obese adults: overcoming the limitations of the skinfold calliper. *American Journal of Clinical Nutrition.* **45**: 717-724.

Kushner, R.F. and Schoeller, D.A. (1986). Estimation of total body water by bioelectrical impedance analysis. *American Journal of Clinical Nutrition.* **44**: 417-424.

Kushner, R.F., Kunigk, A., Alspaugh, M., Andronis, P.T., Leitch, C.A. and Schoeller, D.A. (1990). Validation of bioelectrical impedance analysis as a measurement of change in body composition in obesity. *American Journal of Clinical Nutrition.* **52** (2): 219-223.

Landsberg, L. (1990). The sympathoadrenal system, obesity and hypertension: an overview. *Journal of Neuroscience Methods.* **34** (1-3): 179-186.

Lee, M.M. and Ng, C.K. (1965). Postmortem studies of skinfold calliper measurement and actual thickness of skin and subcutaneous tissue. *Human Biology.* **37**: 91-103.

Leibel, R.L., Edens, N.K. and Fried, S.K. (1989). Physiological basis for the control of body fat distribution in humans. *Annual Review of Nutrition.* **9**: 417-443.

Leiter, L.A. (1986). Obesity: overview of pathogenesis and treatment. *Canadian Journal of Physiology and Pharmacology.* **64** (6): 814-817.

Leonard, H.C. (1971). Obesity. In: *Current Veterinary Therapy IV.* Editor R.W. Kirk, WB Saunders Company, Philadelphia, 63-64.

Lesser, G.T., Deutsch, S. and Marfofsky, J. (1971). Use of independent measurement of body fat to evaluate overweight and underweight. *Metabolism.* **20**: 792-804.

Leung, A.K. and Robson, W.L. (1990). Childhood obesity. *Postgraduate Medicine.* **87** (4): 123-130.

Lewis, L.D. (1978). Obesity in the dog. *Journal of the American Animal Hospital Association.* **14**: 402-409.

Lewis, L.D., Morris, M.L. and Hand, M.S. (1987). Obesity. In: *Small Animal Clinical Nutrition.* 3rd Edition. Editors Mark Morris Associates, Topeka, Kansas, 6/1 - 6/39.

Leymaster, K.A., Mersmann, H.J. and Jenkins, T.G. (1985). Prediction of the chemical composition of sheep by use of ultrasound. *Journal of Animal Science.* **61** (1): 165-172.

Little, G. (1990). The fats of life. *Veterinary Practice Nurse.* **2** (3): 22-24.

Lohman, T.G. (1981). Skinfolds and body density and their relation to body fatness. *Human Biology.* **53**: 181-225.

MacDonald, F.C. (1986). Quetelet index as indicator of obesity. *Lancet.* **i** (8488): 1043.

MacFarlane, W.V. (1976). Water and electrolytes in domestic animals. In: *Veterinary Physiology.* Editor J.W. Phillis, Wright-Scientechnica, Bristol, 463-539.

Marchington, D., Rothwell, N.J., Stock, M.J. and York, D.A. (1983). Diet induced thermogenesis and brown adipose tissue in lean and obese (fa/fa) Zucker rats after adrenalectomy. *Journal of Nutrition.* **113**: 1395-1402.

Markwell, P.J. (1988a). Clinical Small Animal Nutrition. In: *The Waltham Book of Dog and Cat Nutrition.* 2nd Edition. Pergamon Press, Oxford, 97-115.

Markwell, P.J. (1988b). Nutritional management of obesity in dogs and cats. *The Veterinary Times.* **18** (10): 8-9.

Markwell, P.J., van Erk, W., Parkin, G.D., Sloth, C.J. and Shantz-Christienson, T. (1990). Obesity in the dog. *Journal of Small Animal Practice.* **31**: 533-537.

Marshall, J.D., Hazlett, C.B., Spady, D.W. and Quinney, H.A. (1990). Comparison of convenient indicators of obesity. *American Journal of Clinical Nutrition.* **51** (1): 22-28.

Martin, R.J. and Herbein, J. (1976). A comparison of the enzyme levels and in vitro utilization of various substrates for lipogenesis in pair-fed lean and obese pigs. *Proceedings of the Society for Experimental Biology and Medicine.* **151**: 231-235.

Martin, S.L. and Capen, C.C. (1983). The endocrine system. In: *Feline Medicine.* Editor P.W. Pratt, American Veterinary Publications Inc., California, 321-362.

Mason, E. (1970). Obesity in pet dogs. *The Veterinary Record.* **86**: 612-616.

Mattheeuws, D., Rottiers, R., Kaneko, J.J. and Vermeulen, A. (1984a). Diabetes mellitus in dogs: relationship of obesity to glucose tolerance and insulin response. *American Journal of Veterinary Research.* **45**: 98-103.

Mattheeuws, D., Rottiers, R., Baeyens, D. and Vermeulen, A. (1984b). Glucose tolerance and insulin response in obese dogs. *Journal of the American Animal Hospital Association.* **20**: 287-293.

Mayer, J. (1955a). The physiological basis of obesity and leanness. Part I. *Nutrition Abstracts and Reviews.* **25**: 597-611.

Mayer, J. (1955b). The physiological basis of obesity and leanness. Part II. *Nutrition Abstracts and Reviews.* **25**: 871-883.

Mayer, J. (1972). Obesity. *Postgraduate Medicine.* **51**: 66-69.

Mayer, J. (1973). Obesity. In: *Modern Nutrition in Health and Disease.* 5th Edition. Editors R.S. Goodhart and M.E. Shils, Lea & Febiger, Philadelphia, 633-644.

Mayer, J. (1980). Obesity. In: *Modern Nutrition in Health and Disease.* 6th Edition. Editors R.S. Goodhart and M.E. Shils, Lea & Febiger, Philadelphia, 721-740.

Maynard, L.A., Loosli, J.K., Hintz, H.F. and Warner, R.G. (1979). The animal body and its food. In: *Animal Nutrition.* 7th Edition. McGraw-Hill Publishing Company, New Delhi, 9-20.

McCann, J.P. and Reimers, T.J. (1986). Effects of obesity on insulin and glucose metabolism in cyclic heifers. *Journal of Animal Science.* **62**: 772-782.

McLaren, D.S. and Meguid, M.M. (1988). Primary and secondary nutritional disorders. Obesity. In: *Nutrition and its disorders.* 4th Edition. Churchill Livingstone Inc., Edinburgh, 147-156.

McLaughlin, C.L. (1982). Role of peptides from gastrointestinal cells in food intake regulation. *Journal of Animal Science.* **55**: 1515-1527.

McNamara, J.P. and Martin, R.J. (1982). Muscle and adipose tissue lipoprotein lipase in foetal and neonatal swine as affected by genetic selection for high or low backfat. *Journal of Animal Science.* **55**: 1057-1061.

McReynolds, W.E. and Arthaud, V.H. (1970). Estimating fat depth and *longissimus* muscle area by use of ultrasonics in beef cattle. *Journal of Animal Science.* **30** (4): 503-506.

Meijer, J.C. (1980). Canine hyperadrenocorticism. In: *Current Veterinary Therapy VII.* Editor R.W. Kirk, WB Saunders Company, Philadelphia, 975-979.

Meiklejohn, A.P. (1959). Obesity and disease: chairman's opening remarks. *Proceedings of the Nutrition Society.* **18**: 140-141.

Mendez, J., Lukaski, H.C. and Buskirk, E.R. (1984). Fat-free mass as a function of maximal oxygen consumption and 24-hour urinary creatinine, and 3-methylhistidine excretion. *American Journal of Clinical Nutrition.* **39**: 710-715.

Mersmann, H.J., Pond, W.G. and Yen, J.T. (1984). Use of carbohydrate and fat as energy source by obese and lean swine. *Journal of Animal Science.* **58**: 894-902.

Mersmann, H.J. (1985). Adipose tissue lipolytic rate in genetically obese and lean swine. *Journal of Animal Science.* **60**: 131-135.

Mersmann, H.J. (1986). Postnatal expression of adipose tissue metabolic activity associated with a porcine genetic obesity. *Journal of Animal Science.* **63**: 741-746.

Meyer, H., Drochner, W. and Weidenhaupt, C. (1978). Ein beitrag zum vorkommen und zur behandlung der adipositas des hundes. (Occurrence and treatment of obesity in dogs). *Deutsche Tierärztliche Wochenschrift.* **85** (4): 133-136.

Meyer, H. and Stadtfeld, G. (1978). Investigations on the body and organ structure of dogs. In: *Nutrition of the Dog and Cat.* Editor R.S. Anderson, Pergamon Press Ltd., Oxford, 15-28.

Miles, C.A., Pomeroy, R.W. and Harries, J.M. (1972). Some factors affecting reproducibility in ultrasonic scanning of animals. *Animal Production.* **15**: 239-249.

Miller, D.S., Mumford, P. and Stock, M.J. (1967). Thermogenesis in overeating man. *American Journal of Clinical Nutrition.* **20** (11): 1223-1229.

Ministry of Agriculture, Food and Fisheries. (1981). Method no.6. In: *The Analysis of Agricultural Materials.* RB427, M.A.F.F., Her Majesty's Stationery Office, London, 16-17.

Miyauchi, S., Tada, M. and Miki, Y. (1983). Echographic evaluation of nodular lesions of the skin. *Journal of Dermatology.* **10**: 221-227.

Moise, N.S. and Reimers, T.J. (1983). Insulin therapy in cats with diabetes mellitus. *Journal of the American Veterinary Medical Association.* **182**: 158-164.

Morris, M.L., Jr. (1974). Obesity. In: *Current Veterinary Therapy V.* Editor R.W. Kirk, WB Saunders Company, Philadelphia, 90-92.

Morrison, S.D. (1959). Obesity and the control of food intake in experimental animals. *Proceedings of the Nutrition Society.* **18**: 141-148.

Moulton, C.R. (1923). Age and chemical development in mammals. *Journal of Biological Chemistry.* **57**: 79-97.

Mrosovsky, N. (1986). Body fat: what is regulated?. *Physiology and Behavior.* **38** (3): 407-414.

Mueller, W.H. and Wholleb, J.C. (1981). Anatomical distribution of subcutaneous fat and its description by multivariate methods: how valid are principal components?. *American Journal of Physical Anthropology.* **54** (1): 25-35.

Mugford, R.A. and Thorne, C. (1980). Comparative studies of meal patterns in pet and laboratory housed dogs and cats. In: *Nutrition of the Dog and Cat.* Editor R.S. Anderson, Pergamon Press Ltd, Oxford, 3-12.

Muller, G.H., Kirk, R.W. and Scott, D.W. (1989). Structure and function of the skin. In: *Small Animal Dermatology.* 4th Edition. WB Saunders Company, Philadelphia, 1-48.

Mulligan, W., MacLean, J.M. and Preston, T. (1990). Body composition and exercise in racing pigeons. *Research in Veterinary Science.* **48**: 321-326.

Murakami, S. and Miki, Y. (1989). Human skin histology using high-resolution echography. *Journal of Clinical Ultrasound.* **17**: 77-82.

Must, A., Dallal, G.E. and Dietz, W.H. (1991). Reference data for obesity: 85th and 95th percentiles of Body Mass Index (wt/ht^2) and triceps skinfold thickness. *American Journal of Clinical Nutrition.* **53** (4): 839-846.

Myers, S.L., Cohen, J.S., Sheets, P.W. and Bies, J.R. (1986). B-mode ultrasound evaluation of skin thickness in progressive systemic sclerosis. *Journal of Rheumatology.* **13**: 577-580.

Nauss-Karol, C., Triscari, J. and Sullivan, A.C. (1982). The influence of age and genotype on the development of obesity and hyperlipidaemia in Zucker rats. *Federation Proceedings.* **41**: 715 (Abstract).

Neale, G. (1988a). Recognition and management of nutritional disorders. Obesity. In: *Clinical Nutrition.* Heinemann Medical Books, London, 99-108.

Neale, G. (1988b). Nutritional Assessment. In: *Clinical Nutrition.* Heinemann Medical Books, London, 42-82.

Nelson, R.W., Feldman, E.C. and Smith, M.C. (1988). Hyperadrenocorticism in cats: seven cases. *Journal of the American Veterinary Medical Association.* **193** (2): 245-250.

Nelson, R.W., Himsel, C.A., Feldman, E.C. and Bottoms, G.D. (1990). Glucose tolerance and insulin response in normal-weight and obese cats. *American Journal of Veterinary Research.* **51** (9): 1357-1362.

Nestel, P. and Goldrick, B. (1976). Obesity: changes in lipid metabolism and the role of insulin. *Clinics in Endocrinology and Metabolism.* **5**: 313-335.

Newberne, P.M. (1966). Effects of overnutrition on resistance of dogs to distemper virus. *Federation Proceedings.* **25**: 1701-1710.

Nind, F. (1988). Feeding obesity. *Veterinary Practice.* **20** (2): 9.

O'Donnell, J.A. and Hayes, K.C. (1987). Nutrition and nutritional disorders. In: *Diseases of the Cat. Medicine and Surgery.* Editor J. Holzworth, WB Saunders Company, Philadelphia, 15-42.

Pace, N. and Rathbun, E.N. (1945). Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. *Journal of Biological Chemistry.* **158**: 685-690.

Panaretto, B.A. (1963). Body composition in vivo: the composition of living ruminants and its relation to the tritiated water spaces. *Australian Journal of Agricultural Research.* **14**: 944-952.

Panciera, D.L., Thomas, C.B., Eicker, S.W. and Atkins, C.E. (1990). Epizootiologic patterns of diabetes mellitus in cats: 333 cases (1980-1986). *Journal of the American Veterinary Medical Association.* **197** (11): 1504-1508.

Payne, P.A. (1985). Applications of ultrasound in dermatology. *Bioengineering and the Skin.* **1**: 293-320.

Pawan, G.L.S. (1959). Some aspects of metabolism of the obese. *Proceedings of the Nutrition Society.* **18**: 155-162.

Pendergrass, P.B., Bartley, C.M., Nagy, F., Ream, L.J. and Stuhlman, R. (1983). A rapid method for determining normal weights of medium-to-large mongrel dogs. *Journal of Small Animal Practice.* **24**: 269-276.

Peppler, W.W. and Mazess, R.B. (1981). Total body bone mineral and lean body mass by dual-photon absorptiometry. I. Theory and measurement procedure. *Calcified Tissue International.* **33**: 353-359.

Peterson, M.E. and Randolph, J.E. (1989). Endocrine diseases. In: *The cat: Diseases and Clinical Management.* Editor R.G. Sherding, Churchill Livingstone Inc., Edinburgh, 1095-1161.

Pi-Sunyer, F.X. (1988). Obesity. In: *Modern Nutrition in Health and Disease.* 7th Edition. Editors M.E. Shils and V.R. Young, Lea & Febiger, Philadelphia, 795-816.

Pi-Sunyer, F.X. (1991). Health implications of obesity. *American Journal of Clinical Nutrition.* **53** (6 Suppl.): 1595S-1603S.

Planche, E., Joliff, M., de Gasquet, P. and Le Liepvre, X. (1983). Evidence of a defect in energy expenditure in 7-day-old Zucker rat (fa/fa). *American Journal of Physiology.* **245**: E107-E113.

Presta, E., Wang, J., Harrison, G.G., Björntorp, P., Harker, W.H. and Van Itallie, T.B. (1983). Measurement of total body electrical conductivity: a new method for estimation of body composition. *American Journal of Clinical Nutrition.* **37**: 735-739.

Price, J.F., Pearson, A.M., Pfost, H.B. and Deans, R.J. (1960). Application of ultrasonic reflection techniques in evaluating fatness and leanness in pigs. *Journal of Animal Science.* **19**: 381-387.

Pykälistö, O.J., Smith, P.H. and Brunzell, J.D. (1975). Determinants of human adipose tissue lipoprotein lipase: effects of diabetes and obesity on basal and diet-induced activity. *Journal of Clinical Investigation.* **56**: 1108-1117.

Rainbird, A.L. (1988). What do our dogs weigh?. *Pedigree Digest.* **14** (4): 10-11.

Rampone, A.J. and Reynolds, P.J. (1988). Obesity: thermodynamic principles in perspective. *Life Sciences.* **43** (2): 93-110.

Reece, W.O. (1991). Body water. In: *Physiology of Domestic Animals.* Lea & Febiger, Philadelphia, 81-90.

Rocchini, A.P., Moorehead, C., Wentz, E. and Deremer, S. (1987). Obesity-induced hypertension in the dog. *Hypertension.* **9** (Suppl.III): 64-68.

Romsos, D.R., Belo, P.S., Bennink, H.R., Bergen, W.G. and Leveille, G.A. (1976). Effects of dietary carbohydrate, fat and protein on growth, body composition and blood metabolite levels in the dog. *Journal of Nutrition.* **106**: 1452-1464.

Romsos, D.R., Hornshuh, M.J. and Leveille, G.A. (1978). Influence of dietary fat and carbohydrate on food intake, body weight and body fat of adult dogs. *Proceedings of the Society for Experimental Biology and Medicine.* **157**: 278-281.

Roncari, D.A.K., Lau, D.C.W. and Kindler, S. (1981). Exaggerated replication in culture of adipocyte precursors from massively obese persons. *Metabolism.* **30**: 425-427.

Rosenbaum, S., Skinner, R.K., Knight, I.B. and Garrow, J.S. (1985). A survey of heights and weights of adults in Great Britain, 1980. *Annals of Human Biology.* **12**: 115-127.

Rothblum, E.D. (1990). Women and weight: fad and fiction. *Journal of Psychology.* **124** (1): 5-24.

Rothwell, N.J. and Stock, M.J. (1979). A role for brown adipose tissue in diet-induced thermogenesis. *Nature.* **281**: 31-35.

Royal College of Physicians (1983). Obesity. *Journal of the Royal College of Physicians of London.* **17** (1): 5-65.

Rudolph, B.C., Stahly, T.S. and Cromwell, G.L. (1988). Estimation of body composition of neonatal pigs via deuterium oxide dilution: validation of technique. *Journal of Animal Science.* **66**: 53-61.

Ruegg, P.L. (1991). Body condition scoring in dairy cows: relationships with production, reproduction, nutrition and health. *Compendium on Continuing Education for the Practising Veterinarian.* European Edition. **13** (9): E641-E645.

Ruiz, L., Colley, J.R.T. and Hamilton, P.J.S. (1971). Measurement of triceps skinfold thickness. An investigation of sources of variation. *British Journal of Preventative and Social Medicine.* **25**: 165-167.

Rukavina, B. and Mohar, N. (1979). An approach of ultrasound diagnostic techniques of the skin and subcutaneous tissue. *Dermatologica.* **158**: 81-92.

Salans, L.B., Cushman, S.W. and Weismann, R.E. (1973). Adipose cell size and number in nonobese and obese patients. *Journal of Clinical Investigation.* **52**: 929-941.

Schemmel, R., Mickelsen, O. and Gill, J.L. (1970). Dietary obesity in rats: body weight and body fat accretion in seven strains of rats. *Journal of Nutrition.* **100**: 1041-1048.

Scherf, J., Franklin, B.A., Lucas, C.P., Stevenson, D. and Rubenfire, M. (1986). Validity of skinfold thickness measures of formely obese adults. *American Journal of Clinical Nutrition.* **43** (1): 128-135.

Schlundt, D.G., Hill, J.O., Sbrocco, T., Pope-Cordle, J. and Kasser, T. (1990). Obesity: a biogenetic or biobehavioral problem. *International Journal of Obesity.* **14** (9): 815-828.

Schutz, Y., Bessard, T. and Jéquier, E. (1984). Diet-induced thermogenesis measured over a whole day in obese and nonobese women. *American Journal of Clinical Nutrition.* **40** (3): 542-552.

Schwartz, R.S. and Brunzell, J.D. (1981). Increase of adipose tissue lipoprotein lipase activity with weight loss. *Journal of Clinical Investigation.* **67**: 1425-1430.

Schwartz, R.S., Shuman, W.P., Bradbury, V.L., Cain, K.C., Fellingham, G.W., Beard, J.C., Kahn, S.E., Stratton, J.R., Cerqueira, M.D. and Abrass, I.B. (1990). Body fat distribution in healthy young and older men. *Journal of Gerontology.* **45** (6): M181-M185.

Sclafani, A. and Springer, D. (1976). Dietary obesity in adult rats: similarities to hypothalamic and human obesity syndromes. *Physiology and Behavior.* **17**: 461-471.

Scott, D.W. (1987). The skin. In: *Diseases of the cat. Medicine and Surgery.* 1st Edition. Editor J. Holzworth, WB Saunders Company, Philadelphia, 619-675.

Scott, P.P. (1984). Nutrition. In: *Diseases of the cat and their management.* 2nd Edition. Editor G.I. Wilkinson, Blackwell Scientific Publications, Oxford, 1-10.

Scott, R.A., Cornelius, S.G. and Mersmann, H.J. (1981). Effects of age on lipogenesis and lipolysis in lean and obese swine. *Journal of Animal Science.* **52**: 505-511.

Scott, R.S. (1981). Obesity - in search of an explanation. *Journal of the New Zealand Dietetic Association.* **35** (2):12 (Abstract).

Searle, T.W. (1970). Body composition in lambs and young sheep and its prediction in vivo from tritiated water space and body weight. *Journal of Agricultural Science, Cambridge.* **74**: 357-362.

Segal, K.R., Gutin, B., Nyman, A.M. and Pi-Sunyer, F.X. (1985). Thermic effect of food at rest, during exercise, and after exercise in lean and obese men of similar body weight. *Journal of Clinical Investigation.* **76** (3): 1107-1112.

Segal, K.R., Edaño, A. and Tomas, M.B. (1990). Thermic effect of a meal over 3 and 6 hours in lean and obese men. *Metabolism.* **39** (9): 985-992.

Seidell, J.C., Oosterlee, A., Deurenberg, P., Hautvast, J.G. and Ruijs, J.H. (1988). Abdominal fat depots measured with computed tomography: effects of degree of obesity, sex and age. *European Journal of Clinical Nutrition.* **42** (9): 805-815.

Selby, J.V., Newman, B., Quesenberry, C.P. Jr., Fabsitz, R.R., Carmelli, D., Meaney, F.J. and Slemenda, C. (1990). Genetic and behavioral influences on body fat distribution. *International Journal of Obesity.* **14** (7): 593-602.

Serup, J. (1984a). Quantification of acrosclerosis: measurement of skin thickness and skin-phalanx distance in females with 15 MHz pulsed ultrasound. *Acta DermatoVenereologica, Stockholm.* **64**: 35-40.

Serup, J. (1984b). Localized scleroderma (morphea): thickness of sclerotic plaques as measured by 15 MHz pulsed ultrasound. *Acta DermatoVenereologica, Stockholm.* **64**: 214-219.

Serup, J., Staberg, B. and Klemp, P. (1984). Quantification of cutaneous oedema in patch test reactions by measurement of skin thickness with high-frequency pulsed ultrasound. *Contact Dermatitis.* **10**: 88-93.

Shafir, R., Itzchak, Y., Heyman, Z., Azizi, E., Haggai, T. and Hiss, J. (1984). Preoperative ultrasonic measurements of the thickness of cutaneous malignant melanoma. *Journal of Ultrasound in Medicine.* **3**: 205-208.

Sheng, H.P. and Huggins, R.A. (1971). Direct and indirect measurement of total body water in the growing Beagle. *Proceedings of the Society for Experimental Biology and Medicine.* **137**: 1093-1099.

Sheng, H.P., Kamonsakpithak, S., Naiborhu, A., Chuntananukoon, S. and Huggins, R.A. (1977). Measured and calculated fat during growth in the pig and Beagle. *Growth.* **41**: 139-146.

Sibley, K.W. (1984). Diagnosis and management of the overweight dog. *British Veterinary Journal.* **140**: 124-131.

Simm, G. (1983). The use of ultrasound to predict the carcass composition of live cattle - a review. *Animal Breeding Abstracts.* **51** (12): 853-875.

Sinnet-Smith, P.A. and Woolliams, J.A. (1988). Genetic variation in subcutaneous adipose tissue metabolism in sheep. *Animal Production.* **47**: 263-270.

Sisson, S. (1975). Carnivore sense organs and common integument. In: *Sisson's and Grossman's Anatomy of the Domestic Animals.* 5th Edition. Editor R. Getty, WB Saunders Company, Philadelphia, 1741-1783.

Sloan, A.W. (1967). Estimation of body fat in young men. *Journal of Applied Physiology.* **23** (3): 311-315.

Snedecor, G.W. and Cochran, W.G. (1967a). Correlation. In: *Statistical Methods.* 6th Edition. Iowa State University Press, Iowa, 172-198.

Snedecor, G.W. and Cochran, W.G. (1967b). One-way classifications. Analysis of variance. In: *Statistical Methods.* 6th Edition. Iowa State University Press, Iowa, 258-298.

Snedecor, G.W. and Cochran, W.G. (1967c). Multiple Regression. In: *Statistical Methods.* 6th Edition. Iowa State University Press, Iowa, 381-418.

Soll, A.H., Kahn, C.R., Neville, D.M. and Roth, J. (1975). Insulin receptor deficiency in genetic and acquired obesity. *Journal of Clinical Investigation.* **56**: 769-780.

Sonnenschien, E., Glickman, L., McKee, L. and Goldschmidt, M. (1987). Nutritional risk factors for spontaneous breast cancer in pet dogs: a case-control study. *American Journal of Epidemiology.* **126**: 736 (Abstract).

Spitzer, L. and Rodin, J. (1981). Human eating behaviour: a critical review of studies in normal weight and overweight individuals. *Appetite.* **2**: 293-329.

Staff Report (1965). Causes and management of obesity in the small animal. *Modern Veterinary Practice.* **46** (6): 58-62.

Stallings, E.P. (1968). Obesity. In: *Current Veterinary Therapy III.* Editor R.W. Kirk, WB Saunders Company, Philadelphia, 76-78.

Standal, N. and Vold, E. (1973). Lipid mobilization in pigs selected for leanness or fatness. *Animal Production.* **16**: 37-42.

Staten, M.A., Totty, W.G. and Kohrt, W.M. (1989). Measurement of fat distribution by magnetic resonance imaging. *Investigative Radiology.* **24** (5): 345-349.

Steele, N.C., Frobish, L.T. and Keeney, M. (1974). Lipogenesis and cellularity of adipose tissue from genetically lean and obese swine. *Journal of Animal Science.* **39**: 712-719.

Steininger, E. (1981). Die adipositas und ihre diätetische behandlung. (Obesity and its dietetic management). *Wiener Tierärztliche Monatschrift.* **68**: 122-130.

Stogdale, L. and Moore, D.J. (1980). Obesity in a dog with secondary hormonal imbalance. *Journal of the South African Veterinary Association.* **51** (1): 41-45.

Stordy, J.B. (1988). Obesity - management in the treatment of disease. In: *Nutrition in the Clinical Management of Disease.* 2nd Edition. Editors J.W.T. Dickerson and H.A. Lee, Edward Arnold Publishers Ltd., London, 145-157.

Stouffer, J.R. (1963). Relationship of ultrasonic measurements and X-rays to body composition. *Annals of the New York Academy of Sciences.* **110:** 31-39.

Sugden, M.C. and Holness, M.J. (1990). Substrate interactions in the development of insulin resistance in type II diabetes and obesity. *Journal of Endocrinology.* **127:** 187-190.

Swaminathan, R., King, R.F.G.J., Holmfield, J., Siwek, R.A., Baker, M. and Wales, J.K. (1985). Thermic effect of feeding carbohydrate, fat, protein and mixed meal in lean and obese subjects. *American Journal of Clinical Nutrition.* **42:** 177-181.

Talamantes, M.A., Long, C.R., Smith, G.C., Jenkins, T.G., Ellis, W.C. and Cartwright, T.C. (1986). Characterization of cattle of a five-breed diallel: VI. Fat deposition patterns of serially slaughtered bulls. *Journal of Animal Science.* **62:** 1259-1266.

Tan, C.Y., Marks, R. and Payne, P. (1981). Comparison of xeroradiographic and ultrasound detection of corticosteroid induced dermal thinning. *Journal of Investigative Dermatology.* **76:** 126-128.

Tan, C.Y., Marks, R., Roberts, E. and Guibarra, E. (1981). Xeroradiographic and ultrasound techniques in the assessment of skin disorder. In: *Bioengineering and the Skin.* MTP Press Ltd, Lancaster, 215-225.

Tan, C.Y., Statham, B., Marks, R. and Payne, P.A. (1982). Skin thickness measurement by pulsed ultrasound: its reproducibility, validation and variability. *British Journal of Dermatology.* **106**: 657-667.

Tanner, J.M. (1959). The measurement of body fat in man. *Proceedings of the Nutrition Society.* **18**: 148-155.

Taouis, M., Valet, P., Estan, L., Lafontan, M., Montastrue, P. and Berlan, M. (1989). Obesity modifies the adrenergic status of dog adipose tissue. *Journal of Pharmacology and Experimental Therapeutics.* **250** (3): 1061-1066.

Taylor, K.J.W. and Kremkau, F.W. (1985). Basic principles of diagnostic ultrasound. In: *Atlas of Ultrasonography.* 2nd Edition. Editor K.J.W. Taylor, Churchill Livingstone Inc., New York, 1-22.

Thomas, T.R., Crough, L.D. and Araujo, J. (1988). Dietary preparation and per cent fat measurement by hydrostatic weighing. *British Journal of Sports Medicine.* **22** (1): 9-11.

Tokunaga, K., Matsuzawa, Y., Ishikawa, K. and Tarvi, S. (1983). A novel technique for the determination of body fat by computed tomography. *International Journal of Obesity.* **7**: 437-445.

Trayhurn, P. (1984). The development of obesity in animals: the role of genetic susceptibility. In: *Clinics in Endocrinology and Metabolism.* Editor W.P.T. James, WB Saunders Company, Philadelphia, 451-474.

Tremblay, A., Després, J.P., Leblanc, C., Craig, C.L., Ferris, B., Stephens, T. and Bouchard, C. (1990). Effect of intensity of physical activity on body fatness and fat distribution. *American Journal of Clinical Nutrition.* **51** (2): 153-157.

Truswell, A.S. (1985). ABC of nutrition: obesity: causes and management. *British Medical Journal.* **291** (6497): 723-726.

Van Itallie, T.B. (1980). Morbid obesity: a hazardous disorder that resists conservative treatment. *American Journal of Clinical Nutrition.* **33**: 358-363.

Van Itallie, T.B. (1985). Health implications of overweight and obesity in the United States. *Annals of Internal Medicine.* **103** (6,II): 983-988.

Vasselli, J.R., Cleary, M.P. and Van Itallie, T.B. (1983). Modern concepts of obesity. *Nutrition Reviews.* **41** (12): 361-373.

Verryn, S.D. and Geerthsen, J.M.P. (1988). Prediction of mature values of conformation characteristics for young German Shepherd dogs. *Journal of Small Animal Practice.* **29**: 589-595.

Veterinary Record (1989). Feeding a growing pet population. *The Veterinary Record.* **124** (20): 523-524.

Veterinary Record (1991). Pet food manufacterers report further growth. *The Veterinary Record.* **128** (17): 391.

Volz, P.A. and Ostrove, S.M. (1984). Evaluation of a portable ultrasonoscope in assessing the body composition of college-age women. *Medicine and Science in Sports and Exercise.* **16**: 97-102.

Walker, A.D., Weaver, A.D., Anderson, R.S., Crighton, G.W., Fennell, C., Gaskel, C.J. and Wilkinson, G.T. (1977). An epidemiological survey of the feline urological syndrome. *Journal of Small Animal Practice.* **18**: 283-301.

Wang, J., Segal, K.R., Van Itallie, T.B., Kral, J., Gutin, B., Wadden, T.A. and Pierson, R.N., Jr. (1986). Body fat predictions by calliper versus ultrasound in lean and obese adults. *Federation Proceedings.* **45** (3): 600 (Abstract).

Ward, A. (1984). The fat-dog problem: how to solve it. *Veterinary Medicine.* **79**: 781-786.

Wardlaw, A.C. (1985). Correlation, regression and line-fitting through graph points: standard curves. In: *Practical Statistics for Experimental Biologists.* John Wiley & Sons, Chichester, West Sussex, 188-213.

Weatherall, R. and Shaper, A.G. (1988). Overweight and obesity in middle-aged British men. *European Journal of Clinical Nutrition.* **42** (3): 221-231.

Wehberg, K.E., West, D.B., Kieswetter, C. and Granger, J.P. (1990). Baroreflex sensitivity in the canine model of obesity-induced hypertension. *American Journal of Physiology.* **259** (5,II): R981-R985.

Weigle, D.S. (1990). Human obesity. Exploding the myths. *Western Journal of Medicine.* **153** (4): 421-428.

Weingand, K.W., Hartke, G.T., Noordsy, T.W. and Ledeboer, D.A. (1989). A minipig model of body adipose tissue distribution. *International Journal of Obesity.* **13** (3): 347-355.

Weisenburg, C.L. and Allen, C.E. (1973). Adipose tissue metabolism in obese and lean pigs. *Journal of Animal Science.* **37**: 293 (Abstract).

Weits, T., Van der Beek, E.J. and Wedel, M. (1986). Comparison of ultrasound and skinfold calliper measurement of subcutaneous fat tissue. *International Journal of Obesity.* **10**: 161-168.

Wells, P.N.T. (1977). Basic principles. In: *Ultrasonics in Clinical Diagnosis.* 2nd Edition. Editor P.N.T. Wells, Churchill Livingstone Inc., New York, 3-17.

Wells, P.N.T. (1981). Basic physics of ultrasound. In: *Medical Physics.* Proceedings of the International School of Physics Enrico Fermi. Editor J.R. Greening, North-Holland Publishing Company, New York, 398-410.

Westervelt, R.G., Stouffer, J.R., Hintz, H.F. and Schryver, H.F. (1976). Estimating fatness in horses and ponies. *Journal of Animal Science.* **43** (4): 781-785.

Widdowson, E.M., McCance, R.A. and Spray, C.M. (1951). The chemical composition of the human body. *Clinical Science.* **10**: 113-125.

Williams, G.D. and Newberne, P.M. (1971). Decreased resistance to *Salmonella* infection in obese dogs. *Federation Proceedings.* **30**: 572-580.

Wills, J.M. (1988). Obesity in the cat. *Clinical Insight.* **3** (9): 412-414.

Wilkinson, G.T. (1984). Diseases of the endocrine system and metabolic diseases. In: *Diseases of the cat and their management.* 2nd Edition. Editor G.T. Wilkinson, Blackwell Scientific Publications, Oxford, 265-275.

Wolter, R. (1988). Alimentation et obésité chez le chien. (Nutrition and obesity in dogs). *Pratique Médicale et Chirurgicale de l'Animal de Compagnie.* **23**: 111-118.

Womersley, J. and Durnin, J.V.G.A. (1977). A comparison of the skinfold method with extent of overweight and various weight-height relationships in the assessment of obesity. *British Journal of Nutrition.* **38**: 271-284.

Wood, J.D. and Reid, J.T. (1975). The influence of dietary fat on fat metabolism and body fat deposition in meal-feeding and nibbling rats. *British Journal of Nutrition.* **34**: 15-24.

Woods, S.C., Porte, D. Jr., Bobbioni, E., Ionescu, E., Sauter, J.F., Rohner-Jeanrenaud, F. and Jeanrenaud, B. (1985). Insulin: its relationship to the CNS and the control of food intake and body weight. *American Journal of Clinical Nutrition.* **42**: 1063-1071.

Wright, I.A. and Russel, A.J.F. (1984a). Partition of fat, body composition and body condition score in mature cows. *Animal Production.* **38**: 23-32.

Wright, I.A. and Russel, A.J.F. (1984b). Estimation in vivo of the chemical composition of the bodies of mature cows. *Animal Production.* **38**: 33-44.

Yim, G.K.W. and Lowy, M.T. (1984). Opioids, feeding and anorexias. *Federation Proceedings.* **43**: 2893-2897.

Young, C.M., Blondin, J., Tensuan, R. and Fryer, J.H. (1963). Body composition of older women. *Journal of the American Dietetic Association.* **43**: 344-348.

Young, J.B. and Landsberg, L. (1982). Diet-induced changes in sympathetic nervous activity: possible implications for obesity and hypertension. *Journal of Chronic Diseases.* **35**: 879-883.

Ziskin, M.C. (1975). Basic Principles. In: *Diagnostic uses of Ultrasound.* Grune & Stratton Inc., New York, 1-30.