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Kerry William Withers
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**Dietary Omega-6 Fatty Acids
in Protein-Energy Malnutrition.**

**A thesis submitted in fulfilment of the
requirements for the award of the degree**

DOCTOR OF PHILOSOPHY

from



UNIVERSITY OF WOLLONGONG

by

KERRY WILLIAM WITHERS B.A.(Hons), Dip. Ed, M.Sc(Hons)

Department of Biological Sciences

1993

Declaration

This thesis is submitted in accordance with the regulations of the University of Wollongong in fulfilment of the requirements for the degree of Doctor of Philosophy. The work described in this thesis has not been submitted at any other university or similar institution

A preliminary account of part of this work has been published in abstract form (Withers and Hulbert, 1992a;1992b).

Kerry Withers

Abstract

This thesis examines protein-energy malnutrition (PEM) in rats and the influence of omega-6 polyunsaturated fatty acids (PUFA) on this condition.

In the first of three experiments, rats were assigned to one of four diets, which were all isoenergetic and contained 10% (w/w) fat. One group was fed a control diet (containing 22% w/w protein) *ad libitum*, the other three diets were reduced in protein (10% w/w), and at one third the food consumption rate of the control diet rats. These three PEM groups received different amounts of PUFA. Almost all of the PUFA in the diets was linoleic acid. The minimum proportion of dietary energy intake supplied by linoleic acid in the diets was 1.3%. Stearic acid, was chosen to complete the lipid component. Growth, basal metabolic rate and nitrogen and energy balance were determined and tissues analyzed and ion homeostasis examined. Apparent absorption of energy and nitrogen were dramatically reduced in the diet high in stearic acid. A second experiment indicated that substituting coconut oil for stearic acid removed this effect.

In a third experiment, different oils were used, dietary fat content was increased to 15% w/w, the diets were commenced at weaning and the experimental period was extended from six to eight weeks.

Food-restriction lead to a reduced metabolisable energy (as a % of energy intake) and retarded growth. Brain, and the digestive tract were more resistant to growth retardation than the female reproductive organs, skin, fur and spleen. Brain was also less susceptible to changes in composition than other tissues.

PEM reduced apparent absorption of energy and nitrogen, retarding growth to a greater extent than food-restriction. It also increased carcass water content. Increased dietary linoleic acid seemed to ameliorate some of these effects, notably, growth and body water content. Much of the PUFA-induced increased growth was in skin and fur. Net protein utilization improved in PEM, especially with PUFA. The PUFA diet gave rise to no obvious negative effects.

Numbers of sodium pumps in soleus muscle and erythrocytes were not affected by food-restriction or PEM. However, they were reduced in erythrocytes during PEM by PUFA. The extracellular compartment of soleus muscle was increased by food-restriction and to a greater extent by PEM. PUFA tended to reverse this effect.

The increases in growth and protein assimilation, combined with a reduced body water content indicate that increased dietary linoleic acid during PEM is beneficial. The possible implications of this work on rats for human PEM are, that more attention should be paid to the nature of lipids given during recovery from malnutrition and that the mechanistic differences between kwashiorkor and marasmus may be related to essential fatty acid deficiency.

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Chapter 1: Literature Review

Introduction to Protein-Energy Malnutrition

Protein-energy malnutrition (PEM) is a major problem in third world countries, especially during the rapid growth and development stages of young children (Passmore, 1986; Torun and Viteri, 1988). The most distinctive categories of PEM are kwashiorkor and marasmus, which are often regarded as the two extremes of a broad spectrum of malnutrition diseases (Olowookere, 1987). However, other categories, such as marasmic kwashiorkor, nutritional dwarfing and low weight for age/height often constitute a larger proportion of a malnourished population than marasmus and kwashiorkor combined (Waterlow and Alleyne, 1971; Gopalan, 1975; Jaya Rao, 1978). Golden and Ramdath (1987) suggest that only 0.5-2% of children in a chronically malnourished population develop kwashiorkor. In all of these categories, there is growth retardation and a reduction in metabolically-active tissues, mainly skeletal muscle. Because animals undernourished early in life while their cells are still dividing may never attain the same body size as well-nourished individuals (Dobbing, 1964; Winick and Noble, 1966; Widdowson, 1970), it is important to understand the aetiology of malnutrition diseases, so that available resources may be used most efficiently. Much attention was given to kwashiorkor and marasmus in the two decades after World War II, this attention later tended to focus upon less distinctive and more widespread malnutrition diseases (Waterlow, 1984). However, in the 1990's the incidence of severe malnutrition is increasing. The constraints imposed on communities and relief agencies by lack of resources and obstruction by competing and often warring factions suggests that a better understanding of severe malnutrition is urgently required. This understanding should include the aetiology of PEM,

especially kwashiorkor and also the optimum composition of food relief to be transported and distributed to those in need. In this review of the literature, I intend to examine some of these aspects of PEM.

A comparison of Marasmus and Kwashiorkor

Marasmus

Marasmus is a condition of growth retardation, weight loss, muscular atrophy, severe loss of subcutaneous tissue (Truswell, 1984; Vajreswari et al, 1990) and apathy (Passmore, 1986). The term marasmus has been known for centuries (Truswell, 1981; Hendrickse, 1991). It is derived from the Greek "marasmos" and means to "wither" (Concise Oxford Dictionary, 1976). The condition usually results from a reduced food intake or starvation (nutritional marasmus), but may also result from other factors, such as tuberculosis, congenital syphilis and congenital abnormalities of the urinary tract (Pretorius, 1968). In nutritional marasmus, there is also a deficiency of protein, but to a lesser extent than that which is believed to cause kwashiorkor (Pretorius, 1968). Hepatomegally (liver enlargement) is absent and the incidence of hypoalbuminaemia is much less than in kwashiorkor and the fall is much less (James, 1977). Unlike kwashiorkor, liver fat is reduced, rather than increased (Scrimshaw and Behar, 1961). Skin and hair are often normal, although Torun and Viteri (1988) indicate that the hair may be thin, dry and reddish in colour and the skin may also be dry. Oedema is reported to be absent (James, 1977; Vajreswari et al, 1990), although infants without oedema may have an increased % of body water (Smith, 1960; Smith and Waterlow, 1960), especially where body fat has been greatly reduced. This increase is mainly in the extracellular compartment, however, the cells may also be overhydrated (Passmore, 1986; Torun and Viteri, 1988). Truswell (1984) pointed out that

famine oedema resulted from loss of tissues without loss of body water.

Kwashiorkor

The tissue wasting, so obvious in marasmus is obscured in kwashiorkor by oedema in limbs, face and belly (Williams, 1933; Trowell, Davies and Dean, 1952; Truswell, 1984; Passmore, 1986; Olowookere, 1987), the distribution of which may be influenced by gravity (Passmore, 1986). However, there is not a close association between the degree of the oedema and the magnitude of the increase in body water (Passmore, 1986). During the early stages of therapy, there may even be a brief increase in oedema (Passmore, 1986). There is growth retardation (Pretorius, 1968) and in spite of oedema, children with kwashiorkor are underweight (Trowell et al., 1952), although not to the same extent as those with marasmus (Torun and Viteri, 1988). The increase in body water is believed to be largely extracellular (Waterlow and Alleyne, 1971). There is a reduction in bone length and retardation of bone age (Pretorius, 1968; Krueger, 1969). Kwashiorkor is also characterized by a dry scaly skin (Pretorius, 1968; Torun and Viteri, 1988), with the development of skin lesions (Freiman, 1975; Truswell, 1981), hypo- or hyperpigmentation (Williams, 1933; Pretorius, 1968; James, 1977; Passmore, 1986; Golden, 1988a), thin greyish or reddish hair (Trowell et al., 1952; Pretorius, 1968; Freiman, 1975; James, 1977), apathy and irritability (Williams, 1933; Trowell et al., 1952; Pretorius, 1968; Freiman, 1975) and an enlarged and fatty liver (Williams, 1933; Torun and Viteri, 1988). The lipid content of liver may be dramatically increased during kwashiorkor, to up to nine times its normal concentration (Chatterjee and Mukherjee, 1968). In severe cases, fat droplets in hepatocytes coalesce, pushing nucleus and cytoplasm to the cell periphery (Passmore, 1986). However, subcutaneous fat and tissue glycogen are preserved (Trowell et al., 1952; Schwartz and Dean, 1957; Truswell, 1981; 1984; Golden, 1988a; Torun and Viteri, 1988). The colour and texture of hair is influenced by a variety of wasting diseases and environmental factors. This

may account for the variations in dyspigmentation of hair (Williams, 1953).

A variety of endocrine changes have been detected in PEM (Whitehead and Lunn, 1979; Anonymous, 1986). However, the mechanisms involved and the physiological significance of these changes is unresolved e.g., plasma insulin decreases in both kwashiorkor and marasmus (Whitehead and Lunn, 1979) and hypothyroidism appears to develop when PEM is long-term (Anonymous, 1986). Plasma growth hormone concentrations in kwashiorkor are high and negatively correlated with those for plasma albumin. However, it is unclear whether growth hormone concentrations are different from those in marasmus (Whitehead and Lunn, 1979). There are low levels of hepatic export proteins (Golden, 1988a) and anaemia is frequent (Trowell et al, 1952; Freiman, 1975; McLaren and Meguid, 1988). However, "as long as the tissue's needs for oxygen are satisfied by existing capacity for oxygen transport, this should be considered as an adaptive response and not a "functional anemia (i.e., with tissue hypoxia)" (Torun and Viteri, 1988; p 752). However, if increased tissue demands for oxygen, associated with growth during rehabilitation are not able to be met, then functional anaemia will occur (Torun and Viteri, 1988).

It is difficult to give an unequivocal description of kwashiorkor, except in severe cases as the signs and symptoms depend on factors such as the stage of the disease, patient's age and handling by others (Williams, 1973; Hendrickse, 1984, 1988), e.g., the skin may be protected from trauma, by oiling (Williams, 1973). An historical account of the "discovery" of kwashiorkor provides an indication of the problems involved in determining the aetiology of this disease.

It was not until the 1930's, that kwashiorkor began to be recognised as a distinct entity (Hendrickse et al, 1982). Cicely Williams, a pediatrician working

in Ghana introduced to the medical literature "A deficiency disease in infants" (Williams, 1932). In a second publication, "A nutritional disease of childhood associated with a maize diet", she suggested that "some amino acid or protein deficiency cannot be excluded" (Williams, 1933). In a third publication, she introduced the term "kwashiorkor" as a label for this disease. Kwashiorkor is a word of the Ga language of Accra, Ghana, and means "the disease of the deposed baby when the next one is born" (Williams, 1935, 1953). Initially, these articles elicited little response, other than criticism that kwashiorkor was really pellagra (Williams, 1973), a disease also characterised by skin lesions and now known to be caused by a deficiency of nicotinic acid (Passmore, 1986).

Williams' transfer to Malaya in 1936, her subsequent imprisonment during World War II and the effects of the war on communication inhibited progress in the study of kwashiorkor. However, observations made of those suffering from war-time deprivations during the 1940's did stimulate considerable interest in the disease (James, 1977). Unfortunately, confusion between kwashiorkor and pellagra, persisted into the 1950s (Trowell et al., 1952; Williams, 1973). This confusion continued to inhibit progress in the study of kwashiorkor. Even in recent textbooks (Torun and Viteri, 1988) is it still suggested that the skin lesions of kwashiorkor may be confused with those of pellagra.

The increased interest in kwashiorkor also brought criticism of Williams' publications. The disease was found to have been mentioned in earlier literature, although none of these earlier reports were available in the Gold Coast of Ghana and were unknown to Williams at the time of her initial publications (Williams, 1953). Like William's initial publications, these earlier reports had also elicited little response and had introduced a variety of local terms for the disease. Many of these reports originated in Latin American

literature, which was poorly cited at the time (Autret and Behar, 1954). The terms used included "mehinahrshaden" in Germany, "culebrilla" in Mexico, "oedema and ascariasis" in Kenya, "bouffissure d'Annam" in Indochina and "starchy food dystrophy" in Italy (Williams, 1973). Some terms, such as "mehinahrshaden" (damage by cereal flours), "starchy food dystrophy", "starch edema" and "sugar babies" suggested that the disease resulted from an intake of low protein-high carbohydrate foods. Others, such as "syndrome policarencial de la infancia" suggested that it affected mainly young children and involved multiple nutrient deficiencies (Truswell, 1976; Torun and Viteri, 1988). "Culebrilla" (snake-like), was introduced to the literature in 1906 and referred to the serpentine patterns of hypo- and hyperpigmentation. Unfortunately, descriptions of the dermatologic signs published over the next two decades created the impression that the disease was caused by a vitamin deficiency or tropical parasites (Torun and Viteri, 1988).

Criticism of Williams' reports continued into the 1950's, when it also focused on her initial description of the disease and even the origin of the term kwashiorkor. By then, more than 40 terms had been given to the disease (Torun and Viteri, 1988) and Williams' role in describing kwashiorkor and initiating an international awareness of the disease may have been ignored or dismissed in several major United Nations reports. This is apparent in the various accounts of the introduction of the disease and its name to modern medicine given in the literature (Trowell and Davies, 1952; Autret and Behar, 1954; Williams, 1973). Publications, such as that of Trowell et al. (1954) did much to encourage widespread inclusion of kwashiorkor in the textbooks (Passmore, 1986). Truswell (1984), although acknowledging Williams' 1932 report, suggests that it was not until 1952 that medical science became "aware" of kwashiorkor. However, although Williams' publications were not the first concerning the disease, she did introduce its "accepted" label to the literature,

initiate interest in kwashiorkor and contribute significantly to its study. Consequently, many recent texts now include an outline of Williams' contributions when introducing PEM.

Unlike marasmus, which may occur in the first 12 months, before weaning, and even in adults (Scrimshaw and Behar, 1961), kwashiorkor occurs almost exclusively in children (McLaren and Meguid, 1988), usually soon after weaning (Trowell and Davies, 1952; Hendrickse, 1984). Williams (1933) alluded to the possibility of an amino acid or protein deficiency in the aetiology of kwashiorkor. The low levels of serum proteins found in kwashiorkor and their prompt increase with protein supplementation appeared to confirm that protein deficiency was a major factor in its aetiology (Brock et al., 1955; Williams, 1973). Weaning onto a starchy diet low in protein and energy, at a time when energy and protein requirements for growth and development were high was believed to precipitate kwashiorkor (Trowell and Davies, 1952; Williams, 1953; Pretorius, 1968).

The Protein Gap

Unfortunately, in the 1950's, undue emphasis was placed on reports of kwashiorkor from Africa and a variety of places after the Second World War (McLaren, 1974). Subsequently, it was suggested that kwashiorkor was "the most serious and widespread nutritional disorder known to medical and nutritional science" (Brock and Autret, 1952; p 72), although McLaren (1974) suggested that at that time, only about 5% of malnourished children had kwashiorkor. Protein, believed at the time to be the key deficient nutrient was proclaimed as in short supply, world-wide. Thus, was "the protein gap" conceived (McLaren, 1974). The protein gap was touted as an immense problem to overcome and much in the way of resources was diverted away from other

avenues in order to bridge "the protein gap". Similarly, infrastructure for research, as well as that for food production and distribution was focussed on the role of protein deficiency in the aetiology of kwashiorkor (McLaren, 1974).

In an analysis of what he termed "The great protein fiasco", McLaren (1974) admits that the protein gap was eventually closed. Curiously, this was achieved by progressively lowering the recommended dietary protein intake to approximately where it was at the beginning of the century, resulting in a saving of over \$100 billion per year (McLaren, 1974). In reality, what really existed was a "food gap" or "energy gap" (McLaren, 1974; Waterlow and Payne, 1975). Unfortunately, this scaling down of protein requirements has in itself, decreased the importance of dietary protein levels amongst those responsible for large scale planning (Truswell, 1981). It has even been suggested that the protein gap has been artificially abolished and that the present estimates of protein requirements are unrealistically low (Waterlow and Payne, 1975; Waterlow, 1984). However, Ashworth (1985) suggests that many health workers and policy makers still overemphasise the importance of protein-rich foods in treating malnutrition. It is evident from the above, and from analyses such as that of McLaren (1978), "Nutrition planning day dreams at the United Nations", that PEM has not been dealt with effectively by scientists or bureaucrats.

Protein versus Energy

Aside from the "protein gap", the possibility of an amino acid or protein deficiency in the aetiology of kwashiorkor, alluded to, albeit cautiously, by Williams (1933) was subsequently reinforced by others (Brock and Autret, 1952; Trowell and Davies, 1952; Pretorius, 1968) and become widely established as dogma. Plasma proteins are low in kwashiorkor (Truswell, 1981) and the widely accepted mechanism for oedema involved hypoproteinemia (Pretorius,

1968; Read, 1990). In marasmus, hypoalbuminaemia occurs much less than in kwashiorkor and the fall in plasma protein concentration is much less. (James, 1977). Kwashiorkor was held to involve an inadequate protein intake with adequate or even excess energy intake, usually as carbohydrate (Pretorius, 1968; Waterlow and Alleyne, 1971; Williams, 1973; McLaren and Meguid, 1988). This was a widely held view (Hendrickse, 1984), which still persists (Waterlow, 1984; Olorunsogo, 1989; Rossouw, 1989). Lunn and Baker (1983) reported that young baboons allowed free access to a low protein: energy diet develop hypoalbuminaemia. This they concluded was due to an energy excess, rather than to low protein per se. The production of a kwashiorkor-like syndrome in experimental animals requires unrestricted feeding of diets low in protein (Coward and Lunn, 1981).

In "The theory of protein metabolism", Miller and Payne (1963), proposed that if energy intake was very low and protein intake was high or adequate, then dietary protein would be metabolised for the production of energy. Thus, "If the total food intake is inadequate there will be a secondary protein deficiency, because protein cannot be utilized if energy is lacking and extra protein will be of no value" Waterlow and Payne, 1975; p 116). This results in a loss of body protein in adults and a reduced growth rate in the young (WHO, 1973). The catch-up growth of infants recovering from PEM may be greatly increased if their dietary energy intake is increased. The rate of weight gain may be more than 20 times faster than that of healthy children of the same age. "Such rapid rates, are however, only observed when wasting is present, i.e., when there is a greater deficit in weight than there is in height" (Ashworth and Millward, 1986; p 157). The growth is greater in infants with marasmus, than in those with kwashiorkor and may be achieved with "relatively modest protein intakes" (Ashworth et al, 1968; p 600). The higher fat and glycogen content of children with kwashiorkor, also suggests that they are less energy-deficient than

those with marasmus (Waterlow and Payne, 1975; Golden, 1988a) and is thus consistent with the findings of Ashworth and her colleagues.

The work of Gopalan, (1968) suggests that there is no relationship between protein intake and whether children will develop kwashiorkor rather than marasmus. Furthermore, there is not a constant relationship between oedema and hypoproteinaemia (Beattie et al, 1948; Autret, 1955; Passmore, 1986), suggesting that hypoproteinemia is a modifying factor rather than a cause of oedema (Waterlow et al, 1960; Waterlow and Alleyne, 1971). The "causal relationship" held to exist between dietary protein deficiency and kwashiorkor has been widely questioned (Whitehead, 1977; Whitehead and Lunn, 1979; Read, 1990). Hendrickse (1991), suggests that the theory of protein deficiency is unproven and is indeed discredited. According to McLaren (1974), even Cicely Williams had eventually tried to "debunk" kwashiorkor, presumably because of the "development" of this relationship between protein deficiency and kwashiorkor.

The term "protein-calorie malnutrition" was introduced by Jelliffe (1959). Although it was sometimes referred to as "energy-protein malnutrition" (McLaren, 1973, 1974) or "calorie-protein deficiency" (Gopalan, 1975), it is now generally known as "protein-energy malnutrition" or PEM. The failure of protein supplementation in eliminating malnutrition, coupled with a developing awareness of the importance of an adequate energy intake led to these changes (Hendrickse, 1988, 1991). PEM is generally considered to be a continuum between the two disease states of marasmus and kwashiorkor, along which, it is difficult to establish a line of demarcation (Scrimshaw and Behar, 1961; Freiman, 1975; Truswell, 1976, 1984; Passmore, 1986; McLaren and Meguid, 1988). There is overlap of some features of marasmus and kwashiorkor (McLaren, 1974; McLaren and Meguid, 1988) and distinguishing

between the two disorders is sometimes of little practical use anyway, as many children in South Africa, Asia, Central and South America and the Caribbean present with features of both marasmus and kwashiorkor (James, 1977).

Difficulties in the area of malnutrition sometimes arise as a result of loose terminology (Hendrickse, 1988). The high proportion of malnourished children who do not fit the classic definitions of either marasmus or kwashiorkor has encouraged some workers to abandon these terms (Waterlow and Alleyne, 1971). The variety of classification systems for kwashiorkor, especially in the earlier literature made it more difficult to establish its aetiology. The variation in symptoms of both diseases between different geographical locations increased this difficulty e.g., fatty liver during kwashiorkor appears to be more common in some locations than others (Waterlow and Alleyne, 1971). However, even when there is general agreement over distinguishing characteristics such as fatty liver, there is often confusion over the mechanisms which may be involved in the development of these characteristics (Waterlow and Alleyne, 1971).

Although the belief that dietary differences exist between groups prone to kwashiorkor and those which are not still persists (Truswell, 1981; Waterlow, 1984; Rossouw, 1989), kwashiorkor is difficult to reproduce in animals by manipulation of protein and energy intake. As pointed out by Truswell (1981), there have been animal models of kwashiorkor in a number of different species, such as monkeys (Follis, 1957; Ramalingaswami, 1969), rats (Kirsch et al, 1968a,b) and guinea pigs (Worthington et al, 1977). However, Golden (1988a) suggested that in only one study, carried out on baby baboons (Coward and Whitehead, 1972) had kwashiorkor been convincingly reproduced. Differences between a controlled experimental situation in rats and the clinical situation with its added complexities in humans are not unexpected (Pimplikar

and Kaplay, 1981). However, it has been suggested that "the individual features of kwashiorkor had been singly and inconsistently reproduced in animals using a whole variety of, often extreme, experimental manipulations" Golden and Ramdath (1987, p53).

Kwashiorkor occurs after weaning, usually in the second and third years, whilst marasmus may occur before weaning or at any time in life (Passmore, 1986; McLaren and Meguid, 1988). There are regional differences in the time of onset of infant malnutrition (Waterlow and Alleyne, 1971). This temporal distinction, plus the geographical variation in the incidence of kwashiorkor and marasmus (Waterlow and Alleyne, 1971) has been interpreted as support for the view that they are aetiologically distinct diseases (McLaren and Meguid, 1988). However, Waterlow and Payne (1975) report that there was no difference in age between children presenting at hospitals with marasmus or kwashiorkor in Jamaica, Iraq and Jordan. Waterlow (1984) suggested that the pathogenesis of kwashiorkor in children may be the same as that of famine oedema in adults.

Sodium and Potassium in Protein - Energy Malnutrition.

Whole body potassium (WBK) is reduced in malnourished children with oedema, but not in non-oedematous children (Garrow, 1965). This reduction may be a result of diarrhoea (Passmore, 1986). Wharton et al. (1968) reported that diarrhoea was a common problem in kwashiorkor in Kampala. Plasma $[K^+]$ is often low in malnourished children. However, there is not a good correlation between WBK and plasma $[K^+]$ (Garrow, 1965). Plasma $[Na^+]$ is usually normal, although it may be increased by complications such as dehydration resulting from water restriction and decreased by reduced salt intake, excessive water intake or excessive losses in sweat or feces (Passmore, 1986).

During PEM, reductions in $[K^+]$ and organ weight are greater in some tissues than others. Muscle is more susceptible to reductions in $[K^+]$ and organ weight than many other organs (Alleyne, 1975). Furthermore, unlike plasma potassium, there is a significant correlation between muscle potassium and WBK in malnourished infants (Nichols et al., 1969). The brain is very resistant to changes in both weight and composition and there is an increase in brain potassium as a percentage of WBK during PEM (Alleyne, 1975).

Total body sodium content is increased in malnourished children with oedema, although plasma sodium content is often normal or low (Alleyne, 1975).

The Sodium Pump in Protein - Energy Malnutrition

Animal cells maintain high intracellular concentrations of K^+ (120-160 mM) and low intracellular concentrations of Na^+ (< 10 mM) (Metcoff, 1975; Lehninger, 1978). This enables them to maintain intracellular conditions suitable for enzyme activity and the transmembrane ion gradients required for such activities as "action potentials" (Lehninger, 1978). Transmembrane ion gradients are maintained by the "sodium pump", a membrane-bound Na^+ / K^+ ATP-ase which pumps 3 Na^+ ions out of and 2 K^+ ions into the cell, for each molecule of ATP which is hydrolysed (Lehninger, 1978). The sodium pump opposes the continuous leakage of Na^+ into and K^+ out of the cell. If the energetic requirements of the sodium pump are not supplied, Na^+ and also water will accumulate within the cell (Metcoff, 1975).

Metcoff (1975) suggested that the increased intracellular $[Na^+]$ and water and decreased intracellular $[K^+]$ during PEM may be the result of "failure of the

pump". This he suggested was the result of impaired production and utilization of ATP (Metcoff, 1975). However, Welle (1989) reported that there was no correlation between erythrocyte Na^+/K^+ -ATPase activity and resting metabolic rate in humans. He suggested that the erythrocyte was not a good marker of Na^+/K^+ -ATPase activity in tissues with high energy consumption. Furthermore, the energetic cost of sodium pump activity in overall cellular metabolism is controversial. The activity of the sodium pump has been suggested as a significant consumer of energy (Kaplay, 1984). It is estimated to account for up to 50% of a cell's energy usage in some tissues (Whittam, 1961; Whittam and Willis, 1963; Patrick, 1979; Harris et al., 1981; Clausen, van Hardeveld and Everts, 1991). However, Nobes, et al. (1989) reported that the Na^+/K^+ -ATPase accounts for less than 10% of the ATP turnover in hepatocytes from rats. Regardless of the magnitude of the energetic cost of the sodium pump, erythrocytes from children with Kwashiorkor have more than twice the number of sodium pumps per cell than normal or marasmic children (Narayanareddy and Kaplay, 1982) and a 'pump rate' that is 65% higher than that of normal or marasmic children (Willis and Golden, 1988). Increased sodium pumping during kwashiorkor also occurs in leucocytes (Patrick, 1977). Digoxin, a cardiac glycoside which is a specific inhibitor of the sodium pump has been used successfully with diuretics to treat the extracellular fluid overload that develops during high-energy treatment (Patrick 1977). However, during kwashiorkor, plasma has a reduced albumin concentration and thus a reduced binding capacity for digoxin. The resulting increased unbound digoxin may have toxic effects (Passmore, 1986).

Studies in experimental animals reveal that there is a generalized and dramatic increase in sodium transport and in turn an increased demand for cellular energy during PEM (Pimplikar and Kaplay, 1981; Kaplay, 1984). However, it seems anti-intuitive and counter-productive that during a period

of severe protein and energy shortage, cells should make more sodium pumps and use more of their scarce energy to run them.

In studies concerned with the evolution of endothermy, mammalian cells were found to be more permeable or leaky to K^+ and Na^+ than reptilian cells (Else and Hulbert, 1987). This meant that greater sodium pump activity was required in mammals, if they were to maintain transmembrane Na^+/K^+ gradients. More energy was used in tissue from a mammal to maintain these transmembrane ion gradients than in that from a reptile (Hulbert and Else, 1981). Furthermore, a correlation exists between permeability to Na^+ and tissue oxygen uptake (Hulbert and Else, 1990). This phenomenon of increased membrane leakiness in the mammal also extends to proton permeability of liver mitochondrial membranes (Brand et al., 1991). There is evidence that increased cell membrane permeability to Na^+ is also a characteristic of malnutrition, as it has been found in the leucocytes of malnourished children (Patrick and Golden, 1977).

Membrane Leakiness in Protein - Energy Malnutrition

The capacity of muscle and other body tissues to retain K^+ has been shown to be reduced in children with PEM. However, this was not able to be explained by the reduction in cytoplasmic mass associated with PEM (Nichols et al., 1972). Subsequent studies have revealed that intact erythrocytes of children with kwashiorkor are twice as leaky to Na^+ and K^+ (Willis and Golden, 1988), that leucocytes are more leaky to Na^+ during kwashiorkor (Patrick and Golden, 1977) and that transport of Na^+ is dramatically increased in kwashiorkor (Patrick, 1979). Patrick (1980) suggested that oedema was the result of Na^+ retention.

In addition to the sodium pump data, other evidence suggests that there are differences between the membranes of normal children and those with kwashiorkor. The erythrocyte membranes of children with kwashiorkor have a reduced osmotic fragility (Brown et al, 1978; Kaplay, 1978, 1979; Fondu, et al, 1980; Ramanadham and Kaplay, 1982) which may reduce their deformability and thus their survival (Kaplay, 1979; Ramanadam and Kaplay, 1982). An increased ratio of surface area:volume in erythrocytes has been reported in children with PEM (Fondu et al, 1980). Increases in membrane phospholipid content and in the molar ratio of cholesterol:phospholipid have been reported in erythrocytes from children with Kwashiorkor. This ratio may be related to the increased ratio of surface area:volume of erythrocytes in kwashiorkor (Brown et al, 1978). However, Ramanadam and Kaplay (1982) reported a decrease in the ratio of cholesterol:phospholipid and the absence of a relationship between this ratio and osmotic fragility. Acetylcholinesterase, whose activity is often used in the detection of membrane abnormalities is located on the outer surface of the erythrocyte membrane (Kaplay, 1979). However, there is no association between acetylcholinesterase activity and osmotic fragility in human erythrocytes (Kaplay, 1978).

Alternative Theories of Protein - Energy Malnutrition

A variety of causes have been proposed for kwashiorkor and syndromes with similar characteristics, especially in the literature early this century (Autret and Behar, 1954). Read (1990) has outlined some of those under serious consideration at the present time.

The Free Radical Hypothesis

In his "free radical" hypothesis, Golden (1988a) suggests that there is no continuum between marasmus and kwashiorkor and that the two conditions

are aetiologically distinct. He suggests that the features of kwashiorkor could be explained by an imbalance between production and safe disposal of free radicals in the body (Golden, 1988a). All the conditions which may give rise to kwashiorkor also cause an increased flux of free radicals. One of the body's defence mechanisms is the production of free radicals to kill invading microorganisms such as bacteria (Golden and Ramdath, 1987; Muller, 1987). At least 14 different nutrients are involved in protecting the body against free radicals, but in kwashiorkor all the protective pathways are decreased (Golden, 1988a). Zinc is one of these protective nutrients and Sandstead et al. (1965) and Prasad, (1967) have reported low plasma zinc concentrations in Egyptian children with kwashiorkor. Zinc is in low concentrations in malnourished children with skin lesions. Oral and cutaneous supplementation with zinc promotes rapid healing of skin lesions of children with kwashiorkor (Golden et al, 1980). Hansen and Lehmann (1969) reported low serum zinc concentrations in both marasmus and kwashiorkor, although there was no correlation between this parameter and growth retardation. Waterlow et al. (1978) report that plasma zinc concentration is lower in children with kwashiorkor, than in those with marasmus. Furthermore, plasma zinc concentration fell during recovery, suggesting that zinc may have become a limiting nutrient (Waterlow et al, 1978). The stimulation of growth associated with zinc supplementation during the high-energy feeding of a malnourished child (Waterlow et al, 1978) is consistent with this suggestion.

Lipid peroxidation is increased by excess iron (Roberts, 1986). During kwashiorkor, there is an increase in tissue iron, and in turn, a great increase in free radical generation via redox cycling (Golden, 1988a; Golden and Ramdath, 1987). Peroxidation increases membrane leakiness (Golden and Ramdath, 1987). An excessive intake of polyunsaturates such as linoleic acid also induces peroxidation and thus increases vitamin E requirements (A.A.P. Committee on

nutrition, 1976). Failure to supply sufficient vitamin E may result in oedema and skin lesions (Hassan et al, 1966). Vitamin E is a fat-soluble vitamin (alpha-tocopherol is its most active form) which protects cell membrane fatty acids against oxidative damage by free radicals (Roberts, 1986). Selenium also aids in the prevention of peroxidation of membrane lipids (Passmore, 1986) and selenium is reduced in infants with PEM (Burk et al, 1967; Levine and Olson, 1970). Golden's treatment of malnourished children involves the use of vitamin E, selenium and also desferrioxamine (a chelating agent), which reduces free iron and thus reduces free radical formation (Read, 1990).

The Aflatoxin Hypothesis

It has been suggested that the aetiology of PEM involves environmental as well as dietary factors (Hendrickse, 1984; Tolboom et al, 1989). Witchcraft is considered by many in Africa as the real cause of kwashiorkor and similar traditional explanations have also been reported for marasmus in Pakistan (Mull, 1991). Hendrickse (1984) has proposed that kwashiorkor is caused by aflatoxins. Aflatoxins are toxic metabolites produced by fungi. Food in the tropics is frequently contaminated by aflatoxins (Hendrickse 1988). This includes common staples such as maize, cassava and groundnuts (Hendrickse, 1991). Strains of *Aspergillus flavus* fungi are involved in the production of aflatoxins (Hendrickse, 1984). Tropical temperatures and humidity provide suitable conditions for fungal growth and toxin formation (Anonymous, 1984; Hendrickse, 1984, 1991; Read, 1990).

The aflatoxin hypothesis provides an explanation for many of the observations on kwashiorkor (Hendrickse, 1984). There is considerable similarity in the distribution and seasonal fluctuation of aflatoxin levels in food and that of kwashiorkor. There is also considerable overlap in the characteristics of kwashiorkor and those resulting from ingestion of aflatoxins

(Hendrickse, 1984, 1991). Aflatoxins are found more often and in higher concentrations in the sera of children with kwashiorkor than in those with marasmus or in normal children (Hendrickse et al., 1982). Aflatoxin B1 (AFB1) is the most important of these toxins (Anonymous, 1984). Aflatoxinol, the reduction metabolite of AFB1 is frequently found in the livers of children who die from kwashiorkor, but not in those who die from other causes (Hendrickse, 1988). Unstable epoxides of AFB1 form when the capacity of the liver to metabolise and excrete aflatoxins is exceeded. These damage the liver, reducing albumin production (Hendrickse, 1990). The resulting low levels of albumin may lead to the disease (Read, 1990). "When the adaptive mechanisms fail, the concentration of serum proteins and especially albumin, decreases. The ensuing reduction in intravascular oncotic pressure and outflow of water into the extravascular space contribute to the development of the oedema of kwashiorkor" (Torun and Viteri, 1988; p 750).

Other effects attributed to aflatoxins include carcinogenicity, teratogenicity, mutagenicity, inhibition of DNA, RNA and protein synthesis and a variety of effects on lipid metabolism and the immune system (Hendrickse, 1988). Studies in animals indicate that susceptibility to aflatoxins is increased by malnutrition, especially in young animals (Hendrickse, 1988). Oedema and hypoproteinaemia may develop in animals receiving "normal" rations containing aflatoxins. This can be reversed by increased dietary protein (Hendrickse, 1990). Although a reduced capacity to metabolise aflatoxins may not be considered an adaptive process, not all reductions in enzymic capacity are deleterious e.g., the resistance to carbon tetrachloride of rats fed protein-free diets (McLean and McLean, 1965).

Kwashiorkor is more common in tropical regions and marasmus more common in India, middle East and Chile (Truswell, 1981). Hendrickse (1988)

maintains that kwashiorkor has been recorded only in warm humid climates, never in cold temperate climates. Scrimshaw and Behar (1961) outlined a wider distribution of kwashiorkor than this, but as McLaren (1974) suggests, there may have been a gross overestimation of the incidence of kwashiorkor at this time. Even as late as 1984, it was suggested that there was a lack of good epidemiological evidence concerning the incidence of kwashiorkor and marasmus in different geographical locations, although kwashiorkor was believed to predominate in regions where protein intake was lower (Waterlow, 1984).

The increased incidence of kwashiorkor during the "wet" season (Anonymous, 1984) has been interpreted as supporting the aflatoxin hypothesis (De Vries and Hendrickse, 1988; Hendrickse 1984, 1988). Seasonal patterns in the incidence of severe PEM have been reported in the literature. In Lesotho, the pattern correlates with that for the incidence of acute gastroenteritis. However, individual seasonal patterns have been recognized for marasmus and marasmic kwashiorkor, as well as for kwashiorkor (Tolboom et al., 1989). Furthermore, the seasonal increase in incidence of kwashiorkor often coincides with the "hungry season" before harvest (Truswell, 1984; Passmore, 1986). Bad storage conditions and inadequate distribution systems may contribute to post-harvest losses of food, even when production levels were high (Torun and Viteri, 1988). These effects tend to confound attempts to develop a cause and effect relationship between aflatoxins and kwashiorkor. As stated previously, kwashiorkor and marasmus are widely held to be extremes of the spectrum of PEM. If there is a cause and effect relationship between kwashiorkor and aflatoxins, then the long-held view of "PEM" as a spectrum of disease has been "unfortunate" and may have impeded our understanding of the aetiology of kwashiorkor (Hendrickse, 1988, 1991).

It has been claimed that the study of Hendrickse and colleagues (Hendrickse, et al, 1982) has not demonstrated a cause and effect relationship between aflatoxins and kwashiorkor, as only 36% of kwashiorkor patients had detectable aflatoxin levels. Hendrickse and co workers (De Vries et al., 1990) interpret the prolonged fecal excretion of aflatoxins during kwashiorkor as suggesting that there may be difficulty in excreting aflatoxins which accumulate in the liver. However, it is possible that aflatoxins are detectable in some individuals, because their capacity for metabolizing aflatoxins has been impaired by kwashiorkor (Anononymous, 1984; Golden and Ramdath, 1987; Househam and Hundt, 1991). Whitehead and Lunn (1979) suggest that complex interactions between dietary composition, energy intake and environmental stresses such as infections (Whitehead, 1977) may determine whether kwashiorkor or marasmus develops in some malnourished children. Infections such as gastroenteritis, measles and malaria are known to precipitate kwashiorkor (Truswell, 1984). Recently, Househam and Hundt (1991) have reported that in Bloemfontein (S. Africa), kwashiorkor occurs in the absence of aflatoxin exposure. This finding, based on analyses of urine (with some analyses duplicated in Hendrickse's laboratory) is evidence against the aflatoxin hypothesis. Hendrickse acknowledges that kwashiorkor-like symptoms may occur in the absence of aflatoxins, but maintains that kwashiorkor per se is aflatoxin-related (Hendrickse, 1990).

Adaptations During Protein - Energy Malnutrition

The existence of adaptations, operating during malnutrition has been a well-accepted concept, although their mechanistic details were often unclear (Mohan and Narasinga Rao, 1983). During marasmus, adults lose weight, while children stop growing and then lose weight (Golden, 1988a; Truswell, 1984). Weight loss occurs in the form of body water, subcutaneous fat and muscle (Brozek et al., 1957; Montgomery, 1962a; Freiman, 1975; Truswell, 1984;

Passmore, 1986), there is no oedema (Vajreswari et al, 1990). Loss of muscle reduces the cell mass of active metabolic tissue and thus reduces energy requirements (Passmore, 1986).

Increased utilization of glycogen associated with carbohydrate restriction greatly depletes glycogen stores, with a concomitant loss of the 3-4 g water associated with the storage of each gram of glycogen (Passmore, 1986). In starving rats, liver glycogen is rapidly depleted and later replenished to about 20% of original levels. Liver glycogen is also significantly depleted in malnourished children (Alleyne and Scullard, 1969). Muscle glycogen is also low in malnutrition (Alleyne and Scullard, 1969), although, it decreases more slowly than that in the liver (Lawrence and McCance, 1931). This loss of glycogen, water, fat and muscle, further reduces body weight and thus the mechanical work required to move the lighter body about (Passmore, 1986).

During marasmus, there is also a general curtailment of all unnecessary voluntary movement (Passmore, 1986). This may be a result of the general apathy associated with the disease. Thus, there is a general decrease in energy expenditure, both at rest and during physical activity (Passmore, 1986). The reduction in energy expenditure is often interpreted as an adaptation to a reduced food intake (Passmore, 1986). Physical activity promotes skeletal growth (Torun et al, 1981), so a general reduction in physical activity may possibly contribute to a reduction in growth. This is consistent with the reduction in rate of whole-body protein synthesis reported by Schonheyder et al, (1954). Stunted growth has been suggested as an adaptive change to a reduced food supply (Torun et al, 1981). However, in food-restricted rats, it has been reported that physical activity of rats is not reduced (Ahrens and Wilson, 1966; Miller and Payne, 1962; Forsum et al, 1981).

It is not possible to adapt to total starvation (Grande, 1964), at least on a long-term basis. The duration of survival during starvation is believed to depend upon the supply of body fat, the major storage fuel (Grande, 1964; Hoffer, 1988) and is usually limited to about 60-70 days in adults, although one obese individual is reported to have survived for 310 days without food (Barnard et al., 1969). Studies such as the "Minnesota experiment" indicate that a number of the mechanisms which operate during semistarvation may be considered as adaptations to a reduced food intake e.g. in adult men, a reduced level of energy balance is gradually attained during a restricted energy intake regime (Keys et al., 1950). During semi-starvation, the rate of weight loss gradually decreases (Keys et al., 1950; Truswell, 1984). Semistarvation reduces the weight of most organs, with the pattern of weight loss in humans similar to that in other animals. Generally, the brain and spinal cord lose little weight compared to other organs (Keys et al., 1950; Montgomery, 1962a). During semistarvation, the rate of water loss is initially high and gradually decreases. The rate of loss of fat increases rapidly, compared to the loss of protein (Brozek et al., 1957).

The brain normally utilises only carbohydrate as an energy substrate, not protein or fat (Passmore, 1986). Thus, it is essential to maintain a relatively constant blood glucose level. Blood glucose concentration is maintained by liver glycogenolysis, conversion of the lactic acid produced by anaerobic glycolysis in muscle and gluconeogenesis in the liver, using amino acids produced by protein catabolism in skeletal muscle. These amino acids may also be oxidized directly and thus have a sparing effect on blood glucose. Glycerol, produced by hydrolysis of triglycerides (mainly in adipose tissue), can also be converted into glucose. Entry of the fatty acids into the Krebs cycle as acetyl CoA spares the use of glucose for energy. The fatty acids may also be converted to ketone bodies by the liver (Tortora and Anagnostakos, 1987). During starvation and PEM, the brain can utilise these ketone bodies, especially β -hydroxybutyric

acid, thus reducing the need for glucose (Owen et al., 1967; Torun and Viteri, 1988).

Although energy and protein resources may be utilised more efficiently during marasmus, the malnourished individual has a reduced metabolic capacity to respond to stresses such as infection or trauma (Golden, 1988a). Heat production and body temperature are often reduced during malnutrition (Anonymous, 1974). There is also a reduction in metabolism when ambient temperature is reduced (Golden, 1988a). This has been viewed as a poikilothermic response (Anonymous, 1974a). However, in rats, survival time during starvation is decreased, rather than enhanced as environmental temperature is reduced below the lower critical temperature of 30°C (Kleiber, 1961). Conversely, above 35°C, excess heat is produced. Similarly, children with kwashiorkor also respond to high environmental temperatures with pyrexia. They also have defective sweating (Brooke and Salvosa, 1974; Golden, 1988a). These responses cannot be viewed as adaptive strategies, such as those adopted by hibernators (Kleiber, 1961).

Hypothermia in malnourished infants is due more to low heat production than to low heat conservation. The infants do not shiver and brown fat stores, the major source of non-shivering thermogenesis are depleted (Anonymous, 1974). A reduction in heat production and body temperature, coupled with the curtailment of inflammatory and immune responses may decrease resistance to infections. An increased susceptibility to infections may in turn, precipitate diarrhoea. Deficiencies in most trace elements develop with the loss of tissues during malnutrition, as these elements cannot be retained in isolation. Diarrhoea accelerates depletion of these resources, especially of the minerals and may lead to death (Golden, 1988a). The development of hypothermia may also mask the presence of infections

(Waterlow et al., 1978). Some physiological changes essential to permit survival, are referred to as adaptive changes (Grande, 1964). However, in a holistic sense, perhaps the malnourished individual should not always be considered as having become adapted to its situation, in spite of the benefits to several of its component systems.

There is controversy over whether metabolic rate increases, decreases or remains unchanged in malnourished infants (Ashworth, 1969b). During undernutrition, basal metabolism is often markedly decreased, although, not unexpectedly, weight-specific basal metabolic rate is not as markedly decreased (Grande, 1964). Starvation or food restriction reduces basal metabolism or resting energy expenditure in adults (Waterlow and Alleyne, 1971; Truswell, 1984; Foster et al., 1990). This may be considered as an adaptation to a reduced food intake. However, there are exceptions. Adult Indian men have no reduction in basal metabolic rate (BMR) i.e., no adaptation to reduced food intake (McNeill et al., 1987). In children, marasmus reduces BMR. However, the reduction in BMR is reported to be even greater in kwashiorkor than in marasmus (Jaya Rao and Khan, 1974). Montgomery (1962a) reported that weight-specific BMR was approximately normal in infants with marasmus and kwashiorkor. In infants where marasmus was severe, BMR was increased. Montgomery (1962a) suggested that this was because these more emaciated infants had a higher proportion of their body weight composed of the relatively more active brain. Monckeberg et al. (1964) also reported that weight-specific BMR was approximately normal in marasmic children. Gopalan (1975) reported that BMR was not significantly reduced in malnourished children until weight deficit exceeded 40%. Variation in the extent of weight deficit may have contributed to the variation in effects of malnutrition on BMR in other studies.

The variation in the effects of PEM on BMR may be due, at least in part, to a complex interaction of factors, including changes in the mass of "metabolically inert" and "metabolically active" tissue and differential changes in the metabolism of "metabolically active" tissues. Increases or decreases in the quantity of "metabolically inert" tissues, during PEM would include oedema during kwashiorkor and loss of fat during marasmus (Brooke et al., 1974). The reduction in weight-specific BMR during semistarvation is not due simply to a loss of "metabolically-active" tissue. Undernutrition reduces the metabolic rate of at least some "metabolically-active" tissues, such as liver and brain (Grande 1964). Brooke et al. (1974) concluded that oxygen consumption in metabolically active tissues was reduced in malnourished infants, regardless of their clinical classification. Weight specific metabolic rate in malnourished infants increases rapidly during recovery (Montgomery, 1962a). The increase after feeding was considered the result of "specific dynamic action". However, Ashworth (1969b) suggested that it was the result of bursts in growth after each meal, associated with a high cost of protein synthesis.

Adaptations may develop to low intakes of protein, as well as to low intakes of energy. Adaptations to low intakes of energy are more well known (Truswell, 1981), however, complex interactions exist between energy and protein intake and energy and protein metabolism. "The theory of protein metabolism" outlined by Miller and Payne (1963) was aimed at "vulnerable groups of human populations" (Ashworth, 1969a, p842) and has gained wide acceptance amongst those concerned with the treatment of PEM (Waterlow and Alleyne, 1971; Waterlow and Payne, 1975; Truswell, 1981). Scrimshaw and Behar (1961) also suggested that energy intake should be high during treatment of malnutrition to ensure that protein utilization was good. Naismith and Holdsworth (1980) attempted to verify the theory using adult rats, but reported conflicting results. They suggested that the potentially higher growth and

metabolic rates and proportionately smaller fat reserves of the weanlings used by Miller and Payne (1963) may have contributed to the discrepancy between the earlier results and those of Naismith and Holdsworth (1980).

Protein metabolism also adapts to semistarvation, but the adaptation is slow (Grande, 1964). Protein turnover is reduced after 5-6 weeks on a low protein diet, but not by short periods of protein-deprivation or starvation (Waterlow and Alleyne, 1971). Retention of nitrogen increases with higher energy intakes during treatment, especially in marasmus (McLaren and Meguid, 1988).

Protein turnover per Kg body wt per day is higher in young rats than old ones and higher in males than in females (Waterlow and Stephen, 1967, 1968). Waterlow and Alleyne (1971) suggested the existence of a "rough parallelism" between protein turnover and BMR. The high protein turnover in young rats is generally considered to be a result of the the high rate of protein formation associated with their growth. In humans, it is suggested that "the efficiency of total dietary nitrogen utilization is similar in the newborn, adult and elderly and that the differences in protein needs, expressed per Kg body weight, for the various age groups are related to differences in the amount of protein synthesised per unit time" (Young et al, 1975; p 193).

Anabolic and catabolic rates of protein per kg of body weight are the same in recovered children, irrespective of protein intake and both are greatly increased in malnourished children (Picou and Taylor-Roberts, 1969). In an analysis of this finding, Waterlow and Alleyne (1971) suggested that the dilution of active tissue protein by collagen would increase the differences in synthesis and catabolism between malnourished and recovered infants. Collagen comprises a higher proportion of the total body protein in infants dying from malnutrition than that in

well-nourished children (Picou et al., 1966). Waterlow and Alleyne (1971) considered that this may result from a possible decrease in the rate of collagen catabolism during malnutrition. However, it has been suggested "that the protein-sparing effects set in motion by various degrees of food deprivation are better adapted to noncollagen protein than to collagen" (Spanheimer et al., 1991; p 522). In a later analysis of the results of Picou and Taylor-Roberts (1969), Waterlow and Jackson (1981) suggested that the malnourished children were actually in the "recovery phase". They suggested that a reduction in protein turnover may be a major adaptation in reducing energy expenditure in the malnourished state (Waterlow and Jackson, 1981).

The efficiency of growth (weight gain per unit of ingested energy) is greater in marasmus than in kwashiorkor (Waterlow and Alleyne, 1971). However, "as the intake falls, a larger proportion of the N entering the free amino acid pool is taken up into protein and a smaller proportion is degraded to urea" (Waterlow and Alleyne, 1971; p 215). The proportion of the amino acids leaving the total body pool which are recycled or reutilized for protein synthesis is increased from 75% to 90-95% when protein intake is reduced (Waterlow, 1975). The adaptative change is not a reduction in protein turnover, but more an increase in the proportion of the turnover which is reutilized (Torun and Viteri, 1988).

It has been suggested that marasmus involves the protection of the liver, at the expense of muscle tissue and that kwashiorkor develops if this adaptation fails (Gopalan, 1968). Thus, it is a "dysadaptation" during a time of severe energy shortage that leads to kwashiorkor (Gopalan, 1968; James, 1977). However, the mechanisms of the adaptation in marasmus and the dysadaptation in kwashiorkor have not been defined (Waterlow and Alleyne, 1971; Waterlow, 1984; Hendrickse, 1988).

Results generally suggest that in protein depletion, "there is a concentration of protein synthesis in the internal organs at the expense of muscle and skin" (Waterlow et al., 1960; p218). Muscle may act as a buffer for other tissues when protein availability is reduced (Waterlow and Alleyne, 1971). In the rat, skin which contains about 25 % of body nitrogen, may also function as a protein reserve (Waterlow and Stephen, 1966). Golden (1988a) has reported that the viscera and central nervous system are well preserved, at the expense of the skin and intestine. The rapid cellular protein turnover in the skin of the rat, results in a substantial loss after only 3 days on a protein-free diet (Waterlow and Stephen, 1966). However, in general, the increased total turnover rates found in malnourished children have not been reproduced in the rat by Waterlow and Alleyne (1971). Nitrogen may also be lost from skin by sweat and exfoliation (Waterlow and Alleyne, 1971). Furthermore, it has been suggested that the loss of protein in the liver that occurs during kwashiorkor and the loss of protein from muscles in marasmus may be the result of hormonal balances between insulin and cortisol (Whitehead and Lunn, 1979; Truswell, 1981).

Dietary Energy Content

Diets containing large proportions of cassava, starch or sugar are likely to have an inadequate "protein-density", while those consisting of cereals may be inadequate in both protein and energy density (Torun et al., 1981). Although increased tissue synthesis during recovery from severe protein-energy malnutrition increases requirements for all nutrients, it is energy intake which usually limits recovery (Ashworth, 1978, 1980; Waterlow and Alleyne, 1971; Reddy, 1972). Foods associated with PEM are usually high in carbohydrate and are usually cooked in water. Absorption of water, reduces the energy density of the food (Crawford, et al., 1989), creating problems in infants, where a diet

containing sufficient energy may be too voluminous to consume (FAO, 1978). The energy content of lipids is about twice that of proteins or carbohydrates (Schmidt-Nielsen, 1981), so lipid is often included in food supplements given to children with PEM (Torun et al, 1981). This enables them to increase energy intake without exceeding stomach capacity (Waterlow and Alleyne, 1971; Jaya Rao, 1978) and also facilitates the swallowing of some foodstuffs which may otherwise be too gelatinous (Torun et al, 1981).

Increasing the lipid content of food also enables considerable savings to be made in transport costs. The benefits to migratory animals in transporting their stored energy as fat, rather than as carbohydrate are well known. This principle has become more important in the 1990's, where various "human" factors effectively prevent the transport of large volumes of food to undernourished communities. Fat could even be considered as "essential" during early development, as it would provide energy to support brain growth and allow growth of the rest of the body (Crawford, et al, 1989). Increasing the lipid content of catch-up diets is a very successful approach (Ashworth, 1978, 1979, 1980; Ashworth et al, 1968). However, if carried out too quickly, rehabilitation with an energy dense diet may result in problems such as liver enlargement, diarrhoea, increased pulse and pulmonary congestion i.e., "the recovery syndrome" (Patrick, 1977; Ashworth, 1980). Initially, catch-up growth may be rapid, but it decreases to normal, as body weight increases to a level appropriate for that body length (Ashworth and Milward, 1986). The total body water of malnourished children is dramatically increased when their energy intake is increased (Patrick et al 1978). Waterlow et al. (1978) suggested the possibility of a vitamin E deficiency developing during rehabilitation with a high-energy diet. They also suggested that rehabilitation with high-energy diets containing arachis oil or soy-bean formula may increase membrane permeability of leukocytes.

A wide range of lipids are used, including butter, ghee (Ashworth, 1985) and vegetable oils such as coconut oil (Ashworth, 1980; Golden, 1988b), olive oil (Waterlow et al., 1978) and soya, groundnut, sunflower seed, cottonseed and sesame seed oils (Anonymous, 1968). Although absorption of fat is impaired in kwashiorkor (Reddy, 1972; Robinson et al., 1957), steatorrhoea is not worsened by feeding high fat diets (Reddy, 1972). High fat legumes, nuts and oilseeds such as groundnuts, soya and sesame seeds provide both energy and protein. The choice of foods depends on availability, cost and the nature of the staple (Ashworth, 1980).

Essential Fatty Acids and Protein-Energy Malnutrition

Two factors to be considered in the choice of lipid supplements are their essential fatty acid (EFA) content and the EFA status of children with PEM. In this section, I will present a brief introduction to fatty acids, in particular, the EFA. I will also present some evidence from the literature, which suggests that children with PEM may be EFA-deficient.

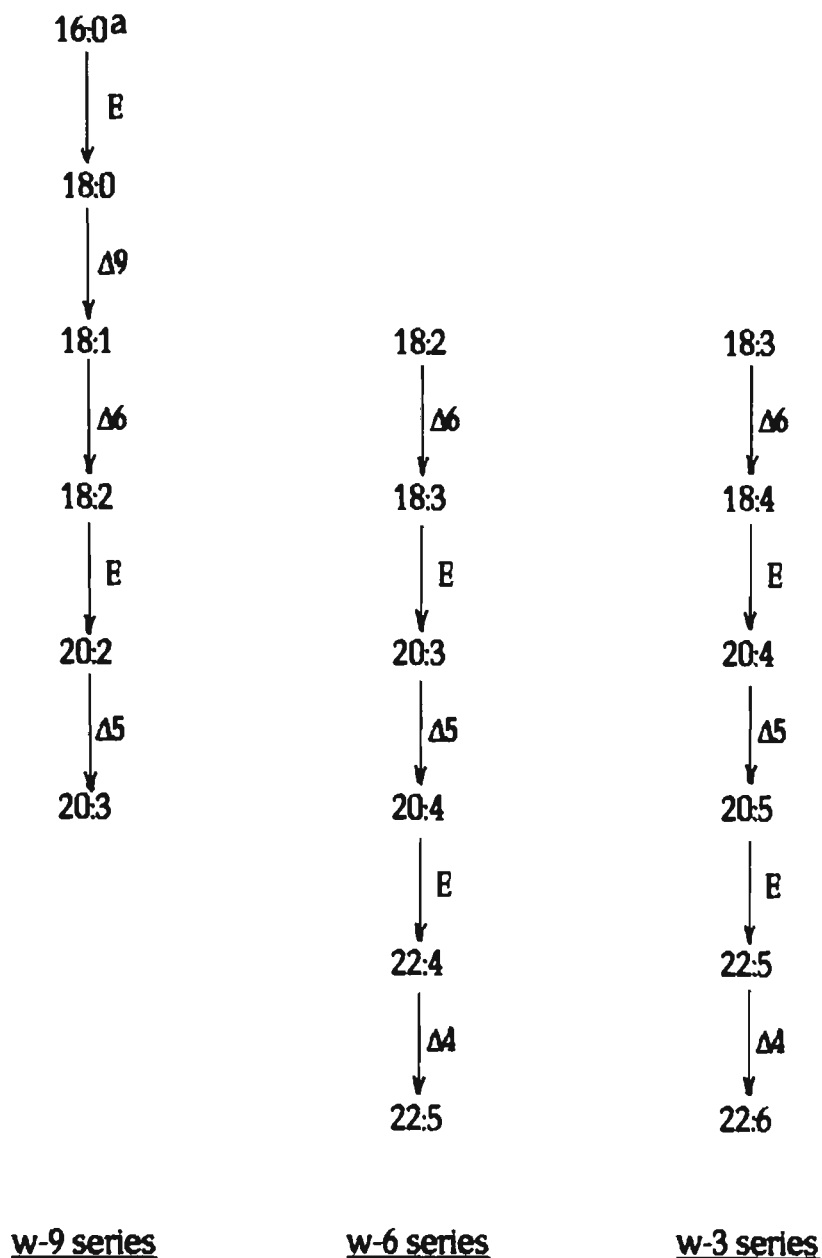
The fatty acids relevant to this study are straight-chain aliphatic monocarboxylic acids. The aliphatic chain between the carboxylic and the methyl end of each fatty acid molecule generally ranges from 8 to 22 carbon atoms in length. Carbon atoms in saturated fatty acids are linked by single bonds, except for the carboxyl group. In monounsaturates, two carbon atoms are linked by a double bond and in polyunsaturates, two or more double bonds are present. Stereochemical isomers resulting from double bonds between carbon atoms are usually of the *cis* configuration, where parts of the molecule lie on the same side of each double bond, rather than on opposite sides (*trans* configuration) (FAO, 1978).

Fatty acid formulae in this thesis are abbreviated in the form xy, w-m (as outlined in FAO, 1978), where x = N° of carbon atoms in the molecule, y = N° of double bonds and m = the position of the first double bond, numbered from the methyl end of the molecule. Thus, linoleic acid (18:2 w-6) and linolenic acid (18:3 w-3), which are necessary precursors for the w-6 and w-3 pathways respectively and are required for the normal functioning of tissues have double bonds 6 carbons and 3 carbons respectively, from the methyl end of the molecule (FAO, 1978). However, neither can be synthesised by humans, as unlike plants, animals cannot insert double bonds in the w-3 or w-6 positions. Both linoleic acid and linolenic acid must be supplied in the diet and are thus dietary EFA (FAO, 1978). Linoleic acid per se has few functions, and must be converted via the Δ -6 pathway to exert its effects. The greater potency of other w-6 fatty acids in reversing the effects of EFA-deficiency indicates that they should also be regarded as EFA (Horrobin, 1992). The EFAs influence membrane properties, such as fluidity, flexibility and permeability. They are also precursors of prostaglandins and leucotrienes. However, their biochemical roles are still unclear (Horrobin, 1992). Linoleic acid also functions in the maintenance of the epidermal water barrier (Wertz et al., 1987).

Metabolism of 18:2 w-6 and 18:3 w-3 by elongases and desaturases produces the w-6 and w-3 families of fatty acids (Fig1.1), which are required for cell structure and prostaglandin synthesis. The most important of these long-chain fatty acids are 20:4 w-6 and 22:6 w-3 (FAO, 1978). In the absence of sufficient EFA, animals desaturate 18:0. However, the resulting 18:1 w-7 is the precursor for another family of polyunsaturated fatty acids, the w-7 fatty acids (FAO, 1978).

Fatty acids are present in a variety of forms. Three fatty acids may be combined with a glycerol molecule to form a triacyl glycerol (formerly known as

Figure 1.1 Pathways for the desaturation and elongation of polyunsaturated fatty acids.



^a Carbon N°:N° of double bonds
 E Elongase (adds 2 carbon atoms)
 Δ Desaturase (inserts C=C double bond)

Animals are unable to synthesise 18:2 ω 6 and 18:3 ω 3, the precursors of the ω 6 and ω 3 pathways respectively. In the absence of these essential fatty acids (EFA), 20:3 ω 9 may be produced. The ratio of 20:3 ω 9 to 20:4 ω 6 is often used as an index of EFA-deficiency.

a triglyceride). Triacyl glycerols are but one example of neutral lipids and are important for energy storage and thermal insulation. Replacement of one of the fatty acids in a triacyl glycerol with a phosphate group produces a phospholipid. The polar phosphate group attached to the non-polar aliphatic chain renders a phospholipid amphiphilic. In an aqueous environment, the charged phosphate group is in contact with water and the hydrophobic aliphatic chain oriented away from contact with the aqueous environment. Phospholipids are thus able to form bilayers, hence their usefulness as structural components of biological membranes (FAO, 1978). Evidence suggests that phospholipid fatty acid composition, along with other factors such as cholesterol and protein content influences the structure and function of biological membranes (Sun and Sun, 1974; Lin et al, 1979; Yeagle, 1989; Andrews and Else, 1992; Couture and Hulbert, 1992; Hulbert, Couture and Brand, 1992; Brand et al., 1992; Brand, Couture and Hulbert, unpublished manuscript). These in turn, are believed to exert a variety of effects concerned with parameters such as energy metabolism (Brand et al., 1991).

Rats fed a fat-free diet, or one poor in EFA develop skin and hair disorders, have reduced growth and increased metabolic rate (Burr and Burr, 1929; Wesson and Burr, 1931; Panos and Finerty, 1954; Rafael et al., 1984). Similar observations have been made in man and other species. The skin disorders develop more rapidly and are more clear-cut in younger animals (Hansen et al, 1958). The effects of EFA-deficiency can be prevented by the dietary intake of small amounts of linoleic acid. The generally accepted minimum requirement for linoleic acid in humans and also in rats is about 1% of dietary energy intake (Holman, 1960; Holman, Caster and Wiese, 1964; Lands, 1986), although Cuthbertson (1976) and Sanders and Naismith (1979) suggested that it is less than 1% and Bourre et al. (1989), that it is about 2.4%. Optimum requirements have also been proposed and these are usually several % higher

than the minimum requirements (Adam, Hansen and Weise, 1958; Weise, Hansen and Adam, 1958). EFA-deficiency also increases the number of sodium pumps in liver and kidney (Lin et al., 1979) and in skeletal muscle (Ayre, 1992) and increases Na^+ / K^+ ATP-ase activity in brain (Sun and Sun, 1974). Vesicles made from phospholipids from EFA-deficient rats are leakier to sodium than those from control rats (Moore et al., 1969).

Initially, less was known about the essentiality of linolenic acid than linoleic acid. However, more is now known about w3 fatty acids in human metabolism than any other group of fatty acids (Simopoulos, 1991), although their function is still controversial. No pathological effects or reduction in fertility, birth weight, survival, growth or organ weight were found in rats, even after 2 or 3 generations of deprivation of dietary w3 fatty acids (Tinoco et al., 1971; Lamprey and Walker, 1976), although there was some evidence of a reduced proficiency in discrimination-learning and exploratory activity (Lamprey and Walker, 1976). Crawford and Sinclair (1972) refuted the finding of Tinoco et al. (1971) that linolenic acid was a non-essential dietary component of the rat. They pointed out, that large changes in the % of w3 fatty acids in tissue phospholipids would be overwhelmed and their significance unappreciated when whole tissue fatty acid analysis is carried out, as by Tinoco et al. (1971). Thus, there are limitations to the use of whole tissue fatty acid analysis to define linolenic acid deficiency (Crawford and Sinclair, 1972). There is now evidence for the essentiality of w-3 fatty acids in brain and retina (Tinoco, 1982; Neuringer and Connor, 1986; Bourre et al., 1989; Connor et al., 1992; Neuringer et al., 1992; Uauy et al., 1992).

At present, there is no clear minimum requirement for w3 fatty acids, although an 18:3 w3 requirement of about 0.3 % of dietary energy in humans has been suggested by Bjerve et al. (1987). Bourre et al., 1989 suggested that a-

linolenic acid should comprise 0.4% of dietary energy. More recently, it has been suggested that an intake of 1% of dietary energy as w-3 fatty acids may be insufficient when supplied as linolenic acid and that preformed docosohexanoic acid (22:6 w3) is required by the preterm infant (Bjerve et al, 1992). Bjerve et al. (1989, 1992) have also suggested that w3 requirements should be expressed in terms of mg/day, rather than % energy intake. Both linoleic and linolenic acids are believed to compete for the same enzyme systems (Horrobin, 1992), which are believed to have a greater affinity for linolenic acid (Mohrhauer and Holman, 1963a,b). A ratio of w-6 : w-3 fatty acids, or of linoleic : linolenic has been recommended, as well as an absolute amount. Lee et al. (1989) suggested a ratio of w-6 : w-3 fatty acids of about 5:1. The results of Bourre et al. (1989) suggest a a value of 6:1, depending on the organ under consideration.

Children with PEM have reduced percentages of EFA in serum (Taylor, 1971; Holman et al, 1981; Chen and Dickerman, 1985) and plasma (Naismith, 1973). In the study of Naismith (1973), infants with PEM were grouped under the category of kwashiorkor, although Truswell (1981) suggested they had marasmic kwashiorkor. In erythrocyte membrane lipids, the proportion of 18:2 w-6 is increased in both marasmus and kwashiorkor and that of 20:4 (w-6) decreased in marasmus (Vajreswari et al., 1990).

It has been suggested that children with PEM are EFA-deficient, even with EFA intakes > 3% of calories as recommended by US National Research Council (Chen and Dickerman, 1985). "In such a situation, EFA would be utilized as an energy source like other non-EFA, rather than serving its EFA functions." (Chen and Dickerman, 1985; p 22). PEM children may indeed be deficient in EFA, as suggested by Chen and Dickerman (1985) and Holman et al. (1981), as well as being deficient in many other nutrients. Indeed, the similarity between the symptoms of kwashiorkor and the effects of EFA-deficiency

(Schendel and Hansen, 1959; Bronte-Stewart, 1961; Naismith, 1962) has prompted suggestions that EFA-deficiency may be a component of the aetiology of kwashiorkor (Bronte-Stewart, 1961; Naismith, 1973; James, 1977). If this is true, then increased dietary EFA may prevent or ameliorate some of the clinical characteristics of kwashiorkor.

Although there are benefits in consuming an energy-dense food when digestive capacity is limited (Waterlow and Alleyne, 1971; Jaya Rao, 1978; Crawford, et al., 1989), fat is not essential as an energy source and may be replaced by carbohydrate (Naismith and Qureshi, 1962). EFA-deficiency may reduce the utilization of both protein (Naismith, 1962) and energy (Wesson and Burr, 1931). Thus, even if a deficiency in EFA is not a major component of the aetiology of kwashiorkor, it may reduce the efficiency with which available energy and protein resources are utilized (Naismith, 1973). Feeding 18:2 w-6 is known to cure the scaly skin condition of kwashiorkor (Vergroesen, 1989). Two questions that arise are whether feeding w-6 fatty acids would ameliorate some of the other symptoms of PEM and whether w-3 fatty acids would also be beneficial. The response of the dermal symptoms of EFA-deficiency to w-3 PUFAs is only about 10% as efficacious as that with linoleic acid (Houtsmuller, 1972). Thus, the response of the skin to w-3 PUFAs during kwashiorkor would probably be incomplete (Vergroesen, 1989).

Decreased humidity increases the severity of dermal symptoms of EFA-deficiency, so that there may appear be a "seasonal" decrease during the moist part of the year (Holman, 1968). However, the increased incidence of kwashiorkor during the "wet" season (Anonymous, 1984) is unlikely to be due to seasonal variations in humidity, as the increased incidence of kwashiorkor coincides with a period of high humidity.

During kwashiorkor, the skin may be protected from trauma by oiling (Williams, 1973). In many tropical countries, oiling of the skin is a cultural tradition (James, 1977). Oiling of the skin with EFA reduces the severity of some symptoms of EFA-deficiency (Press et al., 1974). However, oiling of the skin has been given little attention during the treatment of children with kwashiorkor (James, 1977). It is possible, that the cultural practice of oiling the skin is responsible for some of the differences in the incidence of kwashiorkor between different groups consuming very similar diets. Linoleic acid may be more efficiently utilised when applied cutaneously, rather than ingested (Press et al., 1974). Other cultural differences, such as those concerned with food preparation may also be involved, e.g., some cultural practices in food preparation may influence the amount of nutrients ingested (Naismith, 1973). In many societies, high protein foods such as meat, which are often in short supply, are given to adults, usually men, rather than to children (Truswell, 1981), whose relative needs for protein are higher (Naismith, 1975; Young et al., 1975), or are prepared in highly-spiced meals, which are unsuitable for infants (Truswell, 1984). These cultural and social practices protect the productive members of the family (Torun and Viteri, 1988). The breakdown of cultural practices, a consequence of "Westernization" may also reduce the quality of childrens' diets (Jelliffe 1959). Some practices are consistent with the view that kwashiorkor results primarily from a deficiency in protein.

As pointed out previously, a wide variety of lipids are included in "catch-up" diets. These lipids vary greatly in their fatty acid composition (C.R.C. Handbook of Biochemistry, 1973). Saturated fatty acids are known to promote the onset of EFA-deficiency (Peifer and Holman, 1959; Bronte-Stewart, 1961) and PUFA are hypothesised to increase free radical levels in PEM (Golden, 1988b). The "free radical" hypothesis proposed by Golden, implies that fatty acid composition may be just as important as the availability and energy content of a

particular lipid source (Read, 1990). The practice of adding oils rich in polyunsaturated fatty acids (PUFA) to increase the energy content of food-supplements given to malnourished children could be hazardous without protection against the formation of free radicals (Golden,1988b; Golden and Ramdath, 1987). For this reason, staff at The Tropical Metabolism Research Unit in Jamaica have ceased using peanut oil, which is high in PUFA and low in saturated fatty acids (SFA) and use coconut oil, which is low in PUFA and high in SFA (Read, 1990). It is therefore, of considerable practical significance in both the prevention and amelioration of PEM, to determine the role of dietary EFA in the aetiology of PEM.

Although a possible role of EFA in the aetiology of kwashiorkor has been alluded to, there have been only a few studies of the role of dietary fatty acids in protein malnutrition or in protein-energy malnutrition. Truswell (1975) has suggested that until the role of linoleic acid in PEM is clarified, it would be wise to include adequate linoleic acid in therapeutic diets. Pretorius et al. (1964) examined the effects of dietary fatty acids and reported that sunflower-seed oil had no adverse effects on infants recovering from kwashiorkor. However, compared to other diets with low linoleic acid content there was also no effect on nitrogen, fat or mineral balance or recovery rate, even though about 25% of energy was derived from linoleic acid (Pretorius et al., 1964). Svennerholm et al. (1972) reported that dietary intake of EFA did not influence growth of rats fed high or low protein diets. Schendel and Hansen (1959) reported that the serum fatty acid pattern of infants recovering from kwashiorkor resembled that of EFA deficiency. However, the rehabilitation diet used in their study was fat-free.

Rationale for the Present Study

As pointed out by Golden (1988b), many physiological parameters appear to be altered in kwashiorkor. Consequently, the large number of studies carried over the past 60 years has been concerned with a wide variety of parameters. From these, a vast amount of information has been obtained concerning many aspects of PEM. Unfortunately, there is still little understanding of the mechanistic details underlying the distinction between kwashiorkor and marasmus. Indeed, considerable conflict still exists over the use of classification schemes concerned with parameters believed to cause kwashiorkor and those concerned with the effects of the malaise.

Any explanation of the aetiology of kwashiorkor must account for a wide variety of changes (Golden, 1988a). Many of these changes are concerned with membrane abnormalities, such as increased membrane permeability (Patrick and Golden, 1977; Willis and Golden, 1988), increased sodium pump numbers and 'pump rate' (Patrick, 1977; Pimplikar and Kaplay, 1981; Narayanareddy and Kaplay, 1982; Kaplay, 1984; Willis and Golden, 1988) and membrane-bound enzyme activity e.g., Na^+/K^+ ATPase (Kaplay, 1978) and decreased osmotic fragility (Brown et al., 1978; Kaplay, 1978, 1979; Fondu, et al., 1980; Ramanadham and Kaplay, 1982) and a defect in the anionic charge of the glomerular basement membrane (Golden et al., 1990). There is also a decrease in the affinity of the erythrocyte membrane $\text{Ca}^{2+} + \text{Mg}^{2+}$ -ATPase for Ca^{2+} (Olorunsogo, 1989). It is possible that a number of these changes, especially those concerned with changes in membrane function result from a decrease in EFA. This is an idea that has been alluded to previously, but has not been examined in depth. A deficiency in linoleic acid results in increased permeability to Na^+ of vesicles made from phospholipids isolated from rats (Moore et al., 1969), increased sodium pump numbers in liver and kidney (Lin et al., 1979) and in skeletal muscle (Ayre, 1992), increased Na^+/K^+ ATPase activity in brain (Sun

and Sun, 1974) and decreased osmotic fragility in erythrocytes (Kaplay, 1979). Furthermore, children with PEM have reduced percentages of EFA in plasma (Naismith, 1973). It is possible that a deficiency in EFA develops in children with PEM, even when the % of energy supplied as linoleic acid is above that generally considered adequate (Chen and Dickerman, 1985).

If the mechanistic details involved in protein and energy deprivation were better understood, then it may be possible for those in need to consume a more appropriate type of food. Fats, because of their high energy content, have often included in the food supplied to protein-energy malnourished individuals. However, the significance of their fatty acid composition is unclear. It is not clear whether these fats should, or should not contain high proportions of polyunsaturated fatty acids.

The present study was designed to examine protein-energy malnutrition and dietary fats. It was organized to determine the effects of food-restriction, protein-energy malnutrition, and increasing the proportion of omega-6 polyunsaturated fatty acids in the dietary fat during protein-energy malnutrition. Expansion of the study enabled the effects of age to be included. These treatments were chosen to try to understand protein-energy malnutrition in a rat model and to determine whether PUFA-enrichment during protein-energy malnutrition would have beneficial or detrimental effects.

Chapter 2: Experimental Plan

Introduction

In this study carried out on rats, only the effects of one group of fatty acids, the w-6 EFA will be considered. This enables the presence of 20:3 w9 in tissue phospholipids to be used as a definitive indicator of a deficiency in w-6 EFA. In order to examine the effects of varying linoleic acid (18:2 w6) intake during PEM, stearic acid, a saturated fat of the same carbon chain length (18:0) was initially selected as an appropriate dietary substitute.

This study was designed to determine the effects of linoleic acid enrichment on growth, metabolism and the efficiency with which nutrients are absorbed and utilized. Growth was assessed as both an increase in body weight and body length. Determinations were made of metabolism at both the whole organism and tissue level and of a number of underlying parameters reported to be associated with PEM, including cell membrane permeability and numbers of sodium pumps. The efficiency with which nutrients were absorbed and utilized was determined by measurements of energy and N input (food) and output (urine and feces). Analysis of tissues for protein and sodium and potassium contents and membrane fatty acid composition complements the measurements of functional parameters.

As mentioned above, initially artificial diets were constructed where the proportions of 18:0 and 18:2 w6 were varied. This is experiment 1. However, there was a dramatic reduction in apparent absorption of energy and nitrogen in rats fed the diet containing 7% stearic acid. Another experiment was carried out to determine whether substituting coconut oil, another highly saturated fat

would overcome this problem. This was experiment 2. The results indicated that the use of coconut oil increased the apparent absorption of energy and nitrogen to acceptable levels. Experiment 3 was designed with sunflower seed oil as the source of PUFA and coconut oil as the saturated fat. Experiments 1 and 3 were similar, but with other small differences. Briefly, in experiment 3 the diets were commenced a week earlier (at weaning) and the experimental period was extended from six to eight weeks. A food-restricted group was included and later, a size-matched group. The rats in this group were fed the control diet and killed when they were the same size as the malnourished rats.

The control diet used in Experiments 1 and 3 was the basal diet outlined by the Association of Official Agricultural Chemists (1980, p774), with a variation in its lipid component. This diet was considered adequate. However, when some of the data was presented at a recent conference (Withers and Hulbert, 1992a), the possibility of omega-3 deficiency was suggested (C. Galli, personal communication).

Experiment 1

Twenty four Sprague-Dawley rats were individually housed under 12:12 photoperiod at 25°C in galvanised wire cages (20 by 20 by 22 cm). They were fed a control diet (containing 22% casein) *ad libitum* for one week after weaning. The dams had been fed *ad libitum* with Allied Rat and Mouse Kubes (Allied Feeds, Australia). The young rats were then assigned to one of four dietary groups, each composed of three males and three females. The four diets were isoenergetic and contained 10% fat. One group was fed the control diet (containing 22% casein) *ad libitum*, the other three were fed diets reduced in protein (10% casein) at one third the daily food consumption rate of the control diet rats. These three groups were thus equally protein energy malnourished (PEM), but received different amounts of polyunsaturated fatty acids (PUFA). These differences in PUFA content were produced by varying the proportions of safflower oil, sesame oil and stearic acid. Almost all of the PUFA in the diets was linoleic acid, an 18-carbon chain fatty acid found in high concentrations in safflower oil and in medium concentrations in sesame oil. For this reason, the 18 carbon chain saturated fatty acid, stearic acid, was chosen to complete the lipid component. The minimum proportion of dietary energy intake supplied by linoleic acid in the diets was 1.3%. Linoleic acid is a dietary essential fatty acid. A widely cited minimum requirement for linoleic acid is 1% of dietary energy (Holman, 1960; Lands, 1986). The minimum level of linoleic acid in all of the diets I prepared, exceeded 1% of dietary energy. Thus, none of the diets used in this experiment (or in subsequent experiments) were EFA-deficient by this definition. The diets and their salt and vitamin components are shown in Tables 2.1-2.3.

Fresh food was supplied daily at 9 a.m. for six weeks. In the control group, uneaten food was removed and weighed. Water was supplied *ad*

Table 2.1 Dietary groups in Experiment 1.

	Control diet	Protein-energy malnourished diets		
PUFA content	Medium	Low	Medium	High
Food access	Ad-libitum (Age-matched)	Restricted to 1/3 rate of food consumption of control rats		
N° of rats	6	6	6	6
Casein	22a	10	10	10
Sesame oil	10	3	10	3
Safflower oil	-	-	-	7
Stearic acid	-	7	-	-
Salt mixture	5	5	5	5
Vitamin mixture	1	1	1	1
Cellulose	1	1	1	1
Water	5	5	5	5
Sucrose	56	68	68	68
Calculated energy content of diet	1816b	1788	1788	1788
Calculated % energy as linoleic acid	10.8	1.3	10.5	16.1

a g/100 g

b KJ/100 g

100 i.u. Vitamin E/Kg was added to each diet.

Table 2.2 Composition of salt mixture used in preparation of diets^a

Salt	Amount
NaCl	139.3 ^b
KI	0.79
KH ₂ PO ₄	369.0
MgSO ₄ · 7H ₂ O	57.3
CaCO ₃	381.4
FeSO ₄ · 7H ₂ O	27.0
MnSO ₄ · H ₂ O	4.01
ZnSO ₄ · 7H ₂ O	0.548
CuSO ₄ · 5H ₂ O	0.477
CoCl ₂ · 6H ₂ O	0.023

a Association of Official Agricultural Chemists, 1960, page 680
b grams

Table 2.3 Composition of vitamin mixture used in preparation of diets^a

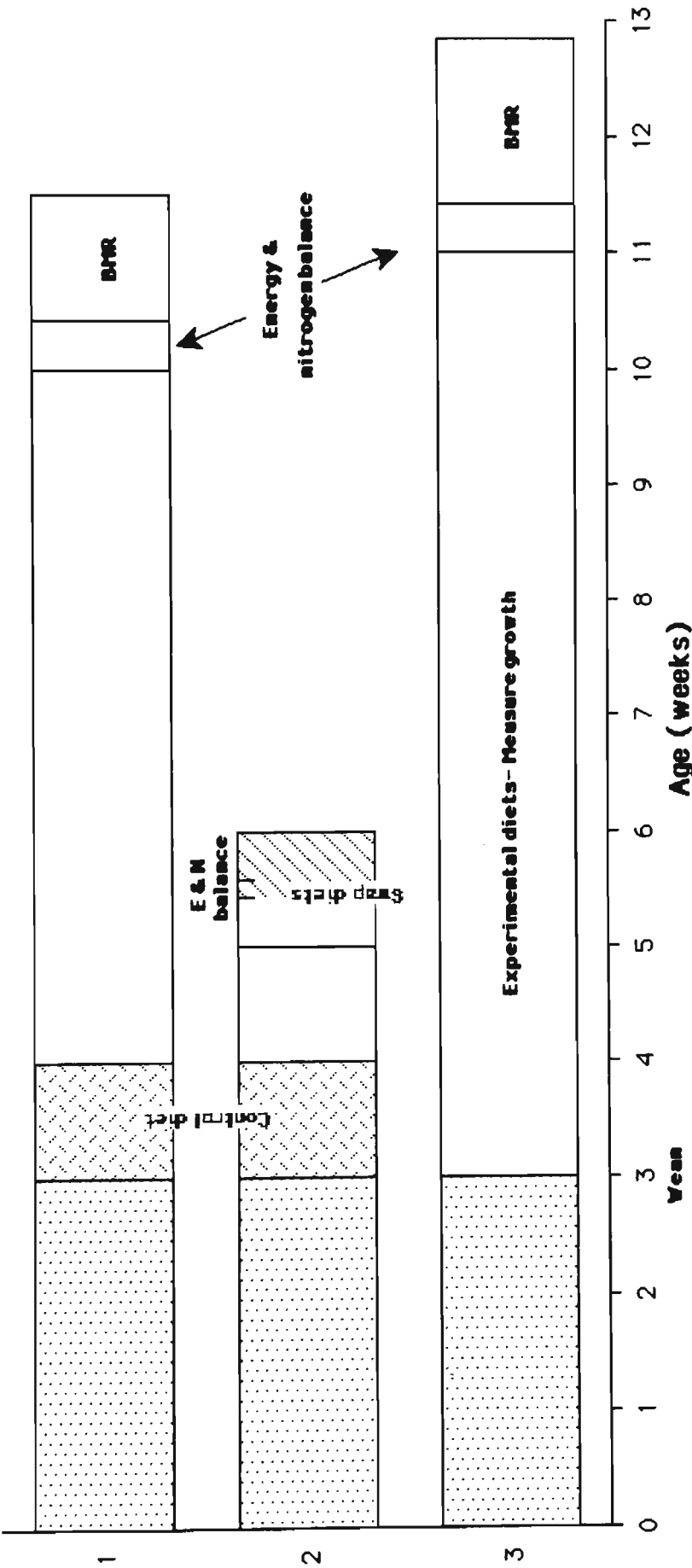
Vitamin	Amount
Menadione (vitamin K)	0.0175 ^b
Choline chloride	7.0000
p-aminobenzoic acid	0.3500
Inositol	0.3500
Nicotinic acid	0.1400
Calcium pantothenate	0.1400
Riboflavine	0.0280
Thiamine HCl	0.0175
Pyridoxine HCl	0.0175
Folic acid	0.0070
Biotin	0.0014
Vitamin B ₁₂	0.000105
Dextrose	to 35g

a Association of Official Agricultural Chemists, 1960, page 680
b grams
Vitamins A and D were added separately to provide
2000 i.u. vit. A and 200 i.u. vit. D respectively per 100 g diet.
Amount of vitamin E used is shown in Tables 2.1 and 2.4.

libitum. The duration of experiment 1 and of subsequent experiments is outlined in Figure 2.1.

Body mass, snout-vent, tail and pes (hind foot) length and were measured each week. At the end of the six week period, basal metabolic rate (BMR) was measured and urine and feces samples were collected for nitrogen and energy balance determination. Collection of urine and feces from each rat was restricted to one 24 hour period, because only a limited number of metabolism cages were available. The rats were then killed by decapitation and tissues removed for analysis and for ion homeostasis experiments outlined in chapter 6.

Figure 2.1 Time chart of Experiments 1-3



Experiment 2

An unexpected finding from Experiment 1 was the dramatic reduction in apparent absorption of energy and nitrogen in rats fed the low PUFA PEM diet, i.e., the diet containing 7% stearic acid (Tables 3.12 and 3.14). This effect created problems, as the aim of the experiment was to assess the effects of PUFA-enrichment during PEM. Unfortunately, the effects of PUFA-enrichment on such parameters as growth and metabolism could not be assessed, if the comparison was made against a dietary fat which apparently inhibited the absorption of both energy and nitrogen. I carried out these energy and N determinations after all other other procedures and analyses had been completed. This was because the energy and N content of food, urine and feces samples in sealed containers remains constant during long-term storage. However, I considered analysis of tissues for phospholipid fatty acid composition was more likely to be influenced by long-term storage, even at low temperature. Kates (1986) suggests that long-term storage renders lipids more susceptible to peroxidation and hydrolysis. Thus, I did not detect the problems associated with the use of stearic acid until all other procedures and analyses had been completed.

These results raised a number of questions. The effects of PUFA-enrichment were to be compared against those of a saturated fat during PEM. When a different saturated fat was selected for future experiments, it would be necessary to determine whether the new saturated fat also reduced the apparent absorption of energy or N.

Experiment 2, a short-term experiment, was carried out to determine the nature of saturated fats to be used in the control group in future dietary manipulations. Coconut oil, another highly saturated lipid source was

compared with stearic acid. Two groups of 6 rats were randomly selected and treated in a similar manner as those in experiment 1. One week after weaning, one group was fed the low PUFA diet used in Expt 1 for ten days. The other group was fed the same diet containing coconut oil instead of stearic acid. During the last 3 days, the rats were placed in metabolism cages and urine and feces were collected. The diets were then reversed for 4 days and urine and feces were once again collected during the last 3 days. The rats were weighed before and after each 3 day period and food was supplied at 6.1 g day^{-1} , the same level as the PEM rats in Experiment 1. Water was provided *ad libitum* and its consumption was also measured. Collection of urine and feces from each rat in Experiment 2 and later in Experiment 3 was able to be carried out over a 3 day period, when a larger number of metabolism cages was available.

Briefly, the results (Table 3.4) indicate that changing the major source of saturated fats from stearic acid to coconut oil increased the apparent absorption of energy from 84% to 97%, a value similar to that of rats fed the control diet in Experiment 1 (Table 3.12). Apparent absorption of nitrogen, 89% with stearic acid and 90% with coconut oil was similar to values obtained for high PUFA PEM rats in Experiment 1 (Table 3.14).

Thus, any changes associated with energy and N balance which may have been detected in the low PUFA group of Experiment 1, may not be due to low dietary PUFA level per se, but to the presence of stearic acid. The results of Experiment 2 indicate that this effect can be minimised if stearic acid is replaced by coconut oil.

Experiment 3

In the design of Experiment 3, some changes were made from the design of Experiment 1. Stearic acid was replaced by coconut oil (so that apparent absorption of energy and N was higher (Experiment 2) and similar to that in the well-nourished rats of Experiment 1). Safflower oil and sesame oil were replaced by sunflower oil and dietary fat content was increased to 15% w/w to facilitate manipulation of greater differences in dietary PUFA content. In experiment 3 the diets were commenced a week earlier (at weaning) and the experimental period was extended to eight weeks. A food-restricted group was included and later, a size-matched group.

Thirty two Sprague-Dawley rats were weaned at 3 weeks and randomly assigned to one of four groups, each consisting of four males and four females. They were housed and fed under the conditions outlined in experiment 1. One group was fed a control diet (containing 22% w/w casein) *ad libitum* and another, the same diet at 1/3 the food consumption rate of the *ad libitum* - fed rats. Two other groups were fed diets containing reduced protein (10% w/w casein) at the same reduced rate. One of these two PEM diets had a much higher PUFA content than the other (62 cf. 6 mole % of total fatty acid). This was achieved by manipulation of the proportions of sunflower oil and coconut oil. Each of the diets, contained 15% w/w lipid and was fed for eight weeks.

During the cell permeability experiments (Chapter six), it was suspected that it may take longer for ^{86}Rb to diffuse through the thicker soleus muscle of larger animals. If this were the case, then the values obtained would not only be a measure of membrane leakiness, but would also be a function of muscle size. For this reason, it was decided to include an extra control group of rats whose body size was the same as that of the malnourished rats. The soleus

muscles in this "size-matched" control diet group were also expected to be similar in size to those of the malnourished rats. A fifth group of eight Sprague-Dawley rats from the same source (Castle Hill, University of Sydney) was weaned at three weeks and fed the control diet until they were approximately the same size as the malnourished rats. The diets of all five groups of rats are shown in Table 2.4. Dietary salt and vitamin components are shown in Tables 2.2-2.3.

Body mass was measured weekly as in Expt 1. However, snout-vent, tail and pes length were measured only at the beginning and end of the dietary period. Nitrogen and energy balance and BMR data were collected as in Expt 1, before the rats were killed by decapitation and tissues removed for analysis and ion homeostasis experiments. Briefly, soleus muscle cell membrane permeability to K^+ was determined using ^{86}Rb as a K analogue and number of ouabain binding sites was determined in both soleus muscle and erythrocytes. Intra- and extracellular volume of soleus muscle was determined using ^{14}C -labelled Inulin and 3H -labelled H_2O .

The fatty acid composition of the diets in experiments 1 and 3 was determined by gas chromatography and is shown in chapter 4 with tissue fatty acid composition (Tables 4.2 and 4.3). Almost all of the PUFA in the diets was supplied as linoleic acid. The calculated % of dietary energy supplied as linoleic acid is included in Tables 2.1 and 2.4.

Table 2.4 Dietary groups in Experiment 3

	Control Diet			PEM	PEM PUFA-enriched
	Size-matched	Age-matched	Food-restricted		
Food access	Ad-libitum		Restricted to 1/3 rate of food consumption of age-matched controls		
Age at end of dietary period (weeks)	4.6	11	11	11	11
Size (g)	81.3	261.0	84.5	76.7	80.7
N°	8	8	8	8	8
Casein	22a	22	22	10	10
Sunflower oil	2	2	2	2	13
Coconut oil	13	13	13	13	2
Salt mixture	5	5	5	5	5
Vitamin mixture	1	1	1	1	1
Cellulose	1	1	1	1	1
Water	5	5	5	5	5
Sucrose	51	51	51	63	63
Calculated energy content of diet	1925b	1925	1925	1896	1896
Calculated % energy as linoleic acid	2.0	2.0	2.0	2.0	20.5

^a g/100 g

^b KJ/100 g

58 mg of Vitamin E/Kg was added to diets other than PUFA-enriched to compensate for the naturally high vitamin E content of sunflower oil.

Experiment 1 was designed to enable the following comparisons to be made:

Comparison of the Control group with the other groups should indicate the effects of PEM.

Comparisons between the PEM groups should indicate the effects of an increased dietary PUFA content during protein-energy malnutrition.

Experiment 3 was designed to enable the following extra comparisons to be made:

Comparison of the Age-matched Control group with the Food-restricted group should indicate the effects of food deprivation.

Comparison of the Age-matched Control group with the PEM group should indicate the effects of protein-energy malnutrition.

Comparison of the Food-restricted group with the PEM group should indicate the effects of an increased dietary protein content during protein-energy malnutrition.

Comparison of the two PEM groups should indicate the effects of an increased dietary PUFA content during protein-energy malnutrition.

Comparison of the Size-matched Control group with the Age-Matched Control group should indicate the effects of age.

Comparison of the Size-matched Control group with the malnourished groups, should indicate the effects of food-restriction and protein-energy malnutrition, after correction for body size and organ size.

Chapter 3: Whole Animal Measurements

Introduction

In this chapter, the effects of food-restriction, PEM and PUFA-enrichment on whole animal parameters were examined. Any effects of these dietary manipulations on growth, may also manifest themselves as changes in whole animal metabolism, which may be measured as basal metabolic rate (BMR). Measurement of parameters associated with energy and nitrogen balance may indicate whether possible differences in growth are related to differences in the efficiency of processes in the gut, such as digestion and absorption, or to changes in the metabolism of absorbed nutrients.

Methods

Measurement of Growth

Rats were lightly anaesthetised with Fluothane (ICI) and body mass, snout-vent, tail and pes length were measured.

Urine and Feces Collection.

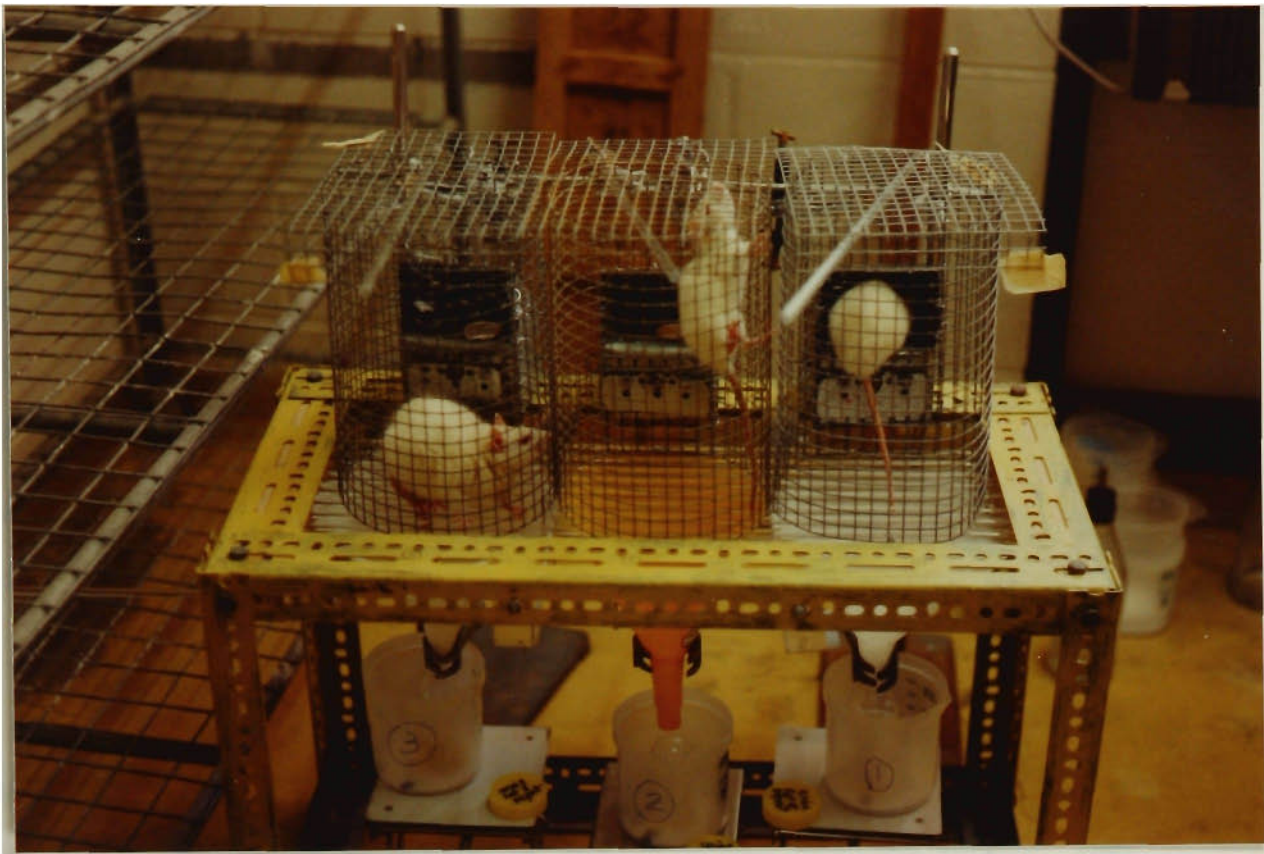
At the end of the dietary period, the rats were placed in metabolism cages where daily output of urine and feces was collected and intake of food and water was measured. The urine was collected in containers to which 20 ml of 2.5% (w/v) HCl had been added to minimise ammonia loss. Urine adhering to the apparatus was washed into the container with distilled water and diluted to 100 ml. Both urine and feces were stored at -20°C. The duration of the collection time for urine and feces was limited to one day in Experiment 1, using the metabolism cages shown in Plate 3.1. This was increased to 3 days in Experiments 2 and 3 to reduce day-to-day variability when other equipment (shown in Plate 3.2) became available.

Plate 3.1

Cages for energy and nitrogen balance experiments showing collection of urine and feces during Experiment 1.

Plate 3.2

Cages for energy and nitrogen balance experiments showing collection of urine and feces during Experiments 2 and 3.



Bomb Calorimetry

The gross energy content (or heat of combustion) of diets and feces (oven-dried at 65°C for 15 h and compacted in a pellet press) and urine (freeze-dried) was measured using a Gallenkamp automatic adiabatic bomb calorimeter. The effective heat capacity of the system was determined using dry compacted benzoic acid. Preliminary trials revealed no weight change when dietary lipids were dried under the conditions mentioned above.

The reproducibility of the system was checked by successive determinations of the gross energy content of safflower oil. Values obtained (Table 3.1) are similar to values in the literature (Schmidt-Nielsen, 1981; Passmore, 1986).

Kjeldahl Nitrogen Determination

The nitrogen content of urine and dried feces and diet samples was determined using the Kjeldahl process. Approximately 1g of food or feces or 15 ml dilute urine was digested in 30 ml concentrated H_2SO_4 in the presence of 3g K_2SO_4 and a 1 g selenium catalyst tablet (BDH). Digestion was carried out in 250 ml flasks heated for 1 h, in batches of six on a digestion stand (Qualtex). One flask in each batch was a blank. Preliminary experiments indicated that 3g was the optimum amount of K_2SO_4 to add under these conditions (Table 3.2).

Under alkaline conditions, the ammonium sulphate $[(\text{NH}_4)_2\text{SO}_4]$ produced during digestion is converted to ammonia $[\text{NH}_3]$. Ammonia determination was carried out using a spectrophotometric procedure based on the Berthelot reaction. Absorbance @ 625 nm was measured using an LKB Biochrom Ultraspec II spectrophotometer. The quantity of indophenol blue produced by the reaction of ammonia and phenate is linearly related to the ammonia concentration. This was evident in the standard curves drawn for

Table 3.1 **Gross energy content of safflower oil measured by bomb calorimetry**

Sample N^o	Energy content (KJ.g⁻¹)
1	39.34
2	39.21
3	39.20
4	39.42
5	39.18
Mean	39.27
SEM	± 0.05

Table 3.2 Kjeldahl nitrogen determination of a casein sample using varying amounts of K₂SO₄.

Mass K ₂ SO ₄ (g)	Nitrogen content (%)
1.000	14.42
2.000	14.91
2.500	15.09
3.000	15.34
4.000	14.51

Table 3.3 Kjeldahl nitrogen determination of a sample of casein.

Sample N ^a	Nitrogen content (%)	Crude ^a protein (%)
1	14.818	92.6
2	14.636	91.5
3	15.179	94.9
4	14.026	87.7
5	14.081	88.0
Mean crude protein (%)		90.94
SD		3.08
SEM		1.38

a N content X 6.25
 Moisture content= 8.15%

each analysis. Standards were prepared using ammonium sulphate which had been dried overnight at 105°C.

The reproducibility of the technique was checked by successive nitrogen determinations of a casein sample (Table 3.3).

Crude protein = % N X 6.25 (Association of Official Analytical Chemists (AOAC), 1980). The crude protein content of the sample casein was calculated as 90.94%, which is an acceptable value, as the moisture content of this sample was determined as 8.15%. Nonprotein nitrogen may be present in some foods, so this parameter is imprecise (WHO, 1973).

Measurement of Basal Metabolic Rate

Each animal was weighed before and after each determination. It was placed inside an airtight cylindrical perspex metabolism chamber (450 mm by 95 mm diam.) on a wire grid over a reservoir of paraffin oil to collect urine and feces. The chamber was kept at a temperature of 30°C by water from a constant temperature water bath recirculating through a water jacket surrounding the chamber.

BMR in Expt.1 was measured by an open-flow technique, as described previously (Withers and Hulbert, 1988). The oxygen content of dried CO₂-free inlet and exit air was measured with a Taylor Servomex type OA 272 Oxygen Analyser and recorded on a National chart recorder (model VP-6521A). Rate of air flow was controlled by a calibrated Brooks flowmeter. BMR in Expt 3 was measured using a modified open-flow technique (Brand, et. al, 1991), where rate of flow was controlled by a calibrated Hastings Teledyne Mass Flow Controller.

Calculation of Nitrogen and Energy Balance Parameters

$$B = I - (F + U)$$

$$A = \frac{(I - F)}{I} \times 100$$

$$BV = \frac{B}{(I - F)} \times 100$$

$$NPU = \frac{B}{I} \times 100$$

where B = apparent nitrogen balance

I = nitrogen intake

F = fecal nitrogen

U = urinary nitrogen

A = apparent absorption of nitrogen

BV = apparent biological value

NPU = net protein utilization

The equations shown above were based on information from the literature (Munro, 1969; Platt et al., 1969; WHO, 1973; Torun et al., 1981). No corrections were made for excretion of endogenous N.

A loss of endogenous nitrogen (N) occurs in skin as hair, nails and desquamated cells and in feces, urine, sweat and breath. There may also be losses via flatus (Bosshardt and Barnes, 1946; Steggerda and Dimmick, 1966; Munro, 1969; WHO, 1973). In some studies, "true" nitrogen balance has been

calculated, after measuring endogenous N loss, or using values from the literature. Many of the values of endogenous N loss from the gut, available in the literature have been obtained from animals fed a protein-free diet, although the validity of this approach has been questioned (Bosshardt and Barnes, 1946; Kleiber, 1961; Torun et al., 1981).

When total fecal N per g food consumed, is plotted as a function of total N intake per g food consumed, it yields a straight line. Extrapolation to the point of zero N intake yields a value for fecal N excretion that is not necessarily the same as that obtained on a N-free diet (Bosshardt and Barnes, 1946). Thus, it would be of dubious benefit to use a value for endogenous N loss from the gut which has been obtained in this way. Fecal N excretion also increases when food intake is restricted (Bosshardt and Barnes, 1946). It was inconsistent with the protocol of the present study and beyond the scope of available resources to determine "true" digestibility of N, using the procedure of Bosshardt and Barnes (1946). Furthermore, both the quantity and source of protein intake are known to influence integumental loss of N in children (Viteri and Martinez, 1981). In view of the above information, I decided not to correct for endogenous N loss and to qualify some parameters as "apparent" rather than "true" determinations. The literature indicates that this is a widely adopted practice.

Physiological Significance of the N Balance determinations

When an organism is in N equilibrium, N intake equals N output i.e., $I = F + U$. This indicates that its protein intake is sufficient for maintenance and repair of body tissue, but not enough for tissue growth (Guthrie, 1983). Nitrogen balance is the difference between N intake and N output (Torun et al., 1981). A negative N balance indicates that an individual's store of tissue protein is decreasing and a positive N balance indicates that it is increasing and

that growth can occur (Guthrie, 1983). Apparent N balance (B), which is an overestimation of "true" N balance, is calculated if the output of endogenous N is ignored.

The apparent absorption (i.e., apparent digestibility) of N (A), is the % of dietary N intake that is absorbed into the body i.e., not excreted in feces. It is a measure of digestive and absorptive efficiency.

The apparent biological value (BV) is the proportion of absorbed N that is retained in the body under specified conditions (Munro, 1969). It is an indication of the efficiency with which the absorbed digestion products (amino acids) are retained for growth, repletion or maintenance (WHO, 1973). It permits comparisons between groups where N intake and absorption are not identical (Schendel and Hansen, 1965), such as the control and PEM groups in the present study.

Net protein utilization (NPU) "is the proportion of ingested N that is retained in the body under specified conditions" (WHO, 1973; p 64). It expresses in a single index, both the digestibility of the protein source and the efficiency with which the absorbed amino acids are utilized (Munro, 1969; WHO, 1973).

Calculations similar to those shown above were also carried out for energy. Energy is used by the body to do work e.g., synthesis of compounds, pumping of ions, muscular contraction, etc. Unfortunately, it is not possible to measure the total amount of work done by the body (Torun et al, 1981). Some of the energy involved in doing work may be "lost" from the body as heat. Thus, energy may leave the body via avenues additional to those also associated with N loss. The amount of both N and energy lost from the body may vary with factors such as environmental temperature and humidity, age and

physical exercise (WHO, 1973). Attempts to determine the efficiency with which absorbed energy is utilized i.e., the proportion of absorbed energy retained by the body in association with tissues or energy stores such as glycogen and fat may lead to gross overestimations. This is because measurements of energy output include only the combustible energy content of urine and feces, whereas the main variable of energy output may be physical activity (Passmore, 1986).

It was inconsistent with the protocol of the present study and beyond the scope of available resources to determine energy balance, although it was possible to calculate metabolisable energy. Metabolisable energy was calculated as $I - (F + U)$. Blaxter (1989) indicates that in the non-lactating mammal, energy balance is metabolisable energy less heat production. Metabolisable energy is that which is available for the demands of maintenance, growth and activity. Metabolisable energy may be synonymous with the ecological term, assimilated energy, (Blaxter, 1989). Both terms are used in ecology (Drodz, 1975; Blaxter, 1989).

Metabolizable energy as a % of energy intake is termed metabolisability (Blaxter, 1989). An alternative term used in ecology is assimilation efficiency, although this term is also used for apparent digestibility (Blaxter, 1989).

Unfortunately, the use of what Blaxter (1989, p33) describes as these "not particularly euphonious" terms, which have not been applied unambiguously throughout the literature results in increased confusion, rather than a sense of order or understanding. In an attempt to avoid this I have expressed metabolisable energy as a % of energy intake and as a % of absorbed energy. These are not analogous expressions for NPU and BV, they indicate the proportion of the energy ingested or absorbed, that is available for the demands of maintenance, growth and activity.

Statistical Analysis

The results of Experiments 1 and 3 were subjected to a 3-way analysis of variance (litter, sex and diet) using the statistical package Genstat. A separate analysis of variance was carried out to include the Age-matched control rats, which were not from the same litters as the other 4 groups. The results of Experiment 2 were subjected to a 1-way analysis of variance.

RESULTS

Experiment 2

Energy and nitrogen balance

As was outlined previously (see page 45), Experiment 1 had a deficiency in its design, not discovered until towards the end of analysis of samples from the experiment. When the 18-carbon saturated fat, stearic acid, was used in the control group in place of the 18-carbon polyunsaturated fat, linoleic acid, it was found that apparent absorption of both energy and N was dramatically reduced. Therefore, a short-term experiment (Experiment 2) was designed to determine whether this effect could be eliminated if coconut oil, another highly saturated lipid source was substituted for stearic acid.

With coconut oil as the major lipid source, apparent absorption of energy was 97%, compared to 83% with stearic acid (Table 3.4; Fig. 3.1). After the diets were switched, the values were 85% with stearic acid and 94% with coconut oil. Apparent absorption of N was 89% with stearic acid and 90% with coconut oil. After the diets were switched, the values were 86% with stearic acid and 90% with coconut oil (Table 3.4; Fig. 3.2). Thus, the apparent absorption of both energy and N during Experiment 2 are increased to levels in control diet rats of Experiment 1, if coconut oil is substituted for stearic acid. The increased volume of feces produced with stearic acid is evident in Plate 3.3.

Although absorption of nutrients was greatly reduced in the low PUFA group of Experiment 1, this was not a problem in the medium and high-PUFA dietary groups. Therefore, the results of Experiment 1 and Experiment 3 will be discussed together.

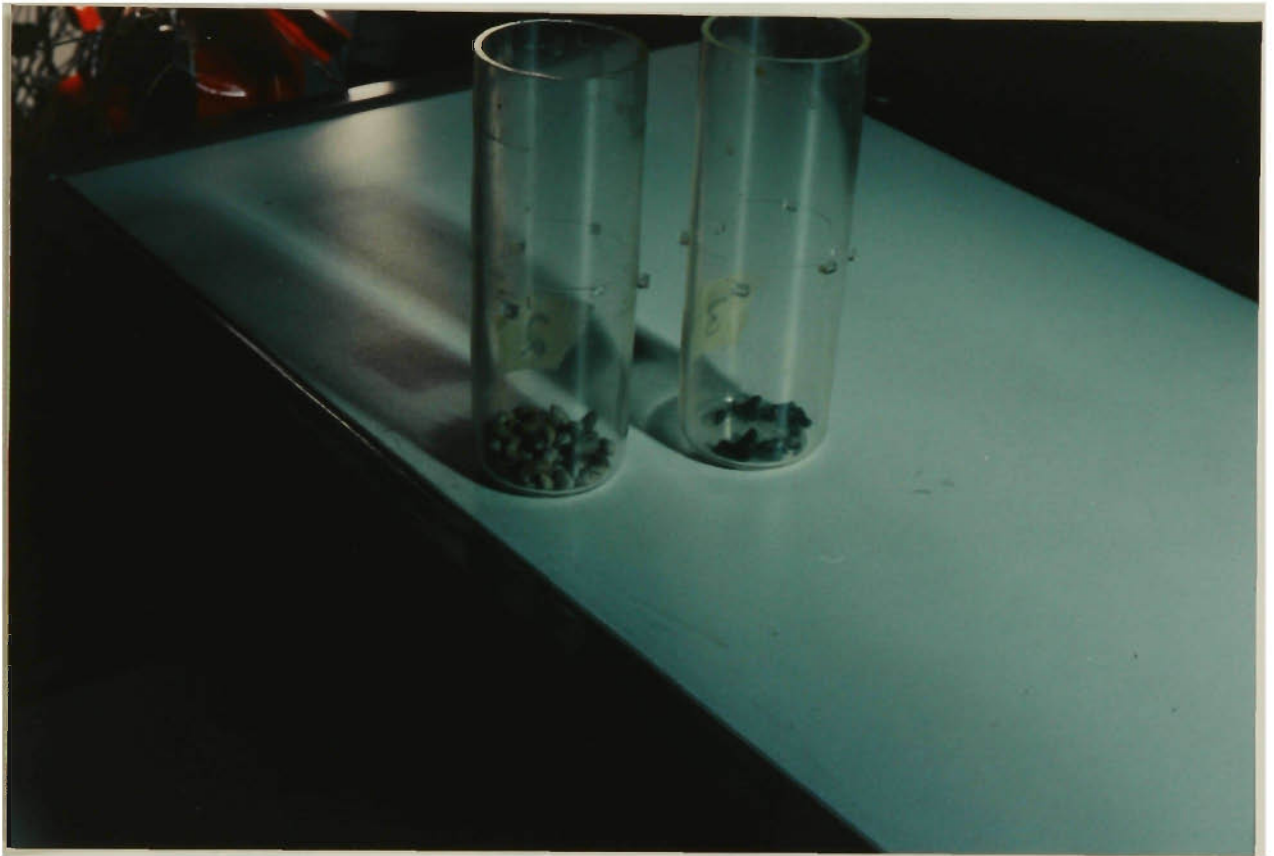


Plate 3.3

Comparison of the effects of stearic acid and coconut oil on volume of feces excreted over three days: Experiment 2.

The feces on the left were excreted by a rat fed a diet containing stearic acid. Those on the right were from a rat fed a diet containing coconut oil.

Table 3.4 A comparison of the effects of stearic acid and coconut oil on apparent absorption of energy and nitrogen: Experiment 2.

Major lipid in diet	1		2	
	Stearic acid	Coconut oil	Coconut Oil	Stearic acid
Change in body mass(g)	-0.90 ±0.80	1.82* ±0.67	-0.89 ±0.57	0.34 ±1.23
Food intake (g.day ⁻¹)	6.10	6.10	6.10	6.10
Water intake (ml.day ⁻¹)	8.70 ±0.43	8.53 ±0.43	7.58 ±0.35	7.35 ±0.49
Energy intake (KJ.day ⁻¹)	106.3	102.3	102.3	106.3
Nitrogen intake (mg N.day ⁻¹)	101.3	100.0	100.0	101.3
Fecal output (g.day ⁻¹)	2.61 ±0.13	0.97*** ±0.09	1.68** ±0.24	3.06 ±0.27
Fecal moisture (%)	29.2 ±2.9	19.5* ±3.0	34.4 ±3.4	39.5 1.2
Fecal energy (KJ.day ⁻¹)	17.6 ±1.2	3.3*** ±0.3	6.3*** ±0.7	16.5 ±1.6
Fecal nitrogen (mg N.day ⁻¹)	11.0 ±1.1	10.0 ±0.7	10.5* ±1.0	14.3 ±1.0
Apparent absorption of energy (%)	83.4 ±1.1	96.8*** ±0.3	93.8*** ±0.6	84.5 ±1.5
Apparent absorption of nitrogen (%)	89.2 ±1.1	90.0 ±0.7	89.7* ±1.0	85.7 ±1.1
N°	6	6	6	5

Diets swapped for trial 2. Values are means ± SEM.

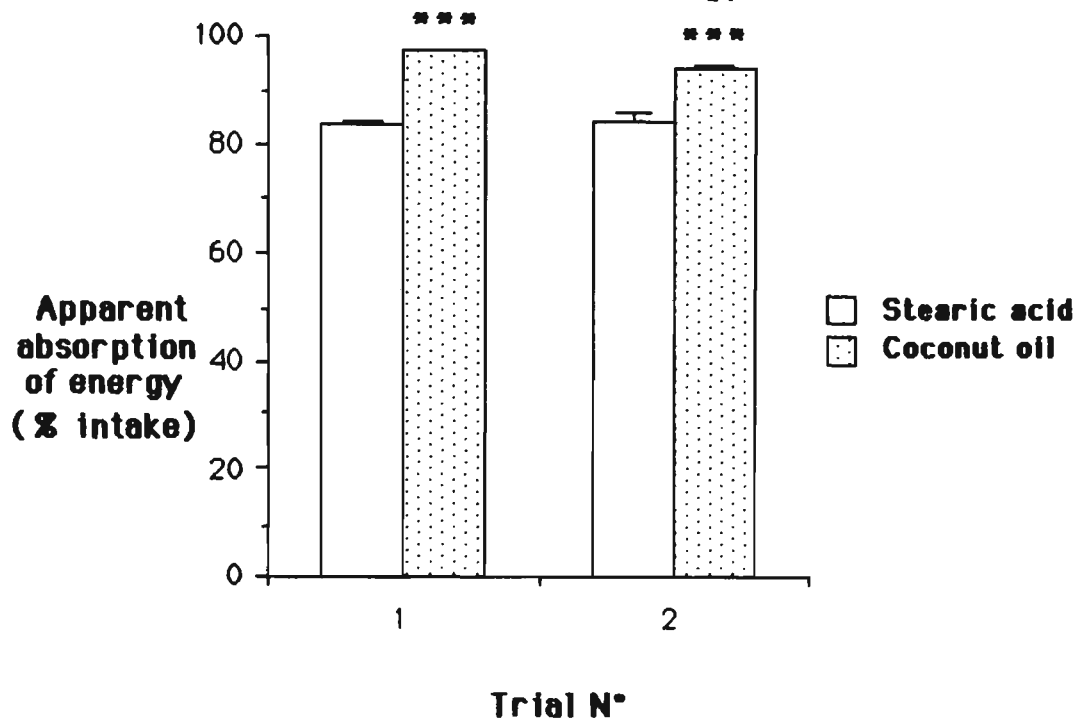
Significant differences from stearic acid detected by ANOVA.

* p<0.05

** p<0.01

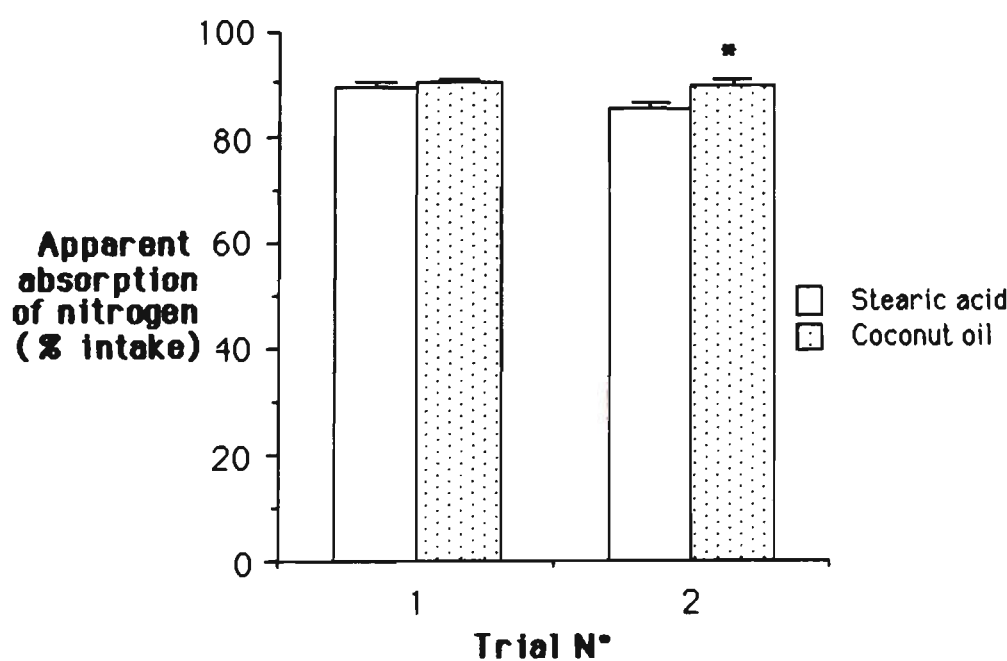
*** p<0.001

Figure 3.1 The effects of stearic acid and coconut oil on apparent absorption of energy: Experiment 2



Values: Mean ± SEM
***** Significantly different from stearic acid (p<0.001)**

Figure 3.2 The effects of stearic acid and coconut oil on apparent absorption of nitrogen: Experiment 2



Values: Mean ± SEM
*** Significantly different from stearic acid (p<0.05)**

Experiments 1 and 3

Food Intake

Food intake of age-matched controls increased from 14 g.day⁻¹ to 19 g.day⁻¹ in Experiment 1 (Table 3.5) and from 10 g.day⁻¹ to 17 g.day⁻¹ in Experiment 3 (Table 3.6), after which it remained relatively constant (Fig. 3.3).

Growth

Growth in body mass of the age-matched controls was similar to that observed when siblings of these Sprague-Dawley rats were fed a standard rat chow (Allied Rat and Mouse Kubes; Allied feeds, Australia). Within both Experiments 1 and 3, PEM and food restriction reduced body mass to a common value during the first week of the experimental diets. Body mass later increased, with progressive divergence between dietary groups (Table 3.8). Food-restriction and PEM significantly retarded growth (Tables 3.7 and 3.9). In experiment 3, growth in both snout-vent length and body mass was retarded significantly more by PEM than by food-restriction (Table 3.9; Fig 3.5). More importantly, growth was greater with increased dietary PUFA during PEM. In experiment 3, this difference was statistically significant for growth in body mass. In Experiment 1, the differences between the medium and high PUFA groups were not significant (Table 3.7; Fig 3.4). Growth in the size-matched controls equalled that of the malnourished rats after only 10 days (Table 3.9).

Metabolic Rate

Food restriction and PEM significantly increased BMR (on a total body weight basis). However, the BMR of food-restricted rats was not significantly



Plate 3.4

Effect of Protein-energy malnutrition on body size of the rat:
Experiment 1.

The rat on the left was protein-energy malnourished. Its littermate
on the right was fed the control diet ad libitum.

Table 3.5 Body mass and food consumption of Control diet rats:
Experiment 1

Age (weeks)	BodyMass (g)		Rate of Food Consumption (g.day ⁻¹)	
4	104.6	±3.1	14.1	±1.6 ^a
5	148.3 ^a	±7.1	15.0	±0.9
6	186.2	±11.2	17.0	±1.0
7	223.9	±16.3	18.4	±1.2
8	250.8	±20.6	19.0	±1.3
9	272.7	±23.9	18.3	±1.3
10	287.7	±26.3	18.8	±1.3

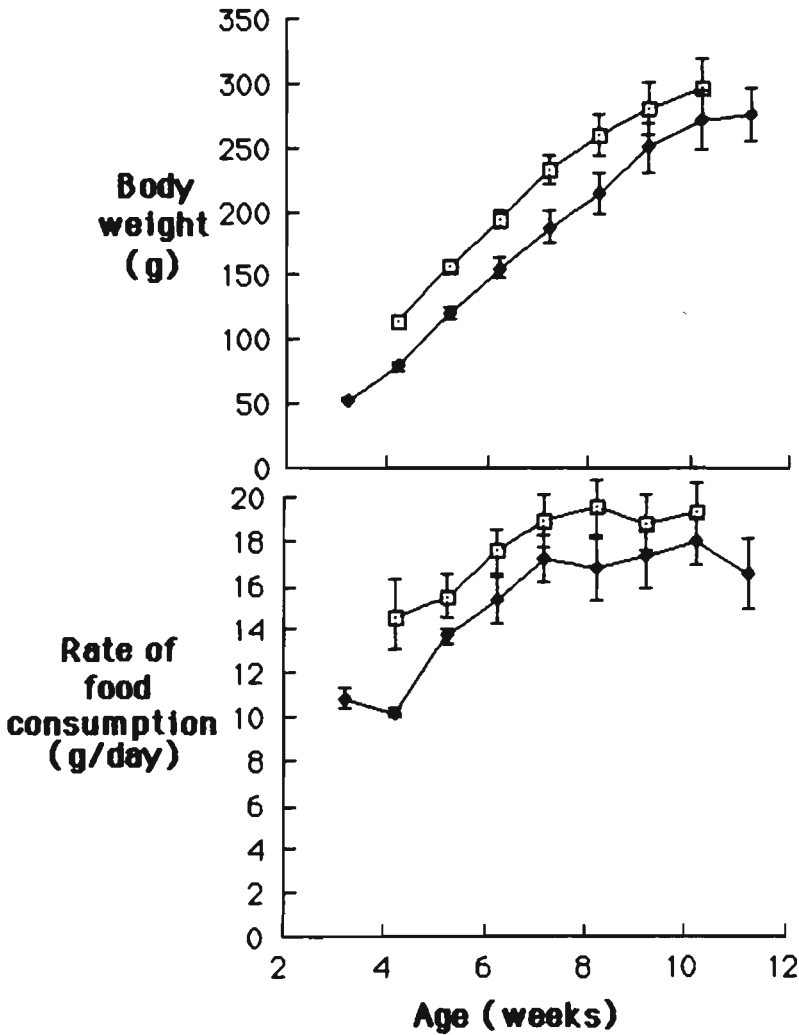
^a Value during each week
All values; Mean ± SEM

Table 3.6 Body mass and food consumption of Control diet rats:
Experiment 3.

Age (weeks)	BodyMass (g)		Rate of Food Consumption (g.day ⁻¹)	
3	44.6	±2.0	-	
4	70.3	±3.2	9.6	±0.2 ^a
5	112.1	±4.8	13.2	±0.3
6	147.3	±8.3	14.8	±1.0
7	178.9	±12.9	16.6	±1.1
8	206.3	±16.4	16.2	±1.3
9	241.6	±19.3	16.73	±1.3
10	262.4	±23.1	17.4	±1.1
11	268.4	±20.7	16.0	±1.5

^a Value during each week
All values; Mean ± SEM

Figure 3.3 Growth and rate of food consumption of control diet rats: Experiments 1 and 3



Values: Mean \pm SEM.

Table 3.7 Effects of PEM and PUFA-enrichment on growth: Experiment 1.

PUFA content	Control	Protein-energy malnourished		
		Low	Medium	High
Change in body mass (g)	183.1 ^{yz} ±23.3	-20.3 ^{yz} ±2.7	-5.8 ^x ±2.5	-3.7 ^x ±2.1
Change in snout-vent (mm)	60.5 ^{yz} ±4.6	-1.4 ^{yz} ±1.8	9.0 ^x ±2.6	9.8 ^x ±1.1
Change in pectoral (mm)	7.0 ^{yz} ±0.5	0.8 ^{yz} ±0.5	2.2 ^x ±0.6	2.0 ^x ±0.7
Change in tail (mm)	65.7 ^{yz} ±5.0	18.6 ^x ±2.3	18.0 ^x ±2.2	19.7 ^x ±1.3
N°	6	5	6	6

Values: Mean ± SEM

x Significantly different from Control ($p < 0.05$).

y Significantly different from High PUFA ($p < 0.05$).

z Significantly different from Medium PUFA ($p < 0.05$).

Table 3.8 Effects of PEM, food-restriction and PUFA-enrichment on body mass:
Experiment 3.

Age (weeks)	Control Diet			PEM	PEM PUFA- enriched
	Size- matched	Age- matched	Food- restricted		
3	45.4 ±1.82	44.6 ±2.0	45.5 ±2.0	45.5 ±2.0	45.2 ±2.2
4	81.0 ^a ±2.1	70.3 ±3.2	35.0 ±1.4	34.7 ±1.4	34.6 ±1.6
5		112.1 ±4.8	40.9 ±1.2	40.2 ±1.2	40.2 ±1.4
6		147.3 ±8.3	50.8 ±1.4	48.1 ±1.4	49.3 ±1.3
7		178.9 ±12.9	61.5 ±1.5	56.9 ±1.5	59.4 ±1.3
8		206.3 ±16.4	70.2 ±1.8	63.0 ±1.8	66.5 ±1.3
9		241.6 ±19.3	78.2 ±2.3	70.8 ±2.3	73.5 ±1.4
10		262.4 ±23.1	82.8 ±1.2	75.1 ±1.2	79.6 ±1.8
11		268.4 ±20.7	84.5 ±1.7	76.7 ±2.5	80.7 ±1.8
N*	8	8	8	8	8

Values: Mean ± SEM
a Body mass at 1.3 weeks
All values (g)

Table 3.9 Effects of PEM, food-restriction and PUFA-enrichment on growth: Experiment 3.

Growth parameter	Control Diet			PEM	PEM PUFA-enriched
	Size-matched	Age-matched	Food-restricted		
Body mass (g)	35.6 ^{axy} ±1.1 ^b	223.7 ^{wyz} ±20.0	38.9 ^{xy} ±1.6	31.1 ^{xz} ±1.6	35.4 ^{xy} ±1.4
Snout-vent (mm)	30.3 ^{cxyz} ±1.4	96.3 ^{wyz} ±6.6	41.9 ^{wxy} ±1.8	36.8 ^{wxz} ±2.2	39.8 ^{wx} ±1.1
Pes (mm)	6.1 ^x ±0.7	13.3 ^{wyz} ±0.9	5.5 ^x ±0.7	5.8 ^x ±0.7	5.8 ^x ±0.5
Tail (mm)	25.8 ^x 1.5	115.9 ^{wyz} ±7.6	31.3 ^x ±2.7	30.3 ^x ±2.0	30.8 ^x ±2.1
N*	8	8	8	8	8

Values are increments in growth

a Mean

b SEM

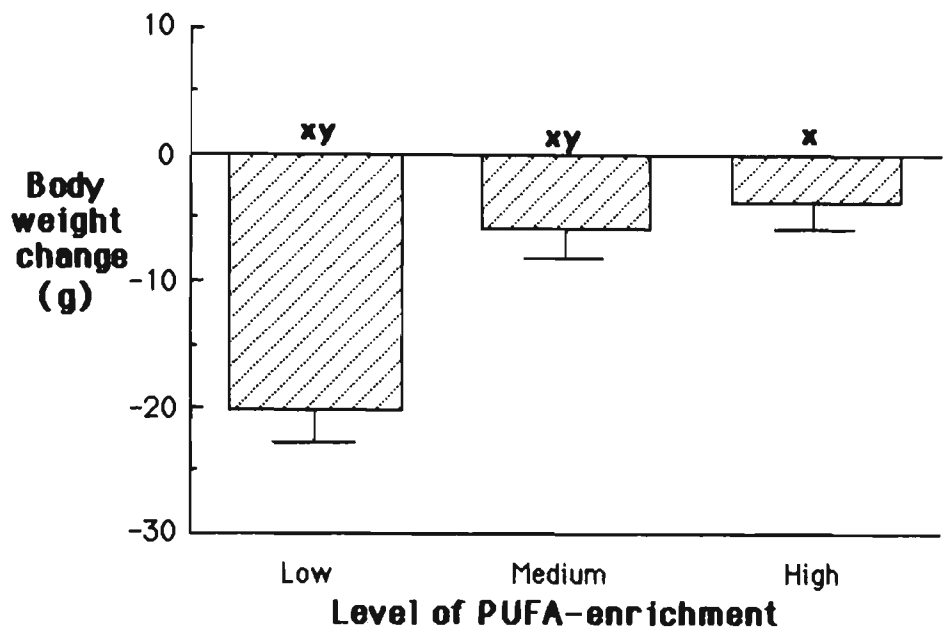
w Significantly different from Size-matched control (p<0.05)

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

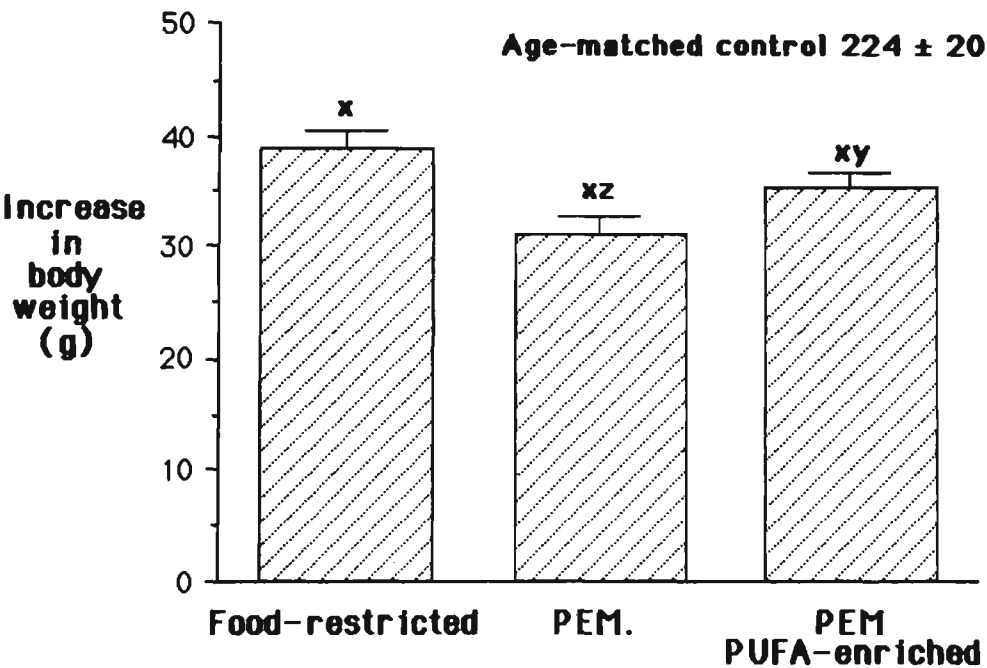
z Significantly different from Food-restricted (p<0.05)

Figure 3.4 The effects of PUFA-enrichment on the decrease in body weight caused by PEM: Experiment 1



Values: Mean \pm SEM
x Significantly different from control ($p < 0.05$)
y Significantly different from High PUFA ($p < 0.05$)

Figure 3.5 Effects of food-restriction, PEM and PUFA-enrichment on growth: Experiment 3



Values: mean \pm SEM
x Significantly different from Age-matched control ($p < 0.001$)
y Significantly different from PEM ($p < 0.05$)
z Significantly different from Food-restricted ($p < 0.05$)

Table 3.10 Effects of PEM and PUFA-enrichment on basal metabolic rate: Experiment 1.

PUFA content	Control	Protein-energy malnourished		
		Low	Medium	High
Body mass (g)	292.7 ^{yz} ±26.0	83.0 ^{xyz} ±0.7	97.4 ^x ±1.1	100.1 ^x ±3.0
BMR (ml O ₂ g ⁻¹ .h ⁻¹)	0.94 ^{yz} ±0.03	1.28 ^{xy} ±0.09	1.20 ^x ±0.05	1.15 ^x ±0.04
N°	6	5	6	6

Values are mean ± SEM

x Significantly different from Control (p<0.05).

y Significantly different from High PUFA (p<0.05).

z Significantly different from Medium PUFA (p<0.05).

Table 3.11 Effects of PEM, food-restriction and PUFA-enrichment on basal metabolic rate: Experiment 3.

	Control Diet				PEM	PEM PUFA- enriched
	Size- matched	Age- matched	Age- matched (lean bodymass)	Food- restricted		
Body mass (g)	78.9 ^x ±3.2	249.6 ^{wyz} ±19.16	175.0	83.5 ^{xy} ±1.7	75.4 ^{xz} ±2.3	78.5 ^x ±2.2
BMR (ml O ₂ g ⁻¹ .h ⁻¹)	1.59 ^{xyz} ±0.09	1.01 ^{wyz} ±0.04	1.39	1.23 ^{wx} ±0.05	1.32 ^{wx} ±0.05	1.28 ^{wx} ±0.05
N°	8	8	8	8	8	8

Values are mean ± SEM

In estimating lean body mass, it is assumed that body water content is 65% of lean tissue.

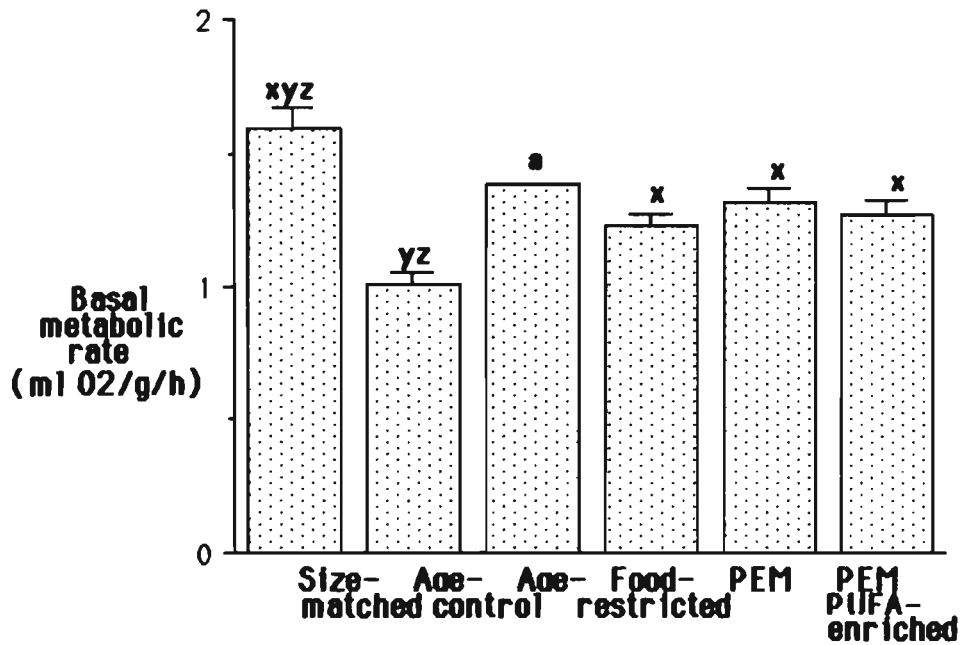
w Significantly different from Size-matched control (p<0.05)

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

z Significantly different from Food-restricted (p<0.05)

Figure 3.6 Effects of food-restriction, PEM and PUFA-enrichment on basal metabolic rate: Experiment 3



Values: Mean \pm SEM

- a** based on estimate of lean body mass
- x** Significantly different from Age-matched control ($p < 0.001$)
- y** Significantly different from PEM ($p < 0.05$)
- z** Significantly different from Food-restricted ($p < 0.05$)

Table 3.12

Effects of PEM and PUFA-enrichment on parameters of energy balance: Experiment 1

PUFA content	Control	Protein-energy malnourished		
	Medium	Low	Medium	High
Body mass change during one day (g)	1.0 ±2.0	-1.8 ±1.5	-0.9 ±0.6	-0.7 ±0.7
Energy intake (KJ.day ⁻¹)	314.1 ±26.7	104.5	101.6	100.4
Fecal output (g.day ⁻¹)	0.9 ±0.2	1.7 ^{xyz} ±0.2	0.6 ±0.1	0.5 ±0.1
Urinary energy (KJ.day ⁻¹)	8.9 ^{yz} ±0.6	1.7 ^x ±0.3	1.7 ^x ±0.4	1.6 ^x ±0.3
Fecal energy (KJ.day ⁻¹)	13.2 ±2.7	47.4 ^{xyz} ±6.1	9.9 ±1.9	7.7 ±1.6
Metabolisable energy ^a (KJ.day ⁻¹)	292.0 ^{yz} ±25.7	55.4 ^{xyz} ±6.2	90.0 ^x ±1.8	91.1 ^x ±1.8
Metabolisable energy (% energy intake)	92.9 ^z ±1.1	53.0 ^{xyz} ±5.9	88.6 ^x ±1.7	90.8 ±1.7
Metabolisable energy (% apparent absorption)	96.9 ±0.3	96.8 ±0.7	98.2 ±0.4	98.3 ±0.3
Apparent ^b absorption (%)	95.8 ^z ±0.9	54.6 ^{xyz} ±5.8	90.2 ^x ±1.8	92.4 ±1.6
N°	6	5	6	6

Values: Mean ± SEM

a Energy intake - (Fecal energy + Urinary energy)

b (Energy intake - Fecal energy).100/Energy intake

x Significantly different from Control (p<0.05).

y Significantly different from High PUFA (p<0.05).

z Significantly different from Medium PUFA (p<0.05).

Table 3.13 Effects of PEM, food-restriction and PUFA-enrichment on parameters of energy balance: Experiment 3.

	Control Diet			PEM	PEM PUFA- enriched
	Size- matched	Age- matched	Food- restricted		
Body mass change during 3 days (g)	19.9 ^{xyz} ±0.5	14.7 ^{wyz} ±3.2	5.1 ^x ±0.8	4.9 ^x ±0.7	4.7 ^x ±0.5
Energy intake (KJ.day ⁻¹)	208.5 ^{xyz} ±8.0	312.0 ^{wyz} ±22.6	112.5 ^{wxy}	109.1 ^{wxz}	107.4 ^{wxyz}
Urinary energy (KJ.day ⁻¹)	7.1 ^x ±0.2	12.1 ^{wyz} ±1.5	6.9 ^x ±0.2	6.2 ^{xz} ±0.2	6.7 ^x ±0.4
Fecal energy (KJ.day ⁻¹)	8.6 ^{xyz} ±0.8	10.5 ^{yz} ±1.1	3.7 ^{wx} ±0.5	4.9 ^{wx} ±0.5	5.3 ^{wxz} ±0.7
Apparent ^b absorption (%)	95.9 ±0.3	96.7 ^y ±0.2	96.7 ^y ±0.4	95.5 ^{xz} ±0.5	95.0 ^{xz} ±0.7
Metabolisable E ^a (KJ.day ⁻¹)	192.8 ^{xyz} ±7.4	289.3 ^{wyz} ±21.6	102.0 ^{wx} ±0.5	98.1 ^{wx} ±0.5	95.4 ^{wz} ±0.8
Metabolisable E (% Energy intake)	92.5 ^{yz} ±0.3	92.7 ^{yz} ±0.5	90.6 ^{wx} ±0.4	90.0 ^{wx} ±0.5	88.8 ^{wxz} ±0.7
Metabolisable E (% Appar. abs.)	96.4 ^{yz} ±0.1	95.9 ^{yz} ±0.5	93.7 ^{wx} ±0.2	94.1 ^{wx} ±0.2	93.5 ^{wx} ±0.4
N ^a	8	8	8	8	8

Values: Mean ± SEM

a Energy intake - (Fecal energy + Urinary energy)

b (Energy intake - Fecal energy). 100/Energy intake

w Significantly different from Size-matched control (p<0.05)

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

z Significantly different from Food-restricted (p<0.05)

Table 3.14 Effects of PEM and PUFA-enrichment on nitrogen balance:
Experiment 1

PUFA content	Control	Protein-energy malnourished		
		Low	Medium	High
N intake (mg.day ⁻¹)	623.1 ±53.0	100.1	106.0	102.3
Urinary N (mg.day ⁻¹)	412.4 ^{yz} ±33.2	70.1 ^x ±8.6	77.1 ^x ±12.9	60.3 ^x ±7.7
Fecal N (mg.day ⁻¹)	27.7 ^{yz} ±5.1	17.6 ^{yz} ±2.2	11.4 ^x ±1.2	11.3 ^x ±2.9
N balance ^a (mg.day ⁻¹)	183.0 ^{yz} ±42.3	12.4 ^y ±7.7	17.5 ^x ±12.6	30.8 ^x ±8.6
Net protein ^b utilization (%)	28.3 ±5.2	12.4 ±7.7	16.5 ±11.9	30.1 ±8.4
Apparent biological ^c value of N (%)	29.6 ±5.5	15.4 ±9.7	18.7 ±13.3	33.3 ±8.8
Apparent ^d absorption (%)	95.7 ^z ±0.6	82.5 ^{xyz} ±2.2	89.3 ^x ±1.2	89.0 ±2.8
N ^e	6	5	6	6

Values: Mean ± SEM.
a N intake - (Fecal N + Urinary N)
b N balance % of N intake
c N balance. 100 / (N intake - Fecal N)
d (N intake - Fecal N). 100/N intake
x Significantly different from Control (p<0.05).
y Significantly different from High PUFA (p<0.05).
z Significantly different from Medium PUFA (p<0.05).

Table 3.15 Effects of PEM, food-restriction and PUFA-enrichment on nitrogen balance: Experiment 3.

	Control Diet			PEM	PEM PUFA- enriched
	Size- matched	Age- matched	Food- restricted		
Water intake (ml.day ⁻¹)	12.0 ^{xy} ±0.8	21.8 ^{wyz} ±1.5	13.7 ^{xy} ±1.1	8.2 ^{wxz} ±0.6	9.2 ^{wxz} ±0.9
Fecal output (g.day ⁻¹)	0.73 ^{xyz} ±0.07	1.22 ^{wyz} ±0.11	0.48 ^{wx} ±0.08	0.47 ^{wx} ±0.05	0.40 ^{wx} ±0.04
Fecal moisture (%)	31.2 ^{xz} ±2.5	41.4 ^{wy} ±2.2	40.4 ^{wy} ±2.4	33.0 ^{xz} ±2.8	25.3 ^{xyz} ±2.5
N intake (mg.day ⁻¹)	448.5 ^{xyz} ±17.3	671.0 ^{wyz} ±48.6	242.0 ^{wxy}	110.4 ^{wxz}	111.9 ^{wxz}
Urinary N (mg.day ⁻¹)	197.6 ^{xyz} ±9.1	439.6 ^{wyz} ±30.0	128.5 ^{wxy}	49.6 ^{wxz} ±3.5	46.6 ^{wxz} ±2.6
Fecal N (mg.day ⁻¹)	23.4 ^{xyz} ±1.7	34.3 ^{wyz} ±2.2	14.6 ^{wx} ±1.5	12.1 ^{wx} ±0.8	12.4 ^{wx} ±1.0
Apparent ^d absorption (%)	94.8 ^y ±0.3	94.8 ^y ±0.4	94.0 ^y ±0.6	89.0 ^{wxz} ±0.7	89.0 ^{wxz} ±1.0
N balance ^a (mg.day ⁻¹)	227.5 ^{xyz} ±20.1	197.1 ^{yz} ±27.6	98.9 ^{wxy} ±7.9	48.7 ^{wxz} ±3.5	53.0 ^{wxz} ±2.6
Net protein ^b utilization (%)	50.2 ^{xz} ±2.9	28.8 ^{wyz} ±2.7	40.9 ^{wx} ±3.3	44.1 ^x ±3.2	47.3 ^x ±2.4
Apparent bio ^c value of N (%)	52.9 ^{xz} ±3.0	30.3 ^{wyz} ±2.8	43.5 ^{wx} ±3.6	49.6 ^x ±3.4	53.2 ^{xz} ±2.6
N ^e	8	8	8	8	8

Values: Mean ± SEM

a N intake - (Fecal N + Urinary N)

b N balance % of N intake

c N balance. 100 / (N intake - Fecal N)

d (N intake - Fecal N). 100 / N intake

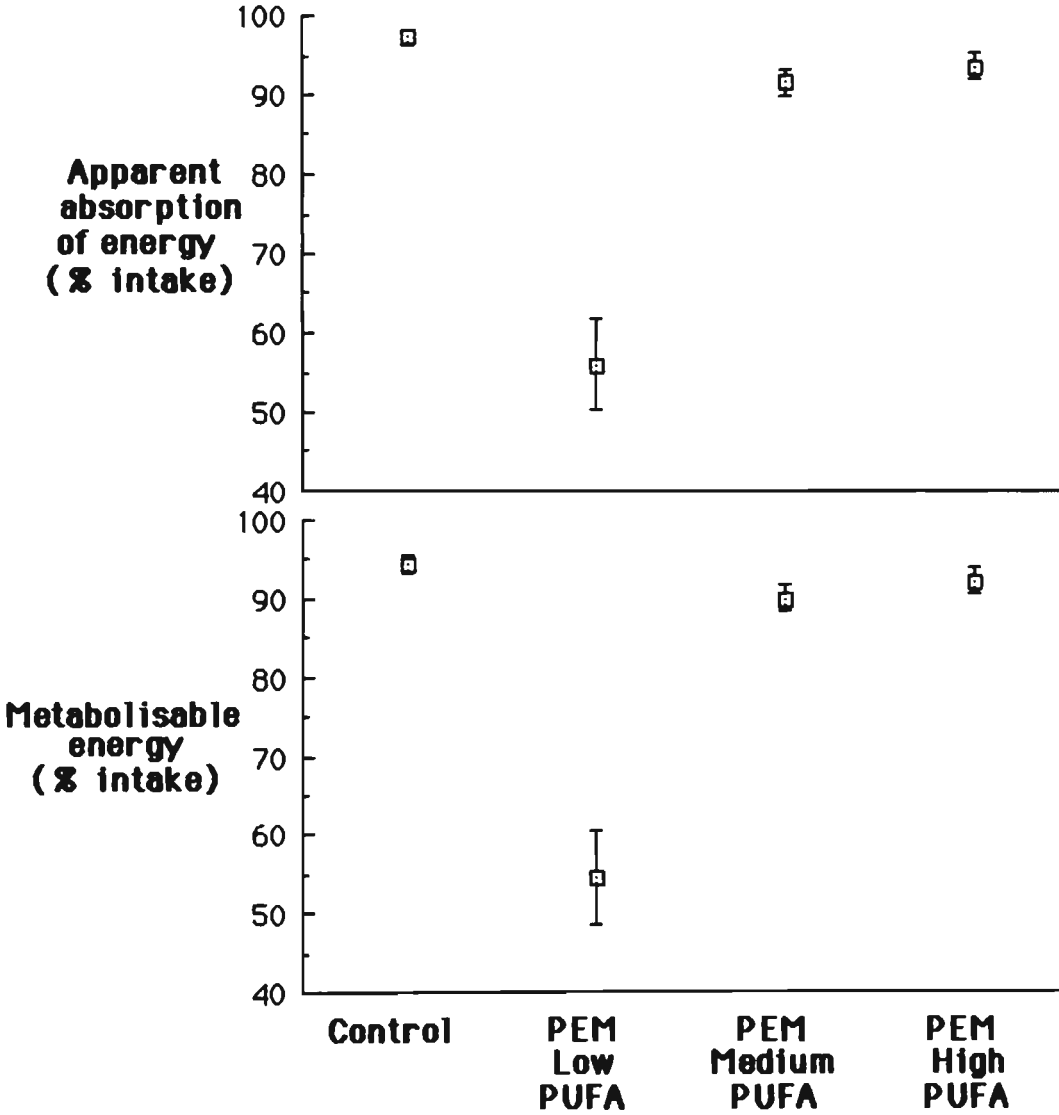
w Significantly different from Size-matched control (p<0.05)

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

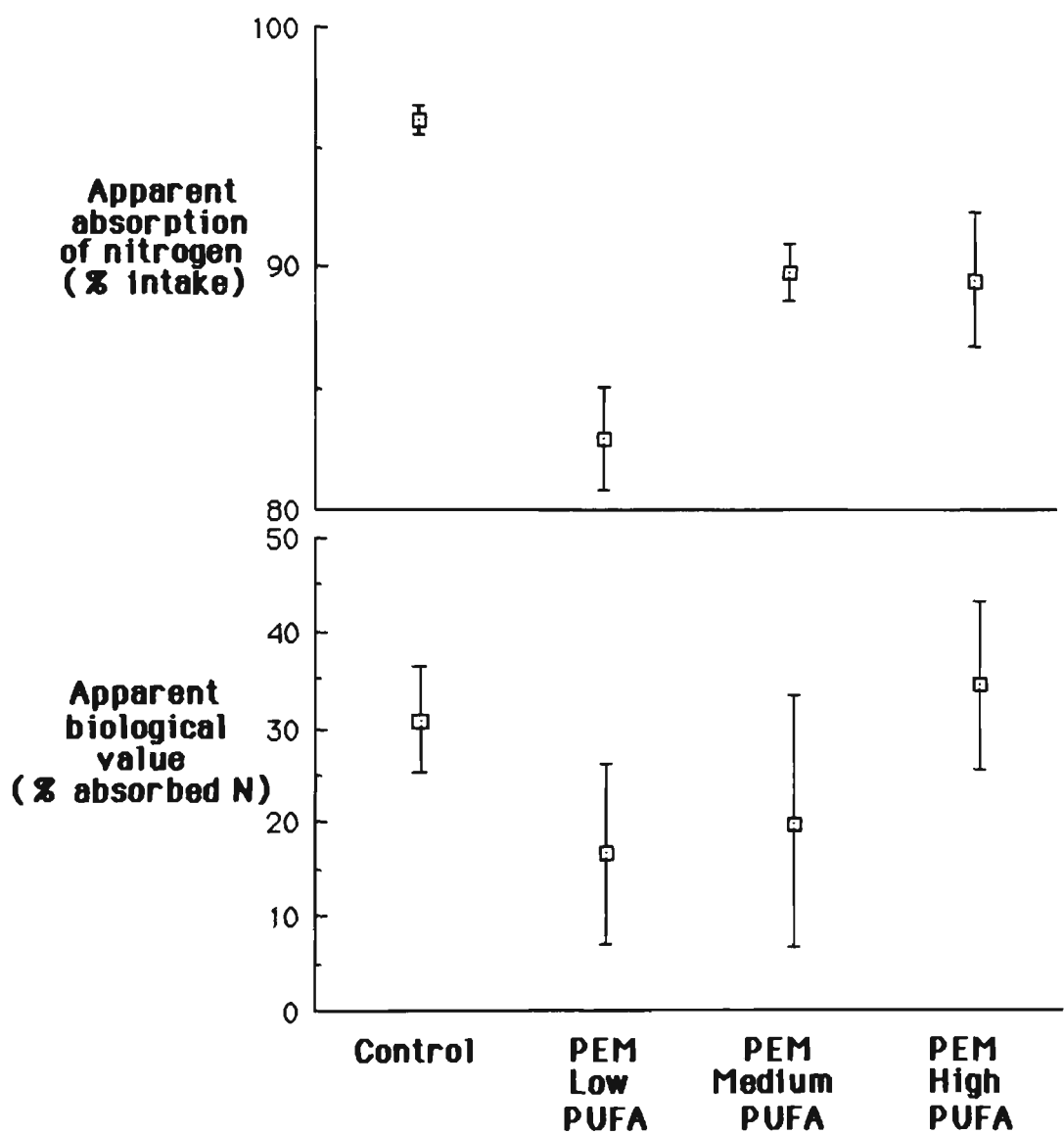
z Significantly different from Food-restricted (p<0.05)

Figure 3.7 Effects of PUFA-enrichment during PEM on apparent absorption of energy and metabolisable energy: Experiment 1



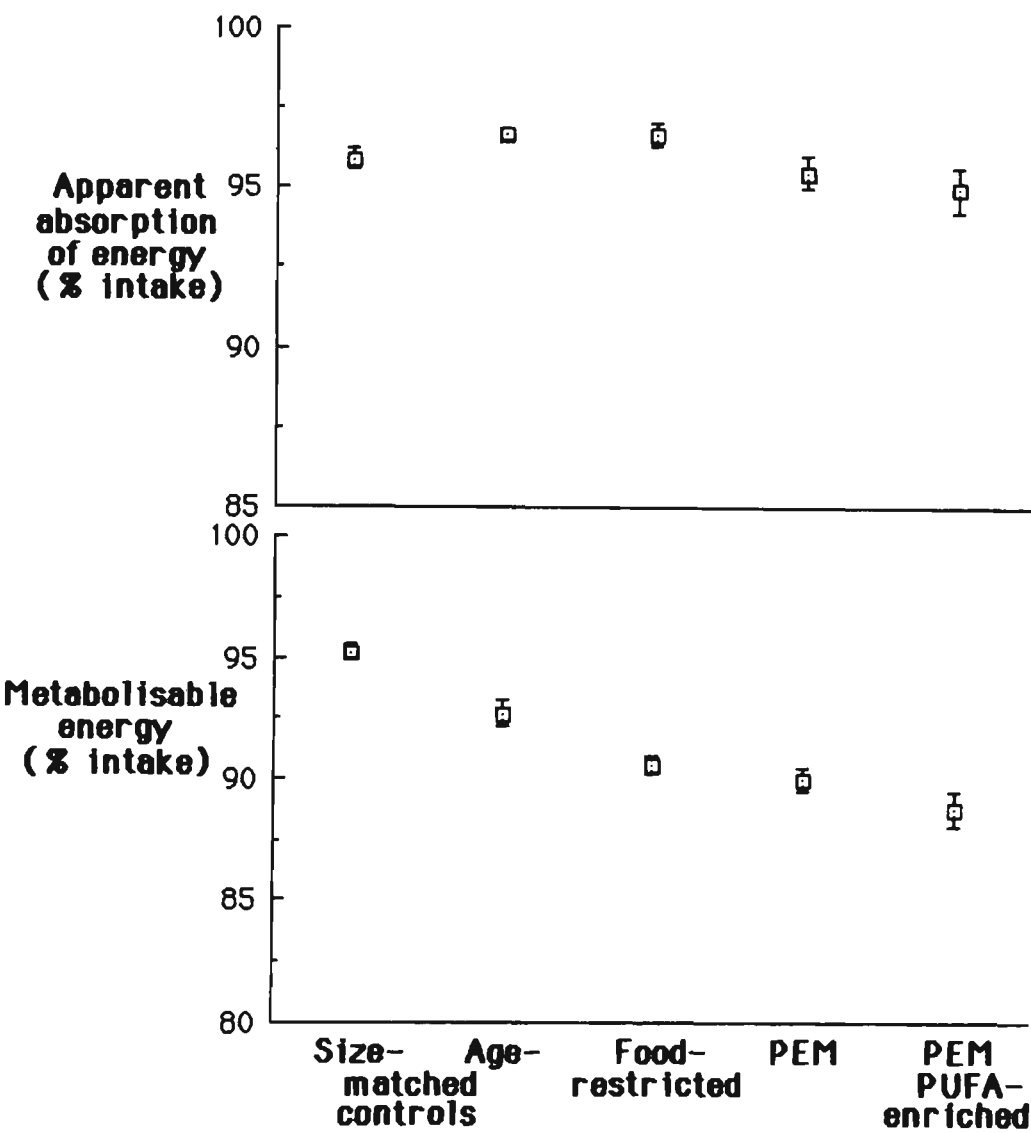
Values: Mean ± SEM

**Figure 3.8 Effects of PUFA-enrichment during PEM
on apparent absorption and apparent
biological value of nitrogen: Experiment 1**



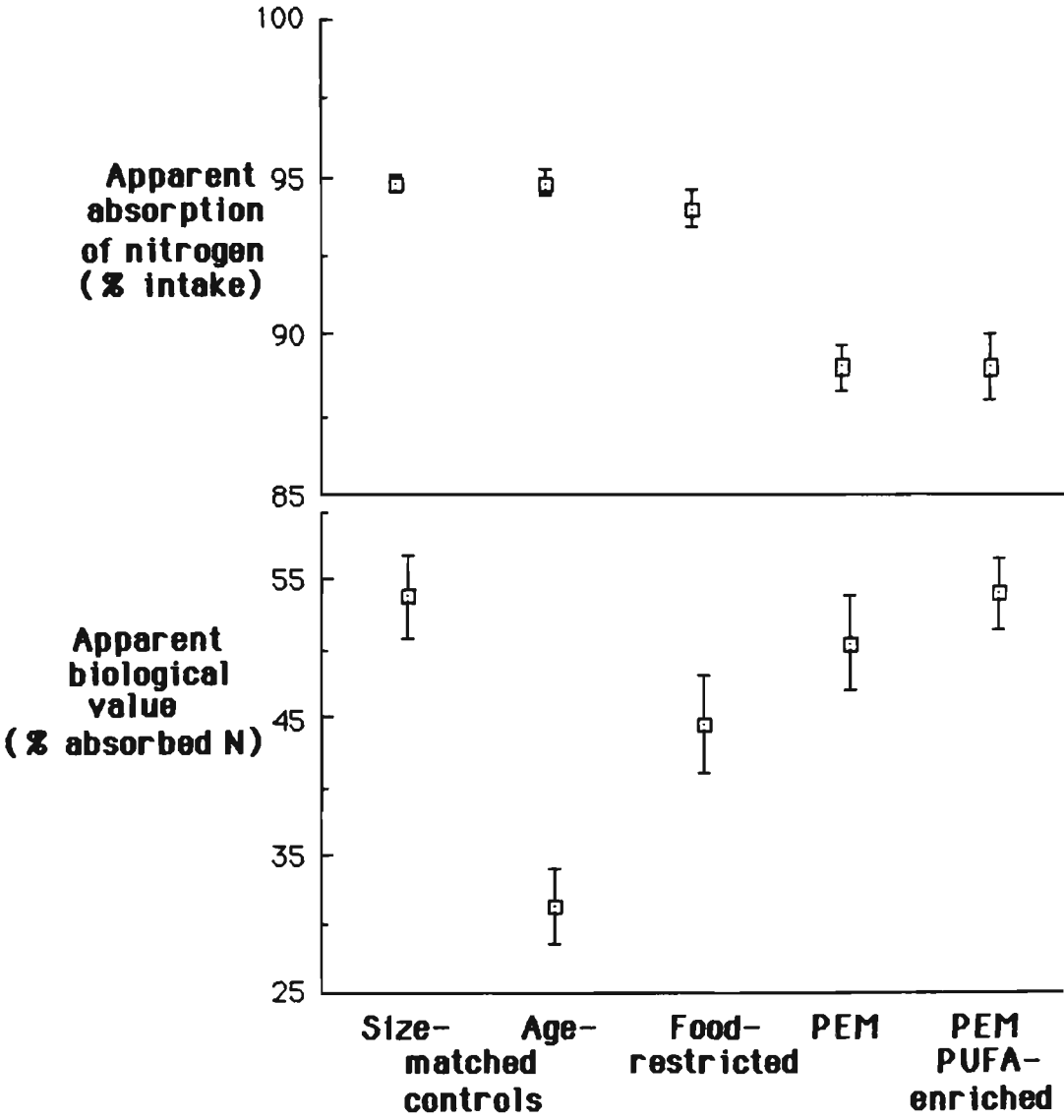
Values: Mean \pm SEM

Figure 3.9 Effects of Food-restriction, PEM and PUFA-enrichment on apparent absorption of energy and metabolisable energy: Experiment 3



Values: Mean \pm SEM

Figure 3.10 Effects of Food-restriction, PEM and PUFA-enrichment on apparent absorption and apparent biological value of nitrogen: Experiment 3



Values: Mean \pm SEM

different from either PEM group (Tables 3.10 and 3.11). Carcass water content was measured in Experiment 3 and found to be very low in the age-matched controls, where there were large fat deposits. Calculation of BMR on a lean body mass basis indicated that food-restriction and PEM decreased weight specific BMR. Increasing the dietary PUFA level of PEM rats reduced BMR in both experiments 1 and 3, although these differences were not significant. Comparison of the size-matched and age-matched controls indicates that BMR decreased with age.

Energy and Nitrogen Balance

PEM reduced apparent absorption of energy from 96-97% to 90-96% and apparent absorption of N from approximately 95% to 89% (Tables 3.12-3.15; Figs 3.7-3.10). Neither food-restriction, nor PUFA-enrichment affected apparent absorption of energy or N, with the exception of PEM rats fed stearic acid, where these parameters were reduced to 55% and 83% respectively. Comparison of the size-matched and age-matched controls indicates that apparent absorption of energy and N did not change with age.

Metabolisable energy of age-matched controls was approximately 290 KJ.day⁻¹, whilst in most malnourished groups it was reduced to about one third of this, at 90-102 KJ.day⁻¹ i.e., similar to the reduction in energy intake (Tables 3.12 and 3.13). The very low metabolisable energy of the low PUFA group of Experiment 1 (55 KJ.day⁻¹), arose mainly from its greatly reduced apparent absorption of energy. The fecal energy output of this group was very large, approximately 5 times higher than that of their PEM littermates.

Metabolisable energy as a % of energy intake, 93% in the age-matched and size-matched controls was reduced to 89-91% by food restriction and PEM.

Some of these reductions were significant.

Within both Experiments 1 and 3, urine energy output of malnourished rats was small and relatively constant, although, it was about 4 times higher in Experiment 3 than in Experiment 1.

Nitrogen balance was positive in all rats (Tables 3.14 and 3.15) i.e., all rats were accumulating N in their tissues. Restriction of both protein and energy intake reduced N balance in both Experiments 1 and 3. Increasing the dietary protein content (food-restricted rats) partially reversed this effect. PUFA-enrichment had a similar but less marked effect in both Experiments 1 and 3 (Tables 3.14 and 3.15). Thus, PUFA-enrichment tended to increase the amount of N accumulating in tissues during PEM. This increased accumulation of N is likely to be associated with increased tissue formation i.e., increased growth.

Net protein utilization of age-matched controls in Experiments 1 and 3 was 28-29%. PEM reduced it to 12-17% in Experiment 1 (except for the high PUFA group which was 30%). In Experiment 3, PEM increased it to 44%. However, unlike the situation for N balance, increasing protein intake did not increase net protein utilization in PEM rats. PUFA-enrichment increased net protein utilization to 47% in PEM rats. Within each of Experiments 1 and 3, the fecal N output of PEM rats was almost identical, thus, increased net protein utilization of PUFA-enriched PEM rats is largely the result of decreased urinary N output. The pattern of apparent biological value of protein (i.e., N balance as a % N absorbed) is similar to that for net protein utilization (Figs. 3.8 and 3.10).

In the size-matched controls, N balance was 30mg N day^{-1} higher and net protein utilization almost twice that of the age-matched controls (Table 3.15).

Discussion

Experiment 2

In Experiment 1, apparent absorption of energy was dramatically reduced when stearic acid (the source of saturated fat) was included in the diet. In Experiment 2, there was almost a complete reversal of a similar effect, measured 4 days after swapping stearic acid for coconut oil. This was a relatively rapid phenomenon. This suggests that in this short-term experiment, it may have been a direct effect of stearic acid per se, rather than a chronic effect of stearic acid on the gut. Interference by stearic acid with apparent absorption of N is not as immediate a phenomenon as with energy (Table 3.4), nor is it as severe as during the long time span involved in Experiment 1. Apparent absorption of both energy and N was increased to levels of control diet rats of Experiment 1 after coconut oil was substituted for stearic acid. The general increase in body mass with the use of coconut oil in the diet and decrease when stearic acid is used (Table 3.4) is consistent with the pattern of energy and N absorption when these two lipids are included in the diets. Although Experiments 1 and 2 were not identical and rigorous comparisons cannot be supported, the above observation is consistent with the finding of Naismith and Qureshi (1962) that the % unsaturation of dietary fatty acids does not affect their absorption by young adult rats.

As was outlined previously, apparent absorption of energy was dramatically reduced in Experiment 1, where stearic acid was included in the diet. The calculated dietary energy intake from stearic acid was 17 KJ.day^{-1} in this group, whereas fecal energy output was 47 KJ.day^{-1} . This output is twice the energy value of the total lipid intake (24 KJ.day^{-1}). Thus, even if none of the energy from stearic acid was absorbed, its presence in the diet has reduced the apparent absorption of energy from other dietary sources. The reduction in

apparent absorption of N in this group indicates that its increased fecal energy content is not due merely to the malabsorption of non-nitrogenous nutrients derived from lipids and carbohydrates, but may also involve the malabsorption of amino acids. Underwood et al, (1967) has reported that that vegetable oil is preferable to butterfat for the treatment of malnourished children, as it provides a better vehicle for the absorption of lipid-soluble nutrients.

Experiment 1 was designed to examine the effects of PEM on a number of parameters and to determine if PUFA-enrichment could counteract any of these effects. Clearly, in Experiment 1, stearic acid was an unsuitable choice as a dietary component to replace linoleic acid.

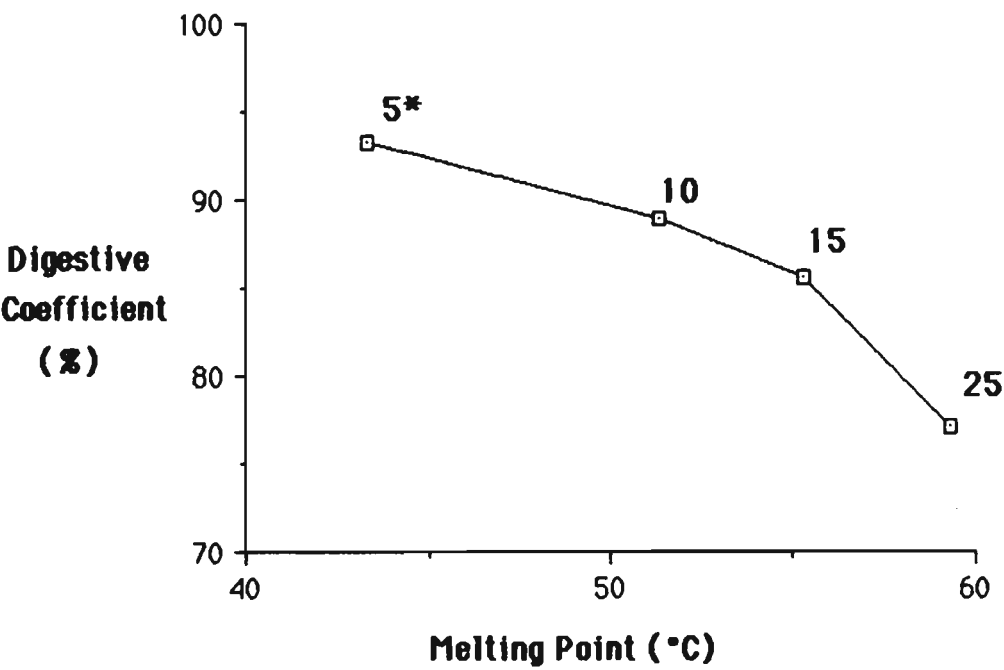
One factor which may possibly contribute to the reduced absorption of energy and N from diets containing stearic acid is the high melting point of stearic acid. Unlike coconut oil which melts at approximately 25°C (CRC handbook of Biochemistry, 1973) and would thus be liquid in the mammalian gut at 37°C. Stearic acid melts at 69.6°C (CRC handbook of Biochemistry, 1973) and, unless dispersed in a low melting point solvent would probably be present in the gut in a semi-solid state. This may interfere with the emulsification and subsequent absorption of stearic acid. The hydrophobic mass of lipid may provide a barrier, firstly between an aqueous solution of digestive enzymes and dietary components, even in the presence of bile and secondly, between digestion products and the absorptive surface of the gut. Increased viscosity of the gut contents may also reduce mixing of the gut contents, effectively producing 'unstirred layers' proximate to the absorptive surface. This would also be expected to reduce apparent absorption.

A search of the literature concerned with digestibility of lipids indicated that this idea had been pursued by others. The apparent absorption of a mixture of olive oil and stearic acid decreases from 93% to 77% when the proportion of stearic acid increases from 5% to 25% (Hoagland and Snider, 1943). Furthermore, (Fig 3.11) drawn from their data shows that an inverse relationship exists between the melting point of each lipid mixture and its apparent absorption. Cheng et al. (1949) found a similar relationship between melting point and digestive coefficient (=apparent absorption; See Drodz 1975; p 349). They also found that factors such as dietary calcium and magnesium levels influence the apparent absorption of lipid mixtures.

Alternatively, it is possible that the general absorptive mechanisms of the gut may not have been greatly affected in either experiments 1 or 2. The reduced apparent absorption of energy and N and increased fecal water loss from rats consuming stearic acid (Experiment 2) may be associated with decreased passage time of food through the gut. Some fats induce a purgative effect (Stobbs, 1989), although diarrhoea was not observed in the present study.

There is considerable variation in the digestibility of fats. Stearic acid is more digestible when ingested as mixed glycerides than as tristearin (Mattil and Higgins, 1945), however, preferential absorption of unsaturated fatty acids over long-chain saturated fatty acids from the gut occurs in the rat (Grigor and Dunkley, 1973). Generally, short-chain fatty acids are better absorbed than long-chain fatty acids, and unsaturated fatty acids are better absorbed than saturated fatty acids (Apgar, et al, 1987). "In the presence of bile, fatty acids are preferentially removed from the sn-1 and sn-3 positions of the triglyceride molecule, leaving a 2-monoglyceride that, having a polar (glycerol) and a nonpolar (fatty acid) end, is itself an excellent emulsifying agent. In all probability, this enhanced ability to participate in micelle formation is

Figure 3.11 Variation of digestive coefficient with melting point of mixtures of olive oil and stearic acid



*** % stearic acid in mixture**
Drawn from data of Hoagland and Snider (1943).

responsible for increased absorption." (Apgar, et al., 1987; p 664). Thus, a fatty acid located in the sn-2 position will be preferentially or more rapidly digested and absorbed. The hydration of unsaturated fatty acids and the preferential absorption of unsaturated fatty acids may be responsible for the higher saturated fatty acid composition of feces compared to the diets fed to rats (Apgar, et al., 1987).

The results of Experiments 1 and 2 indicate that stearic acid was an unsuitable choice as a dietary component because of its effects on apparent absorption of energy and nitrogen. It is possible that malabsorption induced by stearic acid may be involved in other areas of nutrition.

Saturated fatty acids such as palmitic acid are generally regarded as a contributing factor to increased plasma cholesterol (Hegsted, et al., 1965; Keys et al., 1965), although for reasons which are unclear, stearic acid does not exert this effect (Bonanome and Grundy, 1988,1989). Malabsorption of stearic acid has been suggested as one possible explanation for lack of effect of stearic acid on plasma cholesterol (Apgar et al., 1987; Kritchevsky et al., 1987), although other studies suggest that the absorption of stearic acid is similar to that of palmitic acid (Bonanome and Grundy, 1989). However, as acknowledged by Bonanome and Grundy (1989), their method was based on ratios of fatty acids absorbed. Thus, if stearic is poorly absorbed, but also inhibits the absorption of other fatty acids, then it is possible for the ratio of stearic acid to palmitic acid to be similar in dietary and in chylomicron fatty acids, as found by Bonanome and Grundy (1989).

Experiments 1 and 3

The growth pattern of the control rats was characteristically sigmoid, as indicated in the literature (Blaxter, 1989). The pattern of food intake in these rats, initially increasing and then levelling off is consistent with that for growth in body mass (Fig. 3.3). When the control rats were ten weeks old in both Experiments 1 and 3, dietary intake (g food. day⁻¹) was approximately 7% lower in Experiment 3. This is consistent with the 5% higher dietary energy content of Experiment 3, a result of its higher fat content. Similar findings have been reported by others (Harris, 1991).

Food restriction, a 67% reduction in food intake reduced body weight (relative to that of the age-matched controls) by a similar proportion. Other studies in young growing rats indicate that body weight reduction is proportional to the degree of food restriction (Mohan and Narasinger Rao, 1983; Narasinger Rao, 1985). The reduction in body weight was increased by reducing dietary protein content (Fig. 3.5), thus supporting the findings of Mohan and Narasinger Rao (1983). As all groups of malnourished rats consumed the same amount of food, the values of "gross efficiency of food utilization" (i.e., body weight gained/ g food consumed) for each group are implicit in the calculation of change in body weight (Tables 3.7 and 3.9) and have thus not been calculated. A combination of food-restriction and reduced dietary protein content reduced the gross efficiency of food utilization below that found in food-restricted rats. Mohan and Narasinger Rao (1983) reported a similar finding. Although the casein (protein) intake of the PEM rats in the present study was 45% of that of the food-restricted rats, body weight was only 9% smaller. Alling et al., (1974) reported a 50% reduction in body weight of rats when dietary protein content was reduced from 16% to 8% in rats fed *ad libitum* with diets containing 3% of dietary energy as EFA. The reduction in food intake appears to have modulated

the effect of the reduction in dietary protein content. Such an effect would be predicted by Miller and Payne (1963) and Waterlow and Payne (1975), according to the "theory of protein metabolism" (See page 5). An important finding in the present study is that PUFA-enrichment significantly increased gross efficiency of food utilization during PEM.

The BMR of ad lib-fed controls in Experiment 1 is similar to values previously reported for the rat (Schmidt-Nielsen, 1981; Brand et al., 1991), although those in Experiment 3 are slightly higher. The reason for this difference is unclear, although the higher dietary fat content in Experiment 3 may have contributed to increased BMR. Increased metabolic rate has been reported in mice fed a high-fat diet (Richard et al., 1988).

On initial examination, the results appear to suggest that malnutrition increases weight-specific BMR. However, the control rats contained large fat deposits. The metabolic rate of fat is believed to be very small, so these deposits may have reduced the weight-specific metabolic rate of the controls. To deal with this problem, I have estimated the lean body weight of these rats and re-calculated their BMR. I have assumed that body water content is 65% of lean tissue. The calculated lean body mass of the controls is 175g. Re-calculation of weight-specific metabolic rate yields a value of $1.4 \text{ ml O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$. Thus, there appears to have been an adaptive response during food-restriction and PEM to reduce BMR and perhaps reduce energy costs. The existence of such an adaptive response in BMR has been widely accepted (Forsum et al., 1981; Torun and Viteri, 1988), although there are reports where neither food restriction nor PEM have influenced BMR (Mohan and Narasinga Rao, 1983).

During PEM, the aerobic metabolic cost for the whole animal is unaffected by the level of PUFA-enrichment. This indicates that during PEM,

the larger rats on the PUFA-enriched diet are more efficient at using their energy intake and/or they have a smaller mass of metabolically active tissue.

Weight-specific BMR was much higher in the size-matched controls than in the malnourished rats and decreased with age (Table 3.11), although there was a concomitant increase in metabolic rate expressed per rat with age. This has previously been reported by Kleiber et al. (1956). These results are not surprising. Table 3.6 indicates that young rats of the same age as the size-matched controls grow rapidly. As pointed out by Young (1969), a decrease in weight-specific oxygen consumption as body weight increases during growth, has been attributed to the increased proportion of skeletal muscle (Chinn and Hannon 1966) with its lower metabolic rate compared to the viscera (Krebs, 1950; Martin and Fuhrman, 1955 (cited in Young, 1969)). Similarly, the low BMR in malnourished children may be related in part to the increase in relative body proportions of some organs. Brain weight remains largely unchanged by food restriction or PEM. Brain also has a high metabolic rate. In some studies of rats and mice, the *in vitro* oxygen uptake of brain was more than twice that of liver (Kleiber, 1961; Else and Hulbert, 1987; Hulbert and Else, 1981). A large proportion of brain metabolism (over 55%) is "sodium transport-dependent" (Hulbert and Else, 1981; Else and Hulbert, 1987). During starvation, the *in vitro* oxygen uptake of brain, liver and diaphragm muscle are decreased by 50%, 60% and 30% respectively (Kleiber, 1961).

Apparent absorption of energy and to a greater extent N, were reduced by PEM, but not by food-restriction. Thus, during PEM, there was no adaptation to reduced protein and energy intake to improve the efficiency with which available energy and N were absorbed. PUFA-enrichment had no effect on apparent absorption of energy or N. This finding is consistent with that of Naismith and Qureshi (1962), that the % unsat. of dietary fatty acids does not

affect their absorption. Thus, the increased growth resulting from PUFA-enrichment during PEM results from increased retention of absorbed nutrients, rather than improvements in digestive or absorptive efficiency. Apparent absorption of energy and N was reduced to lower levels in experiment 1 than in experiment 2, possibly because the latter measurements occurred over a shorter time span. However, it is unlikely that this difference is an age-related effect, as apparent absorption of energy and N remained relatively unchanged with age in Experiment 3. It is possible that during the 6 week dietary period of Experiment 1, there was a gradual effect of stearic acid on the gut itself. This may have been an indirect effect, mediated via the reduced apparent absorption of energy which precedes it (See Table 3.4), or perhaps by changes in the gut itself, e.g., in fatty acid composition of the gut.

Changes in the weight of the digestive tract (See Ch 5) were compared to changes in apparent absorption of energy and N, to determine whether they could explain the latter. Food restriction reduced the weight of the digestive tract compared to age-matched controls, especially that of the small intestine, but this did not affect the apparent absorption of energy or N. PEM had no significant affect on the weight of either the small intestine or total digestive tract, compared to food restriction. However, PEM reduced the apparent absorption of both energy and N. PUFA-enrichment of PEM rats increased the weight of the digestive tract, especially that of the small intestine, but had no affect on the apparent absorption of energy or N. The weight of the digestive tract of the size-matched controls was significantly smaller than that of the PEM rats, most of this difference arising from the small intestine. However, apparent absorption of energy and N was as high as that in the age-matched controls. Thus, in the present study, there is no clear relationship between the apparent absorption of energy and N and the weight of the digestive tract, in both the normal and malnourished states.

It is possible that PEM reduces the apparent absorption of both nitrogen and energy by reducing the secretion of digestive enzymes. The output of pancreatic enzymes is reduced in children with PEM, (Trowell et al, 1952; Barbezat, 1967). Secretion of gastric acid is also reduced in malnourished children (Gilman, et. al, 1988), although bile output is not reduced in children with PEM (Barbezat, 1967). Karasov and Diamond (1983) predict that the reduction of both sugar and protein intake below minimum energy requirement levels will reduce absorption of both energy and N. If this were the case for the PEM rats in Experiment 3, then both sugar and protein would become caloric sources. Apparent absorption of N was unchanged by food-restriction, but was reduced to 86% by PEM, where it was unaltered by PUFA-enrichment.

Both food restriction and PEM reduce metabolisable energy as a % of energy intake to the same extent (Table 3.13). This reduction is small in relation to the magnitude of the deprivation and during PEM, metabolisable energy as a % of energy intake is not modulated by dietary PUFA content.

The apparent absorption of energy by malnourished rats was greater in Experiment 3 than in Experiment 1. The reason for this increase in apparent absorption and concomitant increase in fecal energy content is unclear, especially as Experiment 3 rats were fed diets of higher energy content (Tables 2.1 and 2.4). The higher urine energy output of Experiment 3 rats may be associated with their higher BMR, compared to those in Experiment 1.

The net protein utilization of the control diet rats decreased with age. The net protein utilization of the size-matched controls was almost twice that of the age-matched controls (Table 3.15). The greater efficiency with which N intake is retained in the younger rats was not the result of a greater apparent

absorption of N (apparent absorption of N in the size-matched controls was the same as that in the age-matched controls). It resulted from a smaller % of the N intake excreted in urine. These results are consistent with the decreased efficiency of protein deposition in growing rats reported by Obled and Arnel (1991). Their physiological significance may be related to the higher protein requirements of early infancy, compared to the end of the first year of life (Naismith, 1975).

The apparent absorption of energy and metabolisable energy as a % of energy intake in size-matched controls are the same as in age-matched controls. However, as energy balance has not been determined, it is unknown whether there was an age-related change in the efficiency with which absorbed energy was retained, as was found for N.

In Experiment 3, apparent absorption of N was not altered by food restriction, but was reduced by PEM. PUFA-enrichment did not improve the apparent absorption of N during PEM. A slight impairment in apparent absorption of N and a high N retention (net protein utilization) was reported in children recovering from kwashiorkor, by Pretorius et al. (1964). In an experiment with similar aims to those of the present study, these authors added sunflower-seed oil to the food of these children. They also found no effect of PUFA-enrichment on apparent absorption of N. However, unlike the present study, they also reported no effect of PUFA-enrichment on N assimilation. In the present study, food restriction increased net protein utilization from 29% to 41%. There is thus a N efficiency adaptation to reduced food intake. However, net protein utilization is not higher in food-restricted rats than in PEM rats, i.e., the adaptation is not modulated by protein intake, at least at these levels of energy and protein intake. The N retention adaptation is enhanced by increased dietary PUFA intake during PEM (Expt. 3). Nitrogen retention has also been

found to be high in acute kwashiorkor and to decrease with treatment (Holemans and Lambrechts, 1955; Hansen et al., 1960; Pretorius et al., 1964; Schendel and Hansen, 1965).

Naismith and Qureshi (1962) reported that N retention in young adult rats was not affected by the degree of saturation or fatty acid composition. Indeed, as a source of energy, fat was completely replaced by carbohydrate in isocaloric diets of weanling rats without affecting N retention (Naismith and Qureshi, 1962). The discrepancy between their results and the increased growth and slight increase in apparent biological value in the present study (Tables 3.9 and 3.15), suggests that the nature of dietary fat may become significant during PEM, even though the efficiency of N retention is increased by PEM (Table 3.15). This suggestion is consistent with the finding of Hill and Holman (1980), that protein deficiency may increase EFA requirements. In a subsequent study concerned with the role of dietary fat in the utilization of protein, Naismith (1962) found that excretion of N in both urine and feces was increased in rats made EFA-deficient. They also found that absorption of energy was not impaired by EFA-deficiency (Naismith, 1962). It is possible that the decreased N retention found in EFA-deficiency may be the result of a disturbance in energy metabolism (Naismith, 1962). Rats fed a fat-free diet, or one poor in EFA have reduced growth and increased metabolic rate (Burr and Burr, 1929; Wesson and Burr, 1931; Rafael et al., 1984). However, the disturbance in energy metabolism is not due to a decrease in energy absorption (Naismith, 1962).

In the study of Pretorius et al. (1964), no significant differences in apparent absorption and retention of nitrogen, calcium, phosphorus magnesium or fat were detected in infants with kwashiorkor when given either milk fat or sunflower seed oil, even though linoleic acid would have constituted 25% calories in the latter group. The results of Pretorius et al. (1964)

are inconsistent with the suggestion of Holman et al. (1981) and Chen and Dickerman (1985) that children with PEM are EFA-deficient and with the general results obtained in the present study with rats.

The major contributor to the increased net protein utilization of the high-PUFA groups of Experiments 1 and 3 was reduced N output via urine. The reduction of some parameters of protein turnover in undernourished children suggests that any adaptation to nutritional deprivation may involve a reduction in protein turnover (Narasinger Rao, 1985). Protein turnover is reported to be lower in malnourished children than in the same children when they recover. Although N balance was positive in all groups of rats, weight losses occurred in some groups in Experiment 1 during the collection of urine and feces. Discrepancies between N balance and body weight data have been reported in the literature. Naismith (1962) suggested that they may be due to losses of fat or water from the body, despite the synthesis of new muscle tissue.

Naismith (1973) suggested that the protein deficiency that leads to kwashiorkor may arise from a severe restriction in energy intake, rather than from the consumption of low-protein foods at adequate or high energy intakes. There is general agreement that successful treatment of PEM usually involves increasing the energy density of food with lipid supplements. Ashworth (1979) has reported low mortality rates during recovery from malnutrition when children are fed milk supplemented with vegetable oil. The lipid is generally regarded as an energy source and little attention has been given to the nature of the lipids. Consequently, a wide variety of lipid supplements has been used, ranging from those high in PUFA e.g., sunflower-seed oil (Pretorius and Wehmeyer, 1964; Pretorius et al., 1964) to those high in saturated fatty acids e.g. coconut oil (Ashworth, 1980).

Recently, Golden (1988b) has suggested that kwashiorkor results from damage caused by free radicals and that increasing the dietary PUFA content during PEM would exacerbate this situation. However, in the present study of PEM in rats, there were no deleterious effects observed in the PUFA-enriched group, which received 20% of its dietary energy as linoleic acid. Furthermore, growth (increase in body weight) was greater if most of the dietary lipid consisted of sunflower oil, rather than coconut oil. Pretorius et. al. (1964) also found no deleterious effects when sunflower-seed oil was fed to children recovering from Kwashiorkor. They also reported that diarrhoea was not increased by sunflower-seed oil. Vitamin E was not added to the PUFA-enriched diets in Experiment 3 of the present study and was not specifically included in the diets prepared by Pretorius et al. (1964). Thus, in both the present study and that of Pretorius et al. (1964), if an increase in free radicals did exert a deleterious effect in the PUFA-enriched groups, then these effects were not immediately obvious. The results of both studies provide no support for Golden's free-radical hypothesis. Contrary to Golden's hypothesis, the present study demonstrates that PUFA-enrichment is beneficial during PEM. Growth is greater with PUFA-enrichment during PEM. This is significant, considering that "One of the main objectives during rehabilitation is to increase the energy intake to achieve maximum weight gain in the shortest possible time." (Golden, 1988b; p105).

Summary.

The percentage of growth retardation in food-restricted rats was approximately the same as the percentage of deprivation that produced it. PEM increased growth retardation.

Apparent absorption of energy, and to a greater extent N, were reduced by PEM, but not by food-restriction. PUFA-enrichment had no effect on apparent absorption of energy or N.

PUFA-enrichment enhanced growth during PEM, especially that of body weight. In doing so, it tended to reverse the extra loss of body weight that occurred over and above that due to food-restriction.

There is no clear relationship between changes in the weight of the digestive tract and those in apparent absorption of energy and N.

The proportion of ingested N retained in the body was greatly increased by food restriction. This adaptation, increased by PEM and further improved by PUFA-enrichment involved an increased retention of N, after it had been absorbed from the gut.

BMR was reduced by food-restriction and PEM. This suggests that the N-retention adaptation may involve decreased energy expenditure. PUFA-enrichment tended to reduce BMR, during PEM, although this effect was very small.

Apparent absorption of energy and N in the size-matched controls was the same as in the age-matched controls.

The proportion of ingested N retained in the body by the size-matched controls was almost twice that of the age-matched controls. This resulted from an increased retention of N, after it had been absorbed.

There were no obvious deleterious effects of PUFA-enrichment, as might be predicted by Golden's free-radical hypothesis. In fact the opposite was found, growth during PEM increased with PUFA-enrichment.

Chapter 4: Fatty Acid Composition of Tissues and Diets

Introduction

Several studies have suggested that children with PEM are EFA-deficient (Holman et al 1981; Chen and Dickerman, 1985). It has also been suggested that that EFA-deficiency may be a component of the aetiology of PEM (Bronte-Stewart, 1961; Naismith, 1973; James, 1977). In this chapter, the effects of malnutrition on the fatty acid composition of phospholipids from selected tissues will be examined. This may indicate whether food-restriction or PEM affects the membrane fatty acid composition of tissues, whether there is evidence of EFA-deficiency and whether these effects may be reversed by PUFA-enrichment.

Methods and Materials

Fatty Acid Analysis of Tissues and Diets

All fatty acid analyses were carried out on phospholipids, except for the diets and plasma, where the fatty acid composition of total lipids was determined. Samples were stored in liquid nitrogen until fatty acid analysis.

Lipids were extracted according to a modification of the method of Folch et al. (1957). Approximately 0.1 g tissue was homogenized in 25 ml chloroform (HPLC grade: Mallinckrodt): methanol (HPLC grade: Unichrom/Ajax) (2:1, v/v) with the antioxidant 2,6-ditert-butyl-p-cresol [BHT] (Sigma) 0.01% using an IKA Ultra Turrax T25 with S25N 8G dispersing rotor @ 2400 rpm. NaCl (5 ml of 0.73%) was added to the filtered (Whatman #1) lipid solution. Lipids were extracted with 3 x 1 ml additions of chloroform. Combined extracts were dried with Na₂SO₄ (anhydrous), filtered (Whatman #1) and excess chloroform evaporated under vacuum, using a Rotavapor-R (Buchi).

Neutral lipids and free fatty acids were eluted through silicic acid columns with 8 ml chloroform and discarded, then phospholipids were eluted with 8 ml methanol into teflon-lined screw-cap test tubes. 2 ml 14% BF_3 in methanol (Merck) was added, the tubes capped and heated at 75°C for 1 h. Samples were cooled with ice and methylation stopped with 3 ml water. Methylated fatty acids were extracted with 3 x 1 ml petroleum spirit, dried with Na_2SO_4 (anhydrous) and filtered (Whatman #1). The purity of fractions collected by this method has been checked in this laboratory.

Impurities were eluted through 60-100 # hydrated florisisil (florisisil+7% water) (Floridin) columns with 8 ml petroleum spirit and methyl esters eluted with 7 ml 5% diethyl ether (Mallinckrodt) in petroleum spirit. Methyl esters were evaporated to dryness under nitrogen and dissolved in 100 μl n-hexane (Nanograde: Mallinckrodt).

In Experiment 1, 1 μl methyl esters in hexane was injected into a Packard 427 gas chromatograph fitted with a 0.22 μm i.d. x 25m fused silica WCOT column (SGE) and flame ionization detector. Column temperature was maintained at 170°C for 20 min and then raised @ $1^\circ\text{C}/\text{min}$ to 195°C . In Experiment 3, 1 μl methyl esters in hexane was injected into a Varian 3300 gas chromatograph fitted with an SGE BPX70 column (25m X 0.22mm I.D.) lined with a fully cross-linked polar stationary phase (0.25 μm) and flame ionization detector. Column temperature was maintained at 170°C for 10 min and then raised @ $2^\circ\text{C}/\text{min}$ to 190°C .

Peak area, retention time and relative composition (weight %) of each fatty acid was determined (threshold value 0.5%) with a Shimadzu C-R3A chromatopac integrator. Fatty acids were identified by reference to standard methyl esters (Alltech, Sigma and Supelco).

Various parameters of fatty acid composition were then calculated. Calculation of "unsaturation index" has been outlined previously (Withers and Hulbert, 1987) and is an indication of the number of double bonds per 100 fatty acid molecules.

Long-term storage may render lipids more susceptible to peroxidation and hydrolysis (Kates, 1986). To determine whether phospholipid fatty acid composition was altered by long-term storage in liquid N₂, liver samples from one rat, (Wistar strain) from the stock colony (Biology Department, University of Wollongong) which were fed a standard rat diet were analysed immediately after decapitation and again after 6 months storage in liquid N₂. Fatty acid analyses of tissue and diet samples in experiments 1 and 3 were carried out after less than 6 months storage in liquid N₂.

RESULTS

Effects of Storage on Fatty Acid Composition

The phospholipid fatty acid composition of fresh liver and that stored for 6 months in liquid N₂ is shown in Table 4.1. There were no significant changes in fatty acid composition after storage in liquid N₂ for 6 months. This indicated that samples may be stored for up to six months before fatty acid analysis.

Fatty Acid Composition of Diets

In experiment 1 all of the dietary PUFA was 18:2 w6 (Table 4.2). In Experiment 3, there was a small amount of 18:3 w3, as well as the 18:2 w6 in some of the diets. However, in these diets, the ratio of 18:2 w6 to 18:3 w3 ranged from 61 to 77 (Table 4.3).

Fatty Acid Composition of Tissues

Food-restriction and PEM affected the concentration of whole families of fatty acids, as well as that of individual fatty acids. The effects were more numerous and of greater magnitude in some tissues than in others. The direction of the change also varied between tissues. Although some of the between-group differences were large, there was considerable variability within each dietary group. Thus, many of the differences between groups are not statistically significant, although the broad effects on whole families of fatty acids suggests that they may be physiologically important. For this reason, I intend to draw attention to these effects where appropriate, even if they are not statistically significant. Although data from the low-PUFA group of Experiment 1 are included with other results, it will be referred to only briefly in the text of

Table 4.1 **Effects of storage in liquid N₂ for six months on fatty acid composition of rat liver phospholipids.**

Fattyacid	Fresh liver		Liver stored in liquid N ₂	
14:0 ^a	0.7	±0.2	0.7	±0.3
15:0	0.4	±0.1	0.1	±0.1
16:0	21.1	±0.5	19.8	±0.6
16:1 w7	0.3	±0.2	0.1	±0.1
d	0.1	±0.1	—	—
e	0.8	±0.1	0.8	±0.0
18:0	22.1	±0.2	22.4	±0.4
18:1 w9	5.4	±0.1	5.6	±0.4
18:1 w7	3.4	±0.1	3.8	±0.2
18:2 w6	14.2	±0.1	14.1	±0.2
20:2	—	—	0.1	±0.1
20:3 w9	0.1	±0.1	—	—
20:3 w6	0.4	±0.1	0.5	±0.1
20:4 w6	25.0	±0.4	26.0	±0.4
22:4 w6	0.2	±0.1	—	—
22:5 w6	0.1	±0.1	—	—
22:5 w3	0.8	±0.1	0.9	±0.1
22:6 w3	4.9	±0.2	5.1	±0.2
% sat FA	44.3	±0.5	42.9	±0.7
% unsat FA	54.8	±0.5	56.2	±0.7
% monoenes	9.1	±0.4	9.5	±0.5
% polyenes	45.6	±0.7	46.7	±0.5
unsat. index	173.4	±2.9	178.6	±2.5
av. chain length	18.1	±0.0	18.2	±0.1
w-3 acids	5.7	±0.2	6.0	±0.2
w-6 acids	39.8	±0.5	40.6	±0.3
w-9 acids	5.6	±0.1	5.6	±0.4
	n=10		n=5	
Values are weight %; Mean ± SEM				
a Carbon N°: N° of double bonds				
d, e unidentified fatty acids				

Table 4.2 **Fatty acid composition of diets: Experiment 1.**

PUFA content Fattyacid	Control		Protein-energy malnourished			
			Low		Medium	High
16:0 ^a	11.5	±1.5	7.9	±0.2	13.2	9.2
18:0	4.1	±0.2	79.7	±1.3	43	2.7
18:1 w9	37.7	±0.3	6.1	±0.5	37.9	20.7
18:2 w6	45.4	±0.4	5.6	±0.5	44.6	67.4
20:0	0.4	±0.4	0.8	±0.1	-	-
22:0	0.6	±0.6	-	-	-	-
	n=2		n=2		n=1	n=1

a Carbon N°: N° of double bonds)
Values are weight %; Mean ± SEM

Table 4.3 Fatty acid composition of diets: Experiment 3.

Fatty acid	Control diet		PEM diet		PEM diet PUFA-enriched	
10:0 ^a	8.0	±0.5	6.1	±1.1	—	
12:0	55.0	±1.3	55.1	±1.1	—	
14:0	15.6	±0.1	17.1	±0.1	—	
16:0	6.8	±0.2	7.1	±0.1	6.6	±0.0
18:0	2.3	±0.1	2.1	±0.1	2.9	±0.0
18:1 w9	6.0	±0.4	6.1	±1.1	28.3	±0.1
18:2 w6	6.3	±1.0	6.1	±1.1	61.4	±0.1
18:3 w3	—		0.1	±0.1	0.8	±0.0
	n=3		n=3		n=4	

^a Carbon N°: N° of double bonds
Values are weight %; Mean ± SEM

this and subsequent chapters. Apparent absorption of energy and N were greatly reduced in this group. I have omitted this group, otherwise, the degree of functional food deprivation is not comparable between deprived groups.

A surprising finding, was that food-restriction and PEM increased, the 18:2 w6 concentration in plasma (Tables 4.4). The pattern of total w6 concentration was more varied, with a tendency for increases in some tissues and decreases or no change in others. An exception was the low-PUFA group of Experiment 1, where PEM reduced both the total w6 and 18:2 w6 concentration in all of the tissues analysed, except for heart, where both parameters were relatively unchanged. However, many of these changes were not statistically significant,

Not surprisingly, PUFA-enrichment increased the w6 content of all tissues, especially their 18:2 w6 content. An exception was the erythrocytes, where w6 content was decreased and w9 content increased. PUFA-enrichment also decreased the w9 concentration, especially that of 20:3 w9.

Food-restriction and PEM increased the concentration of w9 fatty acids in all tissues, except in brain and heart, where it was decreased slightly. Small concentrations of 20:3 w9, an indication of EFA deficiency were detected in liver and gastrocnemius as a result of both food-restriction and PEM. This suggests that these tissues may be marginally EFA-deficient. Small concentrations of 20:3 w9 were also detected in liver and kidney and to a smaller extent heart in the low-PUFA group of Experiment 1, suggesting that these too, were marginally EFA-deficient.

Food restriction had little effect on the % unsaturation (% unsat.), unsaturation index (U.I.) or average chain length (Ave CL), except in plasma,

Table 4.4 Effects of PEM, food-restriction and PUFA-enrichment on plasma fatty acid composition: Experiment 3.

Fatty Acid	Control Diet			PEM		PEM PUFA-enriched	
	Size-matched	Age-matched	Food-restricted				
12:0 ^a	3.8 ±0.6	1.7 ±0.6	2.0 ±0.6	1.6 ±0.2		— ^{wxyz}	
14:0	3.9 ±0.5	2.3 ±0.3	3.2 ±0.2	2.8 ±0.5		0.8 ^{wxyz}	±0.4
14:1 w5	0.7 ±0.2	—	0.3 ±0.3	0.2 ±0.2		0.3 ±0.3	
16:0	22.8 ±0.8	20.2 ±1.2	24.2 ±0.7	23.9 ±1.2		23.9 ±1.6	
16:1 w7	4.0 ±0.4	2.8 ±0.5	2.9 ±0.3	2.9 ±0.3		1.7 ^{wxyz}	±0.3
18:0	14.9 ±0.6	17.0 ^{yz} ±1.2	14.0 ^x ±0.5	13.8 ^x ±0.9		13.1 ^x ±0.4	
18:1 w9	11.6 ^{yz} ±0.9	11.4 ^{yz} ±1.6	15.1 ^{wx} ±0.5	14.6 ^{wx} ±0.5		10.6 ^{yz} ±0.6	
18:1 w7	1.5 ±0.1	1.4 ±0.2	1.4 ±0.1	1.3 ±0.0		0.9 ^{wxyz}	±0.0
18:2 w6	9.3 ^x ±1.0	6.9 ^{wyz} ±0.8	9.6 ^x ±0.1	9.0 ^x ±0.4		19.7 ^{wxyz}	±1.4
20:4 w6	24.8 ±2.7	31.6 ±2.7	25.0 ±0.9	26.1 ±0.6		26.2 ±1.9	
22:0	—	0.7 ±0.4	0.2 ±0.2	0.2 ±0.2		0.2 ±0.2	
22:5 w6	2.5 ±0.2	2.7 ±0.5	2.0 ±0.2	2.7 ±0.2		2.1 ±0.2	
22:5 w3	—	0.4 ±0.3	—	0.5 ±0.2		0.2 ±0.2	
22:6 w3	1.1 ±0.1	0.7 ±0.3	0.2 ±0.2	0.3 ±0.2		0.2 ±0.2	
% unsat. FA	55.4 ±1.0	58.0 ±2.0	56.4 ±0.5	57.7 ±1.1		62.0 ±1.6	
% monoenes	17.7 ±1.1	15.6 ^{yz} ±2.1	19.7 ^x ±0.8	19.0 ^x ±0.3		13.5 ^{wyz}	±0.9
% polyenes	37.7 ±2.0	42.4 ±2.7	36.7 ±1.2	38.7 ±1.1		48.5 ^{wyz}	±2.2
unsat. index	154.4 ±8.8	175.9 ±11.3	149.7 ±4.6	159.8 ±4.4		170.8 ±9.3	
w-3 acids	1.1 ±0.1	1.2 ±0.5	0.2 ±0.2	0.8 ±0.3		0.5 ±0.5	
w-6 acids	36.6 ±2.0	41.2 ±2.4	36.6 ±1.1	37.9 ±0.9		48.0 ^{wxyz}	±1.8
w-9 acids	11.6 ^y ±0.9	11.4 ^{yz} ±1.6	15.1 ^{wx} ±0.5	14.6 ^{wx} ±0.5		10.6 ^{yz} ±0.6	
av chain length	17.8 ±0.1	18.2 ±0.2	17.8 ±0.1	17.9 ±0.1		18.1 ±0.1	
N°	4	4	4	4		4	

Values are weight %; Mean ± SEM
a Carbon N°: N° of double bonds
w Significantly different from Size-matched control (p<0.05)
x Significantly different from Age-matched control (p<0.05)
y Significantly different from PEM (p<0.05)
z Significantly different from Food-restricted (p<0.05)

Table 4.5 Effects of PEM and PUFA-enrichment on fatty acid composition of liver phospholipids: Experiment 1.

Liver PUFA content	Control	Protein-energy malnourished		
		Low	Medium	High
14:0 ^a	—	—	0.2 ±0.2	—
c	0.6 ±0.2	0.2 ±0.1	0.8 ±0.4	0.4 ±0.3
16:0	15.5 ^{yz} ±1.6	20.6 ^x ±0.9	22.1 ^x ±1.2	19.5 ^x ±0.8
16:1 w7	0.4 ±0.2	2.8 ^{xy} ±0.4	1.4 ±0.4	0.9 ±0.5
18:0	27.0 ^{yz} ±1.6	21.4 ^x ±1.5	20.4 ^x ±0.6	22.4 ^x ±0.4
18:1 w9	7.0 ^z ±0.7	13.1 ^{xyz} ±0.9	9.9 ^{xy} ±0.5	6.8 ^z ±0.4
18:1 w7	3.1 ^{yz} ±0.3	2.6 ±0.3	2.2 ^x ±0.1	2.1 ^x ±0.1
18:2 w6	11.5 ^y ±0.7	8.4 ^{xyz} ±0.6	12.8 ^y ±0.6	15.4 ^{yz} ±0.7
18:3 w6	—	0.1 ±0.1	0.2 ±0.1	0.3 ±0.2
20:3 w9	—	1.5 ^{xyz} ±0.2	—	—
20:3 w6	0.5 ±0.2	0.8 ±0.2	0.7 ±0.0	0.3 ±0.2
20:4 w6	30.5 ^{yz} ±0.7	23.1 ^{xy} ±0.9	24.9 ^x ±1.0	26.1 ^x ±0.4
22:4 w6	0.5 ±0.2	0.7 ±0.1	0.6 ±0.1	0.9 ±0.2
22:5 w6	2.1 ±0.7	3.1 ±0.3	2.5 ±0.3	3.0 ±0.5
22:5 w3	—	0.3 ±0.3	—	—
22:6 w3	1.3 ±0.3	1.2 ±0.3	1.5 ±0.2	1.9 ±0.3
% unsat. FA	57.0 ±0.6	57.8 ±1.3	56.6 ±0.9	57.7 ±0.4
% monoenes	10.5 ^z ±0.8	18.5 ^{xyz} ±1.5	13.4 ^{xy} ±0.7	9.8 ^z ±0.8
% polyenes	46.5 ^z ±0.8	39.2 ^{xyz} ±0.4	43.2 ^{xy} ±1.1	47.9 ^z ±0.8
unsat. index	177.6 ^z ±5.0	162.1 ^{xy} ±2.1	165.1 ^x ±5.3	176.9 ^z ±2.0
w-3 acids	1.3 ±0.3	1.5 ±0.1	1.5 ±0.2	1.9 ±0.3
w-6 acids	45.2 ^z ±0.6	36.2 ^{xyz} ±0.5	41.7 ^{xy} ±1.0	46.1 ^z ±0.9
w-9 acids	7.0 ^z ±0.7	14.6 ^{xyz} ±1.1	9.9 ^{xy} ±0.5	6.8 ^z ±0.4
av chain length	18.5 ^z ±0.1	18.3 ^x ±0.0	18.2 ^x ±0.1	18.4 ±0.0
N°	5	5	6	5

Values are weight %; Mean ± SEM

a Carbon N°: N° of double bonds

c Unidentified fatty acid

x Significantly different from Control (p<0.05)

y Significantly different from High PUFA (p<0.05)

z Significantly different from Medium PUFA (p<0.05)

Table 4.6

Effects of PEM, food-restriction and PUFA-enrichment on fatty acid composition of liver phospholipids: Experiment 3.

Fatty Acid	Control Diet			PEM		PEM PUFA-enriched	
	Size-matched	Age-matched	Food-restricted				
12:0 ^a	0.5 ±0.2	0.5 ±0.1	0.2 ±0.2	–	–	0.1 ±0.1	–
14:0	1.7 ±0.1	0.7 ±0.2	1.8 ±1.3	0.5 ±0.1	–	0.2 ±0.2	–
14:1 w5	–	–	1.4 ±1.2	0.2 ±0.2	–	–	–
16:0	19.3 ±1.1	16.1 ±0.5	14.2 ±2.0	20.0 ±2.3	–	16.3 ±1.4	–
16:1 w7	2.9 ^x ±0.2	1.3 ^w ±0.3	2.3 ±0.5	2.3 ±0.8	–	0.7 ^{wyz} ±0.3	–
18:0	23.4 ±1.4	27.3 ±2.1	22.4 ±3.7	22.6 ±1.6	–	27.4 ±1.4	–
18:1 w9	9.9 ^x ±0.9	6.2 ^{wy} ±0.7	8.0 ±1.3	8.2 ^x ±0.3	–	5.5 ^{wy} ±0.4	–
18:1 w7	1.9 ±0.1	2.0 ±0.3	1.8 ±0.1	1.5 ±0.1	–	1.3 ±0.2	–
18:2 w6	6.1 ±0.2	6.9 ±1.4	8.4 ±0.9	7.5 ±0.8	–	10.9 ±1.7	–
20:2 w6	–	0.1 ±0.1	0.7 ±0.7	–	–	–	–
20:3 w9	0.1 ^{yz} ±0.1	– ^{yz}	0.6 ^{wx} ±0.2	0.7 ^{wx} ±0.1	–	– ^{yz}	–
20:3 w6	0.5 ±0.1	0.4 ±0.2	0.8 ±0.3	0.7 ±0.1	–	0.1 ±0.1	–
20:4 w6	23.5 ±1.2	27.3 ±1.8	26.3 ±1.1	22.5 ±1.3	–	25.4 ±1.6	–
22:4 w6	0.7 ±0.2	1.2 ±0.2	0.8 ±0.3	0.9 ±0.1	–	1.7 ±0.2	–
22:5 w6	6.2 ±0.4	7.3 ±0.7	7.4 ±1.1	6.6 ±0.9	–	7.1 ±0.6	–
22:5 w3	0.1 ±0.1	–	0.4 ±0.2	0.1 ±0.1	–	0.1 ±0.1	–
22:6 w3	3.4 ^{yz} ±0.2	2.6 ^y ±0.3	2.1 ^w ±0.2	1.7 ^{wx} ±0.2	–	2.5 ^{wy} ±0.4	–
24:1 w9	–	0.1 ±0.1	0.4 ±0.4	–	–	0.5 ±0.2	–
% unsat. FA	55.1 ±1.2	55.5 ±2.6	61.4 ±4.0	52.9 ±3.3	–	55.9 ±2.7	–
% monoenes	14.7 ^x ±1.1	9.6 ^w ±0.9	13.9 ±3.3	12.2 ±1.1	–	8.0 ^{wyz} ±0.8	–
% polyenes	40.4 ±1.4	45.9 ±3.1	47.5 ±2.1	40.8 ±3.1	–	47.9 ±2.9	–
unsat. index	176.7 ±5.3	191.0 ±11.2	196.3 ±9.5	168.8 ±12.0	–	189.6 ±10.8	–
w-3 acids	3.5 ^{xyz} ±0.2	2.6 ^{wy} ±0.3	2.5 ^{wy} ±0.1	1.8 ^{wxz} ±0.2	–	2.6 ^{wy} ±0.2	–
w-6 acids	36.8 ±1.2	43.3 ±2.9	44.4 ±2.0	38.3 ±2.9	–	45.2 ±2.7	–
w-9 acids	10.1 ^x ±0.9	6.3 ^{wyz} ±0.6	9.0 ^x ±1.8	8.9 ^x ±0.4	–	6.0 ^{wyz} ±0.3	–
av chain length	18.4 ±0.1	18.6 ±0.1	18.5 ±0.1	18.4 ±0.1	–	18.6 ±0.1	–
N ^a	5	5	4	5	–	4	–

Values are weight %; Mean ± SEM

a Carbon N^a: N^a of double bonds

w Significantly different from Size-matched control (p<0.05)

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

z Significantly different from Food-restricted (p<0.05)

Table 4.7 Effects of PEM and PUFA-enrichment on fatty acid composition of brain phospholipids: Experiment 1.

PUFA content	Control		Protein-energy malnourished					
			Low		Medium		High	
15:0 ^a tentative	1.4	±0.9	1.4	±0.7	2.2	±0.8	1.4	±0.9
c	2.8	±0.9	5.6	±2.2	1.2	±0.5	2.2	±0.9
16:0	11.2	±1.1	9.8	±0.9	10.6	±0.4	9.7	±1.0
16:1 w7	0.1	±0.1	0.6	±0.3	—		0.1	±0.1
17:0 tentative	—		0.3	±0.3	—		—	
e	2.0	±1.1	3.7 ^{yz}	±1.4	—		—	
f	4.1	±0.9	3.9	±0.7	4.8	±0.6	4.9	±0.8
g	2.2	±0.5	2.1	±0.1	1.9	±0.1	1.8	±0.3
18:0	24.4	±0.7	22.7 ^{yz}	±1.3	25.4	±0.3	26.4	±1.0
18:1 w9	17.6	±1.3	16.2	±0.9	16.5	±1.4	14.1	±0.4
18:1 w7	3.7	±0.4	3.4	±0.5	3.5	±0.3	2.6	±0.1
18:2 w6	0.9	±0.1	0.4 ^{xyz}	±0.2	0.9	±0.0	1.1	±0.0
20:0	0.1	±0.1	—		—		—	
20:1	1.8	±0.5	1.5	±0.4	1.3	±0.4	1.0	±0.1
20:2	0.2	±0.2	0.1	±0.1	0.2	±0.1	—	
20:4 w6	11.8 ^y	±1.0	11.6 ^y	±0.7	13.2	±0.6	14.9 ^x	±0.6
22:0	—		0.1	±0.1	—		—	
22:4 w6	3.3	±0.4	3.0	±0.2	3.5	±0.1	3.8	±0.3
22:5 w6	2.9	±0.5	2.4	±0.2	2.6	±0.2	3.4	±0.2
22:6 w3	9.7	±1.0	11.2	±0.3	12.3	±0.6	12.5	±0.6
24:0	—		0.1	±0.1	—		—	
% unsat. FA	51.9	±1.3	50.5	±2.0	53.9	±1.0	53.6	±1.9
% monoenes	23.2	±1.9	21.7	±1.7	21.3	±2.0	17.8	±0.6
% polyenes	28.7	±2.7	28.8	±1.7	32.7	±1.1	35.7	±1.6
unsat. index	158.0	±11.7	160.5	±8.1	176.9	±4.1	187.1	±8.0
w-3 acids	9.7	±1.0	11.2	±0.9	12.3	±0.6	12.5	±0.6
w-6 acids	18.8 ^y	±1.8	17.5 ^y	±1.0	20.2	±0.8	23.2 ^x	±1.0
w-9 acids	17.6	±1.3	16.2	±0.9	16.5	±1.4	14.1	±0.4
av chain length	18.7	±0.1	18.8	±0.1	18.8	±0.1	19.0	±0.1
N ^a	6		5		6		5	

Values are weight %; Mean ± SEM

a Carbon N^a: N^a of double bonds

c, e, f, g Unidentified fatty acids

x Significantly different from Control (p<0.05)

y Significantly different from High PUFA (p<0.05)

z Significantly different from Medium PUFA (p<0.05)

Table 4.8 Effects of PEM, food-restriction and PUFA-enrichment on fatty acid composition of brain phospholipids: Experiment 3.

Fatty Acid	Control Diet			PEM		PEM PUFA-enriched	
	Size-matched	Age-matched	Food-restricted				
c ^a	3.8 ±0.1	2.8 ±0.4	3.5 ±0.5	3.1 ±0.3	3.4 ±0.1		
16:0	8.8 ±1.4	10.1 ±2.8	7.3 ±0.5	6.8 ±0.7	7.0 ±0.8		
e	5.2 ±0.5	4.4 ±1.0	5.5 ±0.1	5.1 ±0.3	5.4 ±0.3		
f	2.0 ±0.5	2.5 ±0.4	1.2 ±0.6	2.4 ±0.5	2.4 ±0.6		
g	0.9 ±0.6	1.8 ±0.4	2.2 ±0.7	2.0 ±0.5	1.8 ±0.5		
18:0	26.3 ±4.0	21.3 ±1.1	28.9 ±2.4	21.4 ±1.5	24.4 ±3.3		
18:1 w9	12.6 ±1.4	16.0 ±0.8	13.2 ±1.1	15.4 ±2.0	13.9 ±1.9		
18:1 w7	2.0 ±0.2	3.0 ±0.2	2.1 ±0.1	2.7 ±0.4	2.2 ±0.4		
18:2 w6	0.8 ±0.2	0.3 ±0.2	0.4 ±0.2	0.3 ±0.1	1.0 ±0.0		
20:0 w6	0.2 ±0.2	0.7 ±0.3	—	0.4 ±0.2	0.3 ±0.3		
20:1 w9	0.8 ±0.8	2.8 ±0.8	1.2 ±0.2	2.8 ±1.0	2.0 ±1.2		
20:1 tentative	0.2 ±0.2	0.6 ±0.3	—	0.3 ±0.3	0.4 ±0.4		
20:4 w6	13.4 ±1.4	9.9 ±0.9	12.4 ±0.8	12.8 ±1.6	11.7 ±1.6		
22:0	0.2 ±0.2	0.8 ±0.2	—	0.2 ±0.2	0.3 ±0.3		
22:4 w6	4.7 ±0.5	4.1 ±0.2	4.3 ±0.5	4.9 ±0.4	4.7 ±0.4		
22:5 w6	4.1 ±0.3	4.7 ±0.6	4.7 ±0.8	5.9 ±1.0	4.8 ±1.0		
22:5 w3	0.3 ±0.3	1.3 ±0.5	0.5 ±0.3	0.6 ±0.3	0.9 ±0.4		
22:6 w3	13.0 ±1.2	11.0 ±1.5	12.0 ±2.2	12.1 ±1.5	12.3 ±2.1		
24:1 w9	0.4 ±0.4	1.8 ±0.6	0.5 ±0.3	1.0 ±0.4	1.1 ±0.8		
% unsat. FA	52.4 ±3.6	55.6 ±1.5	51.3 ±3.0	58.7 ±0.6	54.9 ±2.7		
% monoenes	15.9 ±2.8	23.6 ±2.0	17.0 ±1.4	21.9 ±3.7	19.2 ±4.2		
% polyenes	36.4 ±2.6	31.4 ±2.7	34.2 ±3.7	36.6 ±4.0	35.3 ±4.6		
unsat. index	190.3 ±12.8	177.2 ±12.1	182.3 ±19.1	198.5 ±15.8	189.2 ±19.3		
w-3 acids	13.4 ±1.3	12.4 ±1.4	12.5 ±2.1	12.7 ±1.2	13.2 ±1.7		
w-6 acids	23.0 ±1.5	19.0 ±1.6	21.8 ±1.6	23.9 ±2.9	22.2 ±2.8		
w-9 acids	13.8 ±2.6	20.6 ±1.9	14.9 ±1.2	19.2 ±3.4	17.0 ±3.9		
av chain length	19.2 ±0.2	19.2 ±0.1	19.1 ±0.2	19.4 ±0.1	19.3 ±0.1		
N ^a	5	5	4	5	4		

Values are weight %; Mean ± SEM
a Carbon N^a: N^a of double bonds
c, e, f, g Unidentified fatty acids

Table 4.9 **Effects of PEM and PUFA-enrichment on fatty acid composition of kidney phospholipids: Experiment 1.**

PUFA content	<u>Control</u>		<u>Protein-energy malnourished</u>					
			Low		Medium		High	
14:0 ^a	0.1	±0.1	0.6	±0.3	0.4	±0.2	0.3	±0.2
14:1 w5	—		0.1	±0.1	0.1	±0.1	0.1	±0.1
15:0 tentative	2.2	±0.4	2.8	±0.4	3.0	±0.2	3.1	±0.4
c	6.5	±2.7	1.9	±0.5	2.5	±0.6	2.3	±0.7
16:0	14.5	±0.4	13.9	±2.3	16.2	±1.8	15.0	±1.0
16:1 w7	3.3	±2.3	1.8	±0.3	0.8	±0.3	0.2	±0.2
f	1.5 ^y	±0.1	2.2 ^{yz}	±0.2	1.8	±0.1	2.0 ^x	±0.2
g	1.3	±0.1	1.1	±0.1	1.3	±0.1	1.1	±0.1
18:0	20.7	±0.8	24.0	±1.5	21.4	±0.8	21.3	±0.6
18:1 w9	8.2	±0.4	9.6 ^{xy}	±0.7	9.0 ^y	±0.3	7.7 ^z	±0.2
18:1 w7	2.3	±0.1	2.4	±0.2	2.4	±0.1	2.5	±0.1
18:2 w6	9.2 ^{yz}	±0.5	8.8 ^{yz}	±0.5	11.2 ^{xy}	±0.1	13.0 ^{yz}	±0.3
20:3 w9	—		0.8 ^{xyz}	±0.2	—		—	
20:3 w6	0.3	±0.2	0.8	±0.2	0.6	±0.1	0.6	±0.2
20:4 w6	28.5	±1.9	27.4	±2.4	28.5	±2.0	29.5	±0.7
22:4 w6	0.4	±0.2	0.8	±0.3	0.3	±0.2	0.6	±0.2
22:5 w6	0.7	±0.2	0.8	±0.3	0.3	±0.2	0.7	±0.2
22:5 w3	—		0.2	±0.2	—		—	
22:6 w3	0.3	±0.2	0.1	±0.1	0.1	±0.1	—	
% unsat. FA	53.3	±2.6	53.5	±2.0	53.3	±1.7	54.9	±0.7
% monoenes	13.8	±1.9	13.9	±1.2	12.4	±0.7	10.5	±0.4
% polyenes	39.5	±2.6	39.6	±3.2	40.9	±2.3	44.4	±1.0
unsat. index	154.1	±9.7	154.2	±12.5	153.5	±8.9	162.2	±3.8
w-3 acids	0.3	±0.2	0.3	±0.3	0.1	±0.1	—	
w-6 acids	39.2	±2.5	38.4	±2.7	40.8	±2.3	44.4	±1.0
w-9 acids	8.2	±0.4	10.4 ^{xyz}	±0.6	9.0 ^y	±0.3	7.7 ^z	±0.2
av chain length	18.2	±0.1	18.2	±0.2	18.2	±0.1	18.3	±0.1
N°	6		5		6		5	

Values are weight %; Mean ± SEM
a Carbon N°: N° of double bonds
c, f, g Unidentified fatty acids
x Significantly different from Control (p<0.05)
y Significantly different from High PUFA (p<0.05)
z Significantly different from Medium PUFA (p<0.05)

Table 4.10 Effects of PEM, food-restriction and PUFA-enrichment on fatty acid composition of gastrocnemius phospholipids: Experiment 3.

Fatty Acid	Control Diet			PEM	
	Size-matched	Age-matched	Food-restricted		PEM PUFA-enriched
12:0 ^a	1.8 ±0.4	1.8 ±0.8	0.9 ±0.3	0.5 ±0.2	0.1 ^{wx} ±0.1
14:0	2.1 ^y ±0.3	2.1 ^y ±0.4	1.3 ±0.2	0.8 ^{wx} ±0.3	0.5 ^{wx} ±0.1
c	1.1 ±0.2	1.3 ±0.4	1.5 ±0.1	1.7 ±0.6	1.7 ±0.4
16:0	14.5 ^x ±0.5	19.6 ^{wyz} ±1.5	12.2 ^x ±1.4	13.1 ^x ±1.3	14.0 ^x ±1.6
16:1 w7	2.3 ^{yz} ±0.4	1.5 ±0.2	1.2 ^w ±0.1	1.3 ^w ±0.2	0.9 ^{wx} ±0.2
18:0	20.0 ±1.1	24.7 ±3.3	24.1 ±1.3	25.5 ±1.5	24.2 ±1.1
18:1 w9	12.7 ±0.9	9.0 ±0.8	12.2 ±0.6	10.9 ±1.4	11.2 ±1.9
18:1 w7	1.9 ±0.0	2.3 ^{yz} ±0.4	1.6 ^x ±0.2	1.4 ^x ±0.1	1.4 ^{wx} ±0.1
18:2 w6	13.0 ^y ±0.9	10.5 ±0.7	11.8 ±1.1	9.8 ^w ±0.8	14.5 ^{xy} ±1.0
20:3 w9	0.7 ^x ±0.2	— ^{wyz}	0.6 ^x ±0.0	0.6 ^x ±0.1	— ^{wyz}
20:3 w6	0.8 ^x ±0.0	0.4 ^w ±0.2	0.6 ±0.0	0.6 ±0.0	0.2 ^{wyz} ±0.1
20:4 w6	16.0 ±1.2	16.1 ±2.7	18.1 ±1.3	19.7 ±2.3	16.5 ±2.0
22:0	—	0.6 ±0.6	0.3 ±0.3	0.4 ±0.3	0.4 ±0.2
22:4 w6	2.6 ±0.1	1.9 ±0.7	2.5 ±0.3	2.1 ±0.5	2.8 ±0.4
22:5 w6	4.1 ±0.2	6.3 ±1.3	6.3 ±0.7	6.9 ±0.9	5.5 ±0.9
22:6 w3	4.9 ±0.3	1.8 ±0.7	4.0 ±0.8	4.0 ±0.9	4.8 ±1.2
24:1 w9	1.6 ^{xy} ±0.1	0.2 ^{wyz} ±0.2	0.8 ^x ±0.2	0.7 ^{wx} ±0.2	1.2 ^x ±0.2
% unsat. FA	60.5 ±1.5	49.9 ±4.6	59.6 ±2.0	58.0 ±2.1	59.0 ±1.9
% monoenes	18.5 ±1.0	13.0 ±1.2	15.7 ±0.6	14.3 ±1.4	14.6 ±2.0
% polyenes	42.0 ±2.5	37.0 ±5.3	43.9 ±2.0	43.7 ±2.4	44.4 ±3.7
unsat. index	173.1 ±8.1	149.4 ±23.0	180.9 ±11.7	183.0 ±11.3	178.1 ±17.6
w-3 acids	4.9 ±0.3	1.8 ±0.7	4.0 ±0.8	4.0 ±0.9	4.8 ±1.2
w-6 acids	36.5 ±2.1	35.2 ±4.6	39.3 ±1.3	39.1 ±2.3	39.6 ±2.6
w-9 acids	15.0 ±0.7	9.2 ±0.9	13.6 ±0.7	12.2 ±1.6	12.4 ±1.8
av chain length	18.4 ±0.1	18.2 ±0.2	18.6 ±0.2	18.7 ±0.1	18.6 ±0.2
N°	5	5	4	5	4

Values are weight %; Mean ± SEM

a Carbon N°: N° of double bonds

c Unidentified fatty acid

w Significantly different from Size-matched control (p<0.05)

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

z Significantly different from Food-restricted (p<0.05)

Table 4.11 **Effects of PEM and PUFA-enrichment on fatty acid composition of heart phospholipids: Experiment 1.**

PUFA content	Control		Protein-energy malnourished					
			Low		Medium		High	
15:0 ^a tentative	1.8	±0.5	2.3	±0.6	2.5	±0.2	2.1	±0.5
c	4.3	±1.1	2.7	±0.5	6.0	±1.4	3.8	±1.1
16:0	16.3	±4.2	16.4	±7.4	12.2	±2.2	13.0	±2.5
16:1 w7	2.7	±2.7	2.7	±2.5	0.2	±0.2	0.1	±0.1
f	0.9	±0.2	1.8	±0.5	1.5	±0.3	1.3	±0.1
g	0.7	±0.3	1.4	±0.3	1.3	±0.2	0.7	±0.3
18:0	20.2	±0.8	20.1	±1.7	20.7	±0.5	21.1	±0.6
18:1 w9	6.3	±1.1	5.2	±0.6	5.0	±0.4	4.5	±0.3
18:1 w7	3.3	±0.4	2.5	±0.3	2.5	±0.2	2.9	±0.2
18:2 w6	17.4	±1.7	17.9	±2.3	20.7	±1.9	23.6	±0.8
20:3 w9		—	0.1	±0.1		—		—
20:4 w6	17.2	±1.4	19.5	±2.3	20.5	±1.3	18.8	±1.3
22:0	0.3	±0.3		—		—		—
22:4 w6	1.2	±0.3	0.5	±0.3	1.0	±0.3	1.3	±0.4
22:5 w6	5.6	±1.2	4.5	±0.7	3.7	±0.5	4.9	±0.9
22:6 w3	1.8	±0.4	2.4	±0.6	2.3	±0.4	1.9	±0.5
% unsat. FA	55.6	±3.3	55.5	±4.2	55.9	±2.8	58.0	±3.3
% monoenes	12.3	±2.1	10.5	±1.7	7.7	±0.4	7.6	±0.3
% polyenes	43.3	±4.5	45.1	±5.9	48.2	±2.6	50.4	±3.2
unsat. index	159.9	±15.2	164.1	±19.1	167.5	±10.2	170.8	±13.6
w-3 acids	1.8	±0.4	2.4	±0.6	2.3	±0.4	1.9	±0.5
w-6 acids	41.5	±4.1	42.5	±5.3	45.9	±2.3	48.5	±2.7
w-9 acids	6.3	±1.1	5.4	±0.6	5.0	±0.4	4.5	±0.3
av chain length	18.3	±0.2	18.3	±0.3	18.4	±0.1	18.4	±0.2
N°	6		5		5		5	

Values are weight %; Mean ± SEM
a Carbon N°: N° of double bonds
c, f, g Unidentified fatty acids

Table 4.12 Effects of PEM, food-restriction and PUFA-enrichment on fatty acid composition of erythrocyte phospholipids: Experiment 3.

Fatty Acid	Control Diet			PEM		PEM PUFA-enriched	
	Size-matched	Age-matched	Food-restricted				
12:0 ^a	0.5 ±0.5	0.4 ±0.4	0.7 ±0.4	0.5 ±0.3		0.3 ±0.3	
14:0	2.1 ±0.2	1.2 ±0.2	2.2 ±0.3	1.9 ±0.2		2.1 ±0.5	
14:1 w5	-	0.4 ±0.4	0.5 ±0.3	-		0.4 ±0.4	
v	1.0 ^x ±0.1	4.0 ^{wyz} ±0.4	1.0 ^x ±0.1	0.7 ^x ±0.2		1.8 ^{xy} ±0.3	
16:0	23.6 ^x ±1.4	15.1 ^{wy} ±1.8	19.0 ±2.0	21.9 ^x ±1.4		24.9 ^{yz} ±1.1	
16:1 w7	0.7 ±0.1	0.9 ±0.5	1.1 ±0.5	0.9 ±0.1		0.7 ±0.3	
x	0.7 ^x ±0.1	3.9 ^{wyz} ±0.4	0.8 ^x ±0.1	1.1 ^x ±0.6		1.2 ^x ±0.3	
y	3.5 ^x ±0.5	1.9 ^{wz} ±0.2	3.0 ^x ±0.1	2.5 ±0.5		1.7 ^{wz} ±0.2	
z	1.7 ±0.2	0.4 ±0.1	1.5 ±0.3	0.9 ±0.6		0.5 ±0.3	
18:0	16.6 ±0.7	15.0 ±0.9	14.3 ±1.4	15.7 ±1.7		15.4 ±0.9	
18:1 w9	12.2 ^x ±0.3	8.7 ^{wy} ±0.5	10.3 ±0.7	11.6 ^x ±0.3		13.0 ^{yz} ±1.3	
18:1 w7	1.5 ±0.1	1.7 ±0.2	1.2 ±0.1	1.3 ±0.1		1.3 ±0.1	
18:2 w6	5.6 ±0.3	4.5 ±0.6	5.2 ±0.7	5.3 ±0.3		9.2 ^{wxyz} ±0.6	
20:1 w9	1.0 ±0.6	-	0.6 ±0.4	0.6 ±0.4		0.6 ±0.4	
20:4 w6	22.8 ^{yz} ±0.5	33.0 ^w ±1.6	30.8 ^w ±2.8	27.1 ±2.3		20.8 ^{xyz} ±1.9	
22:3 tentat.	-	-	0.2 ±0.2	-		0.5 ±0.3	
22:4 w6	1.7 ^{xyz} ±0.9	4.0 ^w ±0.2	3.5 ^w ±0.5	3.1 ^w ±0.3		2.9 ^w ±0.3	
22:5 w6	2.2 ^x ±0.1	3.7 ^w ±0.4	2.8 ±0.4	2.7 ±0.3		1.1 ^{wxyz} ±0.4	
22:5 w3	0.3 ±0.3	0.3 ±0.2	0.2 ±0.2	0.9 ±0.7		0.2 ±0.2	
22:6 w3	1.6 ±0.8	0.9 ±0.1	1.1 ±0.1	1.2 ±0.2		0.8 ±0.5	
24:1 w9	0.8 ±0.4	-	0.1 ±0.1	-		0.6 ±0.3	
% unsat. FA	50.4 ^{yz} ±2.2	58.1 ^w ±0.8	57.5 ^w ±2.9	54.7 ±1.6		52.0 ^x ±1.9	
% monoenes	16.2 ^x ±0.7	11.6 ^{wy} ±1.4	13.8 ±0.6	14.5 ^x ±0.5		16.8 ^{yz} ±0.8	
% polyenes	34.2 ^{yz} ±1.5	46.5 ^w ±2.0	43.7 ^w ±3.3	40.2 ±2.0		35.3 ^{yz} ±2.6	
unsat. Index	147.3 ^{yz} ±8.6	194.2 ^w ±8.1	183.4 ^w ±14.4	170.8 ±6.6		141.5 ^{yz} ±9.6	
w-3 acids	1.9 ±1.0	1.2 ±0.3	1.3 ±0.3	2.0 ±0.9		0.8 ±0.5	
w-6 acids	32.3 ^{yz} ±0.6	45.3 ^w ±1.8	42.3 ^w ±3.0	38.2 ±2.9		33.9 ^{yz} ±2.3	
w-9 acids	14.0 ^{yz} ±0.9	8.7 ^{wyz} ±0.5	11.0 ^{wx} ±1.0	12.3 ^x ±0.7		14.4 ^{yz} ±0.7	
av chain length	18.2 ^x ±0.2	18.7 ^w ±0.1	18.4 ±0.1	18.4 ±0.1		18.1 ±0.2	
N°	3	4	4	4		4	

Values are weight %; Mean ± SEM

a Carbon N°: N° of double bonds

w Significantly different from Size-matched control (p<0.05)

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

z Significantly different from Food-restricted (p<0.05)

where there was a tendency for U.I. and Ave CL of total fatty acids to be decreased. Unsaturation index is a measure of not only the % of unsaturated fatty acids, but the degree to which they are unsaturated. Determination of the Ave CL may indicate whether there is a general increase in the activity of the elongase enzymes, which increase fatty acid carbon chain length. PEM tended to increase % unsat. in brain and decrease % unsat. in erythrocytes. PEM also increased U.I. in brain and gastrocnemius and decreased U.I. in plasma liver and erythrocytes, as well as decreasing Ave CL in liver. Increased dietary PUFA during PEM reversed the effect of PEM on Ave CL and increased % unsat. in all tissues except erythrocytes, where % unsat. decreased. Increased dietary PUFA increased U.I. in plasma and liver and decreased it in erythrocytes.

Plasma

The % unsat. of total plasma fatty acids was not altered by food restriction or PEM, although it was increased from 58% to 62% by PUFA-enrichment during PEM (Table 4.4). Food restriction and PEM reduced unsaturation index from 176 to 150 and 160 respectively. This occurred through a reduction in 20:4 w6 and increases in 16:0 and 18:1 w9. Increased dietary PUFA during PEM reversed this effect, increasing U.I. to 171, via an increase in 18:2 w6 and decreases in 14:0 and 18:1 w9. Arachidonic acid (20:4 w6) is derived from linoleic acid (18:2 w6). However, PUFA-enrichment did not return 20:4 w6 to control diet levels. Reductions in Ave CL resulting from food-restriction and PEM were small. Food-restriction and PEM increased 18:2 w6 by 2.7% and 2.1% respectively. PUFA-enrichment further increased this by about 10%. The U.I., % unsat. and Ave CL in size-matched controls were similar to that of malnourished rats, with decreased 20:4 w6 and increased 12:0, 14:0 and 16:0. No 20:3 w9 was detected in plasma. The presence of 20:3 w9 in tissues generally indicates that they are deficient in 18:2 w6.

Liver

There was little change in the % unsat. of liver phospholipid fatty acid composition as a result of food restriction or PEM (Tables 4.5 and 4.6). The U.I. was reduced by PEM but not by food restriction. The reduction involved decreases in long chain polyunsaturates and increases in 16:0 and monounsaturates. However, PUFA-enrichment reversed this effect, increasing U.I. back to the level of control diet rats. This was achieved by increases in polyunsaturates such as 18:2 w6, 20:4 w6 and 22:6 w3 and reductions in 16:1 w7 and 18:1 w9, which had been increased by both food-restriction and PEM. PEM reduced Ave CL (Tables 4.5-4.6). Increased dietary PUFA reversed this effect.

Food restriction and PEM increased concentrations of 18:2 w6 and 20:3 w9. PUFA-enrichment further increased the concentration of 18:2 w6, but reduced the concentration of 20:3 w9 to zero. In the size-matched controls, U.I. was slightly lower, although % unsat. was unchanged, as decreases in polyunsaturates were balanced by increases in monounsaturates.

Brain

The % unsat. and U.I. of brain phospholipid fatty acids were increased by PEM (Tables 4.7 and 4.8). Unlike other tissues, the concentration of 18:2 w6 remained unchanged as a result of food restriction or PEM. The concentration of 18:2 w6 was higher in PUFA-enriched rats and size-matched controls. However, the magnitude of these differences was small, as brain has a low 18:2 w6 content. Unlike most other tissues, the 18:1 w9 concentration was reduced in size-matched, food restricted and PEM rats. No 20:3 w9 was detected in brain phospholipids. The fatty acid composition of the low PUFA group in experiment 1 was very similar to that of the control group.

Kidney

The % unsat. and U.I. of kidney phospholipid fatty acids was not affected by PEM (Table 4.9). The concentration of 18:2 w6 increased as a result of PEM, with the highest value recorded for the PUFA-enriched rats. The concentration of 20:4 w6 was relatively unchanged, with only a slight increase in the high PUFA PEM group. There was little change in Ave CL. Unlike most other tissues, PEM reduced 16:1 w7, with the greatest decrease in the PUFA-enriched group. PEM increased 18:1 W9, but this was reversed by increased PUFA-enrichment.

Gastrocnemius Muscle

Food restriction and PEM increased the % unsat. and U.I. of phospholipid fatty acids (Table 4.10). PUFA-enrichment during PEM did not modify these increases, which were largely the result of increases in 18:1 w9, 22:6 w3, 18:2 w6 and 20:4 w6 and decreases in 12:0, 14:0 and 16:0. Small amounts of 20:3 w9 (0.6-0.7%) were detected in the size-matched, food-restricted and PEM rats, but not in the PUFA-enriched PEM group. Food-restriction and PEM did not alter the concentration of 18:2 w6, although it was higher in the size-matched and PUFA-enriched PEM rats. The levels of % unsat, U.I. and Ave CL of the size-matched controls were similar to those of the malnourished groups of rats.

Heart

The % unsat. and U.I. and Ave CL of phospholipid fatty acids were unchanged as a result of PEM (Table 4.11). The concentration of 18:2 w6 was unchanged by PEM, but increased with PUFA-enrichment. The concentration of 16:1 w7 was

reduced in high and medium PUFA level PEM rats. The concentration of 18:1 w9 was reduced by PUFA-enrichment.

Erythrocytes

PEM reduced the % unsat. and U.I. of phospholipid fatty acids (Table 4.12). PUFA-enrichment further reduced the values of these parameters. These changes were largely the result of increases in 16:0, 18:1 w9 and 18:2 w6 and decreases in 20:4 w6 and 22:5 w6. Changes also occurred in proportions of unidentified "fatty acids" which emerged from the gas chromatograph column between 14:1 w5 and 16:0 and also between 16:1 w7 and 18:0. Traces of these unidentified "fatty acids" were also found in other tissues. The concentration of 18:2 w6 was relatively unchanged as a result of food restriction and PEM, with the highest value recorded for the high PUFA PEM rats. The concentration of 20:4 w6 was decreased by PEM, with the lowest value recorded with PUFA-enrichment. The changes in % unsat., U.I. and Ave CL of the size-matched controls are similar to those after PUFA-enrichment, although the level of 18:2 w6 is the same as that of the age-matched controls.

Discussion

Organ Composition

Fatty Acid Composition.

The concentration of 22:6 w3 in liver phospholipid (Tables 4.5-4.6) was lower than that usually found in rats fed a standard rat diet (See Table 4.1). The concentration of 22:5 w6 was also higher. Both of these findings are consistent with the low concentration of 18:3 w3 in the diets (Galli, C., personal communication). Simopoulos (1990) suggested that increased tissue concentrations of 22:4 w6 and 22:5 w6 and a reduced concentration of 22:6 w3 was characteristic of a diet high in linoleic acid and low in of linolenic acid. The low w3 content in the control diets of experiments 1 and 3 indicates that they were both deficient in w3 fatty acids.

The greater growth in body weight with PUFA-enrichment during PEM (Table 3.9) indicates that PUFA-enrichment ameliorates at least one of the effects of PEM. Thus, dietary PUFA content was a growth-limiting factor in PEM, even though the minimum proportion of dietary energy supplied as 18:2 w6 (1.3% in Experiment 1 and 2% in Experiment 3) exceeded the generally accepted minimum requirement of 1% (Holman, 1960; Lands, 1986). If dietary PUFA content is a growth-limiting factor in PEM, then 18:2 w6 may be expected to be reduced in the tissue phospholipids of PEM rats. However, in both Experiments 1 and 3, the concentration of 18:2 w6 in tissue phospholipids increased, or at the very least, did not decrease as a consequence of food restriction or PEM. The concentration of 18:2 w6 was generally higher in food-restricted than PEM rats, although these differences were small. In both

Experiments 1 and 3, PUFA-enrichment increased 18:2 w6 concentrations in all tissues during PEM.

It seems paradoxical that reducing the absolute intake of a fatty acid that cannot be synthesised in the body, should increase its concentration in tissue phospholipids. However, phospholipid fatty acid analysis is a measurement at one instant of a dynamic situation. There is continuous anabolism and catabolism of lipid and protein components of biological membranes *in vivo* (Waterlow et al, 1978; Dawidowicz, 1987). There is also continuous conversion into other fatty acids by desaturases and elongases (See Fig 1.1). An increase in the proportion of 18:2 w6 does not necessarily mean that the absolute amount of 18:2 w6 has increased in the organ. An increase in the proportion of phospholipid 18:2 w6 during PEM may possibly represent a decrease in tissue phospholipid content, desaturation and elongation of 18:2 w6, rate of tissue growth, phospholipid or fatty acid turnover or several of the above. The possible involvement of these mechanisms will be examined in the discussion that follows.

Tissue phospholipid content was not determined in the present study. Phospholipid turnover is rapid in most animal cells (Dawidowicz, 1987). Increases in membrane phospholipid content and in the molar ratio of cholesterol:phospholipid have been reported in erythrocytes from children with Kwashiorkor (Brown et al, 1978). The accumulation of excess fat in the liver during kwashiorkor is mainly in the form of triglycerides (>95% total fat). Phospholipids are slightly reduced, and thus, phospholipid:total lipid ratio is very low. In marasmus, total lipid and phospholipid content of liver remain relatively unchanged (Chatterjee and Mukerherjee, (1968). Although the amount of phospholipids in brain is reduced in children with marasmus, the proportions of phospholipid and cholesterol remain unchanged (Rosso

et al, 1970).

During protein deprivation (not PEM) in adult rats, chain elongation and desaturation activity are unchanged (Gerson and Wong, 1978). However, during protein deprivation in young rats, $\Delta 6$ -, $\Delta 5$ - and $\Delta 9$ - desaturase activity is decreased (De Tomas, Mercuri and Rodrigo, 1980; Narce. et al, 1988). Narce, et al. (1988) have suggested that $\Delta 6$ - and $\Delta 5$ - desaturase activity may be decreased by protein deprivation, only when protein requirements are important, such as during growth. Brenner (1989) suggested that a reduction in $\Delta 6$ desaturase activity in protein-depleted weanling rats (De Tomas et al, 1980) implied that a deficiency in polyunsaturated fatty acids may develop in malnourished children. In rat liver microsomes, $\Delta 6$ desaturase activity is reduced by fasting but increased by EFA-deficiency (Brenner, 1981). Narce, et al. (1988) also reported that the changes they detected in $\Delta 6$ - and $\Delta 5$ - desaturase activity were not strictly paralleled by changes in fatty acid composition in total lipids and microsomal phospholipids of liver and suggested the possible influence of other factors such as elongation, oxidation, substrate availability, removal of product and hormone status.

The w-3, w-6 and w-9 series of fatty acids compete for binding sites of the $\Delta 6$ - desaturase (Fig 1.1). The w-3 series has greatest affinity and the w-9 series least affinity for binding sites of what is believed to be the same enzyme (Mohrhauer and Holman, 1963a; Horrobin, 1992). The low ratio of 18:3 w-3 to 18:2 w6 in each of the diets of Experiments 1 and 3 suggests that competition of 18:3 w3 with 18:2 w6 for binding sites of the $\Delta 6$ desaturase is unlikely to reduce the conversion of 18:2 w6 to 18:3 w6. Similarly, the general absence of increased 18:3 w6 during food restriction or PEM (Tables 4.4-4.12) suggests that product inhibition by 18:3 w6 is unlikely to have inhibited desaturation of 18:2 w6. Furthermore, the general absence of increased 20:2 w6 in phospholipids during

food restriction or PEM suggests product inhibition by 20:2 w6 is unlikely to have inhibited elongation of 18:2 w6 to 20:2 w6. This suggests that a reduction in $\Delta 6$ desaturase activity is a possible reason for absence of a decrease in 18:2 w6 during food restriction and PEM. Sprecher (1989) and Horrobin (1992) suggest that evidence points to the activity of the $\Delta 6$ desaturase being rate limiting in the conversion of 18:3 w-3 and 18:2 w6 to long chain fatty acids.

Tocopherols are naturally occurring antioxidants. Generally, they are found in high concentrations in foods high in PUFA, such as safflower oil (Roberts, 1986). Plasma vitamin E concentration is reduced in malnourished children (Golden and Ramdath, 1987) and the oxidation of plasma free fatty acids has been reported to increase in infants with PEM (Lewis et al., 1967). No determinations of tocopherols were carried out in the diets or tissues of this study. However, the increases in U.I. in some tissues e.g., brain and gastrocnemius (Tables 4.7, 4.8 and 4.10) during food restriction and PEM suggest that the supply of tocopherols to the tissues was adequate in all dietary groups of both Experiments 1 and 3.

Tissue Growth

Body weight and thus organ weight were decreased by both food restriction and PEM. However, PUFA-enrichment during PEM increased growth during the experimental period. Consequently, there was a general increase in organ size, which was significantly higher in skin and fur, spleen and the digestive tract. The effects of PEM in greatly reducing spleen weight have been reported previously (Platt et al., 1969; Ramalingaswami, 1969). Ramalingaswami (1969) claimed that the spleen is more sensitive to reduced protein than the liver. The present results support this view. Different tissues have different periods in which growth spurts occur (Miller, 1969) and the

nature of this growth is also age-dependent (Winnick and Noble, 1965). Thus, the period during which malnutrition occurs determines the nature of the reduction in growth and also the chances of the effects being permanent (Winnick and Noble, 1966). This may influence the effects that dietary restriction has on the chemical composition of different organs e.g. the effects on brain composition would be greatest before weaning and those on muscle composition after weaning.

Liver

The increased concentrations of 18:2 w6 and decreased concentrations of 20:4 w6 (Tables 4.5 and 4.6) suggest that PEM decreases the conversion of 18:2 w6 to 20:4 w6. Furthermore, the absence of 18:3 w6 and low levels of the other intermediate, 20:3 w6, suggest that this decrease probably occurs via a reduction in $\Delta 6$ -desaturase activity. Evidence suggests that the rate limiting step in the metabolism of 18:2 w6 is the $\Delta 6$ -desaturase (Sprecher, 1989). The increased concentration of 18:1 w9 suggests an increase in $\Delta 9$ -desaturase activity relative to that of $\Delta 5$ and $\Delta 6$ -desaturase activity and the decrease in 18:0 is consistent with this view. The $\Delta 9$ -desaturase converts 18:0 to 18:1 w9 (Inkpen et al., 1969). However, a mechanism to explain the increased conversion of 18:1 w9, to 20:3 w9, via an apparently reduced w6-desaturase preceeding the elongase and w5-desaturase, is unclear. Field and Kelleher (1984) found that zinc deficiency reduced the activity of the $\Delta 5$ desaturase enzyme and suggested that this may explain why in liver phospholipids, the concentration of linoleic acid is increased and that of arachidonic acid is decreased. In the present study, zinc from the galvanised cages may have increased the zinc ingested by the rats. All of the rats were seen to chew on the bars of the cages.

EFA deficiency in rats causes structural changes in the intestinal mucosa

(Snipes, 1968) and these are associated with malabsorption of sugars, amino-acids and fat (Snipes, 1968; Imami et al, 1970; Clark et al, 1973). The presence of significant amounts of 20:3 w9 may be used as a practical indication of EFA-deficiency provided that w6 fatty acids are the only polyunsaturates involved (Lundberg, 1980), or the ratio of w6 to w3 fatty acids is high (Mohrhauer and Holman, 1963 b). Fatty acids of the w3 series compete with the w9 fatty acids for the $\Delta 5$ - and $\Delta 6$ - desaturases. The relatively higher affinity of binding sites on these enzymes for w3 fatty acids may prevent the formation of 20:3 w9, even though symptoms of EFA deficiency may still be present (Mohrhauer and Holman, 1963b; Lundberg, 1980). In the present study, differences in tissue w-3 fatty acids between groups were relatively minor. This is hardly surprising, as the diets contained only very small amounts of w-3 fatty acids. The presence of a small proportion of 20:3 w9 in the food-restricted and PEM groups of Experiment 3, where the ratio of w3 to w6 fatty acids was low indicates that these are marginally deficient in 18:2 w6. The level of 20:3 w9 in the low-PUFA group of Experiment 1 exceeded that found in the PEM group of Experiment 3. This may be the result of any of a number of differences between these two groups, such as the % of dietary energy as 18:2 w6 and ingestion of stearic acid. The development of an apparently greater EFA-deficiency in Experiment 1 rats, even though PEM diets were commenced one week later and maintained for a shorter period than in Experiment 3 suggests that stearic acid may be involved.

The role of 18:2 w6 and 20:3 w9 in biological membranes is still unclear. It may be, that the only function of 18:2 w6 is that of a necessary precursor for the formation of prostaglandins, thromboxanes and other biologically active compounds (Lundberg, 1980). However, it is also possible that the activity of some membrane-bound enzymes may depend on the presence of specific fatty acids such as 18:2 w6. Recently, Andrews and Else (1992) reported that an "annular lipid" environment influences membrane-bound sodium pump

activity. The presence of 20:3 w9 is often associated with EFA-deficiency. Lundberg (1980) suggests that the evidence for the impairment of membrane-function by the presence of 20:3 w9 is minimal. Its presence may be no more than an indicator of EFA-deficiency.

Brain

PEM did not alter the w9 fatty acid concentration of brain phospholipids, or their concentration of 18:0. However, food restriction reduced the w9 concentration from 20.6% to 14.9% and increased the 18:0 concentration by about the same amount. The absence of 20:3 w9 in brain phospholipid suggests that, unlike other organs such as liver and kidney, the brain is "selectively-protected" against EFA-deficiency induced by food-restriction and PEM. Mohrhauer and Holman (1963a) report that during EFA-deficiency, less 20:3 w9 is deposited in brain and that brain fatty acid composition is more stable compared to that of other tissues. Sinclair and Crawford (1972) found that most of the 20:4 w6 and 22:6 w3 is deposited before weaning. A greater % of administered 20:4 w6 is incorporated into brain than 18:2 w6 (Sinclair and Crawford, 1973) and desaturation enzyme activity is very low in microsomes of the developing brain (Strouve-Vallet and Pascaud, 1971). Galli et al. (1970, 1971) found that brain weight, lipid content and total phospholipid content were reduced in rats, if EFA-deficiency was induced early in post-natal life or before birth. However, both % unsat and U.I. remained constant. Alling, et al. (1974) found that on a "high" protein diet (16%), the brain weights of rats was only slightly reduced in low dietary EFA intake. Furthermore, these differences were removed when brain weights were related to body weights. The concentration of 18:2 w6 did not change, but the concentration of 22:6 w3 decreased in the very low EFA group. They concluded that the cerebrum was relatively inert to wide differences in the dietary EFA level. Witting et al. (1961)

observed 20:3 in tissues, but not in brain lipids with EFA-deficiency. Although the results of the present study suggest that the brain is more protected than other organs against EFA-deficiency induced by food-restriction and PEM, it should be remembered that, fatty acid analysis was carried out on total phospholipids, rather than on individual classes of phospholipids. It is possible that there may be variations in the proportions of these classes and also in the proportions of the fatty acids within them. Recent studies indicate that dietary manipulation can induce large changes in the fatty acid composition of cardiolipin of brain mitochondria, even in mature rats. Furthermore, these changes do not require EFA deficiency (Dyer and Greenwood, 1991).

Kidney

In kidney phospholipids, PEM increased the concentration of w9 fatty acids and reduced the concentration of 20:4 w6. PUFA-enrichment reversed both of these effects. This trend parallels that found in liver, where 20:3 w9 was also detected. However, other changes such as those of % unsat. and U.I. were minor. The significance of the decrease in 16:1 w7 in PEM, which was more pronounced with PUFA-enrichment is unclear.

Gastrocnemius Muscle

The changes in fatty acid composition of gastrocnemius muscle phospholipids as a result of food restriction were very similar to those from PEM. The presence of 20:3 w9 suggests the presence of a marginal EFA-deficiency in both of these groups. PUFA-enrichment reversed this trend in the PEM rats.

Heart

The % unsat. and U.I. of phospholipid fatty acids were unchanged as a result of PEM, although PUFA-enrichment during PEM did increase these parameters slightly. The general absence of 20:3 w9 in heart phospholipid indicates that, like the brain, the fatty acid composition of the heart appears to be "protected" against PEM-induced EFA-deficiency.

Erythrocytes

The % unsat. and U.I. of phospholipid fatty acids decreased as a result of food restriction and PEM. PUFA-enrichment during PEM further reduced the values of these parameters. These changes were largely the result of increases in concentrations of 16:0, 18:1 w9 and 18:2 w6 and decreases in concentrations of 20:4 w6 and 22:5 w6. The concentration of 18:2 w6 increased slightly as a result of food restriction and PEM, with the highest value recorded for the high PUFA PEM rats. Conversely, the concentration of 20:4 w6 decreased slightly as a result of food restriction and PEM, with the lowest value recorded for the high PUFA PEM rats. The very low concentration of 20:4 w6 resulting from increased dietary PUFA during PEM suggests that fatty acid metabolism in erythrocytes is some way unique amongst the tissues examined in this study. It is unclear whether the reduced concentration of 20:4 w6 represents an increased metabolism of 20:4 w6 or an impairment in the conversion of 18:2 w6 to 20:4 w6. Concentrations of 22:4 w6 and 22:5 w6 are reduced in this group and the concentration of 24:1 w9 is increased, suggesting an impairment in the conversion of 18:2 w6 to 20:4 w6, although the magnitude of these changes is small. The general increase in w9 fatty acids also supports this view. However, no 20:3 w9 was detected in erythrocytes. The high concentration of 18:2 w6 in this group provides no definitive evidence that

conversion to 20:4 w6 is impaired, as this might be expected from a diet containing a high level of 18:2 w6. It is also possible, that the magnitude of the PUFA-enrichment, 20% of dietary energy intake, may have acted to reduce the metabolism of 18:2 w6 via a mechanism such as substrate inhibition.

Changes also occurred in proportions of unidentified "fatty acids" which emerged from the gas chromatograph column between 14:1 w5 and 16:0 and also between 16:1 w7 and 18:0.

The changes in % unsat, U.I. and Ave CL of the size-matched controls are similar to those of the high PUFA PEM rats, although the concentration of 18:2 w6 is the same as that of the age-matched controls.

Vajreswari et al. (1990) reported that in erythrocyte fatty acids of marasmic children, the concentration of 18:1 w9 was increased and that of 20:3 and 20:4 were decreased. Linoleic acid (18:2 w6) was increased in both kwashiorkor and marasmus, but these increases were not significant. They suggested that there may be a defective metabolism of oleic acid (18:1) in both of these syndromes and that the metabolism of arachidonic acid was markedly affected in marasmus. They suggested, that as erythrocytes lack the capacity for de novo synthesis of FA or FA modifications that the FA composition of erythrocyte membrane lipids reflects the FA metabolism of other tissues, particularly in the liver. The ratio of 20:4 w6 to 18:2 w6 reflects the synthesis of 20:4 w6 from 18:2 w6. In their study, this conversion may have been impaired in marasmus, or there may have been a rapid turnover of 20:4 w6 in marasmus (Vajreswari et al, 1990).

Vajreswari and Narayanareddy (1992) fed weanling rats a nutritionally adequate diet containing 20% lipid. The proportions of individual fatty acids of

membrane lipids generally reflected the fatty acid composition of the diets, although % Unsat. remained relatively constant. There was no significant difference in Na^+ , K^+ -ATPase of erythrocyte membranes between rats fed diets containing 20% coconut oil and those fed diets containing 20% safflower oil.

Hill and Holman (1980) found that protein deficiency increased the ratio of 20:3 w9 to 20:4 w6 (the biochemical index of EFA-deficiency) in the liver and heart of rats. In Experiment 3, Food-restriction and PEM resulted in the appearance of a small concentration of 20:3 w9 in liver (Table 4.6). Thus, these treatments increased the ratio of 20:3 w9 to 20:4 w6. This effect was reversed by PUFA-enrichment.

Summary

Food restriction and PEM increased the 18:2 w6 concentration in all of the tissues measured, except brain, where it was relatively unchanged and in gastrocnemius muscle, where it was decreased by PEM.

PUFA-enrichment increased the w6 concentration of all tissues, especially their 18:2 w6 concentration, except for erythrocytes, where w6 concentration was decreased and w9 concentration increased

The concentration of w9 fatty acids increased in all tissues by PEM and food-restriction, except in brain, where it decreased only slightly and in heart, where it decreased slightly.

The effects of food restriction, PEM and increased dietary PUFA on tissue composition were greater in some tissues such as liver, kidney and gastrocnemous muscle than others such as brain and heart. The way in which composition responded to these dietary manipulations also varied between tissues. In liver, PEM, but not food-restriction decreased the w6 concentration of phospholipids, especially of 20:4 w6. In some tissues such as brain, there was little change in any of the parameters.

Chapter 5: Organ Weights and Tissue Composition

Introduction

The effects of PEM on organ weight and various functional parameters such as membrane permeability and sodium pumping varies greatly between organs. Some of these effects are related to changes in tissue Na^+ and K^+ content. In this chapter, the effects of food-restriction, PEM and PUFA-enrichment on organ size and composition were determined.

Methods and Materials

Organ weight and carcass water content

After each rat was killed by decapitation, organs were removed and weighed. Cerebral hemispheres, cerebellum and brain stem were grouped together as brain weight. Testes, epididymis, vas deferens, seminal vesicles prostate gland and urethra were grouped together as male reproductive organs and ovaries, fallopian tubes and uterus were grouped together as female reproductive organs. In Experiment 1, bones of a hind leg femur, patella, tibia and fibula were stripped of tissue and weighed, as were the leg muscles. Tissue samples were stored in a freezer at -80°C . Stomach and intestines were removed, flushed with water, slit with a scalpel, blotted dry and weighed. After decapitation, blood was collected in a plastic weighing tray containing 30 μl heparin solution. Hematocrit was determined in duplicate, using microcapillary tubes and a microcapillary centrifuge. Erythrocytes were separated from the remainder of the blood by centrifugation for 40 secs in a microfuge. Plasma was removed by aspiration and stored at -80°C . Some of the

pellet of blood cells was stored and the remainder was washed (2 X) in ice-cold MgCl_2 and stored at -80°C . In Experiment 3, carcass moisture content was determined, after drying the carcass to constant weight at 65°C and correcting for the moisture content of the organs removed.

Determination of tissue water content

Pre-weighed pieces of tissue were freeze-dried for 24 h in 1.5 ml eppendorf tubes, ground with a dissecting probe and re-freeze-dried to constant weight to determine wet weight-to-dry weight ratios.

Potassium and Sodium Determination

Freeze-dried tissue (approx. 0.02g) or standards (KCl or NaCl) were digested in 2 ml 0.3 M trichloroacetic acid (TCA) and centrifuged at 3000 RPM for 30 min in a Sorval Econospin centrifuge. Aliquots of the supernatant were diluted up to 5 ml with distilled water from a (Millipore Milli Q water System) and Na^+ and K^+ concentrations determined using a flame photometer (Corning 410).

Protein Determination

The method of Lowrey et al. (1951) was used to determine the protein content of freeze-dried tissue.

Analysis of Diets

The moisture content of diets was determined using the same techniques as for the tissues.

RESULTS

Carcass Weight

Not surprisingly, the effects of the dietary manipulations on carcass weight (Table 5.1) closely parallel those on whole body weight (Table 3.9). Carcass water content was increased from 47% in age-matched controls to 67% and 68% by food restriction and PEM respectively (Fig. 5.1). PUFA-enrichment reversed this to 66%, but still increased carcass weight from 71 g to 76 g resulting in a carcass dry weight of 25.6 g. This is 2.9 g or 13% greater than the carcass dry weight of the PEM rats. When the contributions of liver, kidneys, heart, brain and soleus are excluded, PUFA-enrichment reduced carcass % H₂O to 65%, whilst still increasing carcass dry weight from 20.4 g to 23.3 g. This is 2.9 g or 14% greater than that of the PEM rats. Thus, much of the the increased growth induced by PUFA-enrichment was associated with tissues other than those mentioned above. Much of the 20% difference in carcass water content between the control and malnourished rats was due to the higher fat content of the control diet carcasses. These fat deposits would have an extremely low water content.

Organ Weights

Skin and fur

Food restriction reduced body weight by 67% and the weight of skin and fur by 76% (Table 5.3). PEM reduced the weight of skin and fur by 78%. However, PUFA-enrichment during PEM resulted in only a 74% reduction. In the younger (size-matched) rats, the weight of skin and fur weight was larger, both in absolute terms and as a % of body weight than in any of the malnourished groups (Tables 5.2-5.5).

Table 5.1

Effects of food restriction, PEM and PUFA-enrichment on carcass weight: Experiment 3.

	Control Diet			PEM	PEM PUFA- enriched
	Size- matched	Age- matched	Food- restricted		
Carcass ^a Wt(g)	73.2 ^x ±1.5	242.9 ^{wyz} ±17.8	77.7 ^{xy} ±1.4	71.4 ^{yz} ±2.1	75.6 ^{xy} ±1.6
DryWt. ^a (g)	24.4 ^x ±0.8	128.8 ^{wyz} ±12.6	25.5 ^{xy} ±0.6	22.7 ^x ±0.8	25.6 ^x ±0.9
% H ₂ O ^a	66.7 ^x ±0.6	47.4 ^{wyz} ±3.0	67.2 ^x ±0.7	68.3 ^x ±0.4	66.2 ^x ±0.7
Dry Wt. excluding liver, kidneys, heart, brain & soleus (g)	21.4 ^x ±0.8	123.7 ^{wyz} ±12.1	23.2 ^x ±0.6	20.4 ^x ±0.7	23.3 ^x ±0.8
% H ₂ O	65.9 ^x ±0.7	45.5 ^{wyz} ±3.3	66.5 ^x ±0.8	67.7 ^x ±0.5	65.2 ^x ±0.8
N ^a	8	8	8	8	8

Values are Mean ± SEM.

a Includes corrections for organs removed for analysis.

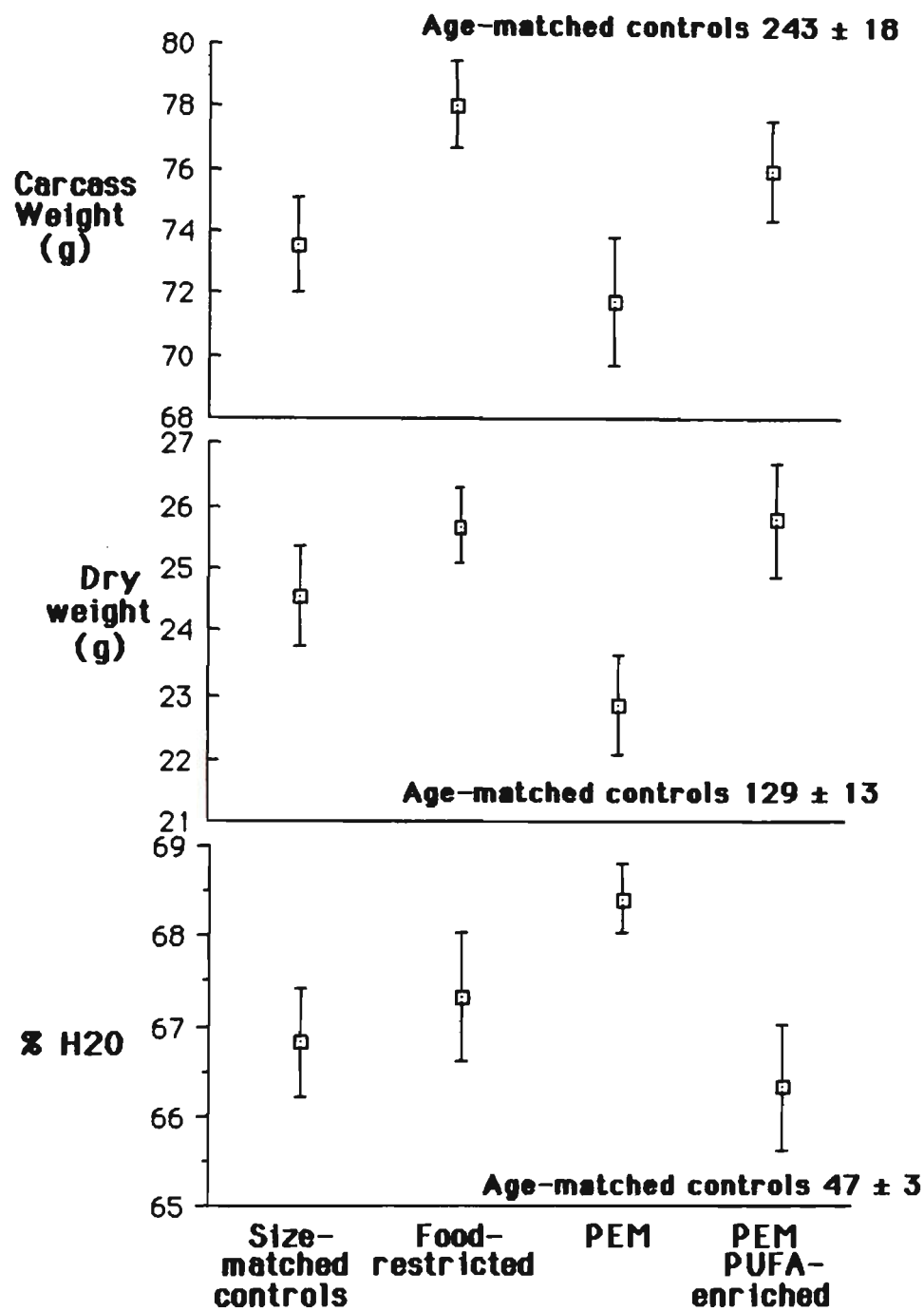
w Significantly different from Size-matched control (p<0.05)

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

z Significantly different from Food-restricted (p<0.05)

Figure 5.1 Effects of food-restriction, PEM and PUFA-enrichment on wet weight, dry weight and water content of carcass: Experiment 3



Values: Mean ± SEM

Table 5.2 Effects of PEM and PUFA-enrichment on organ mass: Experiment 1

PUFA content	Control	Protein-energy malnourished		
		Low	Medium	High
Bodymass	300.33 ^{yz} ±27.28	84.10 ^x ^{yz} ±1.30	101.13 ^x ±1.47	104.00 ^x ±3.72
Liver	11.43 ^{yz} ±1.03	3.03 ^x ±0.18	3.92 ^x 0.30	3.75 ^x ±0.28
Kidney	2.43 ^{yz} ±0.17	1.15 ^x ±0.09	1.07 ^x ±0.04	1.08 ^x ±0.02
Heart	1.12 ^{yz} ±0.13	0.40 ^x ±0.01	0.45 ^x ±0.01	0.48 ^x ±0.03
Brain	1.79 ^{yz} ±0.06	1.58 ^x ±0.01	1.65 ^x ±0.02	1.59 ^x ±0.04
Gastrocnemous muscle	1.88 ^{yz} ±0.25	0.44 ^x ^{yz} ±0.04	0.69 ^x ±0.03	0.77 ^x ±0.04
Hind leg muscle	6.59 ^{yz} ±0.61	1.69 ^x ^{yz} ±0.22	2.56 ^x ±0.26	2.81 ^x ±0.24
Hind leg bones	1.42 ^{yz} ±0.09	0.75 ^x ±0.02	0.82 ^x ±0.03	0.81 ^x ±0.04
Digestive tract	9.42 ^{yz} ±1.14	5.15 ^x ±0.21	5.09 ^x ±0.32	5.23 ^x ±0.38
Skin & fur	44.15 ^{yz} ±4.75	11.12 ^x ±0.45	12.99 ^x ±0.17	13.28 ^x ±0.27
Skinned tail	4.59 ^{yz} ±0.28	1.58 ^x ±0.08	1.66 ^x ±0.05	1.79 ^x ±0.06
Male reproductive	6.84 ^{yz} ±2.72	1.12 ^x ^y ±0.11	2.03 ^x ±0.32	2.70 ^x ±0.34
Female reproductive	0.70 ^{yz} ±0.25	0.13 ^x ±0.04	0.17 ^x ±0.04	0.15 ^x ±0.03
N°	3M 3F	3M 2F	3M 3F	3M 3F

Values are Mean ± SEM; grams
x Significantly different from Control (p<0.05).
y Significantly different from High PUFA (p<0.05).
z Significantly different from Medium PUFA (p<0.05).

Table 5.3 Effects of PEM, food-restriction and PUFA-enrichment on organ mass: Experiment 3.

	Control Diet			PEM	PEM PUFA- enriched
	Size- matched	Age- matched	Food- restricted		
Body mass	81.0 ^k ±1.8	262.3 ^{wyz} ±19.0	85.9 ^k ±1.5	79.0 ^k ±2.1	83.5 ^k ±1.3
Liver	5.17 ^{wyz} ±0.37	9.65 ^{wyz} ±1.28	3.28 ^{wk} ±0.29	3.29 ^{wk} ±0.33	3.87 ^{wk} ±0.41
Kidney	1.19 ^{wyz} ±0.03	2.43 ^{wyz} ±0.16	1.00 ^{wxy} ±0.03	0.91 ^{wk} ±0.02	0.96 ^{wk} ±0.03
Heart	0.49 ^{wyz} ±0.02	1.02 ^{wyz} ±0.07	0.43 ^{wk} ±0.01	0.42 ^{wk} ±0.01	0.41 ^{wk} ±0.01
Brain	1.57 ^k ±0.04	1.90 ^{wyz} ±0.06	1.63 ^k ±0.03	1.60 ^k ±0.03	1.62 ^k ±0.02
Gastrocnemius muscle	0.67 ^{wyz} ±0.03	3.45 ^{wyz} ±0.25	1.09 ^{wxy} ±0.04	0.89 ^{wkz} ±0.03	0.98 ^{wk} ±0.08
Soleus muscle	48.1 ^{kz} ±2.8	161.7 ^{wyz} ±8.2	58.7 ^{bwk} ±1.9	54.8 ^k ±5.3	56.3 ^k ±3.5
Spleen	0.29 ^{wyz} ±0.04	0.51 ^{wyz} ±0.04	0.16 ^{wk} ±0.00	0.14 ^{wk} ±0.00	0.16 ^{wk} ±0.00
Digestive tract ^a	4.75 ^k ±0.22	8.90 ^{wyz} ±0.71	5.30 ^k ±0.17	5.37 ^k ±0.18	5.76 ^{wk} ±0.17
Skin	13.84 ^{py} ±0.76	49.28 ^{wyz} ±4.52	11.97 ^k ±0.37	10.96 ^{wk} ±0.46	12.93 ^{py} ±0.30
Skinned tail	1.16 ^{wyz} ±0.04	5.38 ^{wyz} ±0.46	1.69 ^{wxy} ±0.03	1.43 ^{wkz} ±0.03	1.47 ^{wkz} ±0.06
Male reproductive	0.96 ^{wyz} ±0.04	4.11 ^{wyz} ±0.05	1.72 ^{wk} ±0.11	1.54 ^{wk} ±0.15	1.59 ^{wk} ±0.04
Female reproductive	0.22 ^k ±0.07	0.50 ^{wyz} ±0.07	0.10 ^k ±0.02	0.15 ^k ±0.03	0.13 ^k ±0.04
N*	8	8	7-8	8	8

Values are Mean ± SEM

a Stomach, small intestine, large intestine & caecum

b Inclusion of one atypical muscle increases value to 68.6 ± 10.1

All values (g), except soleus (mg).

w Significantly different from Size-matched control (p<0.05)

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

z Significantly different from Food-restricted (p<0.05)

Table 5.4 **Effects of PEM and PUFA-enrichment on organ mass as a % of body mass: Experiment 1.**

PUFA content	<u>Control</u>	<u>Protein-energy malnourished</u>		
		Low	Medium	High
Liver	3.81 ±0.15	3.60 ±0.22	3.87 0.25	3.64 ±0.33
Kidney	0.82 ^{yz} ±0.03	1.37 ^{xyz} ±0.12	1.06 ^x ±0.03	1.04 ^x ±0.03
Heart	0.37 ^{yz} ±0.02	0.48 ^{yz} ±0.01	0.44 ^x ±0.01	0.46 ^x ±0.02
Brain	0.62 ^{yz} ±0.05	1.88 ^{xyz} ±0.03	1.63 ^x ±0.03	1.54 ^x ±0.06
Gastrocnemous muscle	0.62 ^y ±0.05	0.52 ^{yz} ±0.05	0.68 ±0.03	0.74 ±0.02
Hind leg muscle	2.22 ±0.14	1.99 ^{yz} ±0.27	2.53 ±0.25	2.68 ±0.16
Hind leg bones	0.48 ±0.03	0.90 ^{xyz} ±0.02	0.81 ^x ±0.03	0.77 ^x ±0.02
Digestive tract	3.24 ^{yz} ±0.42	6.14 ^x ±0.30	5.02 ^x ±0.26	5.77 ^x ±0.44
Skin & fur	14.62 ^{yz} ±0.62	13.22 ^x ±0.55	12.86 ^x ±0.20	12.83 ^x ±0.40
Skinned tail	1.56 ±0.10	1.87 ^x ±0.09	1.64 ±0.03	1.73 ±0.09
Male reproductive	1.89 ±0.68	1.37 ±0.14	1.95 ±0.32	2.42 ±0.23
Female reproductive	0.28 ±0.08	0.15 ±0.05	0.17 ±0.04	0.15 ±0.04
N°	3M 3F	3M 2F	3M 3F	3M 3F

Values are Mean ± SEM; % body mass
 x Significantly different from Control (p<0.05).
 y Significantly different from High PUFA (p<0.05).
 z Significantly different from Medium PUFA (p<0.05).

Table 5.5 Effects of PEM, food-restriction and PUFA-enrichment on organ mass as % body mass: Experiment 3.

	Control Diet			PEM	PEM PUFA- enriched
	Size- matched	Age- matched	Food- restricted		
Liver	6.36 ^{xyz} ±0.45	3.62 ^w ±0.34	3.80 ^w ±0.29	4.13 ^w ±0.34	4.60 ^{wx} ±0.42
Kidney	1.47 ^{xyz} ±0.04	0.93 ^{wyz} ±0.03	1.17 ^{wx} ±0.02	1.15 ^{wx} ±0.02	1.15 ^{wx} ±0.03
Heart	0.62 ^{xyz} ±0.03	0.39 ^{wyz} ±0.01	0.50 ^{wxy} ±0.01	0.53 ^{wxz} ±0.01	0.49 ^{wxy} ±0.01
Brain	1.94 ^x ±0.04	0.75 ^{wyz} ±0.05	1.90 ^{xy} ±0.03	2.04 ^{xz} ±0.04	1.94 ^{xy} ±0.03
Gastrocnemous muscle	0.83 ^{xyz} ±0.03	1.32 ^{wy} ±0.04	1.27 ^w ±0.05	1.13 ^{wxz} ±0.03	1.18 ^{wx} ±0.10
Soleus muscle	59.28 ±2.77	62.88 ±3.18	68.76 ^b ±3.41	69.80 ±7.13	67.91 ±4.16
Spleen	0.35 ^{xyz} ±0.04	0.20 ^w ±0.01	0.18 ^w ±0.01	0.18 ^w ±0.00	0.19 ^w ±0.00
Digestive tract ^a	5.88 ^{xy} ±0.25	3.41 ^{wyz} ±0.15	6.18 ^{xy} ±0.20	6.81 ^{wxz} ±0.19	6.95 ^{wxz} ±0.23
Skin & fur	17.05 ^{xyz} ±0.73	18.62 ^{wyz} ±0.43	13.93 ^{wx} ±0.26	13.88 ^{wx} ±0.46	15.48 ^{wxyz} ±0.25
Skinned tail	1.44 ^{xyz} ±0.05	2.05 ^{wy} ±0.08	1.97 ^w ±0.03	1.82 ^{wx} ±0.04	1.75 ^{wxz} ±0.06
Male reproductive	1.22 ^{xyz} ±0.05	0.65 ^{wyz} ±0.06	1.97 ^{wx} ±0.14	1.92 ^{wx} ±0.14	1.89 ^{wx} ±0.06
Female reproductive	0.26 ±0.06	0.23 ±0.03	0.12 ±0.03	0.19 ±0.04	0.16 ±0.04
N°	8	8	7-8	8	8

Values are Mean ± SEM; % body mass, except soleus (% body mass.10³).

a Stomach, small intestine, large intestine & caecum

b Inclusion of one atypical muscle increases value to 80.62 ± 2.57

w Significantly different from Size-matched control (p<0.05)

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

z Significantly different from Food-restricted (p<0.05)

Brain

Food restriction reduced brain weight by only 14%. PEM and PUFA-enrichment resulted in no further change in brain weight. Brain weight was increased from 0.8% to 1.9% of body weight by food restriction and further increased by PEM to 2% of body weight. However, PUFA-enrichment reversed this effect, reducing it to 1.9% of body weight (Tables 5.2-5.5). This was not an effect on brain, but on body weight.

Digestive Tract

Food restriction reduced the weight of the digestive tract by 40% (Table 5.3). The stomach, small intestine and caecum plus large intestine were all reduced by approximately the same degree (Table 5.7). PEM had no further negative effect on the total weight of the digestive tract compared to food restriction, with a decrease in weight of caecum plus large intestine, more than balanced by increases in the weight of stomach and small intestine (Table 5.7). However, PUFA-enrichment during PEM increased the weight of the digestive tract, especially that of the small intestine and stomach (Tables 5.6-5.7).

Hind leg bones and muscles

PEM reduced the weight of hind leg bones from 1.4 g to 0.8 g, a reduction of 43%. PEM reduced the weight of the hind leg musculature from 6.6 g to 2.6g, a reduction of 61%, but only 57% with PUFA-enrichment (Table 5.2). Hind leg muscle as a % of body weight increased from 2.2% in the controls up to 2.7% with PUFA-enrichment rats during PEM. In the low PUFA group, it was only 2 % of body weight (Table 5.4).

Table 5.6 Effects of PEM and PUFA-enrichment on mass of digestive tract: Experiment 1.

PUFA content	Control	Protein-energy malnourished		
		Low	Medium	High
Stomach (g)	1.11 ^{yz} ±0.12	0.71 ^x ±0.10	0.69 ^x ±0.03	0.74 ^x ±0.05
Small & large intestines + caecum (g)	8.31 ^{yz} ±1.05	4.44 ^x ±0.16	4.40 ^x 0.31	4.49 ^x ±0.35
Stomach (%)	0.38 ^{yz} ±0.04	0.85 ^x ±0.12	0.69 ^x ±0.03	0.71 ^x ±0.05
Small & large intestines + caecum (%)	2.86 ^{yz} ±0.39	5.29 ^x ±0.23	4.33 ^x ±0.26	4.36 ^x ±0.40
N°	6	5	6	6

Values are Mean ± SEM; g or % body mass

x Significantly different from Control (p<0.05).

y Significantly different from High PUFA (p<0.05).

z Significantly different from Medium PUFA (p<0.05).

Table 5.7 Effects of PEM, food-restriction and PUFA-enrichment on mass of digestive tract: Experiment 3.

	Control Diet			PEM	PEM PUFA-enriched
	Size-matched	Age-matched	Food-restricted		
Stomach (g)	0.59 ^{xyz} ±0.02	1.15 ^{wyz} ±0.09	0.68 ^{wx} ±0.02	0.72 ^{wx} ±0.03	0.77 ^{wxz} ±0.02
Small intestine (g)	3.51 ^x ±0.21	6.13 ^{wyz} ±0.50	3.68 ^x ±0.14	3.84 ^x ±0.16	4.33 ^{wxz} ±0.15
Caecum + large intestine (g)	0.66 ^{xyz} ±0.02	1.61 ^{wyz} ±0.17	0.94 ^{wx} ±0.05	0.80 ^{wx} ±0.04	0.74 ^{yz} ±0.04
Stomach (%)	0.73 ^{xy} ±0.03	0.44 ^{wyz} ±0.02	0.79 ^{xy} ±0.03	0.92 ^{wxz} ±0.04	0.93 ^{wxz} ±0.03
Small Intestine (%)	4.33 ^{xy} ±0.23	2.36 ^{wyz} ±0.12	4.29 ^{xy} ±0.17	4.87 ^{wxz} ±0.17	5.19 ^{wxz} ±0.19
Caecum + large intestine (%)	0.82 ^{xyz} ±0.03	0.62 ^{wyz} ±0.04	1.10 ^{wx} ±0.05	1.02 ^{wx} ±0.06	0.90 ^{yz} ±0.06
N°	8	8	8	8	8

Values are Mean ± SEM; g or % body mass

w Significantly different from Size-matched control (p<0.05)

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

z Significantly different from Food-restricted (p<0.05)

Gastrocnemius muscle

In Experiment 1, the reduction in weight of gastrocnemius muscle closely paralleled that of hind leg musculature during PEM (Table 5.2) and is thus representative of total hind leg musculature. This is not surprising, as the gastrocnemius is the largest of the hind leg muscles. In the size-matched controls of Experiment 3, gastrocnemius was 0.8 % of body weight i.e., much smaller than the 1.3% of body weight in the age-matched controls. Food-restriction reduced gastrocnemius weight by 68%, leaving gastrocnemius weight at 1.3% of body weight. PEM reduced gastrocnemius weight by 74% and body mass by 70%, reducing gastrocnemius weight to 1.1 % of body weight (Tables 5.3 and 5.5).

Soleus muscle

In experiment 3, food restriction reduced soleus weight by 58%. However, one of the food-restricted rats had a soleus muscle weighing 218 mg. This is well outside the range for the others in this group (47-70 mg) and much higher than the average of the age-matched controls (162 mg). The reason for this atypical value is unclear, especially as the other soleus from the same rat weighed only 47 mg. However, if this atypical weight is omitted from the data, then the weight of the soleus in absolute terms and as a % of body weight becomes 59 ± 2 mg and $69 \pm 3\%$, respectively. The calculated effect of food restriction on soleus weight then becomes a reduction of 64%. PEM reduced soleus weight by 66% (Table 5.3). This resulted in little change in soleus as a % of body weight compared to age-matched controls (Table 5.5). PUFA-enrichment during PEM increased soleus and gastrocnemius weight by 3% and 10% respectively. Unlike the gastrocnemius, the soleus comprised the same % of body weight in the size-matched, as in the age-matched controls.

Tail

In Experiment 1, PEM reduced the weight of the tail (minus its skin) by 61-64% (Table 5.2). In Experiment 3, food restriction and PEM reduced the weight of the tail by 69% and 73% respectively (Table 5.3). PUFA-enrichment had only minor effects on tail weight during PEM, although these effects were positive.

Reproductive organs

Food restriction reduced the weight of reproductive organs in males and females by 58% and 80% respectively. In PEM, they were reduced in males and females by 63-70% and 70-76% respectively (Tables 5.2 and 5.3).

Liver

Food restriction and PEM reduced liver weight by about 66%, but there was no clear effect of PUFA-enrichment on liver weight (Tables 5.2 and 5.3). Liver weight as a % body weight was higher in the size-matched controls (6.4%) than in the age-matched controls (3.6%) (Table 5.5).

Kidney

In Experiment 3 food restriction and PEM reduced kidney weight by 59% and 63% respectively. In Experiment 1, PEM reduced kidney weight by 56%. PUFA-enrichment had little effect on kidney weight (Tables 5.2 and 5.3), or on kidney weight as a % of body weight (Tables 5.4 and 5.5). In Experiment 3, kidney weight was increased from 0.9% to 12% of body weight by both food restriction and PEM. A similar pattern is evident in Experiment 1, except that

PUFA-enrichment increased kidney weight as a % of body weight from 0.8% to 1.0%. In the size-matched controls, kidney weight was increased to 1.5% of body weight.

Heart

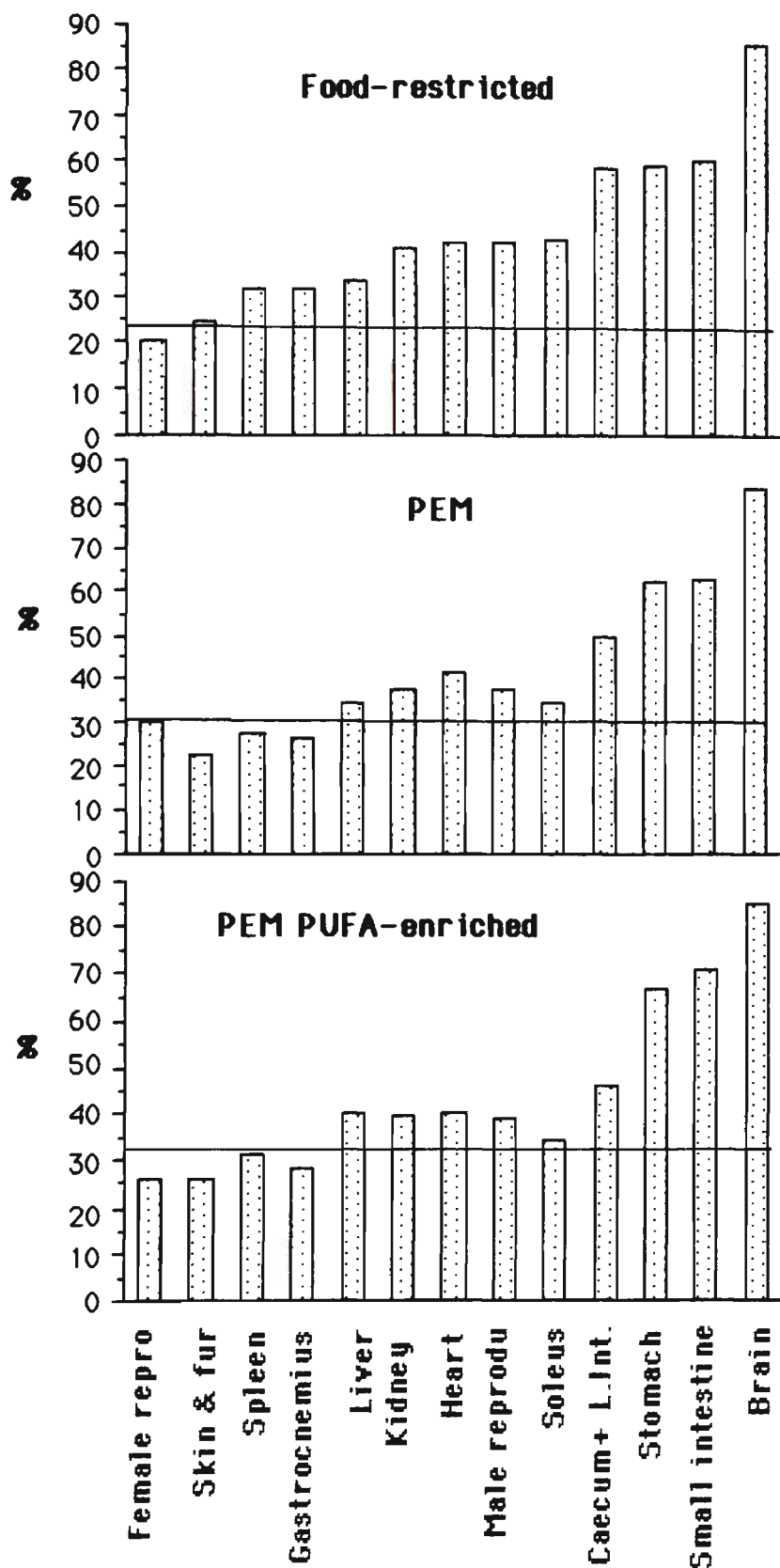
Foodrestriction and PEM reduced the weight of the heart by about 60%. It was larger in the size-matched controls (0.5 g) than in the malnourished rats (0.4 g) (Tables 5.2 and 5.3). There was no effect of PUFA-enrichment. Heart weight as a % of body weight was 0.6% in the size-matched controls, 0.4% in the age-matched controls and approximately 0.5% in the malnourished rats (Tables 5.4-5.5).

Spleen

Spleen weight was reduced by 69% and 73% by food restriction and PEM respectively. PUFA-enrichment reversed the extra reduction in PEM. Spleen weight was 0.2% of body weight in all malnourished groups and in the age-matched controls. In the size-matched controls, spleen weight was twice that of the malnourished rats, which, by definition, were approximately the same body weight (Tables 5.3 and 5.5).

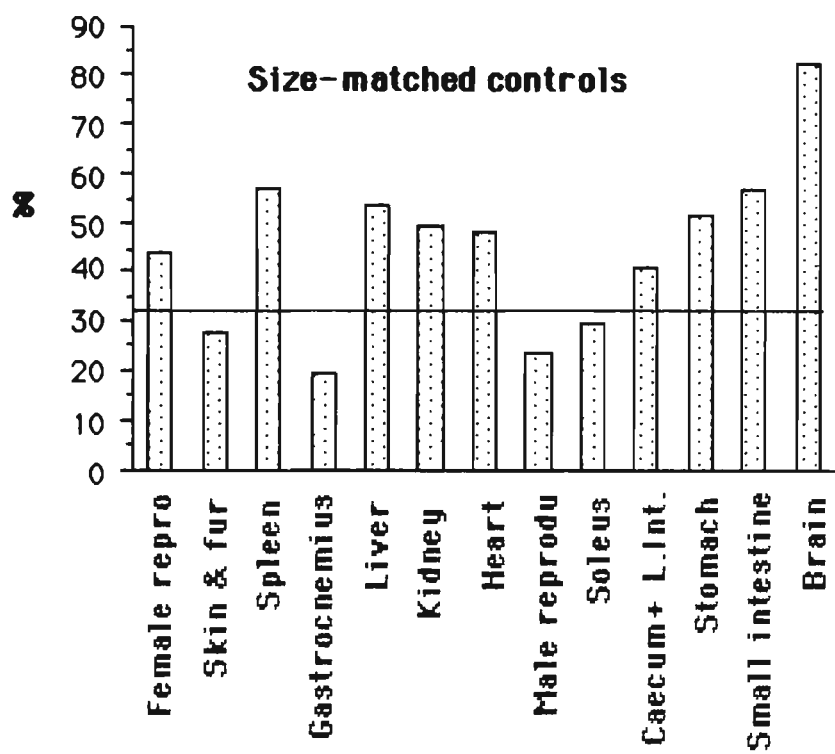
The effects of food-restriction, PEM and PUFA-enrichment on organ weight as a % of body weight are summarised in Figure 5.2. Here, the data has been ranked from organs most affected by malnutrition, to those least affected. Data for the size-matched controls is presented in Figure 5.3. It is clear, that some organs such as female reproductive are greatly affected, while the brain is least affected.

Figure 5.2 Effects of food-restriction, PEM and PUFA-enrichment on organ weight as a % of organ weight in Age-matched controls



Horizontal line denotes body wt. as a % of body wt. in Age-matched controls

**Figure 5.3 Organ weight in Size-matched controls as
a % of organ weight in Age-matched controls**



Horizontal line denotes body wt. as a % of body wt. in Age-matched controls

Organ Composition

Hematocrit was unchanged by food restriction, but was reduced from 42.8% to 40.8% by PEM and to 39.4% by PUFA-enrichment during PEM. The size-matched controls had the lowest hematocrit values, at 37.75% (Table 5.8).

Food restriction increased %H₂O in gastrocnemius muscle, decreased it in brain and soleus muscle and left it unchanged in liver, kidney and heart. PEM increased %H₂O in liver, gastrocnemius and soleus muscles, kidney and heart. Changes in brain were of small magnitude (Tables 5.9-5.18). In general, PUFA-enrichment tended to reduce %H₂O except for liver and heart in Experiment 3.

Food restriction increased the protein content of liver and decreased that of plasma, but the protein content of gastrocnemius muscle was unchanged. PEM did not change protein content of liver, kidney, heart, brain or gastrocnemius muscle, but decreased the protein content of plasma (Tables 5.9-5.17).

Food restriction increased the Na⁺ content of brain, liver and soleus muscle and reduced the K⁺ content of brain and soleus muscle, but not liver. The only measured changes in Na⁺ and K⁺ content caused by PEM, were an increase in soleus muscle Na⁺ content in Experiment 3 and an increase in liver K⁺ content and decrease in heart and brain Na⁺ content in Experiment 1.

When the size-matched controls were compared to the age-matched controls, % H₂O was higher in brain, kidney, gastrocnemius muscle and heart and lower in soleus muscle. Protein concentration was higher in gastrocnemius muscle and lower in plasma. The Na⁺ concentration and Na⁺/K⁺ were lower in liver.

Table 5.8 Effects of PEM, food-restriction and PUFA-enrichment on hematocrit: Experiment 3.

	Control Diet			PEM	PEM PUFA- enriched
	Size- matched	Age- matched	Food- restricted		
Hematocrit (%)	37.7 ^{xyz} ±0.96	42.8 ^w ±1.07	42.1 ^w ±0.60	40.8 ^w ±0.55	39.6 ^{xz} ±0.68
N°	8	8	8	8	8

Values are Mean ± SEM; %.

w Significantly different from Size-matched control (p<0.05)

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

z Significantly different from Food-restricted (p<0.05)

Table 5.9 Effects of PEM and PUFA-enrichment on composition of gastrocnemius muscle: Experiment 1.

PUFA content	Control	Protein-energy malnourished		
		Low	Medium	High
% H ₂ O	74.3 ^z ±0.4	76.0 ^{xy} ±0.1	75.5 ^x ±0.3	75.0 ±0.6
Protein (% Wet Wt)	15.6 ^z ±0.7	13.0 ^x ±0.5	13.5 ^x ±1.3	14.3 ±0.7
Na ⁺ content (μ mol/g Wet Wt ⁻¹)	24.7 ^z ±1.6	31.4 ±4.7	29.1 ^x ±3.5	28.6 ±3.0
K ⁺ content (μ mol/g Wet Wt ⁻¹)	100.6 ±1.3	88.8 ^{xyz} ±5.0	97.8 ^y ±2.8	103.6 ^z ±3.2
Na ⁺ + K ⁺ content (μ mol/g Wet Wt ⁻¹)	125.3 ^y ±2.3	121.1 ^{xyz} ±7.6	127.0 ±5.2	132.3 ^x ±6.1
Na ⁺ /(Na ⁺ + K ⁺)	0.20 ^{yz} ±0.01	0.26 ^{xy} ±0.03	0.23 ^x ±0.02	0.21 ±0.01
Na ⁺ /K ⁺	0.25 ±0.02	0.36 ^x ±0.05	0.30 ±0.04	0.28 ±0.02
N°	6	4-5	6	6

Values: Mean ± SEM.
x Significantly different from Control (p<0.05).
y Significantly different from High PUFA (p<0.05).
z Significantly different from High PUFA (p<0.05).

Table 5.10 Effects of PEM, food-restriction and PUFA-enrichment on composition of gastrocnemius muscle: Experiment 3.

Gastrocnemius	Control Diet			PEM	PEM PUFA- enriched
	Size- matched	Age- matched	Food- restricted		
% H ₂ O	76.4 ^{pyz} ±0.1	74.7 ^{wyz} ±0.2	75.8 ^{wx} ±0.1	75.7 ^{wx} ±0.2	75.7 ^{wx} ±0.2
Protein (% Wet Wt)	18.9 ^x ±0.9	14.9 ^w ±1.1	17.1 ±1.8	15.8 ±1.3	14.3 ^w ±0.8
N°	7-8	8	8	8	8

Values: Mean ± SEM.
w Significantly different from Size-matched control (p<0.05)
x Significantly different from Age-matched control (p<0.05)
y Significantly different from PEM (p<0.05)
z Significantly different from Food-restricted (p<0.05)

Table 5.11 Effects of PEM and PUFA-enrichment on composition of brain: Experiment 1.

PUFA content	Control	Protein-energy malnourished		
		Low	Medium	High
% H ₂ O	77.6 ±0.12	77.7 ±0.2	78.0 ±0.2	77.9 ±0.3
Protein (% Wet Wt)	10.1 ±0.3	9.5 ±0.4	9.8 ±0.4	9.8 ±0.4
Na ⁺ content (μ mol/g Wet Wt ⁻¹)	46.6 ^y ±0.8	42.6 ^x ±0.6	43.3 ^x ±1.6	42.7 ^x ±0.8
K ⁺ content (μ mol/g Wet Wt ⁻¹)	94.4 ±1.3	98.3 ±1.6	96.4 ±1.7	97.5 ±1.2
Na ⁺ + K ⁺ content (μ mol/g Wet Wt ⁻¹)	141.1 ±1.8	140.9 ±2.0	139.7 ±2.6	140.5 ±2.0
Na ⁺ /(Na ⁺ + K ⁺)	0.33 ^y ±0.00	0.30 ^x ±0.00	0.31 ^x ±0.01	0.30 ^x ±0.00
Na ⁺ /K ⁺	0.50 ^y ±0.01	0.43 ^x ±0.01	0.45 ^x ±0.02	0.44 ^x ±0.01
N°	6	5	6	6

Values: Mean ± SEM

x Significantly different from Control (p<0.05).

y Significantly different from High PUFA (p<0.05).

z Significantly different from Medium PUFA (p<0.05).

Table 5.12 Effects of PEM, food-restriction and PUFA-enrichment on composition of brain: Experiment 3.

	Control Diet			PEM	PEM PUFA- enriched
	Size- matched	Age- matched	Food- restricted		
% H ₂ O	79.5 ^{yz} ±0.1	78.0 ^{wz} ±0.2	77.5 ^{wx} ±0.2	77.6 ^w ±0.7	77.6 ^w ±0.1
Protein (% Wet Wt)	7.17 ^{yz} ±0.63	7.51 ^w ±0.57	8.14 ^{wx} ±0.52	7.59 ^w ±0.40	8.30 ^w ±1.09
Na ⁺ content (µmol.gWet Wt ⁻¹)	40.7 ±0.4	41.3 ±1.0	41.5 ±2.0	39.7 ±1.2	39.8 ±0.7
K ⁺ content (µmol.gWet Wt ⁻¹)	92.7 ±1.5	96.4 ±3.4	97.8 ±1.9	96.8 ±1.3	95.7 ±1.1
Na ⁺ + K ⁺ content (µmol.gWet Wt ⁻¹)	133.5 ±1.6	137.7 ±4.4	137.4 ±2.4	135.5 ±1.9	135.5 ±1.3
Na ⁺ /(Na ⁺ + K ⁺)	0.31 ^{yz} ±0.00	0.30 ^{yz} ±0.00	0.29 ^{wx} ±0.00	0.29 ^{wx} ±0.00	0.29 ^w ±0.00
Na ⁺ /K ⁺	0.44 ^{yz} ±0.01	0.43 ^{yz} ±0.01	0.41 ^{wx} ±0.01	0.40 ^{wx} ±0.01	0.42 ^w ±0.01
N°	6-8	8	7-8	7-8	7-8

Values: Mean ± SEM

w Significantly different from Size-matched control (p<0.05)

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

z Significantly different from Food-restricted (p<0.05)

Table 5.13 **Effects of PEM, food-restriction and PUFA-enrichment on composition of soleus muscle: Experiment 3.**

Soleus	Control Diet			PEM	PEM PUFA- enriched
	Size- matched	Age- matched	Food- restricted		
H ₂ O	77.07 ±0.73	78.20 ±0.35	76.77 ±0.65	78.60 ±0.59	78.53 ±1.02
Na ⁺ content (µmol.gWet Wt ⁻¹)	28.00 ±1.42	26.00 ^y ±1.70	29.35 ±2.90	32.65 ^x ±2.26	32.34 ^x ±1.87
K ⁺ content (µmol.gWet Wt ⁻¹)	98.02 ±3.83	93.88 ±2.43	93.67 ±3.15	92.97 ±1.85	91.43 ±2.81
Na ⁺ + K ⁺ content (µmol.gWet Wt ⁻¹)	126.02 ±3.01	119.88 ±2.88	123.02 ±2.24	125.62 ±0.95	123.67 ±2.27
Na ⁺ /(Na ⁺ + K ⁺)	0.22 ±0.01	0.22 ±0.01	0.24 ±0.02	0.26 ±0.02	0.26 ±0.02
Na ⁺ /K ⁺	0.29 ±0.03	0.28 ±0.02	0.32 ±0.04	0.35 ±0.03	0.36 ±0.03
N°	6-8	6-8	6-8	6-8	6-8

Values: Mean ± SEM.

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

Table 5.14 **Effects of PEM and PUFA-enrichment on composition of liver:**
Experiment 1.

PUFA content	Control	Protein-energy malnourished		
		Low	Medium	High
% H ₂ O	69.4 ^z ±0.9	74.4 ^{xy} ±1.2	72.1 ^x ±0.5	71.8 ±0.6
Protein (% Wet Wt)	16.9 ±0.3	15.5 ±0.7	17.4 ±1.1	16.4 ±1.0
Na ⁺ content (μ mol/g Wet Wt ⁻¹)	27.3 ^{yz} ±1.4	35.5 ^{xyz} ±2.7	29.5 ^x ±1.2	29.6 ^x ±1.0
K ⁺ content (μ mol/g Wet Wt ⁻¹)	82.9 ±2.3	74.2 ±5.7	77.7 ±2.8	80.6 ±1.0
Na ⁺ + K ⁺ content (μ mol/g Wet Wt ⁻¹)	110.2 ±2.7	109.6 ±4.2	107.6 ±2.1	110.2 ±1.9
Na ⁺ /(Na ⁺ + K ⁺)	0.25 ±0.01	0.33 ^{xyz} ±0.03	0.27 ±0.01	0.27 ±0.00
Na ⁺ /K ⁺	0.33 ±0.02	0.50 ^{xyz} ±0.07	0.38 ±0.03	0.37 ±0.01
N°	6	5	5-6	4-5

Values: Mean ± SEM
 x Significantly different from Control (p<0.05).
 y Significantly different from High PUFA (p<0.05).
 z Significantly different from Medium PUFA (p<0.05).

Table 5.15 **Effects of PEM, food-restriction and PUFA-enrichment on composition of liver: Experiment 3.**

	Control Diet			PEM	PEM PUFA- enriched
	Size- matched	Age- matched	Food- restricted		
% H ₂ O	66.6 ^y ±0.4	67.3 ^y ±0.7	68.0 ±0.6	68.9 ^{wx} ±0.4	69.4 ^{wx} ±0.4
Protein (% Wet Wt)	11.4 ±1.2	11.8 ±1.0	14.7 ±1.0	12.6 ±1.5	12.7 ±1.0
Na ⁺ content (µmol/gWet Wt ⁻¹)	23.4 ^{xyz} ±1.7	28.5 ^w ±1.8	32.2 ^w ±1.6	29.7 ^w ±1.9	29.9 ^w ±1.2
K ⁺ content (µmol/gWet Wt ⁻¹)	74.2 ±4.5	78.2 ±3.7	84.0 ±3.6	79.0 ±2.8	77.8 ±2.4
Na ⁺ + K ⁺ content (µmol/gWet Wt ⁻¹)	97.59 ±6.14	106.7 ±5.5	116.2 ±4.8	108.7 ±3.9	107.7 ±2.9
Na ⁺ /(Na ⁺ + K ⁺)	0.24 ^{xyz} ±0.01	0.27 ^w ±0.00	0.28 ^w ±0.01	0.27 ^w ±0.01	0.28 ^w ±0.01
Na ⁺ /K ⁺	0.32 ^{xyz} ±0.01	0.36 ^w ±0.01	0.38 ^w ±0.01	0.38 ^w ±0.02	0.39 ^w ±0.02
N°	8	7-8	8	7-8	8

Values: Mean ± SEM.
w Significantly different from Size-matched control (p<0.05)
x Significantly different from Age-matched control (p<0.05)
y Significantly different from PEM (p<0.05)
z Significantly different from Food-restricted (p<0.05)

Table 5.16 **Effects of PEM and PUFA enrichment on composition of kidney:**
Experiment 1.

	<u>Control</u>	<u>Protein-energy malnourished</u>		
PUFA content		Low	Medium	High
% H ₂ O	76.0 ±0.5	78.5 ±1.2	78.1 ±0.9	77.6 ±0.8
Protein (% Wet Wt)	11.6 ±0.3	10.7 ±0.7	11.2 ±0.7	11.3 ±0.7
Na ⁺ content (μ mol.g Wet Wt ⁻¹)	58.0 ±2.9	64.0 ±4.0	55.9 ±3.3	56.9 ±2.4
K ⁺ content (μ mol.g Wet Wt ⁻¹)	70.4 ±1.3	66.1 ±3.6	69.3 ±2.7	70.5 ±3.0
Na ⁺ + K ⁺ content (μ mol.g Wet Wt ⁻¹)	128.4 ±4.0	130.1 ±4.0	125.1 ±5.1	127.5 ±4.9
Na ⁺ /(Na ⁺ + K ⁺)	0.45 ±0.01	0.49 ^{xyz} ±0.03	0.45 ±0.01	0.45 ±0.01
Na ⁺ /K ⁺	0.82 ±0.03	0.93 ^{xy} ±0.06	0.81 ±0.05	0.81 ±0.03
N°	6	5	4-6	5-6

Values: Mean ± SEM.
 x Significantly different from Control (p<0.05).
 y Significantly different from High PUFA (p<0.05).
 z Significantly different from Medium PUFA (p<0.05).

Table 5.17 **Effects of PEM and PUFA-enrichment on composition of heart:**
Experiment 1.

	<u>Control</u>	<u>Protein-energy malnourished</u>		
PUFA content		Low	Medium	High
% H ₂ O	77.0 ±0.2	78.0 ±1.8	77.2 ±0.4	76.5 ±1.6
Protein (% Wet Wt)	13.6 ±0.4	11.0 ^{xyz} ±0.7	12.8 ±0.4	11.9 ±0.8
Na ⁺ content (μ mol/g Wet Wt ⁻¹)	39.8 ^z 1.5	33.5 ^{xyz} ±1.1	37.1 ^x ±0.5	38.9 ±0.6
K ⁺ content (μ mol/g Wet Wt ⁻¹)	75.6 ±1.5	66.7 ±3.1	75.1 ±2.7	77.8 ±5.8
Na ⁺ + K ⁺ content (μ mol/g Wet Wt ⁻¹)	115.5 ^y ±2.2	100.2 ^{xyz} ±3.6	112.2 ±3.1	112.1 ^x ±4.4
Na ⁺ /(Na ⁺ + K ⁺)	0.35 ±0.01	0.34 ±0.01	0.33 ±0.01	0.35 ±0.01
Na ⁺ /K ⁺	0.53 ±0.02	0.50 ±0.03	0.50 ±0.02	0.54 ±0.03
N°	6	4-5	6	4-6

Values: Mean ± SEM.
 x Significantly different from Control (p<0.05).
 y Significantly different from High PUFA (p<0.05).
 z Significantly different from Medium PUFA (p<0.05).

Table 5.18 Effects of PEM, food-restriction and PUFA-enrichment on composition of heart and kidney: Experiment 3.

	Control Diet			PEM	PEM PUFA- enriched
	Size- matched	Age- matched	Food- restricted		
Heart (% H ₂ O)	77.5 ^{xyz} ±0.3	75.9 ^{wy} ±0.2	76.1 ^{wy} ±0.2	76.7 ^{wxyz} ±0.2	76.8 ^{yz} ±0.3
Kidney (% H ₂ O)	76.7 ^{xyz} ±0.1	75.9 ^w ±0.2	75.8 ^w ±0.1	76.2 ^w ±0.2	76.0 ^w ±0.2
N°	8	8	8	8	8

Values: Mean ± SEM

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

z Significantly different from Food-restricted (p<0.05)

Discussion

Carcass Weight

Food restriction, a 67% reduction in food intake retarded growth, so that body weight was 67% smaller. The smaller body weight, combined with the concomitant increase in % H₂O of at least several tissues, reduced carcass dry weight by 80%. PEM reduced growth and increased % H₂O to a greater extent than food-restriction. Garrow et al, (1965) and Halliday (1967) have reported that the bodies of children who died from malnutrition were overhydrated. PUFA-enrichment reversed the extra effects of protein deprivation superimposed on food-restriction. However, the increased growth resulting from PUFA-enrichment during PEM is unlikely to be due to an increased % of body water, as it is associated with a decrease in carcass % H₂O. Furthermore, much of this increased growth is associated with organs other than liver, kidneys, brain, gastrocnemius or soleus muscle, as the PUFA-induced increase in carcass dry weight is 2.9 g, irrespective of whether these organs are included in the calculations. The increased concentration of water in the carcass in the PEM rats is not necessarily an indication of oedema. Total body water (% body weight) has been reported to increase in marasmic children fed high-energy diets, without the appearance of oedema (Patrick et al, 1978).

Organ Weight

Skin and fur was a major component of the PUFA-induced increase in body weight (Tables 5.2 and 5.3). Food restriction reduced the weight of skin and fur in absolute terms and as a % of body weight. PEM further reduced the weight of skin and fur, although not as a % of body weight, because of the

further reduction in body weight of this group. However, PUFA-enrichment increased the weight of skin and fur as a % of body weight over that of PEM rats. This effect was surprising, as body weight was higher in the PUFA-enriched rats than in the PEM rats. However, the weight of skin and fur in the PUFA-enriched PEM rats even exceeded that of the food-restricted rats, both in absolute terms and as a % of body weight. This was unexpected, considering that skin and fur have relatively high protein contents (Waterlow and Stephen, 1966). Ziboh, V. (personal communication) has suggested that the PUFA requirement for skin growth is high. The results of the present study support this suggestion. In a relatively high-protein content tissue, PUFA-enrichment has tended to reverse a weight loss that occurs via food restriction or PEM. The effect of PUFA-enrichment was generally the same in Experiment 1, although the increased weight of skin and fur as a % of body weight in the low PUFA group is a result of its greatly reduced body weight.

Under the conditions of the present study, PUFA-availability is a more significant weight-limiting factor for skin than availability of protein during PEM and food during food-restriction. The present results do not indicate whether the increased growth associated with PUFA-enrichment involves increase of cellular mass, or of other material, such as subcutaneous fat. These results are intriguing, considering the results of Hill and Holman (1980). The dermal signs of EFA-deficiency in rats increase as dietary protein content increases from 5% to about 15%. They remain constant up to 30%, above which, there is a sharp increase. However, in liver phospholipids the triene/tetraene ratio (a biochemical index of EFA-deficiency) increased at low protein levels.

The greater reduction in skin and fur in the low-PUFA group during PEM may increase thermal losses and increase energetic costs. This could occur while trying to maintain a high and constant body temperature when

environmental temperature is below the lower critical temperature, which is 30°C in the rat (Kleiber, 1961). BMR, by definition (Kleiber, 1961) was measured in the thermoneutral zone i.e., between the upper and lower critical temperatures. Thus, differences in BMR may provide an underestimate of the relative differences in metabolic costs arising from increased dietary PUFA during PEM, when the environmental temperature is well below the lower critical temperature.

When considering the energetic costs of a high and constant body temperature where environmental temperatures are low, it should be remembered, that subcutaneous fat is generally depleted in marasmus, but not in kwashiorkor and that in some cases, metabolism has been found to decrease with reduced environmental temperature (See chapter 1). When considering the significance of organ mass for overall protein depletion from the body, Waterlow and Stephen (1966) found that the rat loses more protein in the skin than any other organ. They suggest that in man, it is probably muscle in which this happens. The significance of the effect of PUFA-enrichment on skin and fur for humans is unknown. In some cases it may be unwise to extrapolate from results in animals to man.

Brain Weight

The differences in brain weight as a % of body weight between groups of malnourished rats were modulated by changes in body weight. The brain weight of the malnourished rats is approximately that expected in rats of this size (see size-matched controls). The results appear to suggest the existence of an adaptive mechanism, whereby brain size is selectively protected at the expense of other tissues during malnutrition, although they do not indicate whether this involves the maintenance of a slightly reduced anabolism in brain tissue, protection from increased catabolism, or a combination of both. The

existence of such an adaptive mechanism in brain has been suggested previously (Dobbing, 1968), but has also been discounted (Ramalingaswami, 1969). Although body weight increases by 224% from size-matched controls to age-matched controls, brain weight increases by only 21% during this period of development (Table 5.3). Thus, there is less scope for growth in brain weight, or in differential effects of food restriction, PEM and dietary PUFA, than in other tissues such as liver or kidney. A similarly low growth in brain weight in relation to body weight, especially after weaning has been shown by Donaldson (1908). In some cases, the brain : body ratio has been used as an index of brain maturity. The significance of this parameter has been questioned by Dobbing (1968), as it is not a good indication of brain function.

Digestive Tract

In the present study, increases in the weights of the stomach and small intestine by PUFA-enrichment during PEM were not significant. This is in accord with the finding that PUFA-enrichment did not affect apparent absorption of energy or nitrogen. The 40% reduction in weight of the digestive tract induced by food restriction is much smaller than the corresponding 67% reduction in body weight. PEM tends to increase the weight of the digestive tract more than that of food restriction, especially with additional dietary PUFA. This is evident when digestive tract weight is expressed as a % of body weight, but there is also a tendency for this in absolute terms. This suggests, that during food restriction, and even more so during PEM, that there exists an adaptive mechanism to conserve the weight of the stomach and small intestine, a mechanism which is enhanced during PEM by PUFA-enrichment. In a similar experiment on rats, Pond, Yen and Mersmann (1986) also report that food-restriction increased digestive tract weight as a % of body weight. They suggested that the gastrointestinal tract may have a greater priority than skeletal muscle and adipose tissue for nutrient partitioning during food-restriction.

The reduced stomach capacity during malnutrition, limits the volume of food that a malnourished child can ingest per day. An increased proportion of dietary lipids is often advocated to increase the energy density of food, and thus reduce the volume of food intake to tolerable levels (Waterlow and Alleyne, 1971; Jaya Rao, 1978). The present study indicates that the choice of dietary fatty acids can increase the weight of the digestive tract during PEM, although the increase is not significant. Lumen volume was not measured in the present study.

Hind leg bones and musculature.

PEM is reported to reduce bone length (Pretorius, 1968; Krueger, 1969). To determine the effects of PEM and PUFA-enrichment on the skeletal system, I have measured the weight of the hind limb bones. These bones are relatively large and it is easier to remove tissue from them, than many other parts of the skeletal system. PEM reduced the weight of hind leg bones by 42%, a much smaller reduction than the corresponding 66% reduction in body weight. The results indicate that the weight of hind leg bones is affected, by PEM, but not as much as that of other tissues. They also suggest that PUFA-enrichment has relatively small effect on the weight of hind leg bones during PEM.

The reduction in weight of the hind leg musculature as a result of PEM (61%), was relatively greater than that in hind leg bones. Because of the relatively greater reduction in body weight, hind leg muscle as a % of body weight was increased from 2.2% in the controls up to 2.5% in PEM. Thus, the relative contribution of hind leg muscle to the overall reduction in body weight during PEM is less than that of some other tissues. This occurred, even though skeletal muscle is reported to grow faster than body weight throughout the period of growth (Miller, 1969). It is possible that this relatively greater growth in total body weight in the control rats is related to the deposition of fat, rather

than an increase in metabolically active tissue. The control rats contained large fat deposits when killed.

Gastrocnemius muscle

Gastrocnemius weight as a % of body weight in the younger size-matched rats, was smaller than that in age-matched controls, even though the older rats contained large fat deposits when killed. This indicates that during most of the experimental period, the gastrocnemius muscle was a relatively faster growing muscle, compared to the soleus, which increased in absolute terms, but not in relation to body weight.

Soleus muscle

PEM resulted in a greater % change in gastrocnemius, soleus and total body weight than food-restriction. The weight of the soleus (a slow muscle) was less susceptible than that of the gastrocnemius (a mixed muscle) to both food restriction and PEM and was also less amenable to the beneficial effects of PUFA-enrichment during PEM. That the changes in soleus were small may be associated with the relatively lower growth rate of soleus muscle in relation to body weight, during most of the experimental period. The increase in weight of gastrocnemius muscle from size-matched rats to age matched rats is 415%. The corresponding increase for the soleus is only 236% (Table 5.3). In these respects at least, soleus may not as representative as gastrocnemius muscle, of the change in the weight of hind limb musculature during malnutrition.

Tail

Food restriction and PEM reduced the weight of the tail (minus its skin) by 69% and 73% respectively (Table 5.3). A comparison of the size-matched and age-matched controls (Table 5.5) indicates that tail weight comprises a smaller proportion of body weight in younger rats. This may account for the relatively

smaller reductions in tail weight (61-66%) resulting from PEM in the slightly younger rats of Experiment 1. Dietary PUFA increased tail weight during PEM. However, these improvements were minor, especially when considered as a % of body weight (Tables 5.2-5.5). These results suggest that growth (increase in weight) of the tail is less than that of some other tissues.

Reproductive organs

The effects of food restriction and PEM on reduction in weight of reproductive organs was greater in females than in males (Tables 5.2 and 5.3). The reason for this difference is unclear. There is no clear effect of PUFA-enrichment during PEM increasing the weight of reproductive organs.

Liver, kidney and heart

There was no difference in the effects on liver weight, of food-restriction or PEM. The variability in liver weight was high in Experiment 3, compared to that of other organs, such as kidney and heart (Table 5.3) and may have overwhelmed any possible increase resulting from PUFA-enrichment. The response of kidney and heart weight to food restriction, PEM and PUFA-enrichment are very similar to those detected in liver. The pattern of kidney and heart weight in the age-matched controls is also similar to that detected in liver. It is possible that some of the high variability in weight of liver resulted from differences in liver weight between males and females. Livers from females are generally smaller than those from males, although in the present study, these differences were not significant.

The increase in kidney weight as a % of body weight during PEM (Tables 5.4 and 5.5) has been reported previously by Pimplikar and Kaplay (1981). These authors also reported that liver weight as a % of body weight is also increased during PEM. In the present study, this effect was more marked in Experiment 3.

Organ Composition

The effects of food restriction, PEM and increased dietary PUFA on H₂O, protein, Na⁺ and K⁺ composition were greater in some tissues than others. The ways in which these parameters responded to the dietary manipulations also varied between tissues. In mammalian cells, intracellular concentrations of K⁺ are high (120-160 mM) and intracellular concentrations of Na⁺ are low (< 10 mM) (Metcoff, 1975; Lehninger, 1978). In a sense, K⁺ may be considered as an "intracellular" ion and Na⁺ as an "extracellular" ion. In malnourished children with oedema, whole body Na⁺ content is increased (Alleyne, 1975), and whole body potassium (WBK) is reduced (Garrow, 1965), perhaps as a result of diarrhoea (Passmore, 1986). Expansion of the extracellular space and an increase in the intracellular concentration of Na⁺ both contribute to an increase in whole body Na⁺ content (Waterlow and Alleyne, 1971). In general, an increase in the ratio of Na⁺: K⁺ in a tissue is an indication of increased extracellular volume. The mechanisms by which this may occur are unclear at present. However, they may involve increased membrane permeability, a reduction in ATP available for sodium pumping or a reduction in pumping efficiency, or a combination of these.

Brain, was more resistant than other tissues to the changes in the concentrations of H₂O, protein, Na⁺ and K⁺ induced by food restriction and PEM, even in the low PUFA group of Experiment 1. However, differences between the size-matched and age-matched controls indicate changes in brain H₂O and K⁺ content occur during the growth and development period examined. Malnutrition may cause only small changes in tissue K⁺ concentration, as in the present study. However, malnutrition may still result in a substantial decrease in WBT. Tissue wasting may reduce the capacity for retention of K⁺. However, that capacity for K⁺ may be saturated (Waterlow and

Alleyne, 1971). The apparent lack of effect of the experimental variables on protein content of tissues such as muscle, may be due to a greater loss of active tissue protein than collagen. Montgomery (1962b) report an increase in the collagen content of muscle in infants with kwashiorkor and Halliday (1967) has reported a greater loss of non-collagen protein than collagen protein in malnourished infants.

In the present study, hematocrit was reduced by PEM. Torun and Viteri (1988) suggested that a reduced hematocrit may be considered an adaptive response during malnutrition. The decreased BMR during PEM reported in (Table 3.11) is consistent with the reduced hematocrit. Hematocrit is also reduced in protein-deficient pigs (Platt et al., 1969). Al-Rabi (1962) (cited in Platt, Heard and Stewart, 1969) reported that protein was a limiting factor in hemoglobin synthesis in pigs. However, the major decrease in BMR was with food-restriction (Table 3.11), which had no effect on haematocrit.

Tissue Growth

Body weight and thus organ weight were decreased by food restriction. Figure 5.2 indicates that these malnourished rats were not merely a 1/3 scale model of their well-fed littermates. If they were, then the bar representing organ weight as a % of body weight would be as high as the line representing their body weight as a % of body weight of the Age-matched controls. It is clear that this is not the case. Some groups of organs are more resistant to food-restriction than others. Brain was the organ most highly defended against retardation. This may be related to its rapid growth early in development, compared to that in other organs. At the other extreme, female reproductive organs, skin and fur, spleen and gastrocnemous muscle were greatly affected. Growth in some of these organs may be preferentially retarded to maintain nutrient supply to other more vital organs. Liver, kidney, heart

and male reproductives were less well protected. The pattern of organ weight as a % of body weight is very different in the younger size-matched controls, especially in the reproductive organs and spleen (Fig. 5.3). Different tissues have different periods in which growth spurts occur (Miller, 1969) and the nature of this growth is also age-dependent (Winnick and Noble, 1965). Thus, the period during which malnutrition occurs determines the nature of the reduction in growth and also the chances of the effects being permanent (Winnick and Noble, 1966). This may influence the effects that dietary restriction has on different organs e.g. the effects on brain composition would be greatest before weaning and those on muscle composition greatest after weaning. Reducing dietary protein content (PEM) and PUFA-enrichment produced some alterations in the pattern outlined for food-restriction. The effects of PEM in greatly reducing spleen weight have been reported previously (Platt et al, 1969; Ramalingaswami, 1969). Ramalingaswami (1969) claimed that the spleen is more sensitive to reduced protein than the liver. The present results (Table 5.3) support this view. PUFA-enrichment during PEM increased growth during the experimental period. Consequently, there was a general increase in organ size. This was most evident in some of the organs most affected by PEM, such as skin and fur, spleen and the digestive tract.

Summary

Sprague-Dawley rats were fed control, food-restricted or PEM diets, which were isoenergetic and contained various levels of PUFA, almost entirely as 18:2 w6. PEM reduced growth to a greater extent than food restriction, but PUFA-enrichment reversed this added effect.

Carcass water content was increased by both food-restriction and PEM. PUFA-enrichment tended to reverse this effect, but still increased carcass weight.

Skin and fur accounted for most of the PUFA-induced increase in growth during PEM.

Food-restriction and PEM reduced digestive tract weight, but not in proportion to the corresponding reductions in body weight. PUFA-enrichment increased digestive tract weight, but this had no effect on apparent absorption of energy or nitrogen.

PUFA-enrichment increased liver weight during PEM. However, variability in liver weight was high, compared to most other organs and this increase was not significant. Spleen weight was reduced by food-restriction and to a further extent by PEM. PUFA-enrichment increased spleen weight, but the magnitude of this change was small. In some tissues, notably brain, the effect of food-restriction and PEM were relatively small. The effects of PUFA-enrichment were small, especially in these tissues.

The effects of food restriction, PEM and increased dietary PUFA on tissue

composition were greater in some tissues than others. The way in which composition responded to these dietary manipulations also varied between tissues. In some tissues such as brain, there was little change in any of the parameters. In other tissues, food-restriction and PEM generally increased % H₂O and Na⁺ content and reduced K⁺ content. PUFA-enrichment during PEM had little effect on these parameters.

Chapter 6: Ion Homeostasis Measurements

Introduction

Several studies have indicated the existence of membrane abnormalities during PEM. These abnormalities include increased membrane permeability (Willis and Golden, 1988; Patrick and Golden, 1977), increased sodium pump numbers (Narayanareddy and Kaplay, 1982) and 'pump rate' (Patrick, 1979) and decreased osmotic fragility (Brown et al, 1978; Kaplay, 1978, 1979; Fondu, et al, 1980; Ramanadham and Kaplay, 1982). It is possible that these abnormalities or changes in membrane function during PEM result from a decrease in EFA. In this chapter, I have examined the effects of food-restriction, PEM and PUFA-enrichment on some of these parameters of membrane function. Measurements of oxygen uptake by tissue slices indicate whether metabolism changes occur during PEM. Measurements made with and without ouabain, a specific inhibitor of the sodium pump indicate whether these are related to changes in sodium pump activity. Measurements of membrane permeability to K^+ may indicate whether the cell membranes of major organs such as liver and kidney become more leaky, as do those of human leukocytes during PEM. Measurements of the ouabain binding of tissues indicate whether the number of Na^+ pumps in tissues is altered by the PEM or PUFA-enrichment.

Methods and materials

Measurement of "in vitro" tissue O_2 uptake

The rats were killed by decapitation and liver and kidneys removed and weighed. Liver and kidney slices (approximately 500 μm thick) were prepared with a tissue slicer and placed in Warburg flasks with 3.0 ml of buffered medium (135 mM NaCl, 5 mM KH_2PO_4 , 1.5 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mM Tris (Sigma), 10 mM glucose, pH 7.4). Care was taken to avoid renal papillary

tissue during slicing. The flasks were connected to manometers (B. Braun Warburg Apparatus V166) and flushed for 20 seconds with O_2 . Preparation time from death to the beginning of measurement was kept constant at 60 min and included a 30-min equilibration period. Measurements were taken at 30 minute intervals over 2 hours and rates of O_2 uptake were calculated according to Umbreit et al. (1957). Carbon dioxide was absorbed by a 4 cm^2 filter paper soaked with 1M KOH (0.2 ml) in the alkali cup of each flask. The temperature of the flasks was maintained at 37°C .

Parallel measurements were obtained using buffer containing 1 mM ouabain. The difference in O_2 uptake rates between tissues with and without ouabain (i.e., the "ouabain-inhibitable " O_2 uptake) was taken as "sodium transport-dependent" O_2 uptake. Measurements were carried out in triplicate and a flask containing KOH and buffer without tissue was used as a thermobarometer with each set of measurements. At the end of each experiment, the tissue slices were removed, blotted, and placed in pre-weighed 1.5 ml Eppendorf tubes. They were weighed, and their wet weight determined. Flask constants were determined using water and calculations were simplified by filling the manometers with "Brodie's solution" (density 1.033 gml^{-1}), prepared according to Umbreit et al. (1957).

Measurement of membrane passive permeability to potassium in liver and kidney slices

Membrane permeability or leakiness to potassium was measured using $^{86}\text{Rb}^+$ as an analogue of potassium because of its relatively longer half life than $^{42}\text{K}^+$. The efflux of potassium from liver and kidney slices was measured in the presence of ouabain, using a technique similar to that of Else and Hulbert

(1987). These measurements were carried out simultaneously with those of O_2 uptake, but on different slices. Liver and kidney slices were prepared as for O_2 uptake measurements and incubated for 60 min. in 10 ml of the above buffer solution containing $^{86}Rb^+$ (0.5 or 1.0 MBq/ml) to preload the tissues with $^{86}Rb^+$. During incubation, the tissues were gently aerated with oxygen. After incubation, extracellular $^{86}Rb^+$ was removed by gently washing the slices twice in nonradioactive buffer containing 1 mM ouabain. This concentration had previously been shown to result in maximum inhibition of oxygen consumption of rat liver and kidney slices (A.J. Hulbert, personal communication). The slices were then transferred to 10 ml plastic test tubes containing 8 ml nonradioactive buffer containing 1 mM ouabain. A slow stream of oxygen bubbles from the base of each tube kept the slices gently aerated and mixed the medium. At 5 minute intervals over a 45 minute period, 0.2 ml aliquots of the medium were transferred to counting tubes, after which the slice was transferred to a counting tube. The radioactivity of the aliquots and tissue were measured in a Packard gamma scintillation counter. Back-calculation was used to determine the $^{86}Rb^+$ content of the tissue at each sampling time and at time zero, assuming that mixing was instantaneous. Experiments were carried out in triplicate at 37°C. The least squares method was used to calculate the efflux rate constant from the linear part of a semilog plot of content vs. time.

Measurement of tissue uptake of potassium and membrane passive permeability to potassium in soleus muscle.

The uptake and efflux of potassium from soleus muscle was measured using $^{86}Rb^+$ as an analogue of potassium. After the rats were killed by decapitation, hindlimbs were severed from the carcass at the femur-pelvic junction. The soleus muscle, stripped of visible fat, was removed and placed in

Krebs solution at room temperature (22°C) for 10 minutes. The muscle was then incubated for 40 min. at 37°C in 10 ml of carbogenated Krebs solution containing 0.33 MBq $^{86}\text{Rb}^+$, to load the muscle with $^{86}\text{Rb}^+$. The incubation medium was sampled (3 X 30 μl) prior to adding the muscle. After incubation, surface $^{86}\text{Rb}^+$ was removed by rapidly washing the muscle in nonradioactive Krebs solution containing 10 mM ouabain. This concentration ensured maximum inhibition of sodium pumps in the muscle. The muscle was then transferred to 10 ml plastic test tubes containing 10ml Krebs solution containing 10 mM ouabain. A slow stream of carbogen bubbles from the base of each tube kept the muscle gently aerated. The medium was sampled prior to adding the muscle and at 5 minute intervals over a 45 minute period. After 50 minutes, the muscle was removed, gently blotted and transferred to a preweighed counting tube, after which another medium sample was obtained. These medium samples (200 μl) were transferred to counting tubes. The counting tubes containing muscle were weighed and centrifuged to force the muscle to the bottom of the tubes for efficient gamma counting.

The radioactivity of the aliquots and tissue were measured in a Packard Cobra Autogamma Counter. Back-calculation was used to determine the $^{86}\text{Rb}^+$ content of the tissue at each sampling time and at time zero, assuming that mixing was instantaneous. Experiments were carried out in triplicate at 37°C. The least squares method was used to calculate the efflux rate constant from the linear part of a semilog plot of muscle ^{86}Rb content vs. time. The radioactivity in the tissue at time zero, as determined by back-calculation is also that taken up during the 40 minute incubation period. Rate of uptake of $^{86}\text{Rb}^+$ during the 40 minute period was calculated using the 30 μl samples of medium obtained prior to incubation.

Measurement of Soleus Muscle Ouabain Binding

After the rats were killed by decapitation, hindlimbs were severed from the carcass at the femur-pelvic junction. The soleus muscle, stripped of visible fat, was removed and stored at -80°C . Measurement of the number of ^3H -ouabain binding sites in soleus muscle was carried out using a method adapted from Norgaard et al. (1983). Transverse slices (1 mm) of soleus muscle on Millipore filters in 1.5 ml eppendorf tubes were incubated for 60 minutes at 37°C in a shaking water bath (80 RPM). Each slice was incubated in 200 μl buffer (Tris chloride (10 mM), H_3PO_4 (3 mM), MgSO_4 (3 mM), tris vanadate (1 mM) and sucrose (250 mM). pH was adjusted to 7.58 with tris) containing 0.037 MBq/ml ^3H -Ouabain (Amersham). Total ouabain concentrations of 1.0 μM and 10.0 mM were obtained by the addition of unlabelled ouabain. The samples were pre-washed for 20 minutes at 0°C in 200 μl ouabain-free buffer. The filters were centrifuged at 6500 RPM for 3 minutes in a Stansens Centour centrifuge and washed for 2 minutes (5X) with 200 μl buffer (0°C) containing non-radioactive ouabain. After the weight of each slice was determined, it was soaked overnight in 0.5 ml 1.0 M NaOH for tissue digestion and ^3H activity counted in a 1219 Rackbeta liquid scintillation counter after the addition of 3 ml 'Hi-Ionic Fluor' scintillation cocktail (Packard).

Incubation of samples in 1.0 μM ouabain enabled total binding of ouabain (p mol/g muscle) to be calculated. Incubation of other samples in 10 mM ouabain enabled non-specific binding (i.e., uptake and retention of ouabain not bound to the sodium pumps) to be calculated. Specific binding of ouabain was calculated as the difference between total and non-specific binding (as outlined in Norgaard et al 1983), the latter parameters calculated by reference to 50 μl medium samples (pre-incubation). Incubation and media sampling were carried out in quadruplicate. Preliminary experiments had shown saturation of

binding sites during incubation with 1.0 μM ouabain for 60 minutes. They also showed that 5X 200 μl washes with unlabelled medium containing vanadate was effective in removing most of the unbound ^3H ouabain.

Measurement of Erythrocyte Ouabain Binding

After each rat was killed by decapitation, blood was collected in a plastic weighing tray containing 30 μl heparin solution and mixed. Aliquots (0.5 ml) of heparinised blood were pipetted into Eppendorf tubes. The blood was centrifuged for 40 secs in a microfuge and plasma was removed by aspiration. The cells were washed twice in ice-cold 100 mM MgCl_2 and stored at -80°C .

Measurement of the number of ^3H -ouabain binding sites in erythrocytes was carried out using a modification of the method outlined above for soleus muscle, after reference to Narayanareddy and Kaplay (1982). Erythrocytes (from 0.25 ml blood) were freeze-thawed 5 times to ensure uniform fragmentation and re-suspended and incubated in 10 ml of buffer (tris chloride (10 mM), H_3PO_4 (3 mM), MgSO_4 (3 mM), tris vanadate (1 mM) and sucrose (250 mM). pH was adjusted to 7.58 with tris chloride) containing 0.037 MBq/ml ^3H -Ouabain (Amersham). Total ouabain concentrations of 1.0 μM and 10.0 mM were obtained by the addition of unlabelled ouabain. Media samples (3 X 30 μl) from each were placed in scintillation vials. Erythrocyte suspensions were incubated in scintillation vials at 37°C in a shaking water bath for 20 mins. Aliquots from each vial (250 μl) were deposited onto a Skatron glass fibre filtermat located in a Minifold II 'dot blot' apparatus (Schleicher and Schuell) connected to a vacuum pump. The erythrocyte membranes on the filter paper were washed (4 X 200 μl) with medium containing 1.0 mM or 0.4 μM ouabain. The filter was cut into small squares, placed into scintillation vials with 2 ml Hi-Ionic Fluor scintillant, vortex-mixed and counted in a 1219 Rackbeta liquid scintillation counter.

Preliminary experiments showed incubation for 20 mins at 1.0 mM and 0.4 μ M total ouabain were sufficient for saturation of ouabain binding sites and calculation of non-specific binding of ouabain. They also showed that 4 X 200 μ l washes with medium was effective in removing unbound ^3H ouabain. Previously, the effectiveness of the filter material in retaining erythrocyte membrane fragments had been verified by determining the phospholipid content (Mrsny et al., 1986) of the erythrocyte suspension prior to filtration and also of successive washes from below each filter. CPM of media samples and filters was used to determine total and non-specific binding of ouabain to the erythrocytes. Specific binding of ouabain was calculated as the difference between total and non-specific binding (as outlined in Norgaard et al., 1983), the latter parameters calculated by reference to 50 μ l medium samples (pre-incubation).

Measurement of Extracellular Space.

The soleus muscle, stripped of visible fat, was removed and placed in Krebs solution at room temperature (22°C) for 10 minutes. The muscle was then incubated for 40 minutes at 37°C in 10 ml of (carbogenated) Krebs solution containing $^3\text{H}_2\text{O}$ (3.7×10^{-3} MBq/ml) and ^{14}C -Inulin (3.7×10^{-3} MBq/ml). The muscle was then removed, gently blotted and placed in preweighed counting vials. The vials were reweighed, 0.5 ml of 'Soluene' (Canberra Packard) was added to each vial to solubilize the muscle overnight. The ^3H and ^{14}C activity were counted in a 1219 Rackbeta liquid scintillation counter, after the addition of 5 ml 'Hi-Ionic Fluor' (Packard). Total volume and extracellular volume were calculated as $^3\text{H}_2\text{O}$ and ^{14}C Inulin distribution space respectively. Intracellular volume was the difference between these two volumes.

RESULTS

"In vitro" tissue O₂ uptake

The "in vitro" oxygen uptake of liver and kidney slices in Experiment 1 are given in Tables 6.1-6.2 and Figures 6.1-6.4. Many of the differences are not statistically significant. Total "in vitro" metabolism in kidney ($2.4 \mu\text{l O}_2 \cdot \text{mg wet wt.}^{-1} \text{ h}^{-1}$) is higher than that in liver ($1.5 \mu\text{l O}_2 \cdot \text{mg wet wt.}^{-1} \text{ h}^{-1}$). PEM increased the total "in vitro" metabolism of kidney, an effect which was reversed by PUFA-enrichment. Both of these effects were less marked in liver. Most of the PEM-induced increase was due to increased sodium dependent metabolism. Sodium independent metabolism was unchanged in liver by PEM, although it was increased in kidney. Sodium dependent metabolism increased from 0.4 to $0.5 \mu\text{l O}_2 \cdot \text{mg wet wt.}^{-1} \text{ h}^{-1}$ in liver and 0.7 to $1.2 \mu\text{l O}_2 \cdot \text{mg wet wt.}^{-1} \text{ h}^{-1}$ in kidney. Both of these increases were reversed by PUFA-enrichment. The reversal was statistically significant for kidney ($p < 0.05$). When organ size is included in the calculation of "in vitro" oxygen uptake, the difference between the response of liver and kidney to PEM becomes more distinct. Total "in vitro" metabolism in liver is $16.2 \text{ ml O}_2 \cdot \text{liver}^{-1} \cdot \text{h}^{-1}$. PEM reduces this to $6.1 \text{ ml O}_2 \cdot \text{liver}^{-1} \cdot \text{h}^{-1}$, i.e., about 38% of the control value. Total "in vitro" metabolism in kidney is $5.5 \text{ ml O}_2 \cdot \text{liver}^{-1} \cdot \text{h}^{-1}$. PEM reduces this to $3.4 \text{ ml O}_2 \cdot \text{liver}^{-1} \cdot \text{h}^{-1}$, i.e., about 63% of the control value.

⁸⁶Rb⁺ efflux from liver and kidney

The efflux of ⁸⁶Rb⁺ from both liver and kidney slices was increased by PEM. This increase was not reversed by PUFA-enrichment (Table 6.3; Figs. 6.5-6.6). In both tissues, the lowest values of ⁸⁶Rb⁺ efflux during PEM were in the low-PUFA group.

Table 6.1 Effects of PEM and PUFA-enrichment on metabolic rate of liver: Experiment 1.

PUFA content	Control	Protein-energy malnourished		
		Low	Medium	High
Total "In vitro" Metabolism (μO_2 mg wet wt. h^{-1})	1.52 $\pm 0.2^b$	1.94 ± 0.41	1.65 ± 0.42	1.46 ± 0.13
Sodium Independent Metabolism (μO_2 mg wet wt. h^{-1})	1.17 ± 0.31	1.47 ± 0.46	1.15 ± 0.28	1.06 ± 0.10
Sodium Dependent Metabolism (μO_2 mg wet wt. h^{-1})	0.35 ± 0.12	0.47 ± 0.29	0.50 ± 0.16	0.40 ± 0.11
Sodium Dependent Metabolism (%)	25.49 ± 10.06	17.82 ± 9.16	29.00 ± 3.67	25.90 ± 5.93
Total liver "In vitro" Metabolism (ml O_2 liver $^{-1}$. h^{-1})	16.19 ± 1.30	6.08 ^x 1.66	6.13 ^x ± 0.94	5.47 ^x ± 0.64
N°	5	5	6	6

Values: Mean \pm SEM.

x Significantly different from Control ($p < 0.05$).

Table 6.2 Effects of PEM and PUFA-enrichment on metabolic rate of kidney: Experiment 1.

PUFA content	Control	Protein-energy malnourished		
		Low	Medium	High
Total "In vitro" Metabolism (μO_2 mg wet wt. h^{-1})	2.35 ± 0.41	2.04 ± 0.33	3.24 ± 0.61	2.16 ± 0.31
Sodium Independent Metabolism (μO_2 mg wet wt. h^{-1})	1.70 ± 0.34	1.64 ± 0.37	2.04 ± 0.41	1.74 ± 0.29
Sodium Dependent Metabolism (μO_2 mg wet wt. h^{-1})	0.65 ± 0.21	0.40 ± 0.09	1.21 ^y ± 0.35	0.43 ± 0.15
Sodium Dependent Metabolism (%)	27.95 ± 7.85	21.93 ± 5.85	34.95 ± 7.25	20.74 ± 6.38
Total kidney "In vitro" Metabolism (ml O_2 2 kidneys $^{-1}$. h^{-1})	5.49 ± 0.61	2.46 ^x ± 0.63	3.43 ^x ± 0.59	2.33 ^x ± 0.32
N°	4	5	3	6

Values: Mean \pm SEM.

x Significantly different from Control ($p < 0.05$).

y Significantly different from High PUFA ($p < 0.05$).

Table 6.3

Effects of PEM and PUFA-enrichment on efflux of ^{86}Rb from liver and kidney slices: Experiment 1.

PUFA content	Control	Protein-energy malnourished		
	Medium	Low	Medium	High
Liver ^{86}Rb Efflux Rate Constant (%. min ⁻¹)	1.79 ^y ±0.28	2.06 ±0.19	2.26 ±0.11	2.54 ^x ±0.20
Kidney ^{86}Rb Efflux Rate Constant (%. min ⁻¹)	3.33 ±0.10	2.95 ^y ±0.20	3.60 ±0.18	3.61 ±0.20
N°	5	5	6	6

Values: Mean ± SEM.

x Significantly different from Control (p<0.05).

y Significantly different from High PUFA (p<0.05)

Table 6.4 Effects of PEM, food-restriction and PUFA-enrichment on ^{86}Rb uptake and efflux in soleus muscle: Experiment 3

Soleus	Control Diet			PEM	PEM PUFA-enriched
	Size-matched	Age-matched	Food-restricted		
Soleus weight (mg)	52.6 ^{xz} ±3.4	178.9 ^{wyz} ±11.5	63.9 ^{wx} ±1.8	58.7 ^x ±4.5	64.8 ^x ±5.8
Soleus (% Body Wt)	0.61 ^z ±0.03	0.66 ±0.04	0.90 ^x ±0.16	0.74 ±0.08	0.73 ±0.05
^{86}Rb Uptake (μ mol.g ⁻¹ .min ⁻¹)	0.39 ^x ±0.01	0.23 ^{wyz} ±0.01	0.35 ^x ±0.01	0.39 ^x ±0.03	0.35 ^x ±0.02
^{86}Rb Efflux (%.min ⁻¹)	1.35 ±0.11	0.98 ±0.16	1.24 ±0.22	1.17 ±0.20	1.07 ±0.11
N°	6	6	6	6	6

Values: Mean ± SEM.

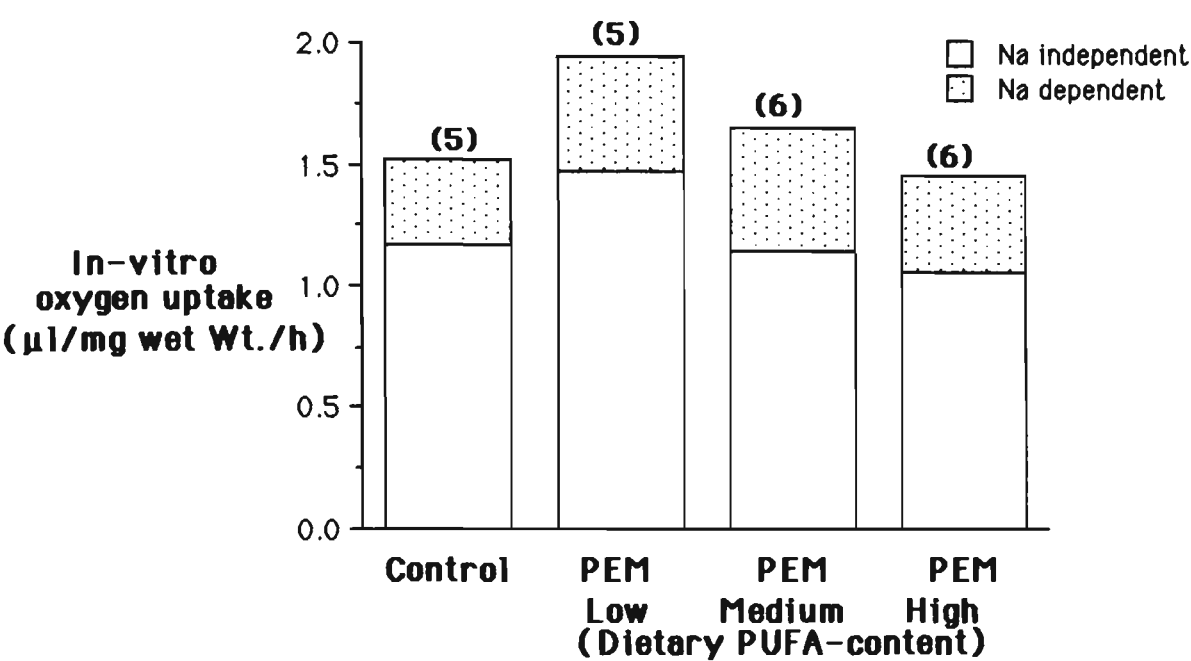
w Significantly different from Age-matched control (p<0.05)

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

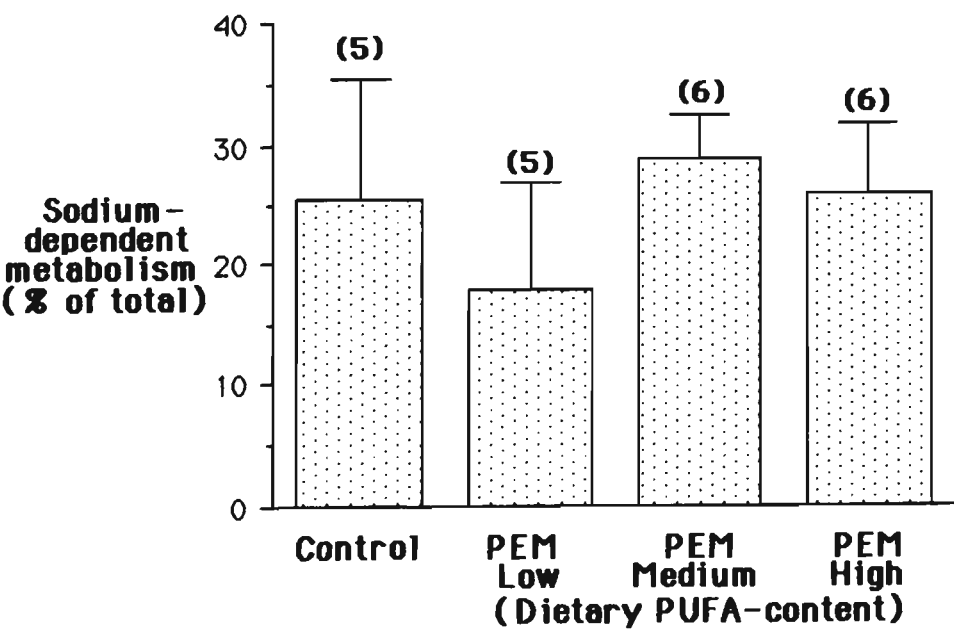
z Significantly different from Food-restricted (p<0.05)

Figure 6.1 Effects of PEM and PUFA-enrichment on in-vitro oxygen uptake of liver slices: Experiment 1



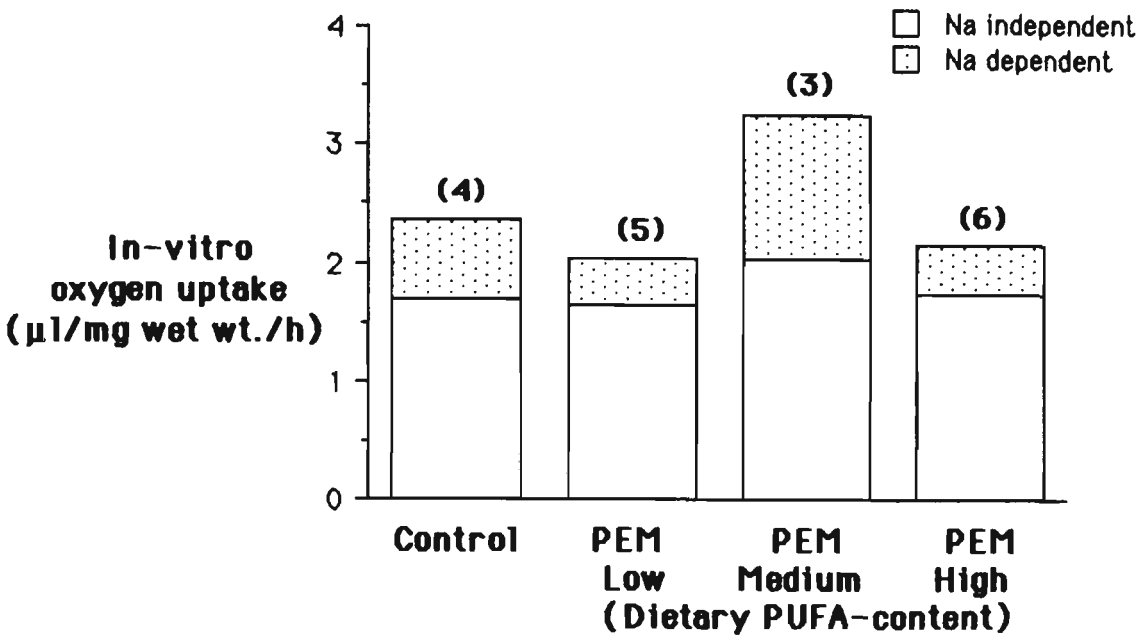
Values: Means; N° of rats shown in parentheses

Figure 6.2 Effects of PEM and PUFA-enrichment on sodium-dependent metabolism as a % of total metabolism in liver slices: Experiment 1



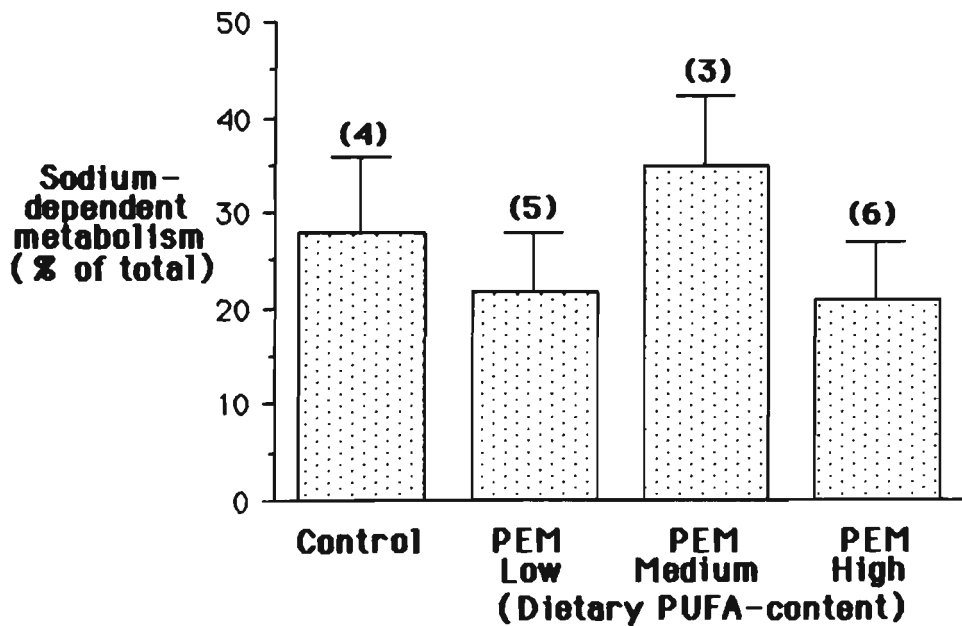
Values: Mean ± SEM; N° of rats shown in parentheses

Figure 6.3 Effects of PEM and PUFA-enrichment on in-vitro oxygen uptake of kidney slices: Experiment 1



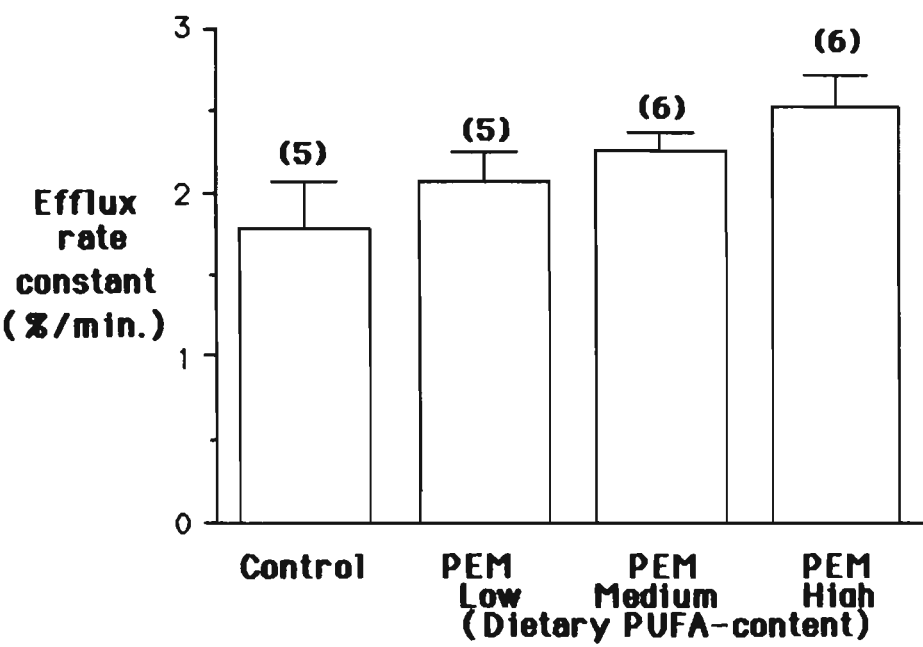
Values: Means; N° of rats shown in parentheses

Figure 6.4 Effects of PEM and PUFA-enrichment on sodium-dependent metabolism as a % of total metabolism in kidney slices: Experiment 1



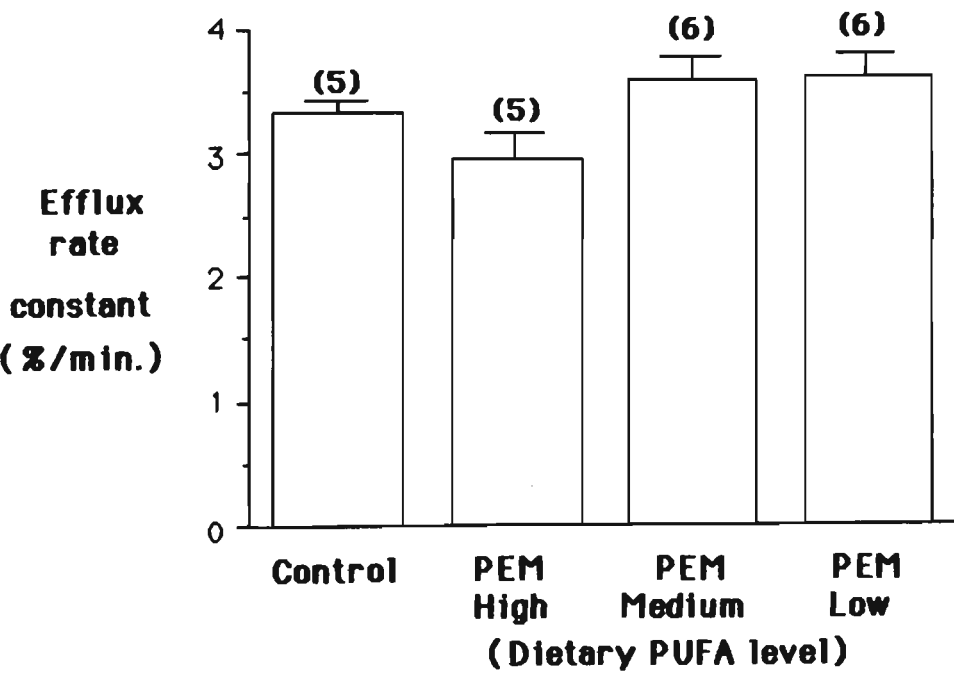
Values: Mean ± SEM; N° of rats shown in parentheses

Figure 6.5 Effects of PEM and PUFA-enrichment on potassium efflux rate constant in liver slices: Experiment 1



Values: Mean \pm SEM; N° of rats shown in parentheses

Figure 6.6 Effects of PEM and PUFA-enrichment on potassium efflux rate constant in kidney slices: Experiment 1



Values: Mean \pm SEM; N° of rats shown in parentheses

Table 6.5 Effects of PEM, food-restriction and PUFA-enrichment on
[³H] ouabain binding of soleus muscle: Experiment 3.

Soleus	Control Diet			PEM	PEM PUFA- enriched
	Size- matched	Age- matched	Food- restricted		
Specific Binding (p mol.g ⁻¹)	291.0 ^{xyz} ±36.2	185.4 ^w ±24.0	189.2 ^w ±23.0	173.6 ^w ±39.7	163.0 ^w ±28.5
N°	5	5	5	3	4

Values: Mean ± SEM.

w Significantly different from Size-matched control (p<0.05)

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

z Significantly different from Food-restricted (p<0.05)

Table 6.6 Effects of PEM, food-restriction and PUFA-enrichment
on [³H] ouabain binding of erythrocytes: Experiment 3.

Soleus	Control Diet			PEM	PEM PUFA- enriched
	Size- matched	Age- matched	Food- restricted		
Specific Binding (p mol.ml ⁻¹ blood)	37.1 ±3.0	30.4 ±5.0	30.3 ±7.8	36.0 ±6.0	20.6 ^{wy} ±1.3
N°	5	5	5	5	5

Values: Mean ± SEM.

w Significantly different from Size-matched control (p<0.05)

x Significantly different from Age-matched control (p<0.05)

y Significantly different from PEM (p<0.05)

z Significantly different from Food-restricted (p<0.05)

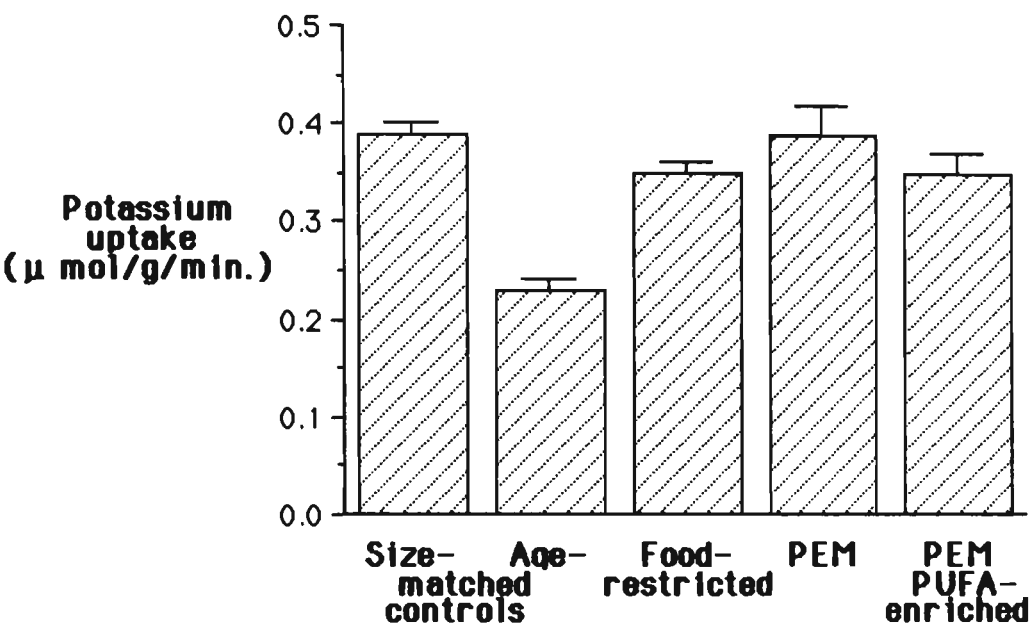
$^{86}\text{Rb}^+$ uptake and efflux from soleus muscle

The uptake and efflux of $^{86}\text{Rb}^+$ by soleus muscle was increased by food-restriction and PEM, but was unchanged by PUFA-enrichment (Table 6.4; Figs. 6.7-6.8). During the $^{86}\text{Rb}^+$ uptake and efflux determinations, it was noticed that higher values were obtained from larger animals. A fifth group of rats was later given ad libitum access to the control diet and killed when approximately the same body weight (and presumably soleus muscle weight) as the malnourished rats. The uptake and efflux of $^{86}\text{Rb}^+$ in size-matched controls was similar to that in food-restricted and PEM rats (Figs. 6.7-6.8). However, there is a significant negative correlation ($R = -0.90$; $p < 0.001$) between potassium uptake and soleus muscle weight (Fig. 6.9). This correlation persists, even without inclusion of the Age-matched controls ($R = -0.80$; $p < 0.001$). Similarly, there is a significant negative correlation ($R = -0.44$; $p < 0.05$) between potassium efflux and soleus muscle weight (Fig. 6.10). This correlation does not persist without inclusion of the Age-matched controls ($R = -0.34$; $p = 0.103$). These correlations suggest that the increased uptake and efflux of potassium may be functions of muscle size, rather than functions of the soleus cell membrane and leakiness and pump rate.

$[^3\text{H}]$ Ouabain binding to soleus muscle

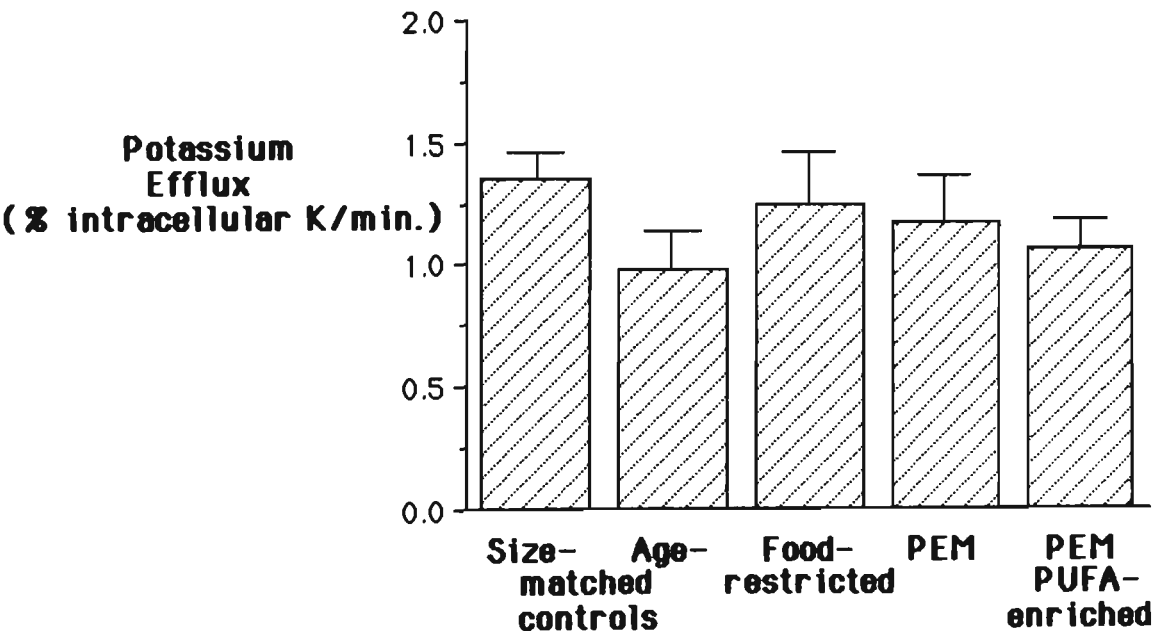
The specific binding of $[^3\text{H}]$ Ouabain to soleus muscle was increased by food restriction, decreased by PEM and unchanged by PUFA-enrichment, although none of these changes were significant (Table 6.5). The specific binding of $[^3\text{H}]$ Ouabain to soleus muscle was higher in the Size-matched than in the Age-matched control diet rats (Table 6.5).

Figure 6.7 Effects of food-restriction, PEM and PUFA-enrichment on potassium uptake of soleus muscle: Experiment 3



Values: Mean ± SEM

Fig 6.8 Effects of food-restriction, PEM and PUFA-enrichment on potassium efflux of soleus muscle: Experiment 3



Values: Mean ± SEM

Figure 6.9 Correlation between soleus muscle mass and potassium uptake: Experiment 3

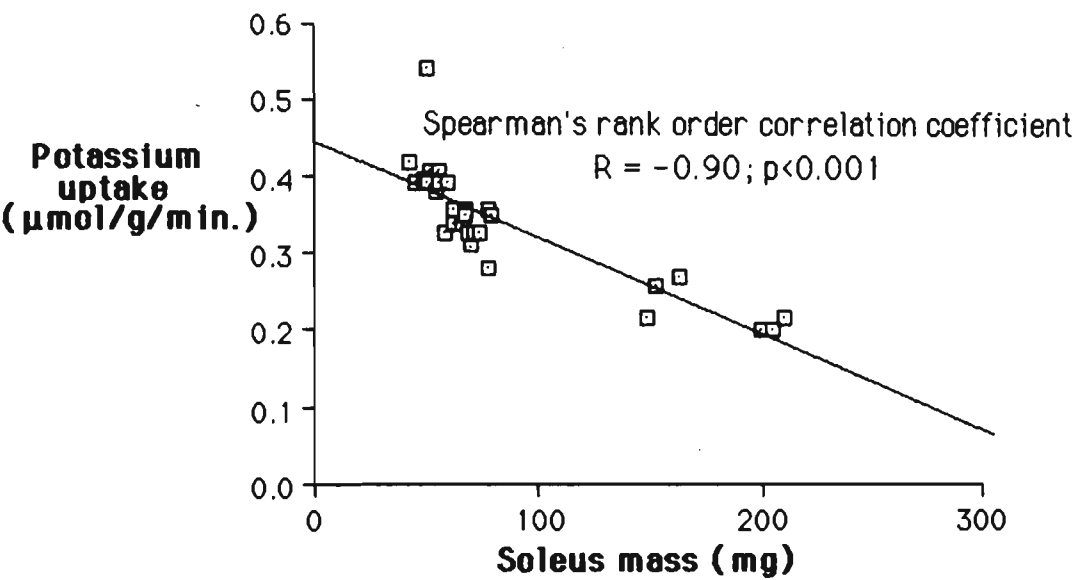
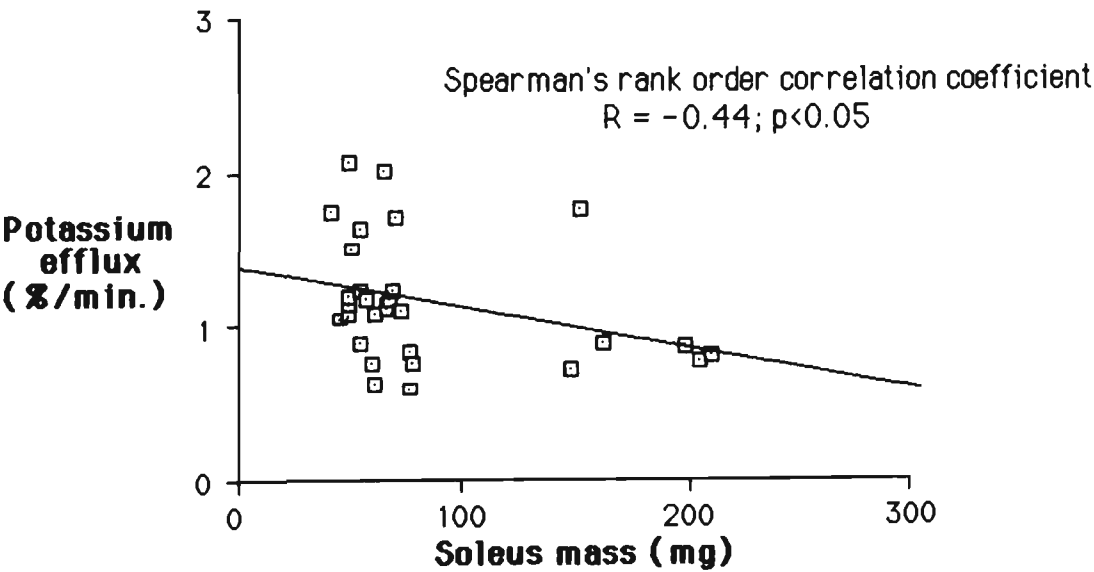


Figure 6.10 Correlation between soleus muscle mass and potassium efflux: Experiment 3



[³H]Ouabain binding to erythrocytes

The specific binding of [³H] Ouabain to erythrocyte fragments was unchanged by food-restriction, but increased (18%) by PEM (Table 6.6). PUFA-enrichment reversed this effect.

Extracellular volume of soleus muscle

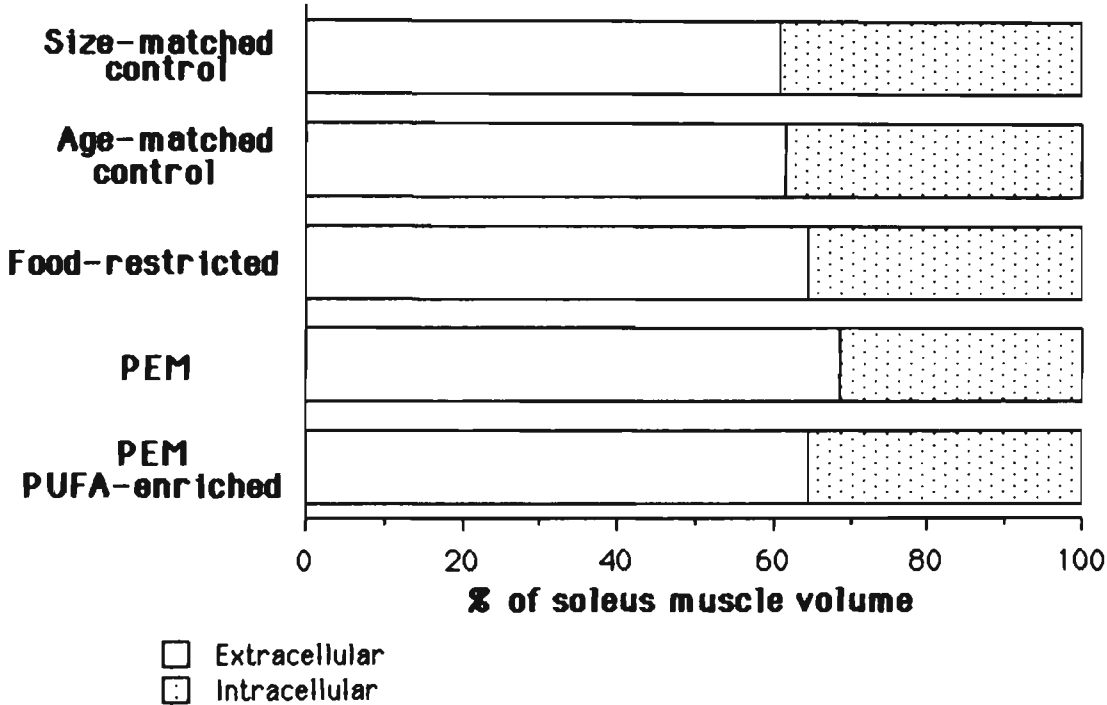
The volume of the soleus muscle was reduced from 106 μ l in control rats to 42 μ l by food-restriction and further reduced to 38 μ l by PEM. Extracellular volume as a % of total muscle volume was increased from 62% in control rats to 65 % by food-restriction and further increased to 69% by PEM (Table 6.7 and Fig. 6.11). PUFA-enrichment tended to reverse both the reduction in total muscle volume and the increase in extracellular volume as a % of total volume. Total soleus volume and extracellular volume as a % of total volume were smallest in the size-matched controls. Although the differences in volume of the soleus and its intra- and extracellular compartments, between the control rats and all other groups were significant, differences between the other groups were not significant.

Table 6.7 Effects of PEM, food-restriction and PUFA-enrichment on extracellular volume of soleus muscle: Experiment 3.

	Control Diet			PEM	PEM PUFA-enriched
	Size-matched	Age-matched	Food-restricted		
Total volume (μl)	37.2 ^x ±4.6	106.0 ^{wyz} ±9.0	42.2 ^x ±2.7	38.4 ^x ±1.8	39.2 ^x ±2.7
Extracellular volume (μl)	23.0 ^x ±4.2	65.0 ^{wyz} ±4.8	27.5 ^x ±2.9	26.4 ^x ±1.8	25.5 ^x ±2.9
Intracellular volume (μl)	14.2 ^x ±1.4	41.0 ^{wyz} ±4.7	14.7 ^x ±0.7	12.0 ^x ±1.5	13.8 ^x ±1.9
Extracellular volume (%)	60.8 ±4.1	61.6 ±1.7	64.7 ±2.8	68.7 ±3.4	64.7 ±4.6
N°	4	4	4	4	4

Values: Mean ± SEM.
z Significantly different from Size-matched control (p<0.05)
x Significantly different from Age-matched control (p<0.05)
y Significantly different from PEM (p<0.05)
z Significantly different from Food-restricted (p<0.05)

Figure 6.11 Effects of food-restriction, PEM and PUFA-enrichment on the extracellular proportion of soleus muscle: Experiment 3



Discussion

The results of the present study support only some of the previous findings that membrane function is affected by food-restriction and PEM. They also indicate that some effects are tissue specific and in some cases they may be reversed by PUFA-enrichment.

As outlined in chapter 2, there were a number of differences between Experiments 1 and 3. These differences were introduced after consideration of the results of Experiments 1 and 2. There were differences between Experiments 1 and 3 in experimental protocol, such as the number of dietary groups, age of the rats when the experimental diets were commenced, duration of diets, lipid content of the diets, fatty acid content of the lipid components of the diets and the experimental parameters measured. Unfortunately, as a result of these differences, when the results of Experiments 1 and 3 are considered together, they do not present a complete picture of the changes involved in protein-energy malnutrition.

Protein-energy malnutrition produced a small increase in the "in vitro" metabolism of liver slices (Table 6.1). This increased metabolism consisted mainly of an increase in ouabain-inhibited metabolism, which implies that sodium pumping was increased in the liver by PEM. Sodium pumping in liver was not measured in this study. The reversal of this effect by PUFA-enrichment, suggests that it may be the result of a deficiency in linoleic acid. However, none of these changes were statistically significant.

Increased membrane permeability to Na^+ and/or K^+ may lead to an increase in sodium pumping (Else and Hulbert, 1987). However, the permeability of liver slices to K^+ was not increased significantly by PEM, nor

was it reduced by PUFA-enrichment (Table 6.3). This suggests that the increase in ouabain-inhibitable metabolism during PEM is not due to an increased membrane permeability to K^+ . This increased metabolism may be due to some other factor, such as an increased permeability to Na^+ , resulting in an increased sodium leak into liver cells. In both Experiments 1 and 3, PEM increased the concentration of sodium in liver. This increase was significant in experiment 1, but not experiment 3.

Protein-energy malnutrition increased the in "in vitro" metabolism of kidney slices (Table 6.2). The effect in the kidney was greater than that in liver, where metabolic intensity was lower. Else and Hulbert (1987) have also reported that total "in vitro" metabolism in kidney was higher than that in liver. The increased metabolism induced by PEM (Table 6.2), consisted mainly of an increase in ouabain-inhibited metabolism, and as for liver, implies that sodium pumping was increased by PEM, although sodium pumping in kidney was not measured in this study. Ouabain-inhibited metabolism was higher in kidney than in liver (Tables 6.1 and 6.2), which is in agreement with the finding of Else and Hulbert (1987). The increased "in vitro" metabolism of kidney was reversed by PUFA-enrichment (Table 6.2). Although some of these changes in metabolic intensity were relatively large, variability within each group was high. The difference in sodium dependent metabolism between the medium and high PUFA groups was the only statistically significant difference ($p < 0.05$) in metabolic intensity.

In Experiment 1, there was no effect of PEM on the concentration of Na^+ or K^+ in kidney. Protein-energy malnutrition produced a slight increase (10%) in the potassium efflux from kidney slices (Table 6.3), but this was not significant. The potassium efflux from kidney slices was unchanged by PUFA-enrichment. As with liver, this suggests that in kidney, the increase in ouabain-

inhibitable metabolism during PEM is not due to increased membrane permeability to K^+ . This small effect may, however, be due to an increased membrane permeability to Na^+ , resulting in a sodium leak into kidney cells.

Measurements were not made of the rate of oxygen consumption of soleus muscle, so the effects of PEM on muscle metabolism in this study are unknown. However, potassium uptake by soleus muscle (an indication of "sodium pumping") was measured in the present study. This was measured using ^{86}Rb as an analogue of potassium. Protein-energy malnutrition increased the potassium uptake of soleus muscle (Table 6.4). This increase was significant ($p < 0.05$). However, the significant negative correlation ($p < 0.001$), between soleus muscle weight and potassium uptake (Fig. 6.9) raises the question of whether this is a real effect. This correlation does not demonstrate a cause-and-effect relationship between soleus muscle weight and potassium uptake. However, it does suggest that the increased uptake of potassium by the soleus during PEM, may be an artifact due to a decrease in muscle size, resulting in an increase in surface area availability of potassium for muscle fibres deep in the muscle. Thus, the increased uptake of potassium by the soleus in the malnourished rats and the size-matched controls (Table 6.4), may be a function of soleus muscle size, rather than a function of soleus cell membrane leakiness and pump rate.

If the the increased uptake of K^+ by the soleus is a real effect, and there is more sodium pumping, then this may tend to increase the intracellular concentration of K^+ and reduce the intracellular concentration of Na^+ . The overall Na^+ concentration in soleus muscle increased significantly (Table 5.13), as did that in gastrocnemous muscle (Table 5.9) in response to PEM. However, it is possible that these increases in the Na^+ concentration of muscle may be due to an increase in the extracellular compartment of muscle. Protein-energy

malnutrition increased the proportion of soleus muscle volume occupied by the extracellular compartment from 62% to 69%, although this increase was not statistically significant. A reduction in the proportion of the soleus muscle occupied by the intracellular compartment would also tend to reduce the effect that any increase in intracellular K^+ concentration would have on overall K^+ concentration in soleus muscle. The concentration of K^+ in soleus muscle and gastrocnemous muscles was relatively unchanged by PEM (Tables 5.9 and 5.13) and is thus consistent with this suggestion. Montgomery (1962b) found that extracellular volume of sartorius muscle in infants was increased from 33% to 52% by kwashiorkor.

Both sodium and potassium are in dynamic equilibrium between intracellular and extracellular compartments. If PEM were to produce no change in the intracellular K^+ and Na^+ concentrations in soleus muscle, then any real increase in sodium pumping induced by PEM has to be counterbalanced by an increased leakage of Na^+ and K^+ . Potassium efflux was increased by food-restriction and PEM (Table 6.4), suggesting that the cell membrane in soleus muscle was more leaky to K^+ . However, these increases were not statistically significant.

Sodium pump numbers in the soleus were measured in Experiment 3. Although the results suggest that there may have been an increase in sodium pumping in PEM, there was no measured increase in sodium pump numbers in soleus muscle. However, recent studies indicate that measurements of sodium pump numbers are not necessarily a good indication of sodium pumping activity (P.L. Else, personal communication).

Erythrocytes from children with kwashiorkor exhibit increases in sodium content, sodium-permeability and sodium pumping, whereas in

marasmus, sodium pumping is reduced (Forrester et al., 1990). Narayanareddy and Kaplay (1982), found that PEM increases the concentration of sodium pumps in erythrocyte membranes from children. However, protein-energy malnutrition in the present study (in rats) produced no change in ouabain binding in erythrocytes (Table 6.6). PUFA-enrichment during PEM reduced the concentration of sodium pumps in erythrocytes. The absence of an effect of PUFA-enrichment on BMR during PEM suggests that reducing the number of sodium pumps in PEM may not be of great physiological significance. The reduced hematocrit in PEM (Table 5.8) reduces the physiological significance of changes in the number of sodium pumps in erythrocytes during PEM. Although about 15-20% of energy turnover in erythrocytes is utilized in maintaining Na^+/K^+ gradients, it has been suggested that their contribution to BMR is very small (Welle, 1989; Clausen et al., 1991).

Overall, the effects of protein-energy malnutrition were not dramatic. There is however, an indication that there is an increase in metabolism to maintain ion homeostasis.

The physiological significance of increased metabolic intensity becomes evident, when organ size is included in the calculation. Total "in vitro" metabolism of the kidneys was reduced from reduced from $5.5 \text{ ml O}_2 \cdot \text{h}^{-1}$ to $3.4 \text{ ml O}_2 \cdot \text{h}^{-1}$ by PEM (Table 6.2). Changes in the metabolism of the renal papillae area and in the proportion of kidney occupied by this area during PEM may vary these values. If the malnourished rats were merely 1/3 scale models of the controls, then a value of about $1.8 \text{ ml O}_2 \cdot \text{h}^{-1}$ would be expected. However, the value of "in vitro" metabolism obtained is 63% of the control value i.e., about twice the "expected" value. These results suggest that PEM increases the proportion of whole animal metabolism contributed by the kidneys. A reduction in whole animal metabolism during PEM (see chapter 3) would

further increase this proportion.

In control rats, the kidneys' contribution to whole animal metabolism is only 34% of that of the liver. In PEM, this is increased to 56% of that of the liver. Thus, while the contribution of the liver to the total "in vitro" metabolism of the animal during PEM decreased in about the same proportion as body weight, that of the kidneys increased greatly. The increase results from both an increase in metabolic intensity of kidney and an increase in kidney weight as a % of body weight. Similarly, if BMR decreases during PEM, then there must be a decrease in metabolic intensity of tissue(s) comprising a high proportion of BMR, or a decrease in size of high metabolic intensity tissue, or both. Pimplikar and Kaplay (1981) have also reported an increase in kidney weight as a % of body weight during PEM.

Metabolism in kidney slices was not increased by PEM when the diet contained stearic acid. These results appear to be inconsistent with the finding that Na^+ / K^+ ATPase activity in kidney was increased by both energy restriction and protein restriction (Pimplikar and Kaplay, 1981). However, in both the present study and that of Pimplikar and Kaplay (1981), the response of the kidney appears to be different to that of the liver. Although the contribution of the sodium pump to cellular metabolism is controversial, evidence suggests that sodium pumping contributes to a greater proportion of total oxygen consumption in kidney cells than in liver cells (Clausen et al., 1991). Other factors responsible for significant components of metabolism may also be changed during malnutrition.

In the present study, PUFA-enrichment during PEM significantly reduced the number of ^3H -ouabain binding sites in erythrocytes, but not in soleus muscle. This indicates that the effects of PEM on tissue sodium pump

concentration may be greater in some tissues than in others. Kjeldsen et al. (1986) reported that food-restriction reduced the concentration of ^3H -ouabain binding sites in rat skeletal muscle by 25%. The continued growth of the food-restricted and PEM rats in the present study may have contributed to the discrepancy between their results and those of the present study, where food-restriction produced no change in the number of ^3H -ouabain binding sites. In the study of Kjeldsen et al. (1986), refeeding reversed the decrease in ^3H -ouabain binding sites in rat skeletal muscle caused by food-restriction.

The concentration of ^3H -ouabain binding sites in skeletal muscle increases dramatically from birth to one month of age in rats and mice (Kjeldsen et al., 1982; 1984). The significantly higher concentration of soleus muscle ouabain binding sites in the one-month old size-matched control rats than in the three-month old age-matched controls (Table 6.5) is consistent with the progressive decrease in concentration of binding sites reported in both rats and mice after they are one month old (Clausen and Everts, 1989). Human skeletal muscle shows no change in concentration of binding sites with age from 0 to 8 years (Kjeldsen and Gron, 1989) or 25 to 80 years (Norgaard, Kjeldsen and Clausen, 1984).

Summary

The effects of food-restriction, PEM and PUFA-enrichment on ion homeostasis vary between tissues.

PEM increases "in vitro" metabolism in both liver and kidney slices. The increases are due mainly to increased "sodium-dependent" metabolism. This effect is greater in kidney, which has a higher metabolic intensity than liver. The increase in metabolism is reversed by PUFA-enrichment in both liver and kidney, which is the only statistically significant difference in metabolic intensity.

The leakiness of the cell membrane to K^+ is increased in soleus muscle by food-restriction and in liver, kidney and soleus muscle by PEM. None of these differences were statistically significant. PUFA-enrichment did not reverse the PEM-induced increases.

The concentration of sodium pumps in soleus muscle is increased by food restriction, decreased by PEM and unchanged by PUFA-enrichment. These differences were not statistically significant. The concentration of sodium pumps in soleus muscle was significantly less in mature than in immature rats.

The concentration of sodium pumps in erythrocytes was unchanged by food-restriction, but increased slightly by PEM. The reversal of this effect by PUFA-enrichment was statistically significant.

The extracellular fraction of soleus muscle volume was increased by food-restriction and further increased by PEM, while soleus muscle volume was decreased. PUFA-enrichment tended to reverse both of these effects. Although

soleus volume was smallest in the youngest rats, their extracellular fraction was smallest. None of these effects was statistically significant.

Chapter 7: General Discussion

This study aimed to examine protein-energy malnutrition and dietary fats. It was organized to determine the effects of food-restriction, protein-energy malnutrition, and increasing the proportion of omega-6 polyunsaturated fatty acids in the dietary fat during protein-energy malnutrition. Expansion of the study enabled the effects of age to be included. A summary of the effects of these dietary manipulations is shown in Table 7.1.

Food-restriction, by definition, reduced the amount of energy available for ingestion. Food-restriction also reduced metabolisable energy as a % of energy intake (Table 7.1) i.e., a smaller proportion of ingested energy was available for the demands of maintenance, growth and activity. Not-surprisingly, food-restriction retarded growth. This effect was evident from the parameters "change in body weight" and "carcass weight". The brain, and to a lesser extent the digestive tract were very resistant to this growth retardation. Components of the digestive tract were equal in their resistance to growth retardation. Other organs, such as the female reproductive organs, skin and fur and spleen were very susceptible to growth retardation. As well as being very resistant to growth retardation, brain was also less susceptible to changes in composition than other tissues.

Some of the changes induced by food-restriction may be considered as beneficial. Basal metabolic rate (BMR) was reduced and net protein utilization (NPU) was increased by food-restriction. Similar findings have been reported previously, in malnourished children (Pretorius and Wehmeyer, 1964; Pretorius et al, 1964; Brooke et al, 1974; Jaya Rao and Khan, 1974). These may be interpreted as adaptive responses, which reduce the energetic costs of

maintenance and increase the efficiency with which N is retained when food intake is greatly decreased. As pointed out previously (see chapter 3), NPU expresses in a single index, both the digestibility of protein and the efficiency with which absorbed amino acids are utilized. Food-restriction had no effect on apparent absorption of either energy or nitrogen, even though the weight of the digestive tract was reduced. Weight of the digestive tract as a % of that in the control diet rats was higher than might be expected, if these animals were merely a one-third scale model of their well-fed littermates (Fig 5.2). Thus, in spite of a weight reduction in the digestive tract, abnormalities at the gut level are unlikely to have contributed to growth retardation. The increased NPU reflects an increase in the efficiency with which absorbed N is retained during food-restriction.

At first glance, the results (Table 6.4) suggest that the rate of uptake of K^+ by the soleus muscle was increased by food-restriction. However, a significant negative correlation was found to exist between rate of K^+ uptake and soleus muscle weight. This suggests that the increased K^+ uptake may be a function of muscle size, rather than membrane permeability and pump rate. Thus, the results provide no definitive evidence that food-restriction increased rate of K^+ uptake in skeletal muscle. I interpret these results as suggesting that rate of K^+ uptake was not increased by food-restriction. The absence of an effect of food-restriction on rate of K^+ efflux from the soleus (Table 6.4), or on the specific binding of ouabain to soleus muscle (Table 6.5) is consistent with this interpretation. The absence of an effect of food-restriction on the specific binding of ouabain to erythrocytes is consistent with the findings of Narayanareddy and Kaplay (1982) that the specific binding of ouabain to erythrocytes in marasmic children is the same as in normal children. Thus, the results of the present study provide no evidence that membrane function is affected by food-restriction. Extracellular volume as a % of total volume in

soleus muscle was increased by food-restriction. However, this increase was not statistically significant.

Food-restriction had little effect on tissue composition. The general effect of food-restriction was a slight increase, rather than a decrease in phospholipid linoleic acid concentration. However, there was a general increase in membrane w-9 fatty acid content and in some tissues, small amounts of 20:3 w9 were detected, suggesting a marginal deficiency in linoleic acid. It is possible that the reduced growth of the internal organs of the malnourished rats is responsible for the maintenance of high concentrations of linoleic acid in phospholipids. Wiese et al. (1962) suggested that the rate of development of EFA-deficiency was related directly to growth and in turn, caloric intake. Adult rats given an EFA-deficient diet will not exhibit the symptoms of EFA-deficiency, unless energy intake is restricted until weight loss occurs. The symptoms appear after the energy intake (of the deficient diet) is increased.

A reduced dietary protein content during food restriction i.e., protein-energy malnutrition (PEM) reduced growth significantly more than food-restriction alone (Table 7.1). However, the pattern of resistance to growth retardation by various organs was similar to that for food-restricted animals. Compared to food-restriction, PEM did not significantly alter BMR or metabolisable energy as a % of energy intake. However, the apparent absorption of energy, and to a greater extent, that of N was significantly reduced, although the absolute weight of the digestive tract was not decreased (but as a % of body weight, it increased significantly). Not-surprisingly, PEM retarded growth to a greater extent than food-restriction. Again, this effect was evident from the parameters of change in body weight and carcass weight. Thus, unlike the situation for food-restriction, abnormalities at the gut level during PEM did

Table 7.1

Effects of age, food-restriction, PEM and PUFA-enrichment on some physiological parameters in rats.

Parameter	Age-compared to Size-matched controls	Food-restricted compared to Age-matched controls	PEM compared to Food-restricted	PUFA-enriched compared to PEM
Growth	+	-	-	+
BMR	(-)	(-)		
Apparent absorption of energy			-	
Apparent absorption of nitrogen			-	
Metabolisable energy (% intake)		-		(-)
Net protein utilization	-	+	(+)	(+)
Carcass weight	+	-	-	+
Carcass water content	-	+	(+)	(-)
N° sodium pumps (soleus)	-			
N° sodium pumps (erythrocytes) (-)				-
% extracellular volume (soleus)		(+)	(+)	(-)

+

Significantly increased (p<0.05)

-

Significantly decreased (p<0.05)

Parenteses

indicate changes that were not statistically significant, but may be physiologically significant

contribute to growth retardation. The higher NPU in PEM indicates a further increase in the efficiency with which absorbed N is retained, above that found in food-restriction, although the effect was not statistically significant, it is probably functionally significant.

The pattern of increased metabolic intensity of major organs, such as liver and kidney during PEM is inconsistent with a pattern of decreased BMR (with respect to lean body mass) during PEM. If BMR decreases during PEM, then there must be a decrease in metabolic intensity of tissue(s) comprising a high proportion of BMR, or a decrease in size of high metabolic intensity tissue, or both. The selective protection against growth retardation of brain, which has a relatively high metabolic intensity suggests that this organ may be influential in determining BMR in these animals. The increased "in vitro" metabolism in liver and kidney was due mainly to an increase in "ouabain-inhibitable" metabolism. This suggests that there is an increase in sodium pumping during PEM and is thus consistent with the findings of Pimplikar and Kaplay (1981).

Compared to food-restriction, PEM did not alter the uptake or efflux of K^+ from soleus muscle, nor the specific binding of ouabain to soleus muscle. Thus, under the experimental conditions of this study, there is no hard evidence that membrane function in skeletal muscle is affected by PEM. As suggested in chapter 6, the increased uptake of K^+ in the malnourished rats may be a function of soleus muscle size, rather than a function of 'pump rate'. PEM did exert some effects on membrane function, although these effects were tissue-specific. PEM increased the specific binding of ouabain to erythrocytes (Table 6.6). This result is in agreement with the finding of Narayanareddy and Kaplay (1982), that the specific binding of ouabain to erythrocytes from children with PEM (kwashiorkor) is greater than that in those from normal children. Extracellular volume as a % of total volume in soleus muscle was further

increased by PEM.

As with food-restriction, PEM had little effect on tissue composition. The general effect of PEM was a slight increase, rather than a decrease in phospholipid linoleic acid concentration, a similar result to that from food-restriction.

Growth during PEM was significantly increased by PUFA-enrichment. Carcass weight and increase in body weight were significantly higher in the PUFA-enriched than in the PEM rats. Again, the pattern of resistance to growth retardation by various organs was similar to that for food-restricted animals. The brain and digestive tract maintained a high resistance to growth retardation, while other organs were more susceptible to growth retardation. A notable exception was skin and fur, which accounted for much of the increased growth with PUFA-enrichment. Although a decreased efficiency at the gut level contributed to the growth retardation during PEM, neither the apparent absorption of energy, or of nitrogen are increased by PUFA-enrichment, even though PUFA-enrichment tended to increase the weight of the digestive tract. Thus, the beneficial effects of PUFA-enrichment arose from the increased retention of absorbed nutrients, rather than from increases in digestive and/or absorptive processes at the gut level, assuming that there is no difference in endogenous nitrogen output between these treatments. Compared to PEM, PUFA-enrichment produced no significant change in BMR. Metabolisable energy as a % of energy intake was, however, reduced by PUFA-enrichment. This effect, which is considered detrimental, was not statistically significant.

PUFA-enrichment during PEM did not alter the uptake or efflux of K^+ from soleus muscle, or the specific binding of ouabain to soleus muscle. Thus, these experiments provided no evidence that membrane function in skeletal

muscle during PEM is affected by PUFA-enrichment. However, PUFA-enrichment significantly reduced the specific binding of ouabain to erythrocytes during PEM (Table 7.1). These results indicate that at least some of the increased specific binding of ouabain to erythrocytes during PEM is able to be reversed by PUFA-enrichment, and may in fact be caused by a deficiency in PUFA. The tendency for extracellular volume as a % of total volume in soleus muscle to be increased by PEM was reversed by PUFA-enrichment. Thus, changes in membrane function induced by both PEM and PUFA-enrichment during PEM are tissue specific.

Comparison of the younger size-matched controls with the age-matched controls enabled some of the effects of age to be determined, although in some cases, these may also include the effects of body size. After the age-matched control rats had grown to a significantly higher body weight, carcass weight was found to be significantly higher, BMR lower (Table 7.1) and there was also a significant decrease in NPU. Experiments 1 and 3 suggest that a nitrogen retention adaptation operates during PEM and that it is enhanced by PUFA-enrichment. An increase in the % of absorbed nitrogen retained in the body, is generally an indication of increased tissue formation i.e., growth. In the younger size-matched controls, where growth was rapid, the proportion of ingested nitrogen retained was twice that in the older age-matched controls. The high efficiency with which ingested N was retained in the younger animals (increased NPU) did not depend on increased efficiency of digestive and absorptive processes, but on an increase in the % of absorbed N that was retained. Neither apparent absorption of energy or N were higher in the size-matched than in the age-matched controls (Tables 3.13 and 3.15). There is little scope for improvement over and above the levels of efficiency in apparent absorption found in the age-matched animals.

The specific binding of ouabain to soleus muscle was significantly higher in the younger rats (Table 7.1). The rate of potassium efflux from the soleus was also higher in the younger rats, although the difference was not significantly significant. Thus, there were differences in the cell membrane between the age-matched control rats and the younger size-matched control rats. However, these differences were tissue -specific, as the specific binding of ouabain to erythrocytes was not significantly higher in the younger rats. As Figure 5.3 indicates, soleus weight comprises a smaller proportion of body weight in the size-matched, than in the age-matched controls. This effect is even more marked in gastrocnemius muscle. Much of the growth in skeletal muscle occurs later in life than when the size-matched rats were killed, so the physiological significance of a greater number of sodium pumps in the soleus muscle of the younger rats may be decreased by their lower ratio of soleus weight to body weight.

It is clear, is that PUFA-enrichment was beneficial during PEM. PUFA-enrichment enhanced growth during PEM, especially growth in body weight. In doing so, it tended to reverse the extra loss of body weight that occurred over and above that due to food-restriction. Water may account for some of this growth, as water is usually about 65% of fat-free body weight. However, the PUFA-induced increase in carcass dry weight during PEM indicates that the increased growth was not just an increase in the amount of water in the body. Furthermore, the % of water in the carcass actually decreased with PUFA-enrichment. During malnutrition, there is generally an increase in the % of water located in the extracellular compartment of many tissues. The tendency for extracellular volume as a % of total volume in soleus muscle to decrease with PUFA-enrichment is consistent with the decrease in carcass water content with PUFA-enrichment.

PUFA-enrichment tended to reverse the PEM-induced increase of "in-vitro" metabolism measured in slices of both liver and kidney. The increased "in vitro" metabolism in liver and kidney was due mainly to an increase in ouabain-inhibitable" metabolism. The reversal of this PEM-induced increase in sodium pumping by PUFA-enrichment, suggests that the increase may have involved a deficiency in linoleic acid.

One factor which may lead to an increase in sodium pumping is an increase in membrane passive permeability to Na^+ and/or K^+ (Else and Hulbert, 1987). However, although PEM tended to increase the membrane permeability to K^+ of liver and kidney in the present study, this effect was not reversed by PUFA-enrichment. Thus, this increase in permeability of the cell membrane is probably not due to a dietary deficiency of linoleic acid, but to some other factor(s). Ouabain-inhibitable metabolic intensity was increased in both liver and kidney during PEM. These increases were reversed by PUFA-enrichment and probably are due to a dietary deficiency of linoleic acid.

As pointed out earlier, the contribution of the sodium pump to cellular metabolism is controversial. Other factors responsible for significant components of metabolism may also be changed during malnutrition. For example, it is possible that the increased leakiness of the cell membrane detected in liver, kidney and soleus muscle in this study and leukocytes in those of Patrick and Golden (1977) may extend to other membranes of the cell. These may include the inner mitochondrial membrane. The proton leak through mitochondrial membranes contributes significantly to cellular metabolism (Brand et al., 1991). This permeability may in turn, depend in part, upon their fatty acid composition (Brand et al., in preparation).

Irrespective of the magnitude of the contribution made by the sodium

pump to overall cellular metabolism in various tissues, the results of the present study support previous findings that membrane function is indeed affected by PEM, but not food-restriction, as reported by Kjeldsen et al. (1986). They indicate that some effects are tissue specific and in some cases may be reversed by PUFA-enrichment. However, they also suggest that the increased leakiness of the cell membrane during PEM is not due to a dietary deficiency of linoleic acid.

For ethical reasons, the present study was carried out in rats, with the possibility that the results might be applied to the human situation. The dietary manipulations used in the present study did not induce in rats, the characteristic symptoms of kwashiorkor found in some malnourished children. This, however is not surprising, as it is reported to be difficult to induce kwashiorkor in experimental animals by dietary manipulations (Golden, 1988a; Golden and Ramdath, 1987).

The advantages in providing high lipid foods to children recovering from malnutrition are well-documented (Ashworth, 1978, 1979, 1985; Waterlow, 1979; Crawford, et al., 1989). This knowledge is becoming even more important in the 1990's, where various "human" factors effectively prevent the transport of large volumes of food to undernourished communities. However, until recently, the fatty acid content of the lipids has been given little attention. It has been suggested that the lipid components of "catch-up" diets should not contain high concentrations of PUFA without protection against the formation of free radicals (Golden, 1988b; Golden and Ramdath, 1987). The use of oils rich in PUFA, such as peanut oil have been discontinued by some, in favour of coconut oil, which is low in PUFA and high in saturated fatty acids (Read, 1990). However, the present study demonstrates that in rats, omega-6 polyunsaturated fatty acids are beneficial during protein-energy malnutrition in that they

promote greater growth than do saturated fatty acids.

It is often unwise to extrapolate directly from the results of studies in animals under laboratory conditions to humans. However, it is possible that increasing the concentration of omega-6 polyunsaturated fatty acids (with adequate concentrations of tocopherols) in the diets fed to children with PEM may also be beneficial. The present study suggests that further research be carried out to determine whether this is the case and also to determine the mechanism of this increased growth. Further research may also determine whether omega-3 polyunsaturated fatty acids have a similar growth promoting effect to the omega-6 polyunsaturated fatty acids during PEM in experimental animals and also in malnourished children.

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