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EFFECT OF DIET ON CARNITINE AND LIPID METABOLISM WITH PARTICULAR REFERENCE TO KWASHIORKOR

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A dissertation submitted to the Faculty of Medicine of the University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science

Johannesburg 1991

This dissertation is dedicated to my parents with thanks for all their love and support.

ABSTRACT

Carnitine plays a role in the transport of activated long chain fatty acyl groups from the site of activation to the site of beta-oxidation in the mitochondria. Endogenous synthesis of carnitine from the amino acids methionine and lysine, takes place mainly in the liver. From there free and acyl carnitine are released into the blood and transported to other tissues. A few studies have indicated that poor nutritional status may lead to carnitine deficiency. There is a high prevalence of kwashiorkor, a protein-energy deficient state, amongst the black population in Southern Africa, but at present little is known concerning carnitine and its possible effects on lipid metabolism. Furthermore there have been relatively few studies concerning the effects of different diets on serum carnitine and lipid. So far no reports have been published concerning the effects of maize meal as a staple diet. This is highly relevant in the South African context.

The aims of the present study were to compare serum carnitine, protein and lipids in black and white South African adults, to evaluate the effect of a high maize diet (low-protein) on healthy adults and to investigate these parameters in children with kwashiorkor as compared with normal children as well as to assess the response to treatment in the nutritionally deprived group.

In the present study no major differences were observed between the different adult population groups; values for normal children were in general within similar ranges. A change to a low-protein diet in healthy white adults produced significant differences in carnitine and the proteins and lipids measured. The values obtained for the kwashiorkor children showed significant changes as compared with normal children. In particular carnitine and total phospholipid concentrations were significantly reduced and the percentage of docosatetraenoic acid (22:4) was increased.

The results obtained suggest that there may be impaired oxidation of lipids in kwashiorkor and the deficiency of carnitine may provide an explanation for the accumulation of fats in the livers of kwashiorkor patients. The decrease in total phospholipid concentration with alterations in the lecithin fatty acid distribution in the children suggest that there may be changes in membrane fluidity and flexibility.

I declare that this dissertation is my own unaided work, except where credit has been acknowledged. It is being submitted for the degree of Master of Science in Medicine to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree in any other University. Ethical clearance was obtained from the Human Ethics Committee, protocol no. 12/4/96.

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CHAPTER 1

1.1 NUTRITION AND NUTRITIONAL DISORDERS

Adequate nutrition is essential to life. The body requires certain essential dietary nutrients from which the tissues and cells can synthesize the numerous compounds required for maintaining its well being. A deficient nutritional status leads to a biochemical imbalance. A prolonged absence of one or more of the essential nutrients in the diet results in disease. The basis for many nutritional diseases is multifactorial and involves the cultural aspects of food preparation as well as social and economic influences. An important aspect of nutrition is the way in which animals respond and adapt to a new diet. In order to survive, animals must adapt to various nutritional conditions and those that fail to adapt will deteriorate and may ultimately die (Peng et al, 1974). Most populations in first world countries subsist on a mixed diet. The pattern of food consumption is varied and almost every meal consists of foods of both animal and vegetable origin. This diversity of food sources is generally regarded as a requirement for a "well-balanced" diet,

which provides all nutrients in adequate amounts. Humans are omnivores but, by necessity, in third world countries many millions of people eat a virtually vegetarian diet. Many forms of edible matter are not used as food; the decision to do so or not depends on social, religious and economic factors and may bear no relation to nutritive value. There is usually one food which is the staple diet of a particular culture and this is nearly always vegetable in origin (for instance wheat, maize, rice, cassava); another form may be labelled as a prestige food, since it is expensive and is, therefore, reserved for special occasions; this is usually meat (Khan-Siddiqui and Bamji 1980; Braunwald et al. 1987). Although this type of diet is deficient in most essential nutrients the most common form of deficiency in developing countries is one of protein and energy. (WHO 1966; Jelliffe 1969; Waterlow 1981; Bhattacharayya 1986).

1.2 NUTRITIONAL REQUIREMENTS

The primary requirement of a diet is to provide enough energy. Animals get their energy from their food in a chemical form which is derived directly or indirectly

from plants. The three food sources which provide this energy are carbohydrates, fats and proteins. An ideal ratio of these substrates in a diet is assumed to be 60% carbohydrate : 25% fat : 15% protein. Some of the energy in food is ultimately converted into heat and its dissipation maintains the temperature of the body. Energy may be expressed quantitatively in units of heat, the unit being the kilocalorie (kcal). Since different foods contain different proportions of the energy providing compounds, appropriate balance must be maintained such that the body's metabolic systems can be supplied with the requisite materials, including the non energy providing nutrients (minerals and vitamins). These nutrients play a vital role in human metabolism, since they are involved in almost every biochemical reaction and pathway known to man. The recommended daily intakes of energy and nutrients vary with age, sex, physiological state and activity (McGilvery and Goldstein 1983). The values given in the World Health Organization recommended intakes provide a basis for assessment of nutritional status (Passmore et al. 1974).

1.3 NUTRITIONAL DISORDERS

Dietary problems are most frequently related to inadequate or excessive intake of the major energy producing components. From a public health point of view, deficient intake of carbohydrates, fats and proteins, which may often occur simultaneously, is one of the most serious nutritional problems in developing countries. It is generally accepted that, in particular, protein deficiency is the underlying cause of many nutritional disorders of the underdeveloped countries. Predominantly, malnutrition in Africa appears in two forms popularly known as kwashiorkor and marasmus and collectively called protein-energy malnutrition. Kwashiorkor may be seen as an inadequacy of protein foods with a relatively high intake of cheap carbohydrates. Marasmus is the result of a low intake of all foods. However, various combinations are very common and the occurrence of the two conditions combined in one child can be referred to as "marasmic-kwashiorkor" (Maletnlema 1986). Generally kwashiorkor manifests itself with the presence of oedema and absence of wasting, while marasmus with wasting and absence of oedema (Bhattacharayya 1986).

1.4 PROTEIN-ENERGY MALNUTRITION

Protein is distinguished from the other classes of major nutrients in that it contains nitrogen. The adequate intake and metabolism of this nitrogen-containing nutrient leads to a well developed body with proper muscle tone and a general feeling of fitness and well-being. Mellinkoff (1957) stated that "unlike sugar, protein is an indispensable food for all higher forms of life. It seems reasonable, therefore, to ask if evolutionary eons have not somehow integrated man's quest for food with assimilation of proteins". During growth, such as in childhood, the body requires an adequate supply of protein to provide amino acids in sufficient quantity and quality to promote development of new tissues. Aside from the growth process, a constant supply of protein is demanded by the body to maintain health.

The evidence that animals are able to adapt to changes in the levels of dietary protein is extensive. In general they respond to dietary alterations by modifying food intake (MacKay et al. 1941; Harper 1965); associated with this are metabolic changes associated with alterations in the activities of specific tissue

enzymes (Allison et al. 1961; Muramatsu and Ashida 1962; Harper 1965; Schirmer and Harper 1970; Mauron et al. 1973) and variations in organ size (Harper 1965). Experimental evidence using animals has shown that a low protein intake causes a shift towards anabolism and reduces the degradation of amino acids (Peng et al. 1974). These animals developed clinical, biochemical and histological disorders similar to those seen in children suffering from kwashiorkor (Kirsch et al. 1968). Meyer and Hargus (1959) suggested that animals fed on a low protein diet ate little because their food intake was limited by an excess of energy intake in relation to protein intake.

As a consequence of inadequate protein intake, Tanphaichitr and Broquist (1973) speculated that a certain degree of carnitine deficiency can be envisaged in populations consuming cereal grain diets. These diets are marginal in lysine and/or methionine, and in kwashiorkor, where a deficiency of essential amino acids is well established (Jelliffe 1959), carnitine is also deficient. Recently there have been several reports regarding the impact of diet on plasma carnitine concentration in various species, such as rats (Seccombe et al. 1978), rabbits (Gillies and Bell 1976),

monkeys and pigeons (Bell et al. 1979). Less has been published on plasma carnitine levels in man. Cederblad (1987) showed that diet can affect plasma carnitine levels and excretion of urinary carnitine in man. In their study seven healthy men were served a high-carbohydrate, low-fat (C) or a low-carbohydrate, high-fat (F) diet for two weeks, ie, one diet regime for four days followed by a three day break and concluded with four days on the other regime. The two regimes contained the same amount of carnitine-rich food. Plasma free carnitine rose significantly from the initial value on F diet and was significantly higher from day three than C diet. Plasma acylcarnitine increased on both diets. Urinary excretion of carnitine increased only on F diet. Renal clearance of both free and acyl carnitine was significantly greater on F diet than on C diet (Cederblad 1987).

1.5 NUTRITIONAL STATUS IN AFRICA

The present food and nutrition situation in Africa can be looked upon as the result of historical events, as well as ecological and socio-economic status. The effects of the industrial revolution in Europe and the

USA on Africa and the colonization of Africa by the Western countries are perhaps the most important factors (Maletnlema 1986).

The food and nutrition problem in Africa may be divided into two major categories:

> Acute shortage of foods leading to mild or severe famine
>
> and
>
> Longstanding moderate food shortages combined with poor utilization and misuse of available foods, leading to chronic malnutrition.

Periodically these two are intensified by the environmental conditions in Africa - lack of water, abundance of pests, vermin, frequent catastrophies including floods and drought and unstable governments which all lead to an increased prevalence of disease (Maletnlema 1986).

If an infectious disease were to kill even one quarter of the number of people that kwashiorkor or marasmus kills on average in a year, an urgent control campaign

would be counted. The relative neglect of the morbidity and mortality associated with malnutrition is partly due to its effects being most frequently seen in young children, and because it is not a communicable disease. Malnutrition tends to be greatly under reported in disease and mortality statistics. This is because many children recorded as having died of diseases such as gastro-enteritis or pneumonia may also have been suffering from malnutrition. A person who is undernourished is more likely to succumb to an infectious disease than one of good nutritional status. It has been shown that infectious diseases of childhood, such as measles and whooping cough, may precipitate the onset of protein-engergy malnutrition; thus, a seemingly healthy child may contract measles and subsequently develop kwashiorkor (Latham 1979).

1.6 KWASHIORKOR

Malnutrition in young children, which predominantly manifests itself as various forms and degrees of energy-protein deficits, has been described in texts dating back to the sixteenth century.

Early in the twentieth century Czerny and Keller (1923-1928) introduced the term <<mehlnährschaden>> for infantile malnutrition resulting from imbalanced food intake (Defekternährung) where starch constituted the main food item. This new clinical syndrome with oedema and skin changes remained largely neglected until Cicely Williams reported h · now classical description in 1933 and used the name kwashiorkor given to it by the people of Ghana, meaning the disease of the displaced child when the next one is born. This recognition by Williams of kwashiorkor in infants marked the beginning of extensive studies leading to great advances in the understanding of the global problem of malnutrition in infants and young children. Kwashiorkor has been reported from many parts of the world under various names (Trowell et al. 1954). Some of these terms are clinically or pathologically descriptive, others are suggestive of nutritional aetiology, and a few are vernacular names used locally (Jelliffe 1966).

Distinction between the major forms of protein-energy malnutrition is generally related to mass. A simplified classification introducing the use of growth charts for clinical purposes has been recommended by

WHO based on the findings of a Wellcome Trust Working Party (Lancet 1970; WHO 1971; DeMaeyer 1976; Waterlow 1976). In this system, all children whose weight is under the third percentile, or less than 80% of standard weight for age, are considered to have protein-energy malnutrition as shown in FIGURE 1. Oedema is the essential feature used in diagnosing kwashiorkor (TABLE I).

TABLE I

WELLCOME CLASSIFICATION OF PROTEIN-ENERGY MALNUTRITION

	Oedema	
Weight % of standard	Absent	Present
80 - 60	Undernourished	Kwashiorkor
< 60	Marasmus	Marasmic-Kwashiorkor

Although there is some controversy in the literature concerning the use of an international standard, studies on children in privileged and under-privileged groups in Africa (including South Africa) in the

FIGURE 1

GROWTH CHART (example of a chart used in clinics and hospitals in S. Africa)

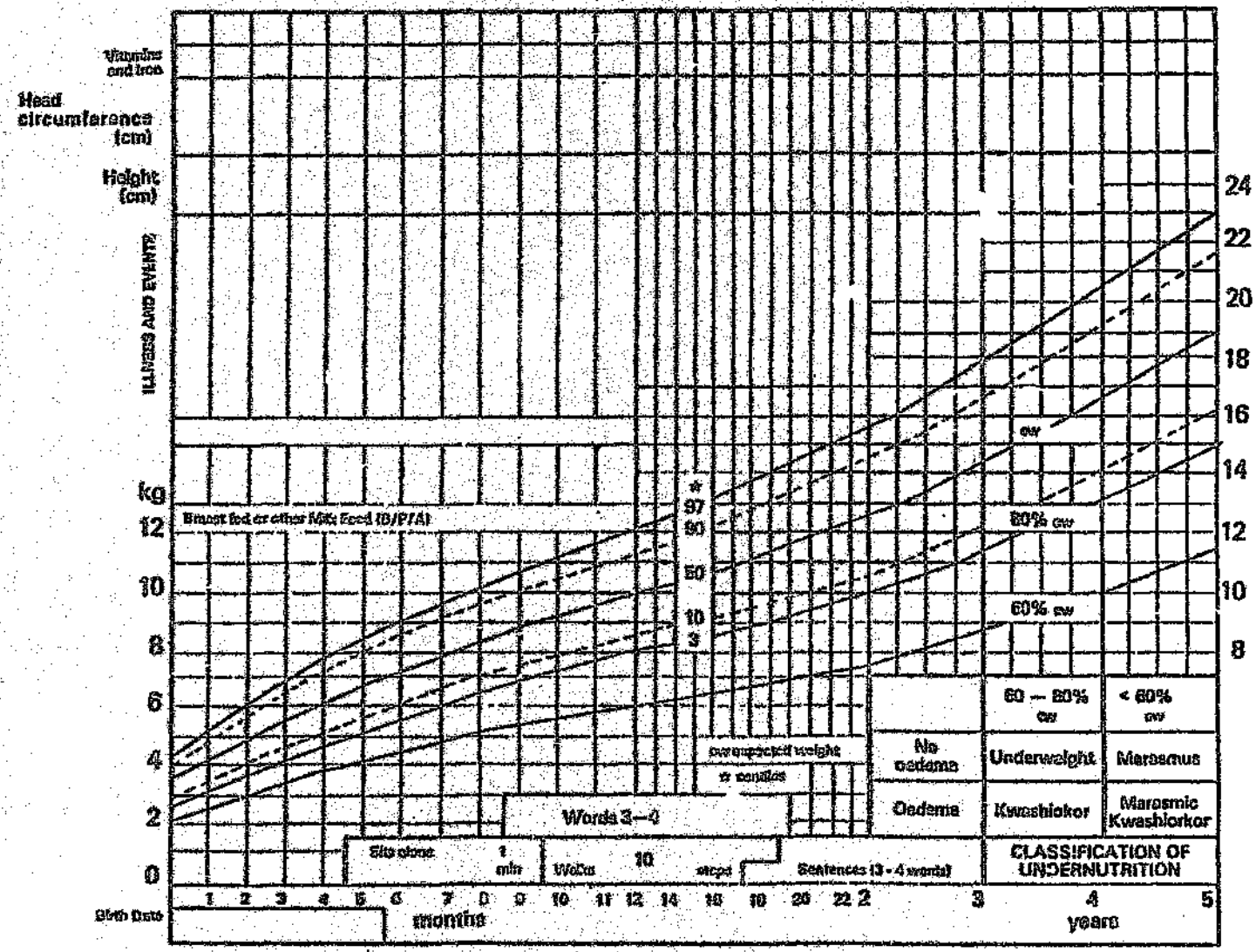
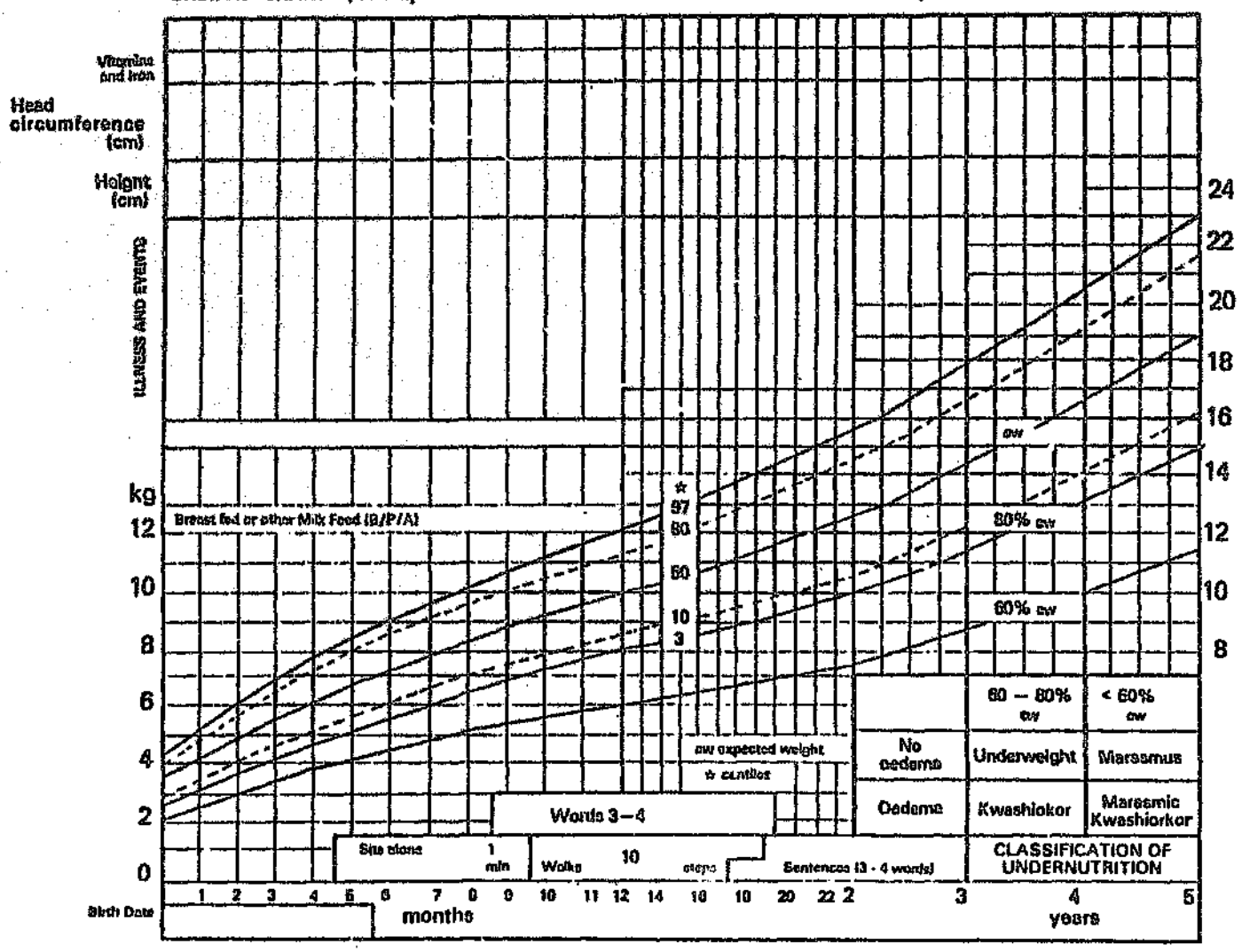


FIGURE 1

GROWTH CHART (example of a chart used in clinics and hospitals in S. Africa)



middle East and in America have indicated that most well-nourished children have growth curves that compare well with the international standards (Khan and Freedman 1959; Ford 1964; Latham 1967; Hassan et al. 1969; Eksmyr 1970; Rea 1971; Wingerd et al. 1971; Amirhakimi 1974). It seems that even though genetic influences must play a part, the role of environmental factors, especially nutritional factors, is paramount (Habicht et al. 1974).

1.7 PROTEINS AND LIPIDS: METABOLIC CHANGES IN RELATION TO NUTRITION AND IN KWASHIORKOR

Nutritional status influences the activities of the major metabolic pathways in the body and in kwashiorkor there are wide ranging effects on protein, carbohydrate, lipid, vitamin and mineral metabolism. Such effects are reflected by reduced concentrations of these compounds in serum; the values generally rise approaching the normal range, following appropriate dietary treatment (Dean and Schwartz 1954; Scrimshaw et al. 1956; Burch et al. 1957).

1.7.1 PROTEINS

Studies by Kirsch and colleagues (1968) who fed rats on a diet containing only 5% mixed protein, showed that these rats developed a condition resembling kwashiorkor. Protein deficiency in rhesus monkeys resulted in a reduction in plasma triacylglycerols and phospholipids, a rise in free fatty acids and a fatty liver (Kumar et al. 1972).

The nitrogen metabolism of children with kwashiorkor has been extensively studied. Trowell was the first to report that hypoproteinaemia was a very constant feature in kwashiorkor (Trowell 1944). Altmann (1948), working in Johannesburg, found an average of 42g/l of total protein in serum from twenty hospitalized children; the albumin:globulin ratio was unity. Anderson and Altmann (1951) reported on the electrophoretic serum protein pattern in malnutrition including kwashiorkor. These workers showed that the gamma-globulin was relatively normal or higher than normal, albumin was low; alpha and beta globulins were normal. Jassen and Le Roux (1950) found an average of about 41g/l of total protein in serum and an albumin:globulin ratio very near unity in forty one

children admitted to a Pretoria hospital. Thirty two children in Curaçao investigated by Van der Sar (1951) had an average of 42g/l for serum total protein, when they were first admitted to hospital, and on discharge the average value was 66g/l; albumin rose from 22 to 45g/l and globulin from 20 to 21g/l. The albumin:globulin ratio therefore changed from 1.1 to 2.2. Dean and Schwartz (1953) showed an average 39.8g/l of serum total protein (ranging from 29 to 51g/l) in thirty three kwashiorkor children admitted to hospital. A rise to about 60g/l of serum total protein was reported on the eighth day after admission. An albumin:globulin average ratio of 0.67 at admission in eighteen kwashiorkor patients was reported by Behrendt in 1949. After treatment the average ratio finally attained was a little over unity. This was only half of that found in normal children (Behrendt 1949). Gitlin and co-workers studied albumin metabolism in kwashiorkor and concluded that hypoalbuminaemia was due to a decreased synthesis of albumin (Gitlin et al. 1958). Oedema, characteristic of kwashiorkor, results in part from the hypoalbuminaemia (Braunwald et al. 1967). An average value of 15g/l of serum albumin for eighteen estimations on kwashiorkor subjects was reported by Dean and Schwartz (1953); a

very slight rise, a little more than 10g/l of serum albumin, from the first to the eighth day after admission into hospital was noted. Some recent studies have verified that the values of serum albumin and total protein in kwashiorkor are decreased (Khan and Bamji 1977; Hammond et al. 1987).

1.7.2 LIPIDS

Lipid disturbances in kwashiorkor have been recognized by various investigators. A decrease in total blood lipids was reported by Schwartz and Dean (1954), Cravioto et al. (1959) and Macdonald et al. (1963). As early as 1945 plasma phospholipids were found to be decreased in children suffering from kwashiorkor (Carvalho et al. 1945). An average serum value as low as 308mg total lipids/100ml in kwashiorkor patients on admission to hospital was reported by MacDonald and co-workers in 1963; these concentrations rose rapidly during treatment to an average of 636mg/100ml. This increase in total lipids, also reported by Schwartz and Dean (1957), was suggested to be due to an increase in cholesterol, triacylglycerols and phospholipids. The serum phospholipid fatty acids in

kwashiorkor children on admission were shown to differ from adult serum phospholipid fatty acids. Palmitic (16:0) and oleic (18:1) acids were higher and linoleic (18:2) and arachadonic (20:4) acids were lower in the malnourished children. MacDonald et al. (1963) suggested that the differences could be due to differences in age and not to malnutrition. Reductions in both alpha-lipoproteins (Cravioto et al. 1959) and beta-lipoproteins (Khalil et al. 1973) have also been reported. In addition to the dietary deficiency of lipids, disturbances in the protein transport system with the concomitant impairment of absorption and increased loss of fat in stools are considered the main factors underlying the disturbances of blood lipids (Khalil et al. 1974). Some degree of steatorrhea has long been recognized as occurring in kwashiorkor. Studies have shown that there is poor absorption during the acute stage (Holemans and Lambrechts 1955), late recovery (Robinson et al. 1957) and even sometimes long after clinical recovery (Mehta et al. 1958). Holemans and Lambrechts (1955) believed this was due to faulty digestion rather than absorption. In Southern India, studies on kwashiorkor patients on a low fat diet, showed faecal fat exceeded that of fat intake, suggesting that some of the fat in

the stools was of endogenous origin (Mehta et al. 1956). Gomez and his associates (1956) found that although fat absorption was poor, larger intakes were associated with greater absorption both in malnutrition and during recovery. However DeMaeyer and Vanderborght (1958) in the Congo showed that fat absorption in kwashiorkor improved during treatment.

Accumulation of fat in livers, as a result of inadequate protein intake has been seen in both animals and man. The livers of rats fed on low protein diets were found to be fatty, owing mainly to the accumulation of triacylglycerols (Litwack et al. 1952; Wikramanayake 1966). This was also seen in rabbits where the increase in liver lipid was more marked when the carbohydrate intake was increased. Fatty change in the liver is a characteristic feature of both experimental and human protein malnutrition (Trowell et al. 1954; Platt 1961; Kirsch et al. 1968). Kumar and co-workers in 1972 showed that rhesus monkeys fed a diet deficient in protein but adequate in all other nutrients and calories developed periportal fatty change.

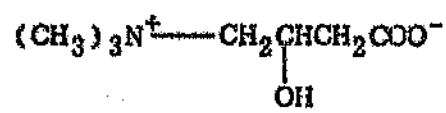
A fatty liver in children suffering from protein-calorie-malnutrition or kwashiorkor is a well known

feature of the disease (Devi et al. 1976). The mechanism of fatty change in the liver in protein malnutrition is not yet clearly understood. It has been hypothesized that fat accumulates because of a reduction in the synthesis of apoprotein very low density lipoproteins, the proteins responsible for the transport of triacylglycerols from liver to fat depots (Truswell et al. 1969; Flores et al. 1970). Other theories have suggested that fatty liver may be due to the accumulation of triacylglycerols associated with a reduction in hepatic phospholipids (Kumar et al. 1972) or defective secretion of hepatic triacylglycerols (Lombardi et al. 1968) or decreased oxidation of fatty acids in the liver (Fritz 1963). In view of the role of carnitine in fatty acid oxidation, lipid accumulation may be also be due to carnitine deficiency (Khan and Bamji 1977).

1.8 CARNITINE: METABOLISM AND METABOLIC CHANGES IN RELATION TO NUTRITION AND IN KWASHIORKOR

Carnitine was discovered at the beginning of this century (Gulewitsch and Kimberg 1905; Kutscher 1905) but it was almost forgotten among biochemists until its importance in fatty acid metabolism was established 50 years later. In the last 30 years, interest in the metabolism and function of carnitine has steadily increased.

The chemical structure of carnitine was determined in 1927 by Tomita and Sendju; it is a quaternary amine, beta-hydroxy-gamma-trimethylamino butyric acid:



In 1959 the essential role of carnitine in the utilization of long chain fatty acids for production of energy was elucidated (Fritz 1959). By the early 1960's carnitine had been shown to be essential for beta-

oxidation. The L-form of this compound was shown to be biologically active in transporting long-chain fatty acids from sites of activation in the cytoplasm to sites of beta-oxidation in the mitochondria of animal tissues (Cran 1968).

Carnitine occurs both in the free form and in the form of esters. In plasma, the esters of carnitine are referred to as acylcarnitines (Bargen-Lockner et al. 1981).

Nutritionists had previously assumed that an individual would synthesize or ingest adequate amounts of carnitine and meet carnitine needs by one or the other or both of these means. Carnitine was never considered a possible essential nutrient for any segment of the healthy population or even for diseased individuals. Clinical investigations of the last decade, however, have clearly demonstrated that carnitine deficiency may occur either as an inborn error of metabolism, or in certain other commonly occurring conditions including malnutrition, diabetes, thyroid disorders, dystrophy, advanced cirrhosis (Rudman et al. 1977) and hyperlipoproteinaemias (Pola et al. 1980).

The carnitine concentration of a tissue at any particular time is dependent on dietary intake, synthesis, transport into and out of the tissues as well as degradation and excretion (Borum 1983).

1.8.1 METABOLISM

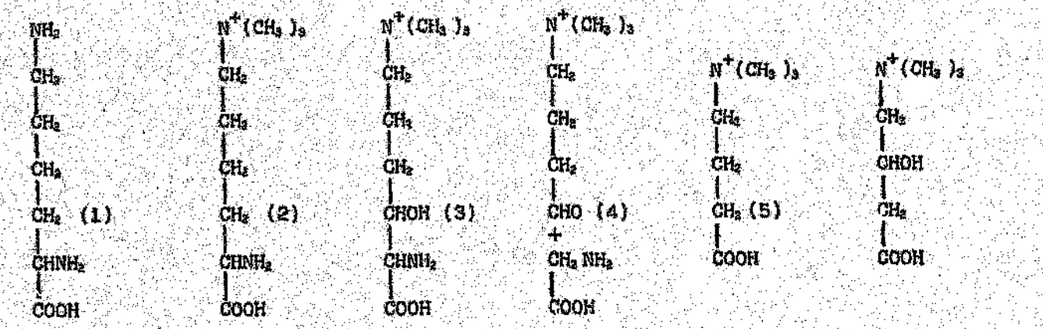
1.8.1.1 Biosynthesis

Two essential amino acids (lysine and methionine), three vitamins (ascorbate, niacin and vitamin B6) and a metal ion (reduced iron) are required for the biosynthesis of endogenous carnitine (Broquist and Borum 1982), in both human liver and kidneys (Tanphaichitr and Broquist 1973). Tissues other than liver and kidneys can synthesize the immediate precursor of carnitine, gamma-butyrobetaine; but lack the last biosynthetic enzyme which hydroxylates gamma-butyrobetaine to form carnitine (Rebouche and Engel 1980). Animals of all ages synthesize gamma-butyrobetaine, but the activity of gamma-butyrobetaine hydroxylase is age dependent. In three human infants, the gamma-butyrobetaine hydroxylase activity was 12% of the normal adult mean; by 2,5

years, the activity was 30% of the adult mean and by 15 years the level was within the standard deviation of the adult mean (Rebouche 1980). The liver conversion of trimethyllysine to butyrobetaine seems to take place in both parenchymal and nonparenchymal cells, whereas the hydroxylation of gamma-butyrobetaine to carnitine occurs only in parenchymal cells (Dunn and Englard 1981). The reaction sequence is shown in FIGURE 2. Reactions (2) and (5) are catalysed by dioxygenases, which utilize alpha-ketoglutarate as a cosubstrate and require ascorbate, ferrous iron and molecular oxygen (Bremer 1983). Reactions (1) and (2) require protein-bound lysine and reactions (3), (4) and (5) require that 3-Hydroxy-6-N-Trimethyllysine, gamma-butyrobetaine aldehyde and gamma-butyrobetaine, respectively, are found in solution (Bieber 1988).

Although a multitude of questions concerning the regulation of carnitine biosynthesis remain to be answered, it is known that the process is regulated by diet, age and hormonal status of the animal (Broquist and Borum 1982).

FIGURE 2
BIOSVNTHESIS OF CARNITINE



REACTIONS DESIGNATED (1-5) ARE CATALYSED BY:

1. Protein (lysine) methyl transferase
2. 6-N-Trimethyllysine, 2-oxoglutarate dioxygenase
3. 3-Hydroxy-6-N-trimethyllysine aldolase
4. γ-Butyrobetaine aldehyde dehydrogenase
5. γ-Butyrobetaine, 2-oxoglutarate dioxygenase

(Modified from Tanphaichitr and Broquist 1973 and Bieber 1988).

1.8.1.2 Transport

Since tissues such as cardiac and skeletal muscle depend upon fatty acid oxidation as a major source of energy but cannot synthesize carnitine, transport of carnitine from its site of synthesis to its site of action is of critical importance to cellular energy metabolism (Borum 1983). Because most tissues have a carnitine concentration that is more than 10 fold higher than that of blood plasma, an active uptake of carnitine must take place. This uptake, however, occurs with widely varying rates. Brooks and McIntosh (1975) showed that the turnover times for carnitine in kidney, liver, heart, skeletal muscle and brain in rats were 0.4, 1.3, 21, 105 and 220h, respectively. Tissue distribution and uptake are at least partially controlled by hormones, particularly in the liver and epididymis (Bremer 1983). Fasting or diabetes mellitus increases the liver concentration of carnitine (McGarry et al. 1975). This increase is particularly great in sheep, where the liver carnitine increases more than 30 fold in diabetic animals (Snoswell and Koundakjian 1972). Compounds structurally related to carnitine that contain the trimethylamine group and a carboxylic group reduce carnitine uptake. Gamma-butyrobetaine is

taken up to the same extent as carnitine (Bohmer et al. 1977). Many cells take up both D- and L-carnitine, although some selectivity for L-carnitine occurs (Bieber 1988). Data from experiments with isolated rat liver cells indicated the existence of a common carrier in the plasma membrane that mediates transport of both carnitine and gamma-butyrobetaine. The liver carrier has a higher affinity for gamma-butyrobetaine than for carnitine, which is consistent with the role of liver hydroxylation of gamma-butyrobetaine produced by other tissues (Christiansen and Bremer 1976).

1.8.1.3 Excretion and degradation

Carnitine is lost mainly by excretion in the urine (Borum 1983). In rats the renal clearance of carnitine is less than 0.051itres/day (Brass and Hoppel 1978). The glomerular filtration rate is approximately 1.71itres/day/100g body weight (Bauman and Phillips 1970). Thus more than 95% of the carnitine in the glomerular filtrate is reabsorbed. This is similar to amino acid reabsorption in the kidneys (Bremer 1983). Both free and acyl carnitines are excreted (Brass and Hoppel 1978). Similar observations have been made in

humans. Normal serum clearance is approximately 1ml/min, and the daily excretion of carnitine is 100-300μmol. Tubular reabsorption in the kidneys was found to be 96-99% (Eans and Biniek 1970; Frohlich et al. 1978). Carnitine is also excreted in milk (Bremer 1983).

In general, carnitine in mammals is not metabolized except by bacteria in the gut, but it is primarily excreted in the urine, both free and in the form of acylconjugates (Bremer 1983). It has been shown that in humans large amounts of carnitine may be excreted as specific conjugates to aid in elimination of specific acylmoieties (Bieber 1988). Small amounts of trimethylamine acetone are found in normal urine (Gruner et al. 1966) and may also be of intestinal or microbiological origin (Bremer 1983). However, in rats and mice there is some evidence that administration of the unphysiological isomer (+)carnitine leads to a far higher excretion of trimethylaminoacetate than does administration of carnitine (Seim et al. 1980). Thus by some unknown mechanism animal tissue seems to metabolize (+)carnitine to trimethylaminoacetate far more efficiently than (-)carnitine (Bremer 1983). Previous studies on the metabolism of labelled racemic

(+ or -) carnitine showed conversion of carnitine to what was identified as methylcholine (Khairallah and Wolf 1965; 1967). Carnitine decarboxylation was stimulated in diabetic and fasting rats, showing that the former were found to have a decreased body pool and a decreased half-life of carnitine (Mehlman et al. 1971). Evidence in rats has shown that degradation of (-)carnitine takes place. However, this has not been shown in humans. Carboxyl-labelled butyrobetaine, which is rapidly converted to (-)carnitine in the body, gave rise to some radioactive CO_2 in rats (Bremer 1962). However, the slow rates of formation of labelled CO_2 make it difficult to exclude participation of intestinal microorganisms. Altogether it is possible that some degradation of (-)carnitine takes place in mammals, but it seems doubtful that this degradation is of any quantitative importance. Most of the carnitine seems to be excreted unchanged in the urine (Bremer 1983).

1.8.2 FUNCTIONS OF CARNITINE

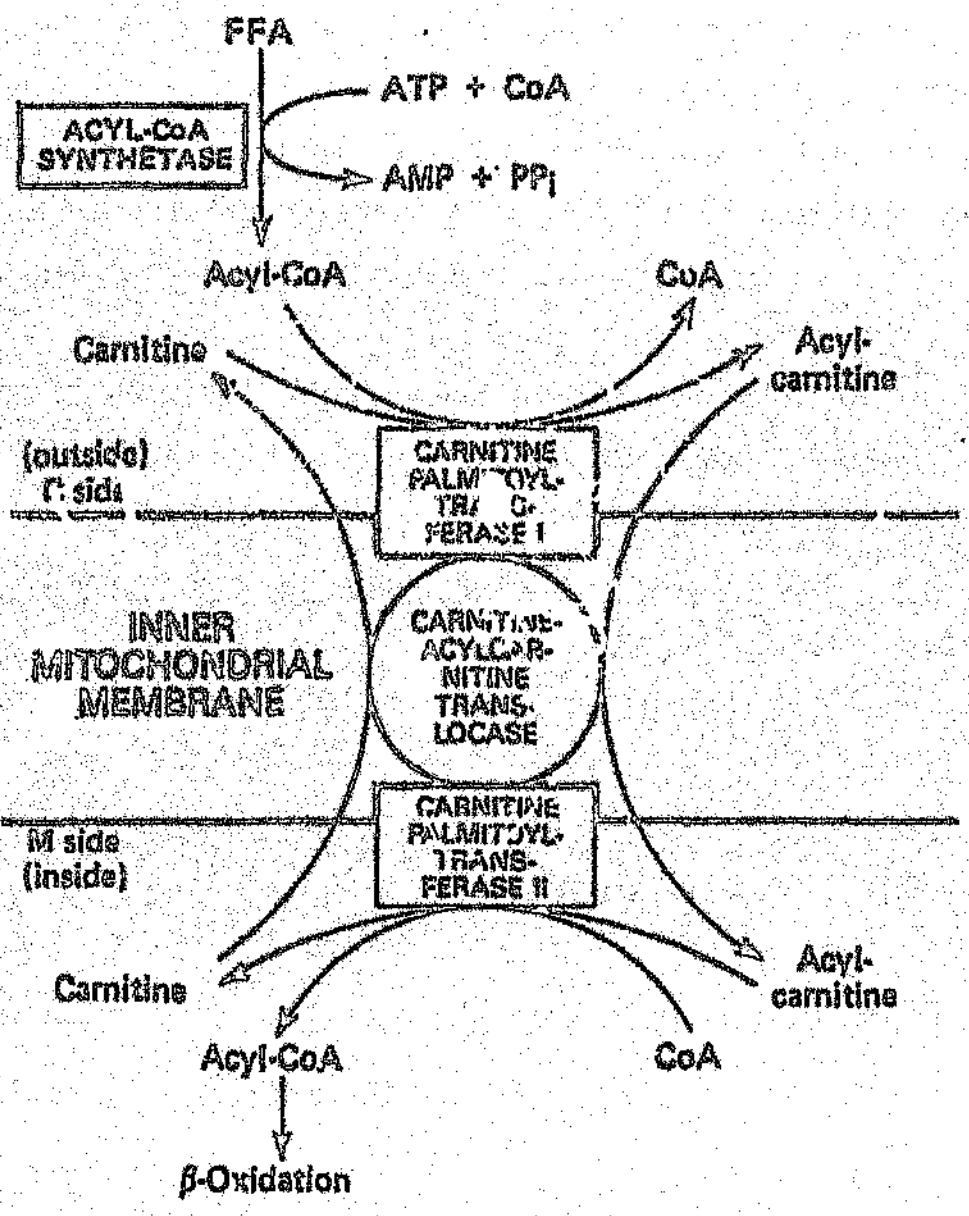
The one universally accepted and major function of carnitine is that it forms part of the shuttle mechanism

whereby long-chain fatty acids are converted into acyl-carnitine derivatives and transported across the mitochondrial membrane as shown in FIGURE 3 . The membrane is impermeable to long-chain fatty acids, as well as to their coenzyme A esters. Once across the membrane, the acyl carnitines are reconverted to their fatty acyl CoA form and undergo beta-oxidation to liberate energy. The enzyme, carnitine acetyl transferase I (CAT I), is associated with the outer side of the inner mitochondrial membrane and allows long-chain acyl groups, such as acylcarnitine, to penetrate the mitochondria and gain access to the beta-oxidation system of enzymes. Carnitine acylcarnitine translocase acts as a membrane carnitine exchange transporter. Acylcarnitine is transported in, and at the same time one molecule of carnitine is transported out. The acylcarnitine then reacts with CoA, catalysed by carnitine acetyl transferase II (CAT II), attached to the inside of the inner membrane. Acyl CoA is reformed in the mitochondrial matrix and carnitine is liberated (Murray et al. 1988).

Carnitine acyl transferase is considered the rate limiting control enzyme of the beta-oxidation pathway of fatty acid catabolism in rat liver (Lumeng et al.

FIGURE 3

ROLE OF CARNITINE IN THE TRANSPORT OF LONG CHAIN FATTY ACIDS ACROSS INNER MITOCHONDRIAL MEMBRANE



(taken from Murray et al. 1988)

1976). However, this was not found to be the case in liver mitochondria of 1-day-old piglets (Bieber et al. 1973). According to McGarry and co-workers, in rats, an increase in the level of substrate (carnitine) for this enzyme resulted from an increase in circulating glucagon. They suggested that the liver carnitine increases under the influence of glucagon by:

- increased hepatic synthesis of carnitine
- increased transfer of carnitine from muscle to liver, or
- increased hepatic uptake from plasma in an analogous way to that of amino acids (McGarry et al. 1975).

While the major function of carnitine is for beta-oxidation, there is evidence of other functions (TABLE II).

TABLE II

SUMMARY OF POSSIBLE FUNCTIONS FOR CARNITINE IN METABOLISM

Established

1. Beta-oxidation of long-chain fatty acids in mitochondria
2. Elimination of selective acyl residues/ detoxification
3. Translocation of acetyl units into mitochondria (Yeast)
4. Modulation of the CoASH/Acyl-CoA ratio (may vary depending on mitochondrial source and the levels of CAT I and the carnitine/acyl carnitine translocase)
5. Reservoir of activated acetyl units (some insect flight muscles, possibly heart and sperm)

Likely, but not established

1. Shuttle chain-shortened products out of peroxisomes

(Taken from Bieber 1988).

It is generally accepted that carnitine is involved in thermogenesis in brown adipose tissue (Borum 1983) and in initiation of ketogenesis (McGarry 1979; McGarry and Foster 1980). Catabolism of branched chain amino acids is important in energy metabolism in many normal physiological and pathophysiological conditions. Carnitine has been shown to play a facilitatory, if not obligatory, role in the catabolism of branched chain amino acids (Bieber et al. 1982).

It has been hypothesized that carnitine may affect gluconeogenesis, because gluconeogenesis is dependent upon fatty acid oxidation and therefore any impairment in fatty acid oxidation leads to hypoglycaemia (Murray et al. 1988). This hypothesis has been supported by the finding that many patients with genetic carnitine deficiency have severe bouts of hypoglycaemia (Sionim et al. 1981).

1.8.3 CARNITINE DEFICIENCY

The term "carnitine deficiency" is applicable only when the number of carnitine molecules (as measured by total carnitine concentrations) is too low to maintain

normal metabolism (Bargen-Lockner et al. 1981). The term carnitine deficiency should never be used to describe a decrease in free carnitine concentrations, when there may be a corresponding increase in acylcarnitine (Broquist and Borum 1982).

With the key role of carnitine in fatty acid oxidation in mind, it is not surprising that disturbances in the function of carnitine may cause pathological phenomena. When carnitine is lacking, a common feature seems to be the accumulation of triacylglycerol in the cell; under these conditions an inhibited fatty acid oxidation diverts fatty acids from oxidation to esterification (lipidosis). Wittels and Bressler (1964, 1965) reported the first example of such a condition. They found that the lipidosis and heart failure in diphtheria could be explained by a loss of carnitine from the heart. It was postulated that carnitine depletion resulted from three factors: inadequate intake of dietary carnitine, inadequate intake of lysine and methionine and the loss of capacity to synthesize carnitine from lysine and methionine (Rudman et al. 1977). Perfused rat liver supplied with oleic acid was treated with 2-tetradecylglycidate, which inhibited fatty acid oxidation. This direct and specific inhibition of

long chain fatty acid oxidation diverted exogenous fatty acids to the esterification pathway and enhanced the secretion of triacylglycerol in the form of very low density lipoprotein (VLDL). The rate of fatty acid oxidation in liver was therefore a major determinant in the production of triacylglycerol-rich plasma lipoproteins (Ide and Ontko 1981).

Carnitine deficiency may be provoked in infants given artificial carnitine free diets, presumably because of a slow endogenous rate of carnitine biosynthesis in the neonatal period (Slonim et al. 1981). Newborn infants normally receive substantial amounts of carnitine from their mother's milk (Borum 1981).

It has been shown that populations consuming cereal grain diets have reduced serum and urinary carnitine concentrations, which are probably related to low carnitine ingested and impaired synthesis of carnitine owing to insufficient intake of lysine and methionine (Tanphaichitr et al. 1980). In a study carried out in India, Khan and Bamji (1977) found that plasma carnitine levels were lower in children suffering from protein energy malnutrition than in healthy controls; after treatment of the malnourished children the values

increased. In a later study serum free carnitine and acylcarnitine were also found to be decreased in African children with kwashiorkor as compared with well-nourished controls (Hammond et al. 1987). These low levels of carnitine, were reported to be associated with reduced intake of carnitine together with deficiency of the precursor amino acids lysine and methionine, giving rise to inadequate endogenous synthesis (Hammond et al.1987). A deficiency of amino acids is well established in kwashiorkor. The results reported by Hammond et al. (1987) showed that serum total proteins and albumin were decreased in kwashiorkor confirming the expected derangement of normal protein profiles. However, these workers showed there was no correlation between serum carnitine and albumin, differing from the correlation reported by Khan and Bamji (1977). It was hypothesized that there is a possibility that mechanisms controlling serum carnitine and albumin levels may be deranged to different extents in kwashiorkor and that markedly suppressed albumin need not necessarily be accompanied by markedly reduced carnitine. Since carnitine plays an essential role in the oxidation of fatty acids (Bremer 1983), the deficiency of carnitine may provide an explanation for the accumulation of fats in the livers of kwashiorkor

patients (Hammond et al. 1987).

1.9 PURPOSE OF THE STUDY

There have been very few reports on serum carnitine and phospholipid concentrations in adults of different population groups. Several investigators have however indicated that diet influences values for serum protein, carnitine and lipids, but not many studies have been undertaken to investigate the effect of a low-protein diet on these parameters.

Kwashiorkor is prevalent among children in the Black population of Southern Africa. To date there appears to be very little information concerning the metabolism of carnitine and phospholipid in nutritionally deprived children in this region. Studies of serum carnitine may aid in the understanding of metabolic changes occurring in kwashiorkor and may be of potential value as regards therapy (Hammond et al. 1987).

The aims of this study were:

1. to compare serum carnitine, protein and lipid concentrations in black and white South African adults,
2. to evaluate the effect of a high maize diet (low-protein) on healthy adults,
3. to investigate the above biochemical parameters in children with kwashiorkor and to assess their response to treatment.

CHAPTER 2

2.1 SELECTION OF SUBJECTS

Ethical clearance for this study was obtained from the Human Ethics Committee (12/4/86) of the University of the Witwatersrand. Two to three millilitres of blood was obtained from black children and from rural and urban adult blacks. Ten millilitres of blood was collected from adult whites by voluntary permission of the donors. The blood was centrifuged at 2 000rpm, the serum removed and kept at -20^{0}C until the biochemical analyses were carried out.

2.1.1 NORMAL ADULT SUBJECTS

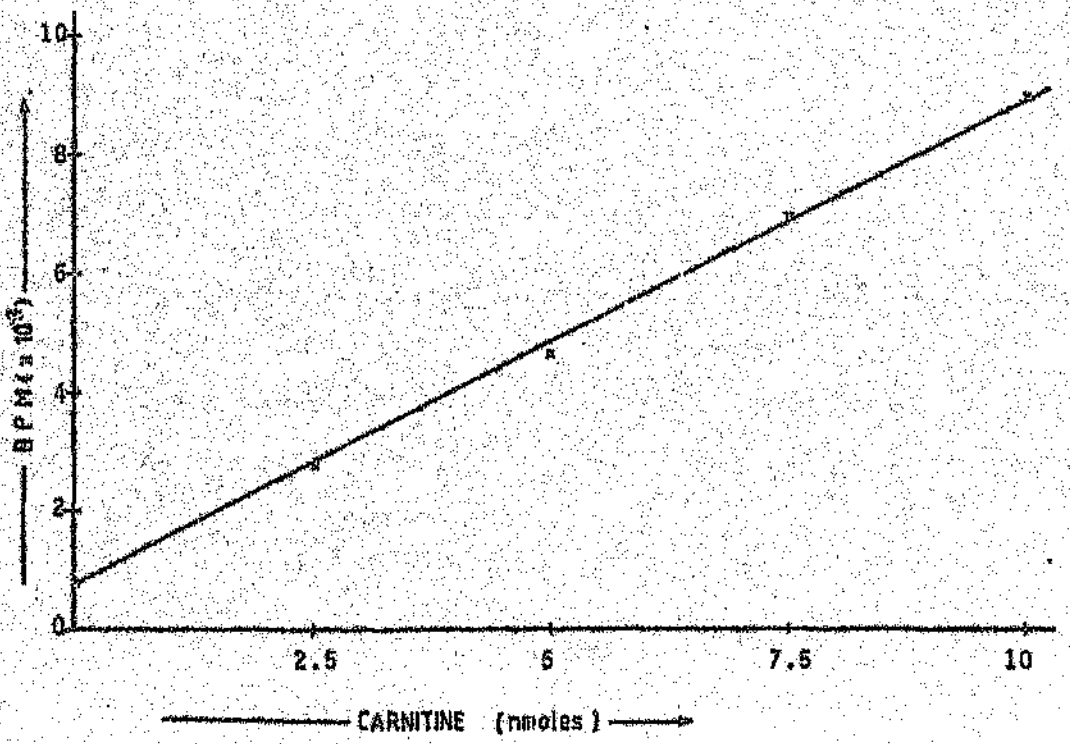
Blood samples were obtained from different population groups:

a) twelve white blood donors (six females and six males)

b) twenty-eight urban blacks (eight females and twenty males)

c) ten rural blacks (all males)

FIGURE 4
CARNITINE STANDARD CURVE



Protein standard (5g/l), (Sigma, USA): 0.5g dry human albumin dissolved in dictilled water and made up to 100ml.

2.2.2.2 Method

The following were pipetted into clean 10ml test tubes:

	Test(T)	Standard(S)	Blank(B)
Serum	0.2ml	---	---
Albumin solution(5g/l)	---	3.0ml	---
Distilled H_2O	2.8ml	---	3.0ml

To each tube 5ml of biuret reagent was added, the solutions mixed and incubated at 37^0C for 10 min. After cooling the solutions were read spectrophotometrically at 540nm using distilled water to zero the instrument. Since it was only possible to obtain small serum samples from children and certain of the rural adult population, the method was adjusted in these cases using one quarter volumes throughout.

2.1.2 NORMAL ADULT SUBJECTS ON A LOW-PROTEIN DIET

Eight healthy white subjects (six females and two males; aged from twenty to twenty three years) were maintained on a low protein diet for three weeks (appendix A). This low protein diet was taken from a book on low-protein cooking by Bounds (1983). It was adjusted to include a high mealie content in place of a low red meat content, in order to be as close as possible to the low-protein, high mealie-pap diet fed to black babies and young black children by parents in the lower socio-economic group.

Fasting blood samples (10ml) were collected before the low-protein diet was commenced and after the first, second nd third week of diet. Two months after resuming a normal diet blood samples were again obtained from each subject.

2.1.3 CHILDREN WITH KWASHIORKOR

Blood was collected from twenty two black children (ages ranging from nine months to four years), who had been clinically diagnosed as suffering from kwashiorkor

and admitted to various hospitals in the Transvaal. Six additional samples from the initial twenty two patients were obtained after the first week and samples from three of these six after the second week of hospitalization and treatment. Details of these patients are given in Appendix B. The treatment given is summarized in Appendix C.

2.1.4 CONTROL CHILDREN

Blood was obtained from a rural control group (Elim, Malamulele and Tshilidzini hospitals) comprising fifteen children (7 females and 8 males) of the same age range and race group as those with kwashiorkor; they had no nutritionally related symptoms and showed no evidence of muscle or liver disease.

2.2 BIOCHEMICAL INVESTIGATIONS

All chemicals and solvents used were of analytical reagent grade. All solvents were obtained from Merck, W. Germany, and distilled water was used throughout. All investigations, except for the lecithin fatty acids

in the children with kwashiorkor where insufficient serum was available, were carried out in duplicate.

2.2.1 SERUM CARNITINE

Principle

A radioisotopic method (McGarry and Foster 1976) was used to measure serum levels of carnitine. This involved determination of [^{14}C]-acetyl carnitine formed in the following reaction:

Carnitine + [^{14}C]-acetyl CoA --> [^{14}C]-Acetyl-carnitine + Co ASH

Reaction is catalysed by carnitine acetyltransferase.

[^{14}C]-Acetyl-carnitine was separated from [^{14}C]-acetylCoA with Dowex 1-X8. In this system the quantity of [^{14}C]-acetyl-carnitine formed (not absorbed to the resin) was stoichiometrically related to the amount of carnitine present.

Free carnitine was estimated using untreated serum samples and total carnitine was determined after alkaline hydrolysis. the difference giving the acylcarnitine concentration.

2.2.1.1 Reagents

Acetyl CoA (lithium salt) [Sigma Chemical Co, St Louis, USA]: 4mg dissolved in 20ml of 120mM Tris-HCl buffer pH 7.8; stored in 3ml aliquots at -20^0C.

^{14}C-Acetyl CoA, specific activity 53mCi/mmol (Amersham, UK)

Carnitine acetyl transferase (EC 2.3.1.7), activity 400U/ml (Boehringer Mannheim Corp, W Germany): 0.1ml made up to 2ml with 120mM - Tris-HCl buffer, pH 7.3.

Carnitine Standard, L-carnitine chloride (Sigma Chemical Co, St Louis, USA): 8mg carnitine dissolved in 200ml of 120mM Tris-HCl buffer, pH 7.3. (10nmol/50µl). Stored in 20ml aliquots at -20^0C. Working standards: For use one aliquot of the

stock carnitine standard was thawed and diluted to 2.5, 5, 7.5 and 10nmol/50µl.

Sodium tetrathionate (Riedel-De Haen AG): 13.4mg/24ml of reaction mixture (see page 45).

Dowex 1-X8 anion exchange resin (BDH, Poole, England); Cl^- form; 200-400 Mesh.

Preparation: (Cederblad and Lindstedt 1972) 100g of Dowex was suspended in 220ml of ethanol:acetone (1:1). The resin was filtered dry in a Büchner funnel, resuspended in water and stirred with a glass stirrer. It was washed once with 1M sodium acetate and filtered, resuspended in 0.5M NaOH and filtered. The resin was washed twice more by suspension in 0.5M NaOH followed by filtration. It was then suspended in 3M HCl. Following filtration and an additional wash with 0.5M HCl, the resin was washed with distilled water until the filtrate was neutral (pH approximately 7). Finally the resin was air dried to a constant weight. It was then kept in a dry closed plastic container until needed. Just prior to use a por-

tion of the prepared Dowex was added to 7.4ml H_2O to make up a volume of 10ml.

2.2.1.2 Assay

Assay for free carnitine

Carnitine assays were carried out in 10ml glass conical centrifuge tubes. The tubes were washed in Contrad after each assay.

The basic reaction mixture contained, in a volume of 24ml: 21.6ml of 120mM Tris-HCl buffer, 13.4mg sodium tetrathionate, 2.4ml of prepared acetyl CoA and 12µl 1-[^{14}C]acetyl-CoA. From this mixture 1ml was pipeted into each tube and 50µl of either serum or standard was added. The final volume was 1.05ml and this contained 120µmol of Tris-HCl buffer, pH 7.3; 2µmol of sodium tetrathionate; 25nmol acetyl CoA; 0.025µCi 1-[^{14}C]acetyl-CoA, and 50µl of either serum or standards. Reactions were initiated by addition of 50µl (1U) of carnitine acetyltransferase suspension and the mixtures allowed to stand for 30 min at room temperature. The reaction was terminated by the addition of 0.3ml of a continuously stirred slurry of the prepared Dowex

resin. The mixture was vortexed for 1-2 min and placed on ice. This step was repeated twice at 10 min intervals and the tubes were then centrifuged at 2 000rpm for 10 min. Finally, 0.5ml of the supernatant fluid was placed in a scintillation vial with 10ml Aquagel and counted in a LKB liquid scintillation counter. A blank from which enzyme had been omitted allowed correction for nonspecific supernatant radioactivity. In this system the quantity of 1-[^{14}C]acetylcarnitine formed (not adsorbed to the resin) was stoichiometrically related to the amount of carnitine present. The standards were found to be linear in the concentration range used - an example is given in FIGURE 4.

Assay for total (free plus esterified) carnitine

Alkaline hydrolysis of the acylcarnitine present in the serum sample was carried out by adding 0.1ml of 1M Tris base and 50µl of 0.4M KOH to 50µl of serum, the mixture (pH approx. 13) was incubated for 1 hour at 37^{0}C. After incubation, 0.2ml of 0.575M HCl was added to return the pH to 7.3, followed by the addition of 1ml of the reaction mixture described above, the procedure

was repeated as described for free carnitine. The values for total carnitine obtained from the standard curve were corrected for volume differences. Concentrations of acylcarnitine were obtained by subtracting the value for free from that for total carnitine.

2.2.2 SERUM TOTAL PROTEINS

Principle

Total serum protein concentration was measured using the Biuret reagent. The principle of this method is that copper in an alkaline solution reacts with the peptide linkages of amino acids in the protein producing a violet colour.

2.2.2.1 Reagents

Biuret Reagent; (Weichselbaum formula) CSML Diagnostics, Johannesburg. The reagent is supplied ready for use.

2.2.3 SERUM ALBUMIN

Principle

Certain anionic dyes have protein-binding properties. The binding of the dye by the protein causes the wavelength at which maximum absorption occurs to differ from that of the free dye. The intensity of absorption of light of the bound dye is related to the amount of dye converted. Bromcresol green has a high affinity for albumin while being substantially free of pigment interference. In the presence of an excess of dye, the intensity of absorption of the albumin-dye complex is proportional to the concentration of albumin present.

2.2.3.1 Reagents

Albuxact (CSML Diagnostics, Johannesburg) This contained bromcresol green, lactate buffer pH 4 and surfactant. One volume of albuxact was diluted with 4 volumes of distilled water for use.

Quality control serum [QC] (Burroughs Wellcome UK). When reconstituted with 5ml distilled H_2O this contained 35-38g/l albumin.

2.2.3.2 Method

The following were pipetted into clean 10ml test tubes:

	Test(T)	Std(S)	Blank(B)
Albuxact	5.0ml	5.0ml	5.0ml
Serum	0.01ml	---	---
Standard (QC)	---	0.01ml	---

After mixing, the absorbences of tests and standard were measured immediately against the blank at a wavelength of 630nm.

2.2.4 SERUM PROTEIN ELECTROPHORESIS

Principle

The serum proteins were separated on an agarose gel at a pH of 8.6 using the Beckman Paragon electrophoresis system. .Electrophoresis is the migration of charged particles in an electrolyte solution which occurs when

an electric current is passed through the solution. Various protein components of a mixture, such as serum, at pH values above or below their isoelectric points will migrate at varying rates in such a soluti be-cause they possess different surface charges. At a pH of 8.6 the albumin molecules, which are smaller and more highly charged, exhibit the most rapid rate of migration towards the anode, followed by the globulins. These are designated in order of decreasing mobility as alpha$_1$, alpha$_2$, beta and gamma globulins; the latter two tend to move towards the cathode during the electrophoretic run. Migration of a charged particle towards one of the electrodes of the field depends upon the electric charge and size of the particle, the strength of the electric field, and the nature of the medium used to support the particle during the migration process.

2.2.4.1 Reagents

Serum protein electrophoresis: Paragon system from Beckman, Palo Alto, USA.

an electric current is passed through the solution. Various protein components of a mixture, such as serum, at pH values above or below their isoelectric points will migrate at varying rates in such a solution because they possess different surface charges. At a pH of 8.6 the albumin molecules, which are smaller and more highly charged, exhibit the most rapid rate of migration towards the anode, followed by the globulins. These are designated in order of decreasing mobility as $alpha_1$, $alpha_2$, beta and gamma globulins; the latter two tend to move towards the cathode during the electrophoretic run. Migration of a charged particle towards one of the electrodes of the field depends upon the electric charge and size of the particle, the strength of the electric field, and the nature of the medium used to support the particle during the migration process.

2.2.4.1 Reagents

Serum protein electrophoresis: Paragon system from Beckman, Palo Alto, USA.

Preparation of reagents and samples was carried out as given in Appendix D.

2.2.4.2 Method

Electrophoretic procedure: The Paragon system is composed of electrophoresis cells, a power supply, sample applicator, a wet processor station and a dryer. The components supplied with the kit are: agarose gels, E-2 barbital buffer, Paragon blue stain, templates and blotters.

Application of samples: The agarose gel was removed from its foil package and placed on a paper towel. It was blotted gently with a gel blotter and the blotter discarded. A template was carefully placed on the "B" position on the gel. Samples (3 to 5µl) were applied across each template slot. Five minutes after application of the last sample the template was gently blotted.

Electrophoretic run: The gel was then placed into the Gel Bridge Assembly, aligning positive (+) and negative (-) sides of the gel with the corresponding positions marked on the Gel Bridge Assembly. The assembly was

placed in the Paragon electrophoresis cell, containing the pH 8.6 buffer, and covered. The cell was inserted into the power supply, the voltage set to 100 volts, and run for 25 minutes.

Fixing and staining: The gel was removed, placed into a gel frame and suspended in acid-alcohol solution I for 3 minutes to fix the protein. After drying the separated proteins were stained and the background cleared using the following sequence:

Paragon blue stain	3 min
Acetic acid solution I	2 min
Acid-alcohol solution II	2 min
Acetic-acid solution II	2 min

The stained film was dried and scanned using a Gelman densitometer at 600nm.

2.2.5 SERUM LIPOPROTEIN ELECTROPHORESIS

2.2.5.1 Reagents

Lipoprotein electrophoresis Paragon system from Beckman, Palo Alto, USA.

Preparation of reagents and samples was carried out as described in appendix E.

2.2.5.2 Method

The serum lipoproteins were separated electrophoretically at pH 8.6 using an agarose gel medium supplied with the Beckman Paragon lipo electrophoresis kit. Serum samples were not frozen before use. When referring to electrophoretic separations of lipoproteins, the fraction with the greatest anodic mobility is designated as alpha lipoprotein, the next two as pre-beta and beta lipoproteins, respectively, and the fraction remaining at the origin as chylomicrons. The alpha, pre-beta, and beta fractions are also referred to as high density (HDL), very low density (VLDL), and low density (LDL) lipoproteins respectively.

The procedure was essentially as described above for serum protein electrophoresis.

2.2.8 SERUM PHOSPHOLIPIDS

Total serum phospholipids were extracted into an organic solvent and this extract used for measurements of phosphorus (for total phospholipid) and thin layer chromatographic separation for isolation of lecithin and analysis of the lecithin fatty acids.

2.2.8.1 Reagents

Sulphuric acid (5M): To 350ml water, 139ml conc. H_2SO_4 were carefully added, the solution was cooled and made up to 500ml with water.

Ammonium molybdate: 0.4g ammonium molybdate tetrahydrate was dissolved in 100ml water. The solution was freshly prepared every two weeks and kept at 4^0C.

Ascorbic acid : 12mg in 2ml water. Prepared freshly for use.

Stock Standard Phosphate solution: 5mg P/100ml distilled water: 22mg of potassium dihydrogen phosphate (KH_2PO_4, mw=136.09) was dissolved in 100ml water in a volumetric flask. The solution was stored at 4^0C. (100µl of this solution contained 5µg P).

Thin layer chromatographic (TLC) plates (Macherey-Nagel, Duren, W. Germany): silica gel G, 0.25mm thick, 20 x 20 cm plastic-backed plates.

Lecithin and sphingomyelin mixtures (Sigma Chemical Company, ST Louis, USA): Chloroform solutions with lecithin:sphingomyelin ratios of 2:1, 4:1 or 6:1 and concentrations of lecithin of 2, 4 and 6mg/ml.

Sodium methylate methanol (Saarchem, Johannesburg, RSA). Purchased ready for use at a concentration of 0.2M.

2.2.6.2 Methods

Extraction of serum phospholipids

The procedure was based on that of Folch et al. 1957.

Methanol (1ml) was added to 0.2ml serum, the solution vortexed and left to stand for 5min. A further 1ml methanol was added, the mixture vortexed and left to stand for 5min. Chloroform (2ml) was added, the tube vortexed and left to stand for a further 10 min. After centrifugation for 5 min at 2 000rpm, the supernatant was transferred to a clean test tube and 8ml of 0.1M sodium chloride added. The mixture was vortexed and centrifuged at 2 000rpm for 5 min. The addition of sodium chloride solution alters the equilibrium of the mixture giving rise to two phases. The lower (chloroform) phase containing the phospholipids was transferred to a conical centrifuge tube and evaporated to dryness under nitrogen at 50^0C; the residue was dissolved in 1ml chloroform and then divided into two aliquots, one for the analysis of total phospholipid, and the other for analysis of lecithin fatty acids.

Serum total phospholipid

Principle

The total phosphorus can be determined after destruction of the organic matter. Total phospholipid is digested with sulphuric acid and peroxide. The resulting solution is treated with ammonium molybdate, which couples with phosphorus to form a yellow phosphomolybdate. On reduction with ascorbic acid a blue compound is formed. The intensity of the blue colour is proportional to the amount of phosphorus present.

Method

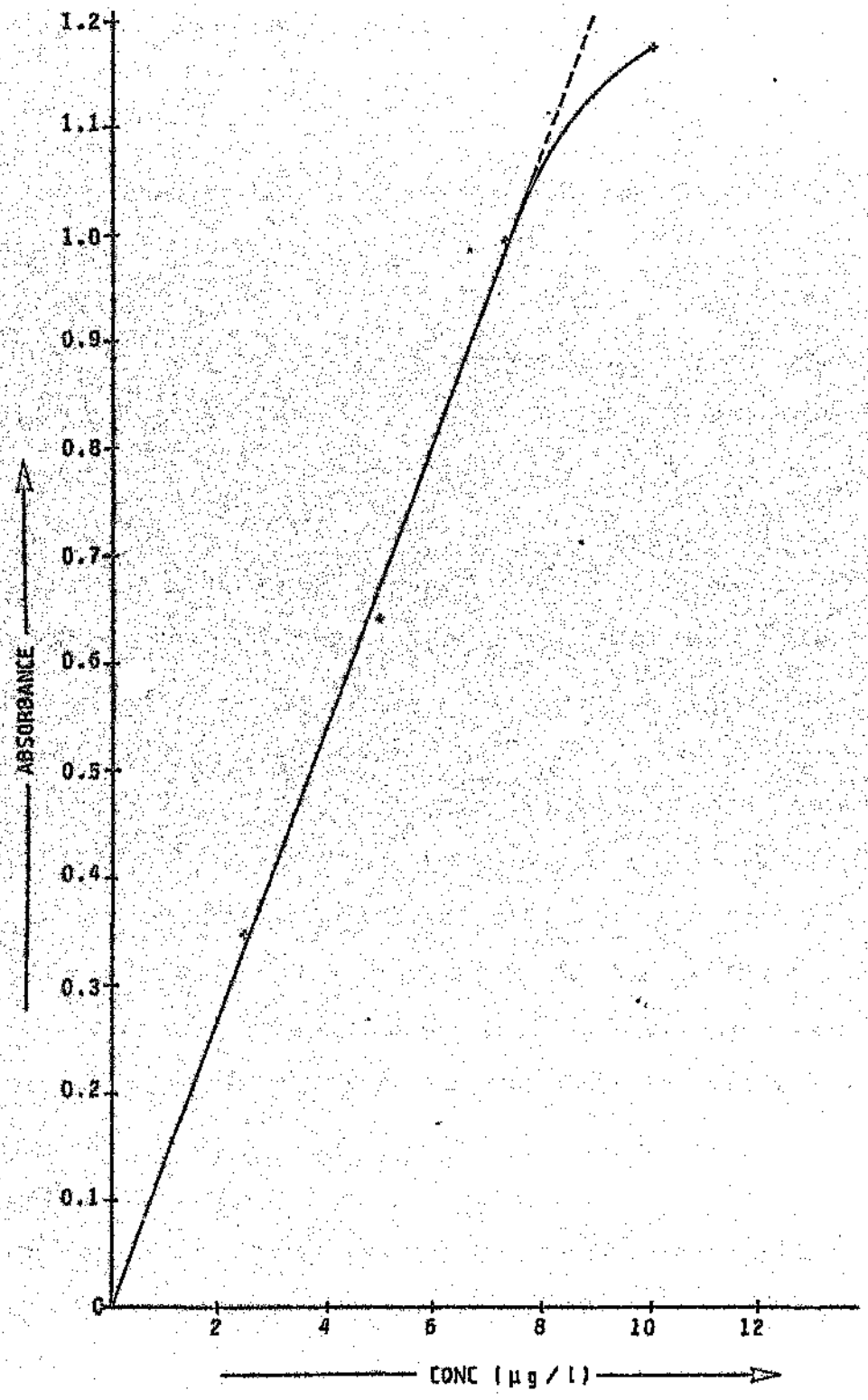
This was a modified Bartlett (1959) procedure.

One aliquot of the chloroform solution (0.5ml) was taken to dryness under nitrogen at 40^0C in an acid washed 20ml pyrex test tube. Four phosphorus standards (50, 100, 150 and 200µl) containing 2.5, 5, 7.5, and 10µg of phosphorus, respectively and a control (0.1ml

water), were included with each batch of samples. The standard curve was found to be linear for this range of concentration (FIGURE 5). To the dried extract, the standards and the control, 0.3ml of 5M H_2SO_4 and 0.1ml 30% (V:V) H_2O_2 were added and well mixed. After heating for 30 min at 220^{0}C, the tubes were removed, the test tube rack tilted so that the tubes were at about a 30 degree angle, and a further 0.1ml H_2O_2 added. After heating for an additional 30 min at 220^{0}C, the tubes were removed and cooled for 10 min, 1ml of water was added to each tube and the tubes vortexed for 2 min to ensure all the phosphorus had gone into solution. The solutions were transferred into 10ml conical tubes for centrifugation at 3 000rpm for 5 min. To acid-washed test tubes containing 0.5ml of 0.4% ammonium molybdate tetrahydrate and 0.05ml of 0.6% L-ascorbic acid solution, 0.5ml of the supernatant from each tube was added and each solution vortexed for 10 sec. The tubes were covered with glass bubbles or marbles and heated for 15 min at 100^{0}C. After cooling for 10 min, the marbles were removed and the tubes centrifuged at 1 000rpm for 2-3 minutes. The supernatants were read at 700nm against a blank containing 0.5ml ammonium molybdate, 0.05ml ascorbic acid and 0.5ml H_2O. Total phosphorus was multiplied by 23.6 (MW lecithin/MW

FIGURE 5

STANDARD CURVE FOR PHOSPHORUS ESTIMATION



phosphorus) to obtain the total phospholipid concentration expressed as phosphatidylcholine.

Analysis of serum lecithin fatty acids

a) Thin layer chromatographic separation of serum phospholipids

Principle

Phospholipids may be separated by adsorption thin-layer chromatography according to the type and number of functional groups, irrespective of chain length and degree of unsaturation (Mangold 1961). Depending on the solvent system, the phospholipids separate in order of polarity. In the solvent system used in this study, [chloroform:methanol:acetic acid:water (50:25:7:3)] the phospholipids separate in order of decreasing polarity into phosphatidyl ethanolamine, phosphatidyl serine, lecithin, sphingomyelin and lysolecithin, respectively.

Method

One of the aliquots obtained from the above extraction of serum phospholipids was dried under nitrogen at

40^0C. The dried phospholipid extract was reconstituted with 100µl chloroform and spotted on a silica gel G plastic-backed plate (0.25mm layer). The plates were chromatographed for one hour, air dried for a few minutes and finally dried at 100^0C for 15min. The bands were visualized with iodine vapour, the lecithin fraction outlined with a pencil and the plate heated at 100^0C until the iodine had evaporated. Lecithin and sphingomyelin standards were chromatographed at the same time for identification of the bands.

b) Saponification and methylation of lecithin fatty acids

The lecithin band was carefully scraped off the thin layer chromatographic plate into a suitable tube and then treated as follows: methanol (1ml) was added to the silica gel followed by 2ml of 0.2M sodium methylate in methanol. The tube was heated for 15 min at 50^0C with frequent mixing. After cooling, 4ml of water was added. The methyl esters were extracted with 2ml hexane. The hexane extraction was repeated twice. Anhydrous sodium sulphate was added (approximately half a spoon spatula) to remove residual water and the tube centrifuged at 2 000rpm for 5 min. The supernatant was

transferred to a clean tube. The hexane solution was evaporated to dryness under nitrogen at 60^0C. The residue was dissolved in 10µl hexane and 0.5µl was injected into a gas chromatograph.

c) Gas chromatographic analysis

Principle

A flame ionization detector was used. An advantage of this detector, which was recognized early in its development, was that it responds to ions produced by "active" carbon atoms. These may be defined as those carbon atoms which do not have a carbonyl function, or which do not form a carbonyl function on thermal breakdown of the molecule in the flame. The latter process may of course be sensitive to a number of factors such as the operating parameters of the flame or the design of the jet and the relative weight or molar responses between individual detectors to halogenated or similar polyatomic molecules of low molecular mass (Ackman 1964). In gas liquid chromatography (GLC), a non volatile liquid, the stationary phase, is coated on an inert solid support (column) and a gas passes through as the mobile phase.

Method

Nitrogen was used as the mobile phase, and two polar stationary phases were used. For the diet study, the methyl esters were separated on a 2m open column, 4mm internal diameter, packed with 5%DEGS-PS on 100/200 Supelcoport (Packard Instruments, SA) at a nitrogen flow rate of 23ml/min. The rest of the study was carried out on a 30m mega bore column packed with SP2330 (Supelco, USA) at a total nitrogen flow rate of 25ml/min (5ml/min of nitrogen through the column and 20ml/min of nitrogen as a make up gas). A Packard 427 gas chromatograph was used. The methyl esters were separated isothermally at an oven temperature of 160^0C for both p ases. The injection port was at 180^0C and the detector at 200^0C. Both columns are highly polar and the methyl esters separate out in order of increasing chain lengths. The results were recorded and integrated on an SP4100 integrator. Under the conditions described 12 fatty acids were regularly encountered and adequately separated in all the groups studied, except in children with kwashiorkor (14 fatty acids were separated). Other minor or occasional components were included in the total integration, but were not included in the calculated report because of

their infrequent occurrence or their very low concentrations.

Identification of fatty acids

The following methyl ester fatty acid standard mixtures were used: PUFA-2 (animal source), RM_3, GLC 50, NHIF, rapeseed oil (Supelco SA, Gland, Switzerland), 68A, 06C', 3C'(Nu Check, Elysian, Minnesota, USA).

In order to identify the fatty acids correctly, it is first necessary to measure retention volumes of homologous series of known compounds. In this case the standards used contained zero, one, two, three, etc., double bonds. A straight line is obtained when $\log_{10}$ retention time is plotted against the number of carbon atoms in the chain. Plotting is simplified by the use of semilogarithmic paper. A family of parallel straight lines is obtained, one for each homologous series. Thus the points on the line nearest the x-axis represent saturated fatty acid esters with different carbon numbers. the points on the next line up represent the homologous series containing one double bond, and so on. The retention volumes of the unknown are then determined, and the logarithms of these values

are plotted on the grid. The line on which the point falls reveals the degree of saturation. The position of the point on the line shows how many carbon atoms are in the molecule (Burchfield and Storrs 1962). Prior to analysis of the samples, all the standards were analysed and the graphs drawn. As there can be slight variations in retention volumes, day to day analysis of two of the standards were carried out routinely and a full set of standards was analysed every 3 to 4 weeks.

2.3 STATISTICAL ANALYSIS

The Mann-Whitney test was used for analysis of the results between the white, urban and rural black subjects, in children with kwashiorkor and in age and sex-matched children with no nutritional disorder (controls).

The data for the adult subjects on a low protein diet was analysed using the Wilcoxon test. The values compared were basal level, 1st, 2nd and 3rd weeks on the low protein diet, and two months after resuming normal

diet. The significance value used for all analyses was $p \leq 0.01$.

CHAPTER 3

3.1 CARNITINE

3.1.1 EFFECT OF STORAGE

Serum from a normal white adult was divided into 0.5ml portions and placed in plastic tubes. One portion was analysed immediately. Three aliquots were frozen at -20^0C. The fifth portion was kept at 4^0C for 4 days and then frozen. Portion six was kept at room temperature for 4 days and then frozen. Eight days after the last samples were frozen, portions 2, 5, and 6 were analysed for free carnitine. Portion 3 was tested after 2 months at -20^0C and portion 4 was tested after 5 months at -20^0C. The results are shown in TABLE III. There was no change in the serum free carnitine level in aliquots 2, 5 and 6 compared with aliquot 1 (fresh serum). Aliquot 3, which had been frozen for two months, showed a slight decrease in the concentration of free carnitine (approx. 10%). Storage for 5 months at -20^0C produced a marked decrease in the serum free carnitine level (portion 4). These results

showed that serum could be safely frozen for a month without marked alterations in the concentration of free carnitine. The majority of the serum samples were kept for less than one month, the kwashiorkor samples were kept frozen for a maximum of one and a half months. Serum carnitine appears to be stable at room temperature or at 4^0C for several days prior to freezing.

TABLE III

EFFECTS OF STORAGE ON THE CONCENTRATION OF FREE CARNITINE

STORAGE CONDITIONS	FREE CARNITINE
1. Fresh	50.0
2. Eight days at -20 C	49.9
3. Two months at -20 C	44.4
4. Five months at -20 C	35.4
5. Four days at 4 C and 4 days at -20 C	49.7
6. Room temperature for 4 days and frozen for an additional 4 days	49.1

Results are expressed as μmol/l

3.1.2 SERUM VALUES

Carnitine results for the different adult population groups, the well fed and kwashiorkor children are depicted in FIGURES 6a and 6b; mean values (±SD) are listed in TABLE IV and the individual results given in Appendices F, G, H, I and J. The values for the different adult populations fell within a similar range. No sex-related differences were found in these adult groups.

The concentrations of both free and acyl carnitine were significantly lower ($p<0.0005$) in children with kwashiorkor than in age- and sex-matched controls. No sex-related differences were apparent in either group. The serum carnitine concentrations in the well-fed children were comparable with those of the normal adult population group.

When serum carnitine concentrations were measured in six of the kwashiorkor patients after 4-5 days on a normal diet, the values for free and acyl carnitine were similar to those in untreated subjects. In three of these children studied after 2 weeks of treatment, a

FIGURE 6a

VALUES FOR SERUM FREE CARNITINE IN ADULTS OF DIFFERENT POPULATION GROUPS, IN WELL-NOURISHED CHILDREN AND CHILDREN WITH KWASHIORKOR (△= FEMALES; □ = MALES)

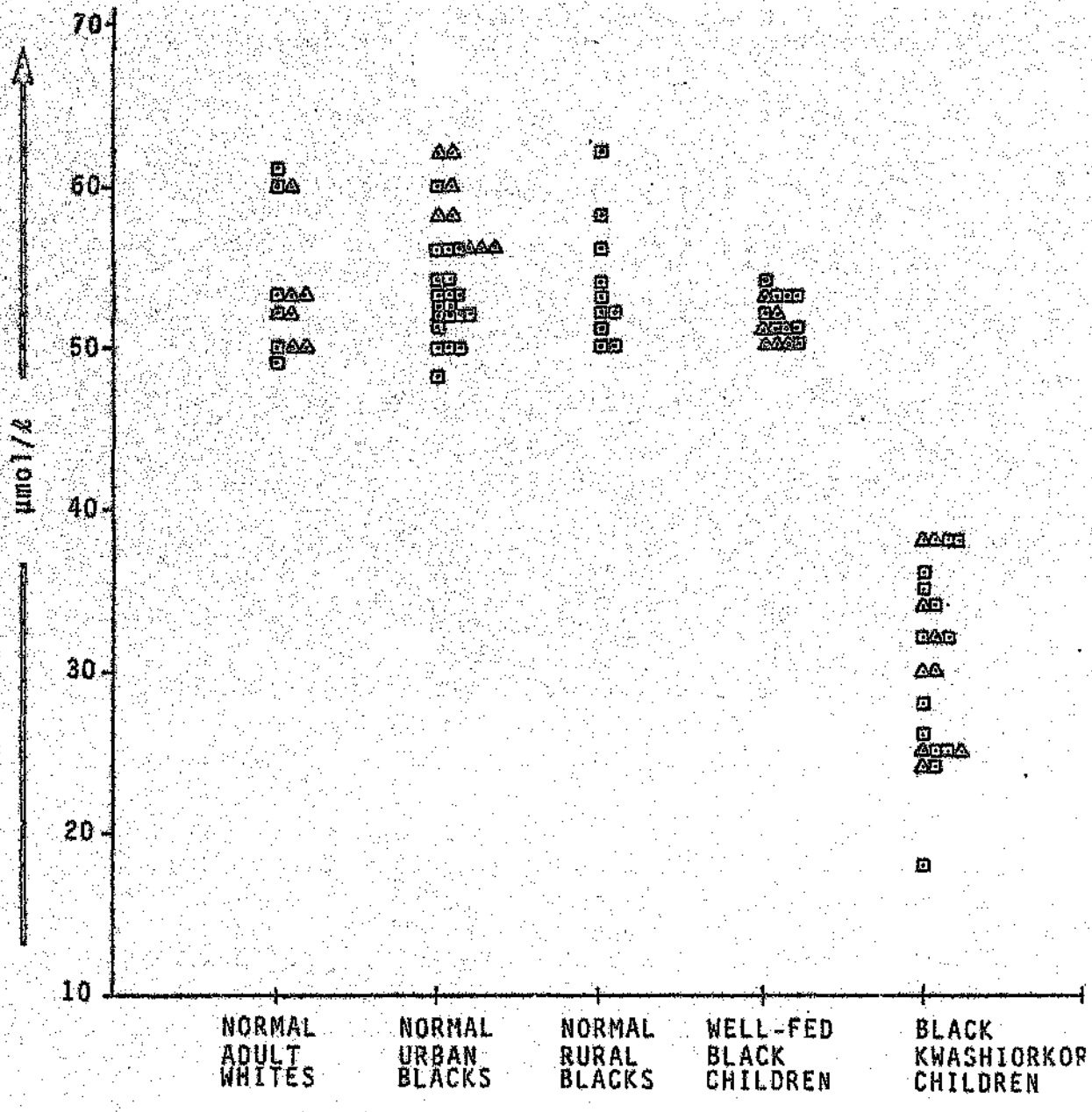


FIGURE 6b

VALUES FOR SERUM ACYLCARNITINE IN ADULTS OF DIFFERENT POPULATION GROUPS, IN WELL-NOURISHED CHILDREN AND CHILDREN WITH KWASHIORKOR (△= FEMALES; ⊡ = MALES)

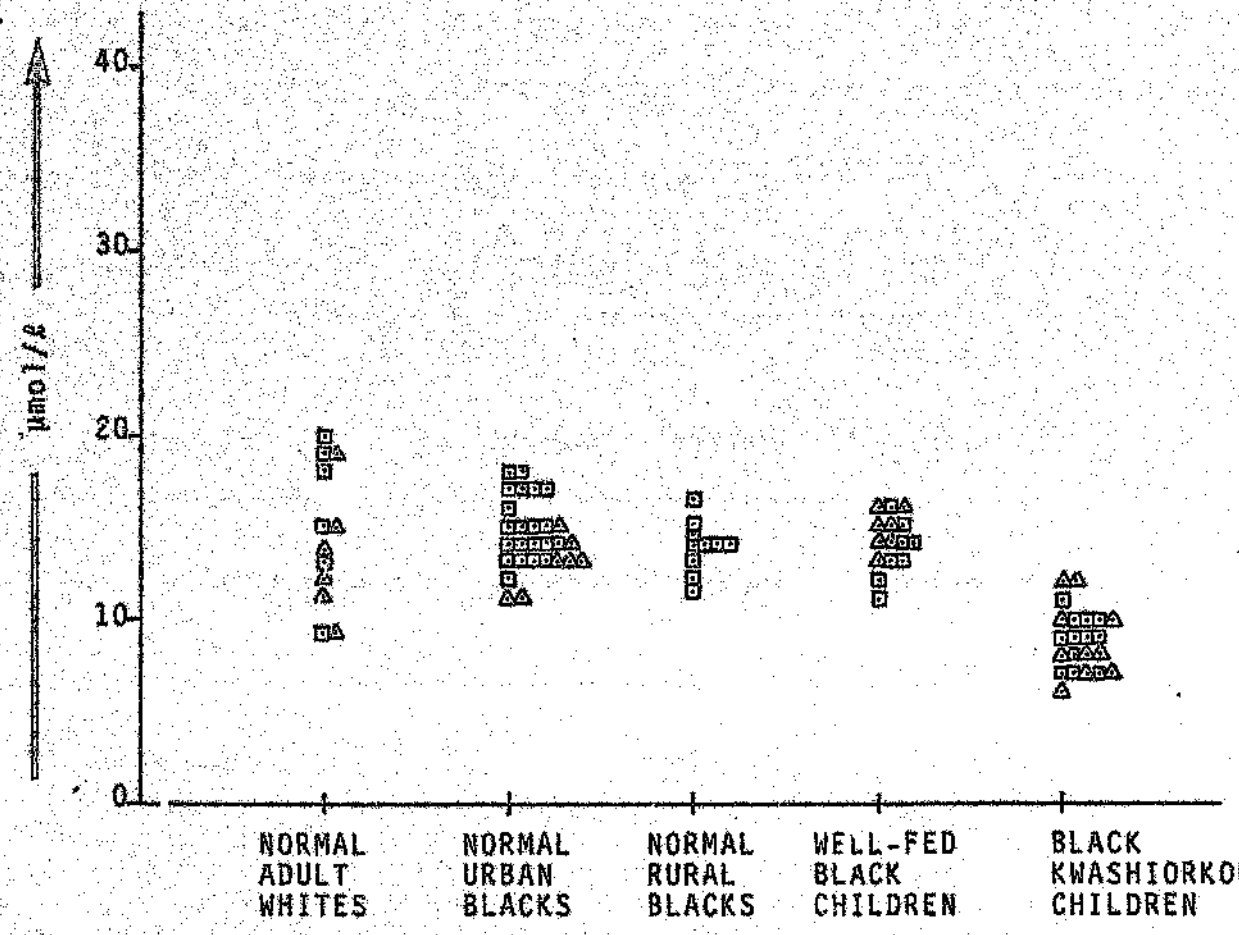


TABLE IV

SERUM CARNITINE CONCENTRATIONS IN ADULTS OF DIFFERENT POPULATION GROUPS, IN CHILDREN WITH KWASHIORKOR AND IN WELL-NOURISHED CHILDREN

	FREE CARNITINE	TOTAL CARNITINE	ACYLCARNITINE
Whites (n=12)	53.7±4.5	68.5±4.0	14.8±3.9
Blacks urban (n=28)	54.6±3.6	69.3±3.0	14.5±1.9
rural (n=10)	54.1±3.6	68.4±3.5	14.1±1.3
Well fed children (controls) (n=15)	51.7±1.3	65.8±1.8	14.1±1.4
Kwashiorkor (n=22)	30.3±5.8	39.0±6.5	8.7±1.8

Results are expressed as μmol/l

marked increase in free carnitine was observed, but for acylcarnitine there was little change. The results are depicted in FIGURES 7a and 7b and the mean values given in TABLE V.

The carnitine results in eight white adult subjects maintained on a low-protein diet for three weeks are depicted in FIGURE 8 and listed in Appendix K. A change to a low-protein diet resulted in a significant decrease ($p<0.01$) in serum free carnitine after the period of three weeks. Values measured after two months on normal diet were comparable with initial values.

3.2 TOTAL PROTEIN AND ALBUMIN

The results obtained for serum proteins and albumin in the different adult population groups, well-nourished children and in those with kwashiorkor are depicted in FIGURES 9a, 9b and the mean values ($\pm$SD) are listed in TABLE VI.

Both the serum total protein and albumin concentrations were within the locally determined (SAIMR) reference

FIGURE 7a

CONCENTRATIONS OF FREE CARNITINE IN KWASHIORKOR CHILDREN WHO HAD BEEN PLACED ON AN ADEQUATE DIET FOR TIME PERIODS INDICATED

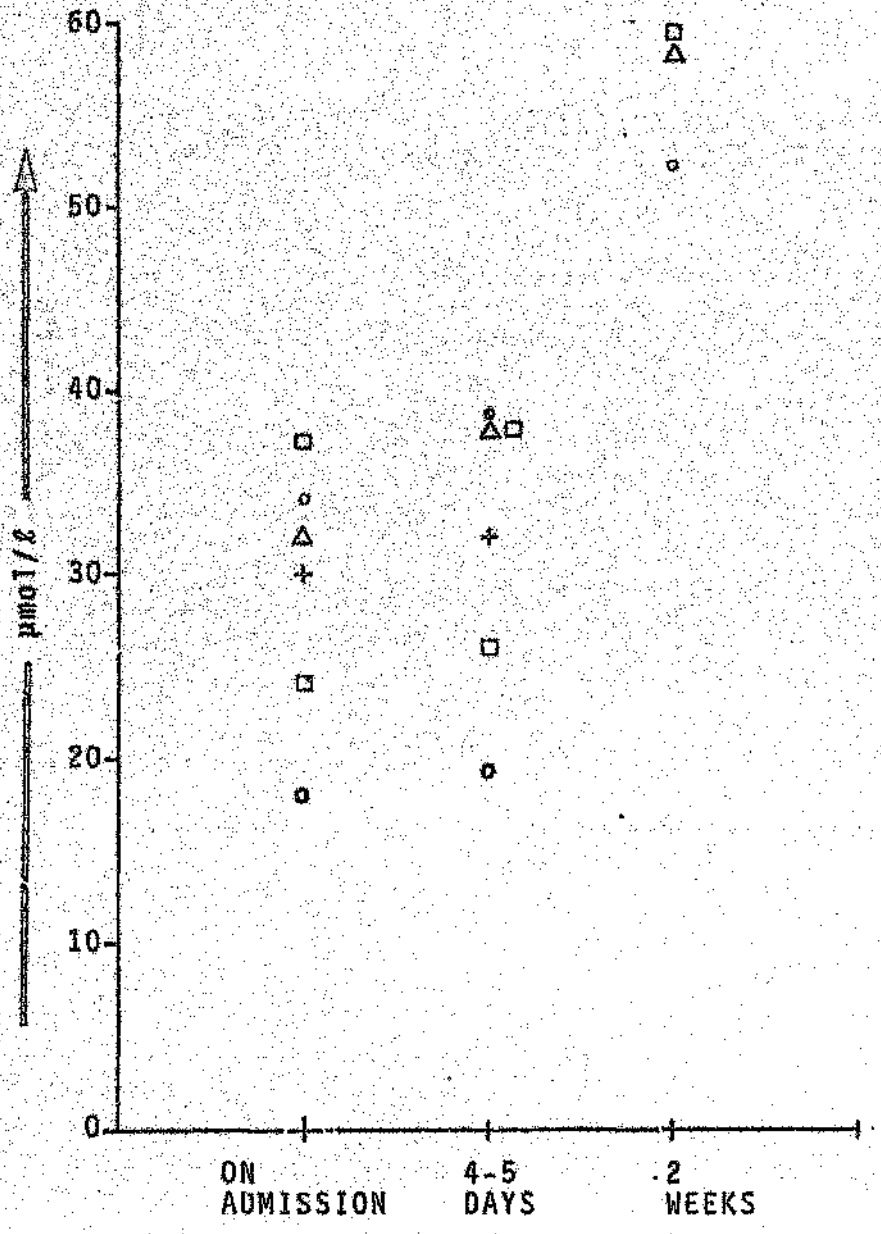


FIGURE 7b

CONCENTRATIONS OF ACYLCARNITINE IN KWASHIORKOR CHILDREN WHO HAD BEEN PLACED ON AN ADEQUATE DIET FOR TIME PERIODS INDICATED

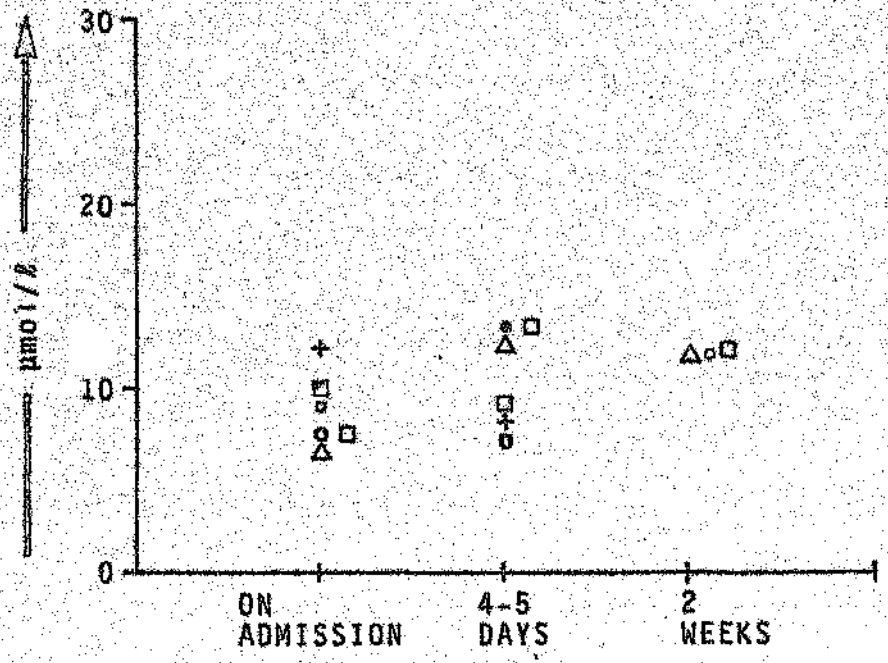


TABLE V

MEAN VALUES FOR SERUM CARNITINE IN THE FOLLOW UP OF KWASHIORKOR PATIENTS AND IN THE CONTROLS

	FREE CARNITI	TOTAL CARNITINE	ACYLCARNITINE
on admission (n=22)	30.3±5.8	39.0±6.5	8.7±1.8
1st week treatment (n=6)	31.7±7.8	42.3±10.3	10.5±2.7
2nd week treatment (n=5)	56.3±3.8	67.7±3.4	11.5±0.3
CONTROLS (n=15)	51.7±1.3	65.8±1.8	14.1±1.4

Results are expressed as µmol/l

FIGURE 8

MEAN VALUES FOR SERUM CARNITINE IN EIGHT SUBJECTS MAINTAINED ON A LOW-PROTEIN DIET FOR THREE WEEKS.

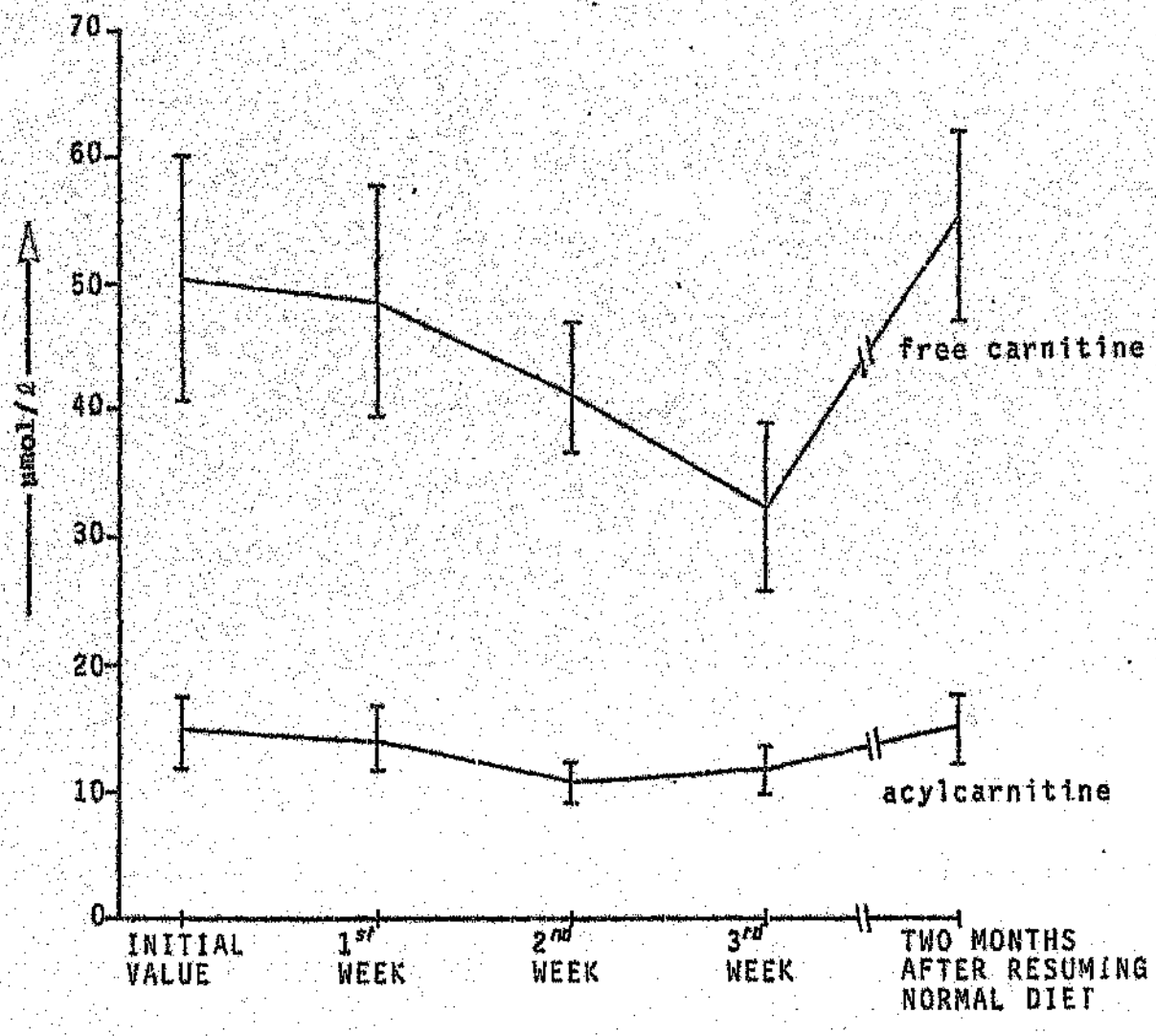


FIGURE 9a

SERUM TOTAL PROTEINS IN ADULTS OF DIFFERENT POPULATION GROUPS, IN WELL-NOURISHED CHILDREN AND CHILDREN WITH KWASHIORKOR
(△= FEMALES; □ = MALES)

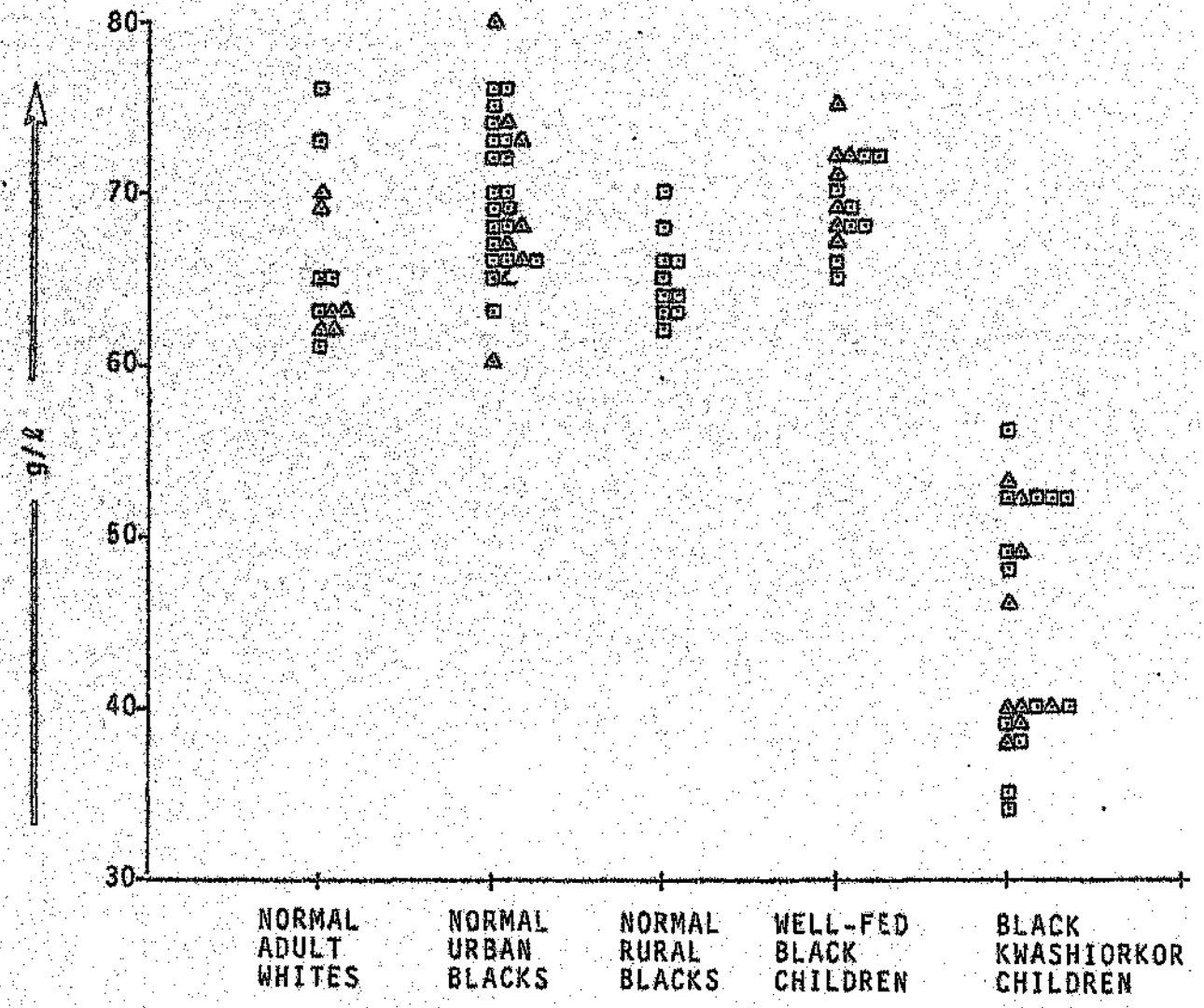
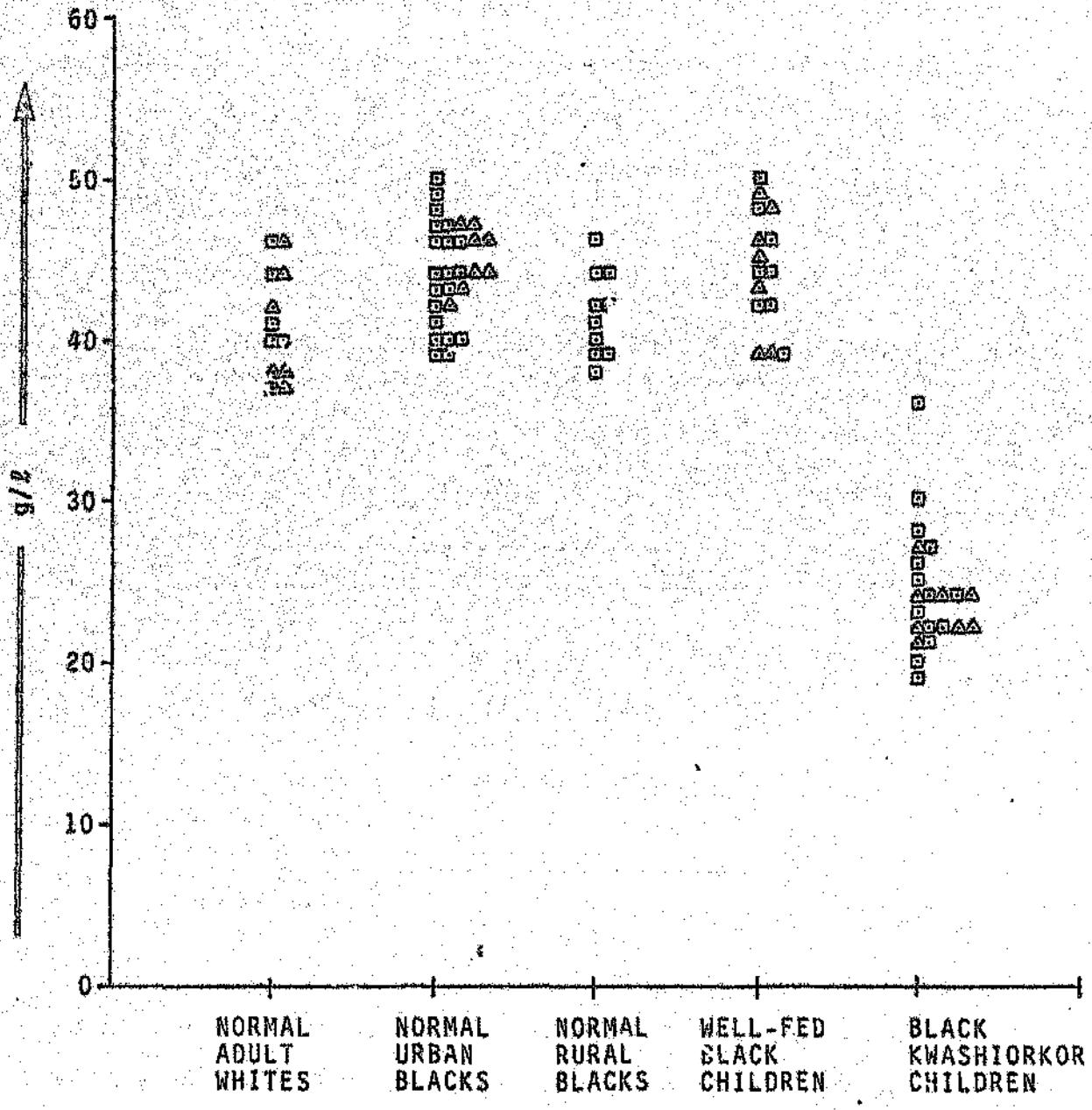


FIGURE 9b

SERUM ALBUMIN IN ADULTS OF DIFFERENT POPULATION GROUPS, IN WELL-NOURISHED CHILDREN AND CHILDREN WITH KWASHIORKOR
(△ = FEMALES; ⊡ = MALES)



ranges for ambulant white and black adults (Whites: total proteins 55-85g/l; albumin 35-50g/l; Blacks: total proteins 60-83g/l; albumin 30-55g/l). No significant differences between the three groups were noted and there were no sex-related differences. The individual estimations for serum total proteins and albumin for the white population group can be found in Appendix L and for the black population in Appendix M.

TABLE VI

SERUM TOTAL PROTEIN AND ALBUMIN CONCENTRATIONS IN DIFFERENT POPULATION GROUPS, IN CHILDREN WITH KWASHIORKOR AND IN CONTROL CHILDREN

		PROTEINS	
		TOTAL	ALBUMIN
Whites	(n=12)	66.0 ± 4.9	41.1 ± 3.3
Urban blacks	(n=28)	69.7 ± 4.5	44.2 ± 3.0
Rural blacks	(n=10)	66.1 ± 2.4	41.5 ± 2.6
Controls	(n=15)	69.6 ± 2.7	44.2 ± 3.7
Kwashiorkor	(n=22)	44.8 ± 6.9	24.3 ± 3.8

Results are expressed as g/l

Total protein and albumin were significantly decreased in the kwashiorkor group as compared with controls

($p<0.0005$). The individual values for the serum proteins are shown in Appendices N and O. The values for the controls were comparable with those of the adults.

In the kwashiorkor children who had been treated there was a significant increase ($p<0.005$) in both total protein and albumin after one week of treatment (FIGURES 10a and 10b and TABLE VII).

TABLE VII

MEAN VALUES FOR SERUM TOTAL PROTEIN AND ALBUMIN IN CONTROL CHILDREN AND IN KWASHIORKOR PATIENTS

	TOTAL PROTEIN	ALBUMIN
On admission (n=22)	44.8±6.9	24.3±3.8
1st week treatment (n=6)	59.5±4.5	31.6±2.1
2nd week treatment (n=3)	63.9±2.3	36.9±1.0
CONTROLS (n=15)	69.6±2.7	44.2±3.7

Results are expressed as g/l

FIGURE 10a

CONCENTRATIONS OF TOTAL PROTEIN IN KWASHIORKOR CHILDREN WHO HAD BEEN PLACED ON AN ADEQUATE DIET FOR TIME PERIODS INDICATED

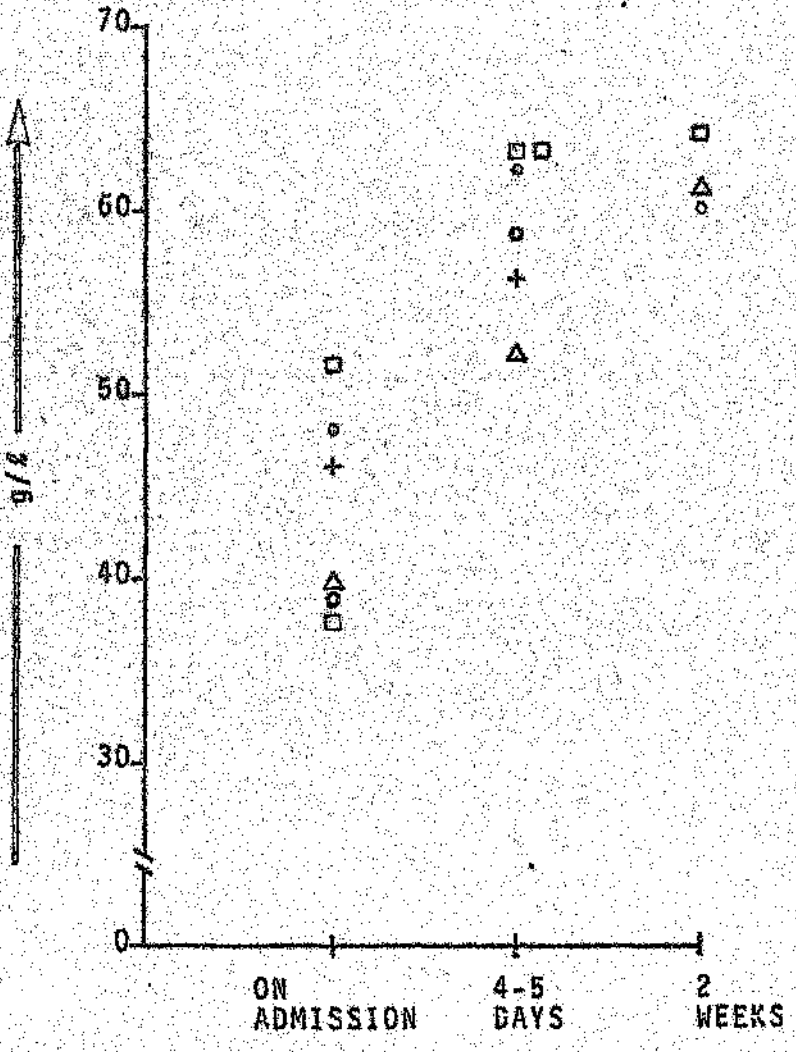
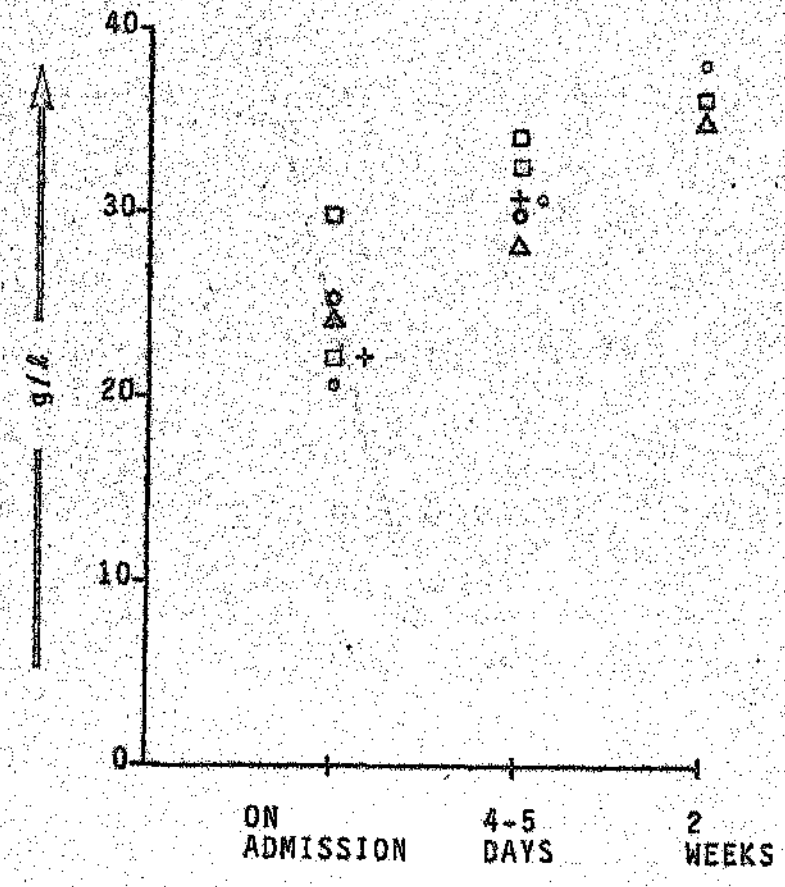


FIGURE 10b

CONCENTRATIONS OF ALBUMIN IN KWASHIORKOR CHILDREN WHO HAD BEEN PLACED ON AN ADEQUATE DIET FOR TIME PERIODS INDICATED



After two weeks on treatment, although values were markedly higher than the initial values, they still remained lower than in the control children.

The values obtained for serum proteins (mean ±SD) in the subjects on a low-protein diet are listed in TABLE VIII and depicted in FIGURE 11. Both total protein and albumin decreased over the three week period; by the third week the values were significantly lower ($p<0.01$). The concentrations had returned to the initial values when estimated two months after resuming normal diet. Individual values for the subjects on a low protein diet can be found in Appendix P.

TABLE VIII

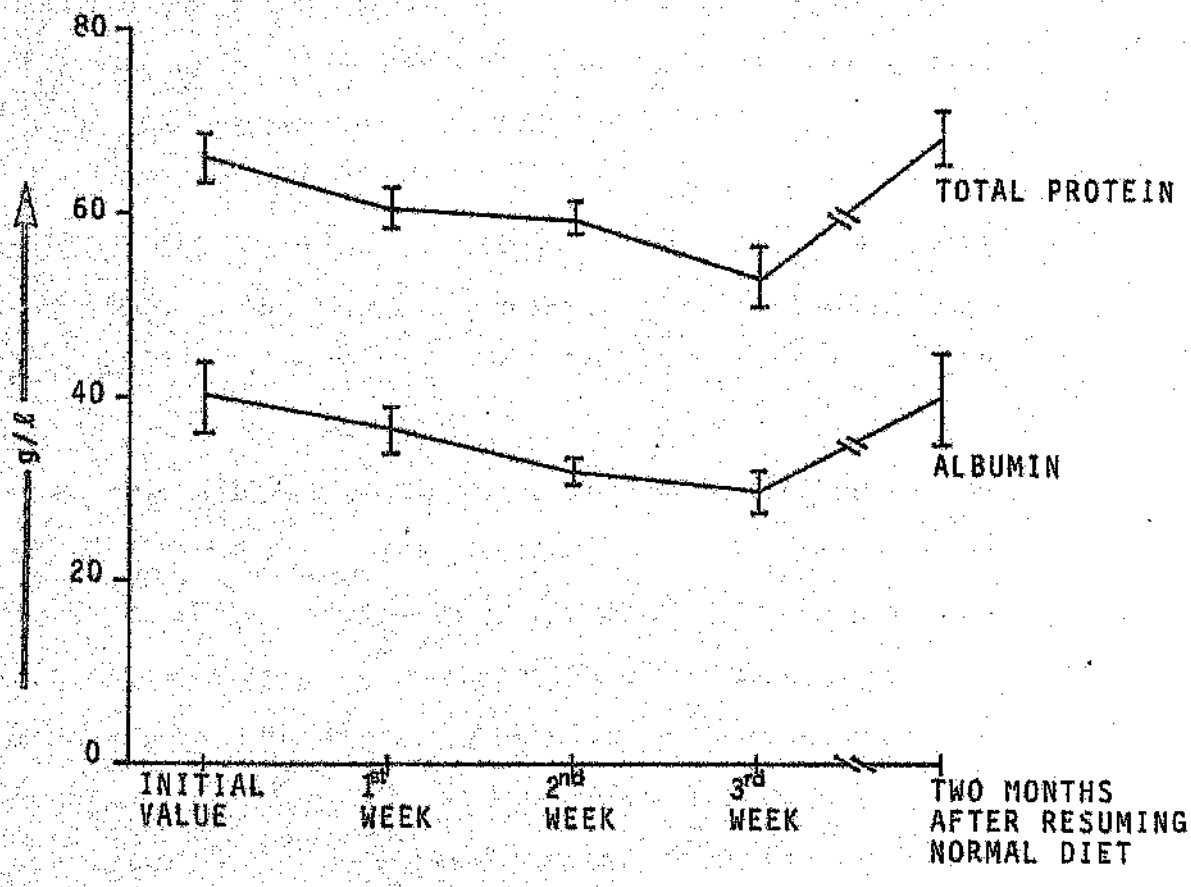
MEAN VALUES FOR SERUM TOTAL PROTEIN AND ALBUMIN IN EIGHT SUBJECTS MAINTAINED ON A LOW-PROTEIN DIET FOR THREE WEEKS.

PROTEINS	INITIAL VALUE	1ST WEEK	2ND WEEK	3RD WEEK	TWO MONTHS AFTER RESUMING NORMAL DIET
Total protein	66.4±2.2	61.3±1.8	59.8±1.5	53.0±2.7	68.9±2.0
Albumin	40.4±4.2	37.4±1.8	32.5±1.7	30.4±2.3	40.6±4.9

Results are expressed as g/l

FIGURE 11

MEAN VALUES FOR SERUM TOTAL PROTEIN AND ALBUMIN IN EIGHT SUBJECTS MAINTAINED ON A LOW-PROTEIN DIET FOR THREE WEEKS



3.3 ELECTROPHORETIC SEPARATION OF PROTEINS

Individual values for the serum albumin, $alpha_1$, $alpha_2$, beta and gamma globulins are listed in Appendices Q (white population), R (urban black population), S (rural black population), T (control children) and U (children with kwashiorkor). The mean ($\pm$SD) concentrations for the protein fractions in the groups studied are summarised in TABLE IX.

Values fell within a similar range for the adult population groups and no sex-related differences were apparent.

Values for control children showed no significant differences from those for adults. In the kwashiorkor group the albumin and beta globulin fractions were significantly lower ($p<0.0005$) than in the controls, whereas the the $alpha_1$ globulin was significantly higher ($p<0.001$). No significant differences were found for the $alpha_2$ and gamma globulin fractions relative to controls.

TABLE IX

CONCENTRATIONS OF SERUM PROTEIN FRACTIONS IN ADULTS OF DIFFERENT POPULATION GROUPS, IN CHILDREN WITH KWASHIORKOR AND IN CONTROL CHILDREN

		PROTEINS				
		ALBUMIN	GLOBULINS			
			α_1	α_2	β	γ
Whites	(n=12)	41.3 ± 4.3	1.5 ± 0.5	5.6 ± 1.5	8.1 ± 1.3	9.5 ± 2.0
Urban Blacks	(n=28)	42.2 ± 3.4	1.8 ± 0.6	6.2 ± 1.4	7.8 ± 1.3	11.7 ± 1.9
Rural Black	(n=10)	39.2 ± 3.9	1.8 ± 1.1	6.1 ± 0.8	7.8 ± 1.4	9.4 ± 1.5
Controls	(n=15)	41.5 ± 2.6	1.9 ± 0.7	6.6 ± 0.9	8.3 ± 1.1	11.0 ± 2.1
Kwashiorkor	(n=22)	19.2 ± 3.9	3.3 ± 1.2	7.3 ± 1.6	3.9 ± 1.1	11.0 ± 2.6

Results are expressed as g/l

The values measured in children with kwashiorkor, who had been placed on an adequate diet are listed in TABLE X (Details Appendix U). After one week on an adequate diet, albumin and the alpha$_2$ and beta globulins showed an increase as seen in TABLE X. After two weeks,

TABLE X

MEAN VALUES FOR SERUM PROTEIN FRACTIONS IN THE KWASHIORKOR PATIENTS WHO HAD BEEN PLACED ON AN ADEQUATE DIET FOR THE TIME PERIODS INDICATED AS COMPARED WITH CONTROLS

	PROTEINS				
	ALBUMIN	GLOBULINS			
		α_1	α_2	β	γ
on admission (n=22)	19.2±3.9	3.3±1.2	7.2±1.9	3.9±1.1	11.0±2.6
1st week treatment (n=6)	30.3±7.0	3.5±1.4	9.5±2.0	7.6±1.8	10.0±1.8
2nd week treatment (n=3)	37.0±1.8	2.2±0.3	6.4±0.3	8.6±0.1	10.7±1.6
CONTROLS (n=15)	41.9±2.6	1.9±0.7	6.6±0.9	8.3±1.1	11.1±1.2

Results are expressed as g/l

abumin appeared to increase further with $alpha_1$ globulin decreasing and the remaining globulins virtually at the values seen for control children. Due to the very small number of samples obtained no statistical analysis was able to be carried out. Appendix U_1 illustrates the electrophoretic separation of serum proteins on agarose gel for adults of different population groups, kwashiorkor children and their controls.

Individual values (g/l) for the protein fractions in the eight subjects on a low-protein diet for 3 weeks are listed in Appendix V. TABLE XI shows the mean values obtained during the study. After the first week a significant decrease was found in the $alpha_1$ ($p<0.01$) and $alpha_2$ globulins ($p<0.01$), these were still decreased after the second week; by the third week the values were similar to the initial values for all fractions, except for the albumin, which was now significantly lower ($p<0.01$). Two months after resuming normal diet all results were similar to the initial values.

TABLE XI

MEAN VALUES FOR SERUM PROTEIN FRACTIONS IN EIGHT SUBJECTS MAINTAINED ON A LOW-PROTEIN DIET FOR THREE WEEKS

	PROTEINS				
	ALBUMIN	GLOBULIN			
		α_1	α_2	β	γ
INITIAL	39.0 ±3.6	2.3 ±0.5	7.2 ±1.0	8.5 ±1.5	9.6 ±2.2
1ST WEEK	40.7 ±1.7	0.9 ±0.3	5.0 ±0.7	6.7 ±0.8	7.9 ±1.4
2ND WEEK	35.4 ±1.6	1.1 ±0.4	5.2 ±1.0	7.7 ±0.8	10.0 ±1.5
3RD WEEK	27.4 ±2.2	2.1 ±0.5	5.2 ±0.9	8.5 ±2.0	9.9 ±2.1
TWO MONTHS AFTER RESUMING NORMAL DIET	42.8 ±2.8	1.6 ±0.4	6.9 ±0.5	8.7 ±0.6	9.1±2.1

Results are expressed as g/l

3.4 LIPOPROTEIN ELECTROPHORESIS

The mean percentages for the serum lipoprotein fractions in the three population groups are listed in TABLE XII. The whites showed a significantly higher ($p<0.01$) value for chylomicrons as compared with the black population groups (urban and rural). There was a significant difference ($p<0.01$) in LDL between urban and rural blacks, the latter having a lower percentage

of this lipoprotein. The individual values for lipoproteins in the white, urban and rural black groups are listed in Appendices W, X and Y. In the urban black population there appeared to be sex-related differences; chylomicrons were not detected in the males, whereas a mean of 0.4% was found for the females. Appendix Y_1 illustrates the elecrophoretic separation of the lipoproteins in the different adult population groups.

TABLE XII

PERCENTAGES FOR SERUM LIPOPROTEINS IN ADULTS OF DIFFERENT POPULATION GROUPS

NORMAL ADULTS	CHYLOMICRONS	LDL	VLDL	HDL
WHITES (n=12)	0.4 ±0.5	57.3 ±4.4	20.0 ±2.3	22.3 ±3.2
URBAN BLACKS (n=28)	0.1 ±0.3	56.9 ±3.1	19.0 ±4.1	22.1 ±2.7
RURAL BLACKS (n=10)	-	53.6 ±3.6	21.5 ±3.3	24.7 ±3.9

Results are expressed as %

The individual values for serum lipoproteins in the well-nourished children are listed in Appendix Z. These are similar to the values found for the lipoprotein fractions in the normal adult population. As the majority of the serum samples collected from

children with kwashiorkor had been stored at -20^0C no lipoprotein electrophoresis was possible.

Densitometric scanning of the lipoprotein fractions in the eight subjects on a low protein diet showed the percentages listed in TABLE XIII; individual values are given in Appendix AA. After the first week, the VLDL fraction increased ($p<0.001$) and the HDL fraction decreased ($p<0.001$). After the second week of diet, VLDL showed a decrease ($p<0.01$) while HDL increased compared with the first week ($p<0.01$), the chylomicrons were decreased ($p<0.01$) as compared with the initial values; by the third week they had all returned to the initial values.

TABLE XIII

MEAN PERCENTAGES FOR SERUM LIPOPROTEINS IN EIGHT SUBJECTS MAINTAINED ON A LOW-PROTEIN DIET FOR THREE WEEKS.

	CHYLOMICRONS	LDL	VLDL	HDL
Initial value	0.8±0.8	56.3±5.5	15.7±6.1	26.9±4.2
1st week	0.1±0.2	57.4±3.7	34.3±3.1	8.0±3.5
2nd week	0.08±0.1	56.0±2.2	24.8±3.1	19.1±3.6
3rd week	0.4±0.1	56.2±2.4	16.2±2.4	27.6±1.5
Two months after resuming normal diet	0.9±0.7	58.4±5.3	14.5±4.1	26.3±2.9

Results are expressed as %

3.5 PHOSPHOLIPIDS

3.5.1 TOTAL PHOSPHOLIPID CONCENTRATION

The results for serum total phospholipid in the three population groups, the well nourished children and kwashiorkor children are depicted in FIGURE 12; mean values (±SD)are listed in TABLE XIV.

TABLE XIV

SERUM TOTAL PHOSPHOLIPID CONCENTRATION IN THE DIFFERENT ADULT POPULATION GROUPS, IN CHILDREN WITH KWASHIORKOR AND IN CONTROL CHILDREN

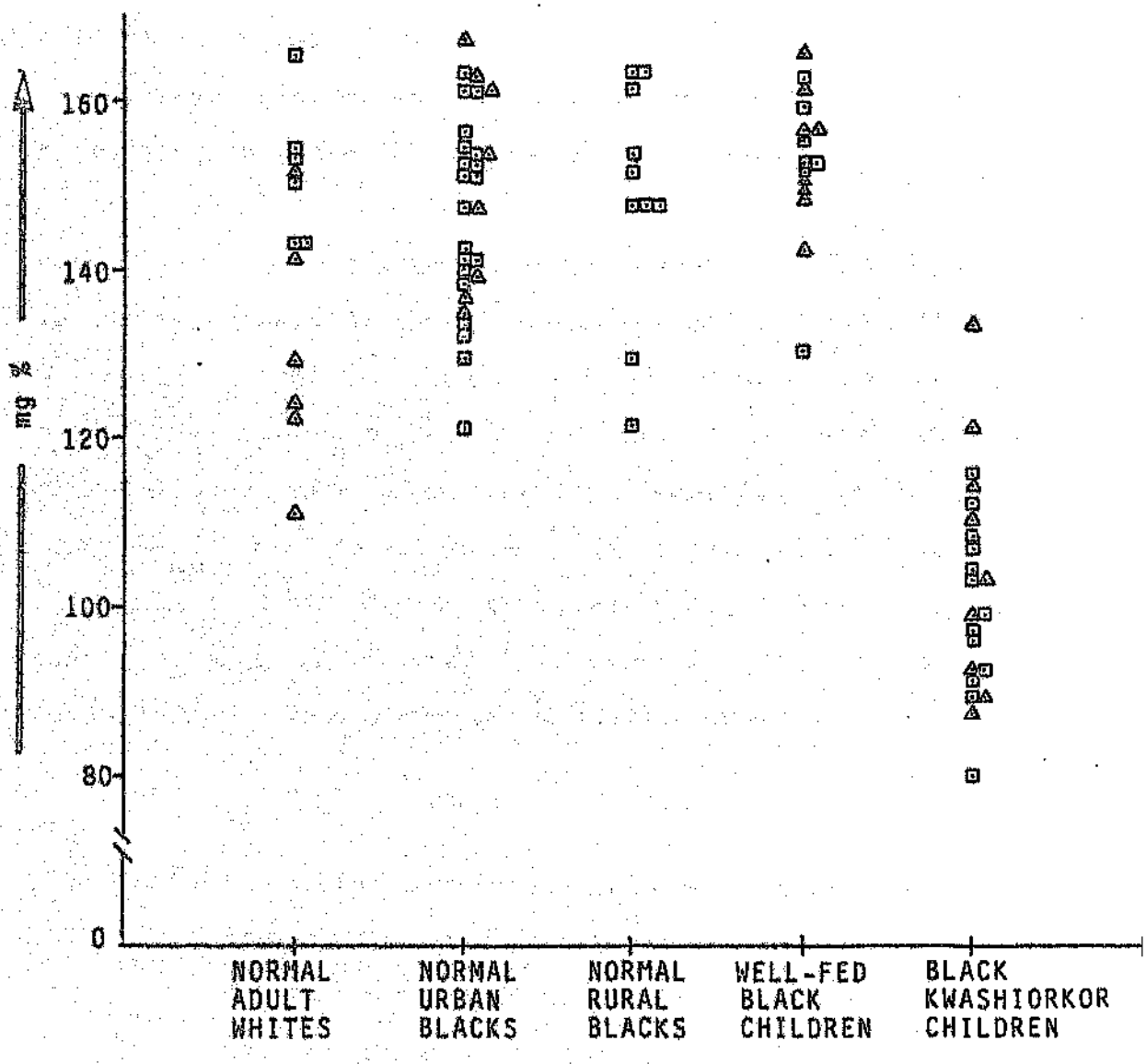
WHITES (n=12)	140.6 ±16.1
URBAN BLACKS (n=28)	147.2 ±11.5
RURAL BLACKS (n=10)	148.6 ±13.7
CONTROLS (n=15)	152.7 ±8.6
KWASHIORKOR (n=22)	102.1 ±12.6

Results are expressed as mg%

There was no significant difference between the values for the adult population groups (Appendix BB).

FIGURE 12

SERUM TOTAL PHOSPHOLIPID IN THE DIFFERENT ADULT POPULATION GROUPS, IN WELL-NOURISHED CHILDREN AND CHILDREN WITH KWASHIORKOR
(△ = FEMALES; ⊡ = MALES)



The total phospholipid was significantly decreased in the kwashiorkor group as compared with controls ($p<0.0005$). The values for the controls were similar to those obtained for the adult population group (details for kwashiorkor children and controls are given in Appendix CC). Total phospholipid was not able to be measured in the samples collected from the kwashiorkor children who had been placed on an adequate diet owing to the small volume of serum obtained.

Results for total phospholipids in the eight subjects on the three week diet diet are depicted in FIGURE 13; mean values ($\pm$SD) are listed in TABLE XV and individual results in Appendix DD. After the second week the phospholipids were significantly decreased ($p<0.01$), they continued to decrease after the third week ($p<0.01$) as compared with the first week. Two months after resuming normal diet the values for total phospholipid were similar to the initial values.

TABLE XV

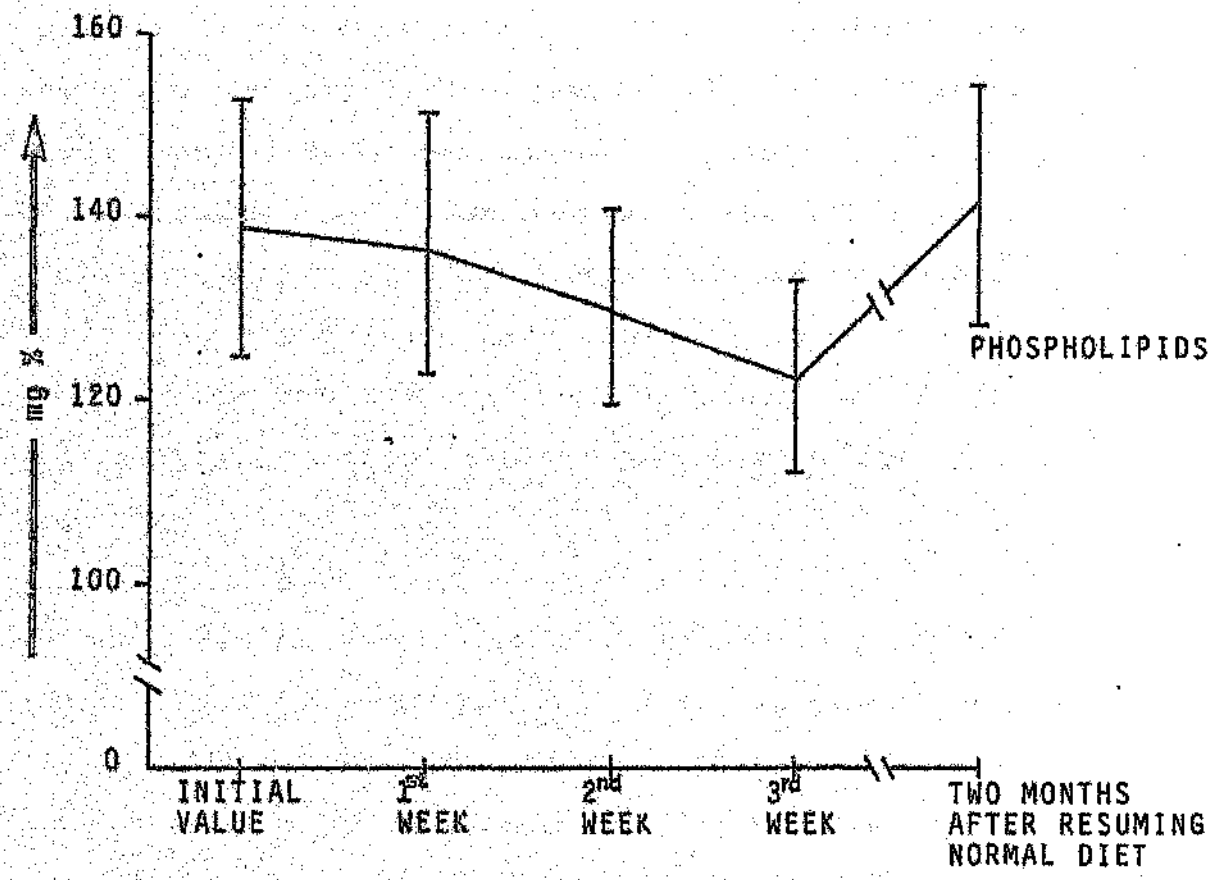
TOTAL PHOSPHOLIPID CONCENTRATIONS IN EIGHT SUBJECTS MAINTAINED ON A LOW-PROTEIN DIET FOR THREE WEEKS

PHOSPHOLIPIDS	INITIAL VALUE	1ST WEEK	2ND WEEK	3RD WEEK	TWO MONTHS AFTER RESUMING NORMAL DIET
	139.1 $\pm$14.0	136.9 $\pm$14.1	129.9 $\pm$11.3	122.8$\pm$11.0	141.4 $\pm$12.8

Results are expressed as mg%

FIGURE 13

MEAN VALUES FOR SERUM TOTAL PHOSPHOLIPID IN EIGHT SUBJECTS MAINTAINED ON A LOW-PROTEIN DIET FOR THREE WEEKS



3.5.2 LECITHIN FATTY ACID DISTRIBUTION

The mean values (±SD) for the adult population groups are shown in TABLE XVI, the individual results in Appendices EE, FF_1, FF_2 and GG. There were significant differences between the three groups. The white population group had an increased ($p<0.001$) linolenic acid (18:3;omega-6) with a concomitant decrease ($p<0.001$) in eicosapentaenoic acid (20:5) compared with the other two population groups, whereas the urban black population group showed a higher ($p<0.01$) stearic acid (18:0) compared with the other adult groups and a lower ($p<0.01$) eicosatrienoate (20:3) compared with the whites. The rural black population group showed increases ($p<0.001$) in palmitoleic (16:1) and arachidonic (20:4) acids compared with the other two groups.

In the urban black population group sex-related differences were found in palmitic (16:0) and arachadonic (20:4) fatty acids. The palmitic acid (16:0) was significantly ($p<0.001$) higher in the females whereas the arachidonic acid (20:4) was significantly ($p<0.001$) higher in the males (Appendices FF_1, FF_2).

TABLE XVI

LECITHIN FATTY ACIDS IN THE ADULT POPULATIO' GROUPS

FATTY ACIDS	16:0	16:1	18:0	18:1	18:2	18:3ω6	18:3ω8	20:3	20:4	20:5	22:5	22:6
WHITES (n=12)	32.0 ±2.03	0.21 ±0.08	15.1 ±1.39	13.7 ±1.16	25.0 ±2.76	0.11 ±0.08	0.25 ±0.13	2.78 ±0.54	6.53 ±1.00	0.29 ±0.11	1.66 ±0.71	2.22 ±0.51
URBAN BLACKS (n=28)	30.6 ±3.19	0.40 ±0.32	17.2 ±1.88	12.8 ±1.62	26.2 ±3.49	0.01 ±0.03	0.23 ±0.13	2.31 ±0.46	6.93 ±1.73	0.55 ±0.22	1.08 ±0.52	1.65 ±0.56
RURAL BLACKS (n=10)	29.5 ±2.15	0.79 ±0.33	15.5 ±0.70	11.2 ±1.86	27.6 ±3.02	nd nd	0.44 ±0.32	2.69 ±0.52	9.26 ±0.92	0.67 ±0.19	0.84 ±0.41	1.32 ±0.51

Results are expressed as %

The results for lecithin fatty acid methyl esters in the well-fed and kwashiorkor children are listed in TABLE XVII, the individual percentages in Appendices HH and II, respectively. There were significant differences between the kwashiorkor and well-fed children. The kwashiorkor children showed significant ($P<0.001$) decreases in stearic (18:0), linoleic (18:2), and eicosatrienoic (20:3) acids, with significant ($p<0.001$) increases in palmitoleic (16:1), docosatetraenoic (22:4) and docosapentaenoic (22:5;omega-6) acids. Both 22:4 and 22:5;omega-6 were not detected in the well-nourished children. The lecithin fatty acids in the well-fed children were comparable with those of the normal adult population group.

TABLE XVIII shows the means for serum lecithin fatty acids in the eight subjects on a low protein diet and the individual results are listed in Appendix JJ. After the first week the 16:0 ($p<0.01$) and 18:3;omega-6 ($p<0.001$) were significantly increased, while the 18:0 ($p<0.001$), 18:1 ($p<0.02$), 18:3;omega-3 ($p<0.001$), 20:3 ($p<0.01$), 20:5 ($p<0.01$) and 22:5 ($p<0.01$) showed a decrease. Palmitoleic acid (16:1) was not detected during the period of the diet. After the second week the 16:0 had increased ($p<0.001$) further but 18:0 ($p<0.01$) and 18:3;omega-3 ($p<0.03$) had increased as compared with the first week, with a concomitant decrease ($p<0.01$) in 22:5 and 22:6. At the end of the third week all fatty acids were significantly different from the initial values. The decreases in 18:0 ($p<0.01$), 18:1 ($p<0.01$), 18:2 ($p<0.01$), 18:3;omega-3 ($p<0.001$), 20:3 ($p<0.001$), 20:4 ($p<0.001$), 20:5 ($p<0.01$), 22:5 and 22:6 ($p<0.01$) and the increases in 16:0 ($p<0.01$) and 18:3;omega-6 ($p<0.001$) were significant as compared with the initial value. Two months after resuming their usual diet there was no apparent difference in lecithin fatty acid distribution as compared with the initial findings.

TABLE XVII

LECITHIN FATTY ACIDS IN WELL-NOURISHED CHILDREN AND CHILDREN WITH KWASHIORKOR

FATTY ACIDS	16:0	16:1	18:0	18:1	18:2	18:3ω6	18:3ω6	20:3	20:4	20:5	22:4	22:5ω6	22:5ω3	22:
KWASHIOR-KOR (n=22)	31.6 ±2.82	3.21 ±1.54	8.00 ±1.25	15.9 ±3.40	17.8 ±2.9	0.29 ±0.22	0.55 ±0.24	1.78 ±0.94	7.88 ±2.45	0.76 ±0.45	8.98 ±3.35	0.73 ±0.41	1.89 ±1.01	0 ±0.
CONTROLS (n=15)	31.0 ±2.15	0.84 ±0.47	16.0 ±0.88	11.6 ±1.93	26.3 ±1.86	0.06 ±0.04	0.27 ±0.27	3.11 ±0.77	7.04 ±1.68	0.50 ±0.14	nd nd	nd nd	1.12 ±0.	2. ±0.

Results are expressed as %

TABLE XVIII

LECITHIN FATTY ACIDS IN EIGHT SUBJECTS ON A LOW-PROTEIN DIET

FATTY ACIDS	16:0	16:1	18:0	18:1	18:2	18:3ω6	18:3ω3	20:3	20:4	20:5	22:5	22:6
Basal	20.8 ±5.13	0.39 ±0.32	18.0 ±1.54	12.9 ±1.61	27.9 ±2.62	0.19 ±0.18	0.39 ±0.09	3.46 ±0.34	10.1 ±3.44	0.79 ±0.39	1.21 ±0.51	3.68 ±1.39
1st week diet	28.7 ±2.56	nd	13.2 ±1.39	10.4 ±1.46	27.5 ±3.67	7.57 ±6.92	0.10 ±0.08	2.48 ±0.41	7.68 ±1.20	0.33 ±0.15	0.52 ±0.20	2.47 ±0.41
2nd week diet	34.3 ±3.63	nd	16.8 ±1.70	10.9 ±0.71	25.0 ±4.09	2.39 ±1.80	0.34 ±0.25	2.20 ±0.52	6.34 ±2.09	0.15 ±0.08	0.18 ±0.07	1.24 ±0.62
3rd week diet	29.4 ±2.65	nd	14.0 ±2.47	9.92 ±1.34	21.2 ±4.05	16.3 ±8.62	0.06 ±0.08	1.79 ±0.57	5.03 ±0.87	0.23 ±0.12	0.09 ±0.19	1.68 ±1.20
2 months after resuming normal diet	21.3 ±5.00	0.42 ±0.35	18.1 ±1.66	12.9 ±1.70	27.6 ±2.91	0.39 ±0.36	0.43 ±0.08	3.52 ±0.44	10.3 ±2.91	0.84 ±0.40	1.42 ±0.50	3.16 ±1.77

Results are expressed as %

CHAPTER 4

4.1 METHODS

This study is concerned with the measurements of serum carnitine, proteins and lipids. The methods used for total protein (Biuret), albumin (dye-binding) and for lipids (Folch extraction for total lipids, Bartlett procedure for total phosphorus, thin-layer chromatographic separation of phospholipids and gas-liquid chromatography for lecithin fatty acids) have all been widely used and are well validated.

As far as carnitine is concerned, the first reports of the measurement of this compound appeared in the early nineteen fifties. As knowledge of the role of carnitine in metabolism increased, it became clear that measurement of this compound could be of clinical significance. The first methods for the assay of carnitine were based on the separation of quaternary amines, followed by crystallization of the isolated carnitine by standard methods of organic chemistry. Later a method based on determining the effectiveness

of samples containing carnitine to promote normal growth of the mealworm, Tenebrio molitor, was published (Carter et al. 1952). In this rather cumbersome biological assay, the amount of carnitine in an extract was determined by comparing the growth of the worm in the presence of the extract with the growth in the presence of standard carnitine solutions. With the discovery of the carnitine acetyltransferase enzymes, a new era in the assay of carnitine began, involving spectrophotometric techniques (Williamson and Corkey 1969). These tests were relatively insensitive and subsequently a radioisotopic method was introduced by Cederblad and Lindstedt in 1972. In this method the reaction mixture containing carnitine, labelled acetyl-CoA and carnitine acetyltransferase was incubated in the presence of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) to ensure completion of the reaction, and then passed through a Dowex 2X8 column which binds labelled acetylCoA but does not bind acetylcarnitine. This method was designed to detect picomole quantities of free and esterified carnitine in small volumes of plasma. The introduction of this radioisotopic method resulted in improved accuracy and precision, providing an important impetus to carnitine investigations. Limitations in this method were, however, apparent when

nonlinear standard curves were obtained, unless a considerable excess of radioactive material was used. Modifications and improvements of this method have been published, but the basic procedure has remained the same. Bohmer and co-workers (1974) used Tris buffer and short columns adapted to fit directly over scintillation vials, but the problem of non-linear response remained. A major modification of the method of Cederblad and Lindstedt was contributed by McGarry and Foster (1976). In their procedure, tetrathionate was included as a sulphydryl reagent, the reaction terminated by adding a slurry of Dowex 1X8, followed by centrifugation to remove the ion exchange resin before quantification of the acetylcarnitine produced. This procedure overcame the problem of non-linear response. The McGarry and Foster (1976) assay yields a linear response over a wide range of carnitine concentrations. In addition, measurement of free and acyl carnitine in very small volumes of plasma without extraction or prior deproteinization is possible even in the presence of excess acylcarnitine. Further radioisotopic assays were subsequently published by Parvin and Pande (1977), Seccombe et al. (1978) and Brass and Hopel (1978), but with little significant improvement over the method of McGarry and Foster (1976).

4.2 NORMAL ADULT SUBJECTS

In the normal white adult population serum concentrations for free and total carnitine (53.7±4.5 and 68.5±4.0µmol/l, respectively) were consistent with values published for adult human plasma. These values were 51±10.5µmol/l for total carnitine (Cederblad and Lindstedt 1972) and 55.7±1.99 for free and 65.7±2.55µmol/l for total carnitine (McGarry and Foster 1976).

The values obtained for urban and rural blacks were similar to those obtained for whites. There were no significant differences between the different population groups (urban blacks: 54.6±3.6 and 69.3±3.0µmol/l; rural blacks: 54.1±3.6 and 68.4±3.5µmol/l for free and total carnitine respectively).

Serum total proteins and albumin showed no significant differences between white, urban and rural black populations. The values were within normal reference ranges.

With respect to lipoproteins, the white population group showed a significantly higher percentage of chylomicrons (0.4±0.3%) as compared with the urban black population (0.1±0.3%). This could be due to the time at which the samples had been collected. For the black population samples were collected very early in the morning whereas for the whites, samples from blood donors were collected during the afternoon. Sex differences were observed in the chylomicron concentrations in the urban black population group (females 0.4%, males not detected). The occurrence of sex differences in lipids and lipoproteins has been reported (Carlson and Hardell 1977). In the urban black population group, the males showed a higher level (19.9±4.0%) of VLDL than the females (16.6±3.8%). However, sex-related differences were not seen in the white group.

The phospholipid concentrations of the different adult population groups were within published reference ranges (125-275 mg%, Tietz 1986).

There were a few differences in the lecithin fatty acids in the different population groups. The whites had an increased (0.11±0.08%) linolenic acid

(18:3;omega-6) with a concomitant decrease (0.29±0.11%) in eicosapentaenoic acid (20:5) compared to the other two population groups (18:3;omega-6: 0.01±0.03% and not detected; 20:5: 0.55±0.22% and 0.67±0.19% for urban and rural blacks respectively). Whereas the urban black population group showed a higher (17.2±1.88%) stearic acid (18:0) compared to the other population groups (15.1±1.39% for the whites and 15.5±0.70% for the rural blacks) and a lower (2.31±0.46%) eicosatrienoic acid (20:3) compared to the whites (2.78±0.54%). The rural black population group showed increases in palmitoleic (16:1) and arachidonic (20:4) acids (0.79±0.33 and 9.26±0.92% respectively) compared with the other two groups (16:1: 0.21±0.03, 0.40±0.32% and 20:4: 6.53±1.00 6.93±1.73% for whites and urban blacks respectively). Differences in the ecithin fatty acids, especially the essential fatty acids, seen in the adult population groups in our study, could be attributed to the different dietary habits as has been suggested by Holman et al. (1979). These workers showed that differences in the fatty acid composition of the total phospholipids could also be associated with sex and age.

The major phospholipid in serum is lecithin (phosphatidylcholine), which comprises approximately 70% of all serum phospholipids. There have been relatively few studies on lecithin fatty acids in different population groups for comparison with the results in this study. Each phospholipid molecule contains two fatty acid molecules, the chain length of which can vary between 12 and 22 and many can be unsaturated containing between 1 and 6 double bonds. Thus large numbers of isomers of each phospholipid can exist and many of these can be incorporated into membranes. The significance of the different types of fatty acids which are found in membranes as components of phospholipids is not clear (Wills 1985). It can be postulated that the exact composition is important as the fluidity of membranes is dependent on the degree of unsaturation of the fatty acids forming the membrane. Changes in the serum lecithin fatty acid composition could point to alterations in membrane phospholipid fatty acid composition and may affect membrane fluidity and hence membrane function.

4.3 NORMAL ADULT SUBJECTS ON A LOW-PROTEIN DIET

The present study clearly showed that a change to a low protein diet, deficient mainly in meat, affected plasma carnitine levels. The values for total carnitine decreased after a period of three weeks from 65.6±10.0 to 43.7±7.4μmol/l. It would appear that there was an inadequate dietary supply of amino acid precursors, lysine and methionine. The daily requirements of the human body for carnitine are unknown, and little effort has been made to approximate even usual dietary intake (Lennon et al. 1986). However, it has been reported that meat and dairy products are the major sources of dietary carnitine, with the highest concentration found in red meat (Broquist and Borum 1982). Vegetables contain a negligible amount of carnitine, and thus vegetarians have only endogenous carnitine (Slonin et al. 1981). The lack of information on carnitine consumption may be due to the dismissal of dietary intake as playing a significant role in overall carnitine metabolism, since it can be synthesized endogenously, from lysine and methionine (Bremer 1961; Tanphaichitr and Broquist 1973). However dietary carnitine should not be excluded as playing an important role in meeting the body's nutrient needs (Lennon et al. 1986). An

analogy which may be drawn is the role played by dietary cholesterol in overall body cholesterol metabolism (Mitchell 1978). The amount of dietary cholesterol consumed and absorbed may change the amount of cholesterol synthesized by the liver. It is therefore also possible that the amount of dietary carnitine absorbed may modulate the amount of hepatic carnitine synthesized (Lennon et al. 1986).

Consistent with the low protein intake were the reduced values for serum total protein (from 66.4±2.2 to 53.0±2.7g/l) and albumin (from 40.4±4.2 to 30.4±2.3g/l) after three weeks. A change to a low protein diet also showed alterations in the concentrations of the protein and lipoprotein fractions in young adults. An expected decrease was seen in the albumin fraction (from 39.0±3.6 to 27.4±2.2g/l) throughout the diet. The $alpha_1$, $alpha_2$ and gamma globulins had decreased after the first week of diet (from 2.3±0.5 to 0.9±0.3, 7.2±1.0 to 5.0±0.7 and 9.6±2.2 to 7.9±1.4g/l respectively), and with the exception of the albumin fraction the other components returned to basal levels by the third week. The decrease in the $alpha_1$ globulin, mentioned above, appears to fit in with the decrease in HDL (from 26.9±4.2 to 8.0±3.5%), which was

seen after a week on the diet, as the HDL apoprotein runs in the alpha$_1$ globulin region. This initial decrease could be due to reduced availability of cholesterol and also reduction of proteins; the main function of the HDL is to transport important proteins, such as apoprotein C-II which regulates the activity of the enzymes involved in lipoprotein metabolism (Wills 1985). An interesting finding was a marked increase in VLDL (from 15.7±6.1 to 34.3±3.1%) after the first week. The initial increase in VLDL could be due to the low protein intake leading to an increased mobilization of lipid from adipose tissue and subsequent increased release of VLDL from the liver (Tietz 1986). After the second week on the diet the VLDL showed a decrease (from 34.3±3.1 to 24.6±3.1%), while HDL increased (from 6.0±3.5 to 19.1±3.6%), and by the third week the homeostatic mechanisms came into play and both had returned to basal levels.

Total phospholipid decreased over the period of the diet (from 139.1±14.6 to 122.6±11.0mg%). The reason is not clear but it could be due to the low meat intake and hence decreased fat intake.

Changes in the lecithin fatty acids in young adults were seen. In particular those fatty acids which are indicative of animal product consumption eg. palmitoleic (16:1), eicosapentaenoic (20:5) and docosahexaenoic (22:6) acids were decreased (from 0.39±0.32% to not detected, from 1.21±0.51 to 0.09±0.19%, from 3.68±1.39 to 1.68±1.20% respectively). A marked increase in the 18:3;omega-6 (gamma linolenic acid) was seen after the third week of diet (from 0.19±0.18 to 16.3±8.62%). This is an essential fatty acid and is mostly of plant origin. Melchert et al. (1987) in their study on vegetarians (these subjects had been vegetarians for a minimum of five years) reported increases in the linoleic (18:2) and alpha linolenic (18:3;omega-3) acids but no increase in gamma linolenic (18:3;omega-6) acid. It would appear that there are no reports of the effect of a short term low-protein diet on lecithin fatty acids for comparison. It is of interest to note that lecithin fatty acid distribution had returned to the basal level after two months. It would be of value to follow the lecithin fatty acids in normal adults on a low-protein diet for a longer period and to assess the distribution on discontinuation of the diet at shorter time intervals.

4.4 CHILDREN WITH KWASHIORKOR

The findings reported here showed that both free and acyl carnitine were decreased in black South African children with kwashiorkor (30.3±5.6µmol/l for free and 8.7±1.8µmol/l for acylcarnitine) as compared with well-nourished black children (51.7±1.3µmol/l for free and 14.1±1.4µmol/l for acylcarnitine). This is consistent with the observations of Hammond and co-workers (1987) who reported 32.6±6.2µmol/l for free and 8.1±2.2µmol/l for acylcarnitine for South African children admitted to hospital with kwashiorkor. In a study carried out in India, Kahn and Bamji (1977) reported that total carnitine levels were lower in a group of children suffering from kwashiorkor (21±5µmol/l) than in healthy controls (90±6µmol/l). After treatment of these malnourished children for four weeks the values for total carnitine increased to 42±6µmol/l (Kahn and Bamji 1977).

In this study although the number of follow-up samples obtained was relatively small there was an increase in both free and acyl carnitine after two weeks of treatment on an adequate diet. After one week there had been no significant change in the free and acyl car-

nitine, but the three subjects who had been placed on an adequate diet for two weeks showed increases in free and acyl carnitine(from 30.5±5.8 to 56.3±3.8µmol/l and 8.7±1.8 to 11.5±0.3µmol/l respectively). The number of samples in the present study is too small to reach any definite conclusion, but it does appear that at least two weeks of treatment is required for the free carnitine to return to normal. Altmann in 1948 suggested that treatment of the order of four to five weeks may be required for the carnitine to return to normal. It is possible that a certain time period may be required to allow synthesis of carnitine in the liver prior to its transfer to peripheral tissues.

In the children with kwashiorkor subsisting mainly on maize, the low concentrations of carnitine may be associated with a reduced intake of carnitine itself, together with deficiency of precursor amino acids methionine and lysine, giving rise to inadequate endogenous synthesis. Newborns and infants are incapable of synthesizing the necessary amount of carnitine and without an adequate supply from food, symptoms of deficiency can occur (Sionim et al. 1981). Children suffering from kwashiorkor are found deficient in a wide variety of other nutrients such as iron and ascor-

bic acid (Lindstedt and Lindstedt 1970). Probably as a result of low protein intake, the activities of many enzymes involved in the biosynthesis of carnitine are low (Kahn and Bamji 77). As carnitine plays an essential role in fatty acid oxidation (Bremer 1983), a deficiency could be associated with the development of a fatty liver frequently seen in children with kwashiorkor; a common feature seems to be accumulation of triacylglycerols in the cell (Bremer 1983). It is possible that administration of carnitine may be helpful in correcting this defect, as previously mentioned by Hammond et al. (1987). However, one must bear in mind that the low concentrations of serum carnitine may not necessarily be indicative of reduced tissue concentrations, but could result from enhanced tissue uptake. A possible mechanism whereby this could occur would be increased active transport into carnitine dependent tissues perhaps as a result of induction of transport proteins in response to altered serum levels of certain hormones as suggested by Hammond et al. (1987). It would be of interest to measure tissue levels, but unfortunately this is not possible as biopsies are rarely, if ever, carried out on these patients.

The assay for serum carnitine is a valuable one. Many disease states associated with low carnitine levels, such as organic acidurias, may benefit from administration of oral doses of carnitine (Borum 1983). It is possible that carnitine therapy may alleviate some of the lipid metabolism disorders seen in numerous disease conditions, such as dyslipidaemias and hyperlipidaemias (Bremer 1983). It was reported by Bohmer et al. (1978) and Battistella et al. (1978) that plasma and muscle carnitine levels fall below normal in uraemic patients on maintenance haemodialysis. The fact that hypocarnitinaemia may play an important role in the pathogenesis of dyslipidaemia in uraemic patients on maintenance haemodialysis was suggested by the improvement seen as a result of carnitine treatment of these patients (Bougneres et al. 1979). Bagdade and co-workers (1979) reported that approximately one quarter of patients on dialysis displayed a type IV hyperlipidaemia. It was subsequently shown this could be reversed by administration of carnitine (Bertoli et al. 1981).

Total protein and albumin were found to be decreased (44.8±6.9 and 24.3±3.8g/l) in the kwashiorkor group as compared with controls (69.6±2.7 and 44.2±3.7g/l respectively). The results in kwashiorkor patients for whom follow up studies were possible showed that treatment increased the total protein and albumin concentrations, although the values for total protein still remained lower (63.9±2.3g/l) (after two weeks of treatment) than in the controls (69.6±2.7g/l). This was possibly due to the very diminished amounts of protein present on admission and it would seem that a longer period of treatment was needed. In a study carried out in 13 kwashiorkor children and 9 children with nutritional oedema by Anderson and Altmann (1951) at the Baragwanath Hospital in Johannesburg, it was found that a normal total serum protein concentration was reached only after three to four weeks on a diet with a high protein content, by which time clinical recovery also occurred. The levels of serum proteins in our kwashiorkor patients confirmed the expected derangement of normal protein profiles. As early as the 1940's Trowell (1944), Gillman and Gillman (1945) and Altmann (1948) reported diminished amounts of albumin and total protein and suggested this could account for the obvious symptoms of kwashiorkor such as

oedema and hepatomegaly. The oedema of kwashiorkor may also occur as a result of alteration of renal function (Davies 1956), changes in the secretion and metabolism of the adrenal glands (Sinclair 1948) and changes in the production and inactivation of posterior pituitary antidiuretic hormone (Gopalan and Waterlow 1955).

The serum protein fractions in the kwashiorkor children, in our study, showed decreases in the albumin fraction (19.2-2.9g/l) and in the beta globulin fraction (3.9±1.1g/l), with an increase in the $alpha_1$ globulin (3.3±1.2g/l) as compared with the well-nourished children. No significant change from the controls was seen in the gamma globulin fraction. There appears to be lack of consistency in publications dealing with changes in the individual protein fractions in kwashiorkor, especially with regard to the $alpha_2$, beta and gamma globulin fractions. However, a decrease in the albumin fraction in kwashiorkor was seen throughout (Anderson and Altmann 1951; Schrimshaw et al. 1955; Politzer and Wayburne 1957; Edozien 1960). Scrimshaw et al. (1955) using a technique of free electrophoresis in an Antweiler micro-electrophoresis apparatus, found that the outstanding features of the

protein pattern in malnourished children were the low absolute and relative amounts of albumin, compared with the normal values and the high absolute and relative amounts of gamma globulin. This pattern was also reported by Politzer and Wayburne (1957). However, in a later study by Edozien (1960) in 146 kwashiorkor children, using paper electrophoresis, the alpha$_1$, alpha$_2$, and beta globulins were decreased, with no significant variation from the controls in the gamma globulins. In 1960 Edozien reported that in children with protein energy malnutrition the albumin and beta globulin fractions were found to be affected relatively more than the other protein fractions; the results of the present study are relatively consistent with these findings. However, a raised alpha$_1$ globulin was found in contrast to the decrease reported by Edozien (1960). A relationship between low values for beta globulins and the presence of fatty liver in malnourished children was observed by Flores et al. (1970). These workers found that in children with fatty livers (kwashiorkor), the beta globulins were lower than in children without fatty livers (marasmus and normal children). The individual serum protein fractions differ in amino acid composition. Albumin and beta globulins contain more sulphur and sulphur-containing

amino acids than do alpha$_2$ and gamma globulins (Edsall 1974). In kwashiorkor there is qualitative as well as quantitative deficiency of amino acids (Edozien 1960). The sulphur containing amino acids, which are mainly supplied by the animal proteins in the diet, may be very deficient in kwashiorkor patients since the intake of animal proteins such as milk and meat is usually very limited. The pattern of amino acid deficiency in kwashiorkor may, therefore, play an important role in determining the serum protein spectrum; a deficiency of sulphur containing amino acids may be one of the limiting factors in the synthesis of albumin and beta globulin (Edozien 1960). Among their many functions, plasma proteins transport inorganic substances. In kwashiorkor, it has been shown that there is a failure in the total iron binding capacity of the serum (Lahey et al. 1958) which is related to the decrease in beta globulin concentration.

The lack of significant correlation between serum carnitine levels and albumin in serum from kwashiorkor children was consistent with the observations of Hammond and co-workers (1987), although Khan and Bamji (1977) showed otherwise. It is possible that the mechanisms controlling serum carnitine and albumin

levels are deranged to different extents in kwashiorkor and markedly depressed albumin levels need not necessarily be accompanied by markedly reduced serum carnitine values (Hammond et al. 1987).

In this study, the levels for serum total phospholipid (102.1±12.6mg%) in the kwashiorkor children were decreased compared to the controls (152.7±8.6mg%). These values were consistent with the findings of Schwartz and Dean (1957). In South African Black children they reported 145mg% for children with kwashiorkor and 200mg% for well nourished children. The results of serum total phospholipid in the controls (152.7±8.6mg%) were comparable with those of the adult population group in this study (Whites: 140.8±16.1; Urban blacks:147.2±11.5; Rural blacks:148.6±13.7mg%). A diet leading to kwashiorkor is known to be rich in carbohydrate, low in fat and poor in protein (Hafiez et al. 1970). Such a diet was reported to produce low levels of blood lipids (Cravioto et al. 1959; Schwartz and Dean 1957). As previously mentioned, phospholipids form part of cell membranes and their shortage may be one of the causes of the decreased cell membrane production observed in protein energy malnutrition (Teichberg 1982). This hypolipidaemia together with

the intense accumulation of fat in the liver and abundance of fat in the depots suggests the presence of a defect in lipid metabolism affecting its transport from depots and liver to the blood stream (Hafiez et al. 1970). Abnormalities of lipid metabolism in kwashiorkor are well documented (Coward 1971). In children with kwashiorkor frequent findings are fatty infiltration of the liver and a reduced metabolism of serum lipids (Schwartz and Dean 1957; Devi et al. 1976). The mechanism of lipid changes in the liver in protein malnutrition is not yet clearly understood. It has been hypothesized that fat accumulates in the liver probably due to a reduction in the proteins responsible for the transport of triacylglycerols from the liver to fat depots (Truswell et al. 1969; Flores et al. 1976). Among other possibilities, a decrease in the oxidation of fatty acids in the liver may also contribute to the lipid accumulation (Fritz 1963). In view of the role of carnitine in fatty acid oxidation, lipid accumulation may, in addition, result from carnitine deficiency (Khan and Bamji 1977; Hammond et al. 1987).

In this study the main alterations in the lecithin fatty acid composition in children with kwashiorkor compared with well-nourished children were increases in

the percentages of palmitoleic (16:1), oleic (18:1), docosatetraenoic (22:4) and docosapentaenoic (22:5;omega-6) acids, with decreases in stearic (18:0), linoleic (18:2) and eicosatrienoic (20:3) acids. The increases were from 0.84±0.47 to 3.21±1.54% (16:1), 11.6±1.93 to 15.9±3.40% (18:1), not detected to 8.98±3.35% (22:4) and not detected to 0.73±0.41% (22:5;omega-6) and the decreases were from 16.0±0.88 to 8.00±1.25% (18:0), 26.3±1.86 to 17.8±2.98% (18:2) and 3.11±0.77 to 1.78±0.94% (20:3).

It has been reported that fat formed from carbohydrate may give rise to an increase in palmitic and palmitoleic acids and decrease linoleic acid percentages in the total fatty acids (Hirsh et al. 1960; MacDonald et al. 1963). As lecithin accounts for 60-70% of the total phospholipid this could explain the changes in these fatty acids in the children with kwashiorkor. The decrease in essential fatty acid intake would be expected to enhance the linoleic acid decrease. This is borne out by the marked decrease in linoleic acid found in these children. Possible consequences of the decrease in the essential fatty acids in kwashiorkor are not fully understood. As essential fatty acids are precursors of prostaglandins a deficiency may interfere

with a number of regulatory processes (Koletzk et al. 1986). One of the most common clinical symptoms in protein energy malnutrition (PEM), the dry scaly dermatitis with a tendency to superinfection and increased water permeability, resembles the same lesion occurring in other forms of essential fatty acid deficiency (Holman 1971; Nichols et al. 1974). In 1986 Koletzk et al. reported the fatty acid composition of plasma phospholipids in 17 Nigerian children with PEM of whom 9 had kwashiorkor. The total phospholipid fatty acid results reported by these workers do not show a decrease in linoleic acid in PEM compared with well-nourished controls, but do show a decrease in arachidonic acid. In our study the lecithin fatty acid results show a decrease in linoleic acid but no decrease in arachidonic acid in kwashiorkor compared with well-nourished children. A literature search has not produced any publications on lecithin fatty acid composition in children with kwashiorkor for comparison with the results in this study. It is known that the fatty acid distribution in the different lipid classes alters with disease (Sanders et al. 1978). Insufficient work has been done on the phospholipid fatty

acids in PEM. It is possible that marasmus and kwashiorkor could have a different fatty acid distribution.

Holman and co-workers (1979) reported that docosatetraenoic acid (22:4) was present in children aged 0 - 4 months at a concentration of approximately 4%. This fatty acid was also reported to be present in the adult population group (males ±2.0%; females ±2.3%) Holman et al. (1979). Under the conditions of our assay no 22:4 was found in the adult population group and in the well-nourished children, but in the children with kwashiorkor this fatty acid was present in relatively large amounts (9%). To our knowledge it is the first time that this has been reported. Docosatetraenoic acid forms part of the elongation and desaturation chain of the fatty acids. Apart from this, the function of this acid is not known. Changes in the intake of dietary polyunsaturated fatty acids can be expected to modify physiological functions by changing membrane structure, modifying membrane fluidity, and modulating signal transduction (Johnston 1985). Kwashiorkor children have increased red cell fragility (Coward 1971), which suggests that membrane fluidity is altered, which in turn suggests that the

phospholipid fatty acid composition is altered. In this study, kwashiorkor children showed a decrease in the concentrations of total phospholipid and the lecithin fatty acid distribution was altered.

4.6 CONCLUSION

The fine homeostatic balance between the different biochemical parameters studied was shown to be closely dependent on the nature of the diet. The present study clearly showed that a change to a low-protein diet affected the plasma carnitine, proteins and lipids. For proteins and lipids, changes were seen after the first week on the diet and for carnitine, changes were seen after the third week. This shows how the normal biochemical status changes and how dependent we are on diet.

Carnitine is a scientifically exciting molecule and its biochemistry is still being unravelled by a number of research groups. Its role in the metabolism of long chain fatty acids is now well established and its usefulness in the treatment of a range of deficiency syndromes is well accepted.

The results obtained for serum carnitine suggest that there may be impaired oxidation of lipids in kwashiorkor. The deficiency of carnitine may provide an explanation for the accumulation of fats in the livers of these patients. Measurements of tissue carnitine concentrations could prove informative, but liver biopsies would not be able to be performed in these children. Further studies of carnitine in relation to progression of the disease need to be carried out on a larger number of kwashiorkor children and during a longer period of treatment in hospital. In addition, the value or otherwise of the administration of carnitine needs to be established.

As expected, in kwashiorkor the lipids were deranged, of particular significance was the increased percentage of docosatetraenoic acid (22:4), in the lecithin fatty acid pattern seen in these children. It appears to be the first time this has been reported. As the exact composition of the phospholipids is important in membrane fluidity and flexibility, it would be of interest to study red cell membrane fluidity in these children and to determine the red cell membrane phospholipid fatty acid composition.

APPENDIX A

LOW-PROTEIN DIET

A typical day's menu followed this simple regime (no red meat allowed).

Breakfast
Choose from
- muesli made from cereal flakes, nuts, seeds, dried or fresh fruit;
- dried fruit compote or fresh fruit with yoghurt followed by wholemeal toast;
- porridge;
- sandwiches;
- corn flakes

Lunch or light evening meal
Choose from
- soup (vegetable), as much bread as one likes;
- salad or mixture of different salads with roll or wholewheat or spbread;
- sandwiches (cheese and tomato, egg);
- mealies/mealiepap/sweet corn (as much as one likes);
- or many vegetables as one likes;
- fresh fruit, yoghurt;
- pizza

Main meal
Choose from
- *chicken/fish (small portion) and vegetables, potatoes, mealies/mealiepap;
- fruit, milk;
- pizza/pasta

Fruit juices allowed. Alcoholic drinks allowed in moderation.
*Chicken allowed twice a week. Fish allowed twice a week.

Appendix B

DETAILS OF PATIENTS DIAGNOSED WITH KWASHIORKOR

	AGE	SEX	ETHNIC GROUP	HOSPITALS	OTHER ILLNESSES	1ST WEEK	2ND WEEK
1	11m	M	Venda	Elim	Gastro-enteritis	x	x
2	±1y	M	Shangaan	Malamulele	Gastro-enteritis		
3	1y	M	Sotho	Baragwanath	Gastro-enteritis		
4	1y	M	Zulu	Baragwanath	Marasmic-Kwashiorkor		
5	1y2m	M	Zulu	Baragwanath	Measles		
6	1y4m	M	Shangaan	Elim	Gastro-enteritis		
7	1y5m	M	Shangaan	Malamulele	-		
8	1y9m	M	Xhosa	Baragwanath	-	x	x
9	2y	M	Shangaan	Malamulele	Diarrhoea	x	x
10	2y	M	Venda	Elim	Diarrhoea	x	
11	2y	M	Shangaan	Elim	Gastro-enteritis	x	
12	2y3m	M	Shangaan	Elim	Bronchitis, pneumonia		
13	3m	M	Ndebele	Tshilidzini	Gastro-enteritis		
14	10m	F	Venda	Elim	-		
15	1y	F	Sotho	Elim	Diarrhoea		
16	1y	F	Tswana	Baragwanath	Measles		
17	1y4m	F	Sotho	Baragwanath	Gastro-enteritis		
18	1y6m	F	Shangaan	Malamulele	Gastro-enteritis		
19	±2y	F	Shangaan	Elim	Gastro-enteritis	x	
20	±2y	F	Venda	Elim	Pneumonia (slight), diarrhoea		
21	2y	F	Sotho	Elim	-		
22	4y	F	Zulu	Baragwanath	Retarded, gastro-enteritis		

Appendix C

ROUTINE TREATMENT OF KWASHIORKOR IN SOME RURAL HOSPITALS OF THE NORTHERN TRANSVAAL, AND AT BARAGWANATH HOSPITAL

A. Medicines

1. Ferro-drops: 0,6ml daily x 14 days

2. Potassium chloride: 10ml daily x 7 days

3. Aprovit orally - 200 000 U on admission
100 000 U the next day
100 000 U on discharge

B. Nutrition (slight variations from hospital to hospital)

Baragwanath Hospital
Start: S26 formula (humanized milk, low protein 1.2 g/l); progress to Lactogen (high in protein); later infant diet supplemented with vitamin A, K etc.

Hospitals of Northern Transvaal (at least seven feeds daily)

1. Mild cases (no diarrhoea)
Milk or F mixture: 150ml/kg/day
Fresh fruit juice: 100ml/day
Add solids as soon as child can eat them and reduce milk accordingly.

2. Severe cases (+ diarrhoea)
Day of admission: oral rehydration 150ml/kg
Day one: as for mild cases

NB: If the child refuses all feeds put up a drip of half Darrow solution (150ml/kg/day).

C. Education of infant's mother (usually occurs in rural hospitals)

D. Mantoux - skin test vs TB, Tuberculin test

Appendix D

PREPARATION OF SUPPLIED REAGENTS FOR SPE.

* <u>B-2 Barbital buffer, pH 8.6</u>
  * 1 500ml distilled water
  Each compartment (2) of Paragon Electrophoresis Cell was filled with 45ml B-2 Barbital Buffer .
  90ml of B-2 Barbital Buffer in cell is critical.

* <u>Acid-Alcohol solution (I,II)</u>
  1 000ml distilled water
  * 600ml methanol
  * 150ml glacial acetic acid

* <u>Paragon Blue stain (0.5%)</u>
  * 1 000ml of 5% Acetic acid solution

* <u>Serum Dilution</u>
  1 Part Serum + 4 parts Buffer

Containers of Paragon Wet processor filled with the following solutions in sequence stated below:

#1 - Acid-Alcohol solution I	300ml
#2 - Paragon Blue stain solution	300ml
#3 - Acetic Acid solution, 5% I	300ml
#4 - Acid Alcohol solution II	300ml
#5 - Acetic Acid solution, 5% II	300ml

NOTE

Each of these solutions may be used to process a total of 4 gels. The containers should then be emptied and refilled with fresh solutions.

Appendix E

PREPARATION OF SUPPLIED REAGENTS FOR LPE.

* B - 2 Buffer, pH 8.6
  + 1500ml distilled water
  90ml of Buffer in electrophoresis cell critical
  45ml of Buffer use poured into each reservoir of cell.

* Fixative solution
  450ml distilled water + 450ml Reagent Grade Ethanol
  + 100ml Glacial Acetic Acid

* Lipo Stain working solution
  210ml Reagent Grade Ethanol + 1ml stain stock solution*
  + 90ml distilled water.
  Stand 30 minutes before use.

*Stain stock solution -
Lipo stain solvent poured into lipo stain bottle. Mixed for 2 to 5 minutes. Stock stain aged 2 to 3 days. This improves stain intensity.

* Destaining solution (I,II)
  600ml Reagent Grade Ethanol + 400ml distilled water.

4 Containers of Paragon wet processor filled with the following solutions in sequence listed below:

#1 - fixative solution	300ml
#2 - Lipo stain working solution	300ml
#3 - Destaining solution I	300ml
#4 - Destaining solution II	300ml

#1, #3, #4 are reusable for four gels; #2 must be prepared daily

Appendix F

SERUM CARNITINE CONCENTRATIONS IN THE WHITE ADULT POPULATION GROUP

	CARNITINE (μmol/l)		
SAMPLE NO	FREE	TOTAL	ACYL
1	50.0	69.3	19.3
2	49.0	68.9	19.9
3	60.0	69.3	9.3
4	53.6	72.0	18.4
5	52.0	65.6	13.6
6	62.0	77.3	15.3
Mean	54.4	70.4	16.0
SD	5.4	3.9	4.1
7	53.2	68.8	15.6
8	50.0	61.3	11.3
9	50.0	69.3	19.3
10	52.0	64.0	12.0
11	53.0	66.7	13.7
12	60.0	69.3	9.3
Mean	53.0	66.6	13.5
SD	3.7	3.3	3.5
Overall			
Mean	53.7	68.5	14.8
SD	4.5	4.0	3.9

SAMPLES 1-6: MALES
SAMPLES 7-12: FEMALES

Appendix G

SERUM CARNITINE CONCENTRATIONS IN BLACK URBAN ADULT POPULATION GROUP

	CARNITINE (μmol/l)		
SAMPLE NO	FREE	TOTAL	ACYL
1	51.2	64.8	13.0
2	53.2	63.8	14.8
3	52.8	66.5	13.7
4	54.4	69.7	15.3
5	52.6	66.5	13.9
6	53.0	67.0	14.0
7	52.0	65.4	13.4
8	52.0	65.4	13.4
9	56.2	73.8	17.6
10	50.2	66.2	16.0
11	56.0	70.2	14.2
12	60.2	76.2	14.0
13	48.6	66.7	18.1
14	50.2	66.9	16.7
15	50.6	68.0	17.4
16	52.2	69.7	17.5
17	54.2	67.2	13.0
18	52.0	66.5	14.5
19	53.0	69.6	16.6
20	56.0	71.2	15.2
Mean	53.0	68.3	15.2
SD	2.6	2.9	1.6
21	62.0	74.7	12.7
22	56.0	69.3	13.3
23	56.4	70.2	13.8
24	60.0	73.4	13.4
25	58.0	73.2	15.2
26	62.0	72.8	10.8
27	58.2	69.4	11.2
28	56.4	68.4	12.0
Mean	58.6	71.4	12.8
SD	2.5	2.4	1.4
Overall Mean	54.6	69.3	14.5
SD	3.6	3.0	1.9

SAMPLES 1-20: MALES
SAMPLES 21-28: FEMALES

Appendix H

SERUM CARNITINE CONCENTRATIONS IN RURAL BLACKS

SAMPLE NO	CARNITINE (µmol/l) FREE	TOTAL	ACYL
1	50.4	65.0	14.6
2	54.4	66.2	11.8
3	56.2	70.4	14.2
4	50.2	68.9	16.7
5	58.4	72.0	13.6
6	62.0	76.2	14.2
7	52.6	67.0	14.4
8	53.0	68.0	15.0
9	51.2	63.6	12.4
10	52.8	67.0	14.2
Mean	54.1	68.4	14.1
SD	3.8	3.5	1.3

ALL MALES

Appendix I

SERUM CARNITINE CONCENTRATIONS IN WELL-NOURISHED CHILDREN

SAMPLE NO	CARNITINE (μmol/l) FREE	TOTAL	ACYL
1	51.6	64.0	12.4
2	51.6	[illegible].6	16.0
3	50.0	[illegible].8	13.8
4	52.8	65.4	12.6
5	51.6	62.8	11.2
6	53.2	68.1	14.9
7	53.6	67.0	13.4
8	53.2	67.6	14.4
Mean	52.2	65.8	13.6
SD	1.2	2.1	1.5
9	50.8	66.5	15.7
10	50.4	66.5	16.1
11	50.0	64.4	14.4
12	53.6	68.1	14.5
13	52.0	67.0	15.0
14	50.4	63.8	13.4
15	51.2	65.0	13.8
Mean	51.2	65.9	14.7
SD	1.2	1.5	1.0
Overall			
Mean	51.7	65.8	14.1
SD	1.3	1.8	1.4

SAMPLES 1-8: MALES
SAMPLES 9-15: FEMALES

Appendix J

SERUM CARNITINE CONCENTRATIONS IN CHILDREN WITH KWASHIORKOR ON ADMISSION TO HOSPITAL AND DURING TREATMENT.

	CARNITINE (μmol/l)								
	ON ADMISSION			TREATMENT 1ST WEEK			2ND WEEK		
SAMPLE NO	FREE	TOTAL	ACYL	FREE	TOTAL	ACYL	FREE	TOTAL	ACYL
1	34.0	43.1	9.1	38.0	51.1	13.1	52.0	63.8	11.8
3	36.0	45.2	9.2						
4	36.0	33.3	7.3						
5	25.0	32.5	7.5						
6	25.0	33.0	8.6						
7	38.0	47.9	9.9						
8	37.6	45.3	7.7	38.0	50.9	12.9	59.0	70.2	11.2
9	32.0	38.8	6.8	37.6	50.5	12.6	58.0	69.2	11.6
10	18.0	25.6	7.6	19.6	26.7	7.1			
11	24.0	34.1	10.1	25.6	34.6	9.2			
12	32.0	43.0	11.0						
13	35.2	45.2	10.0						
Mean	30.1	38.7	8.7						
SD	6.2	6.8	1.3						
14	38.4	45.3	6.9						
15	25.0	31.9	6.9						
16	38.0	47.9	9.9						
17	24.0	30.6	6.6						
18	34.0	41.6	7.6						
19	30.4	42.6	12.2	32.0	40.0	8.0			
20	32.0	43.6	11.6						
21	30.0	40.4	10.4						
22	25.0	30.9	5.9						
Mean	30.6	39.4	8.7						
SD	5.6	6.6	2.4						
Overall Mean	30.3	39.0	8.7	31.7	42.3	10.5	56.3	67.7	11.5
SD	5.8	6.5	1.8	7.8	10.3	2.7	3.8	3.4	0.3

SAMPLES 1-13: MALES
SAMPLES 14-22: FEMALES

Appendix K

VALUES FOR SERUM CARNITINE CONCENTRATIONS IN EIGHT SUBJECTS (A-H) MAINTAINED ON A LOW-PROTEIN DIET FOR THREE WEEKS.

	CARNITINE (μmol/l)														
	INITIAL			1ST WEEK			2ND WEEK			3RD WEEK			2 MONTHS AFTER RESUMING NORMAL DIET		
	FREE	TOTAL	ACYL	FREE	TOTAL	ACYL	FREE	TOTAL	ACYL	FREE	TOTAL	ACYL	FREE	TOTAL	ACYL
A	64.0	77.3	13.3	62.0	72.0	10.0	48.0	59.7	11.7	37.6	51.2	13.6	64.4	77.3	12.9
B	58.0	74.7	16.0	52.0	66.1	14.1	46.8	56.0	9.2	38.8	50.7	11.9	63.2	76.3	13.1
C	50.0	67.9	18.0	48.0	62.4	14.0	47.0	60.0	13.3	32.4	46.4	14.0	56.0	76.3	20.3
D	38.4	51.5	13.1	38.2	51.2	13.0	36.0	45.9	9.9	33.2	45.3	10.1	48.0	63.5	15.5
E	39.6	52.3	12.1	39.2	52.3	13.1	37.2	46.9	9.7	28.0	39.5	11.6	47.6	62.9	15.3
F	44.0	62.9	18.9	42.0	60.0	17.6	38.0	50.0	12.0	22.0	33.1	11.1	46.0	63.5	17.5
G	60.0	74.7	14.7	59.6	77.3	17.7	40.0	49.1	9.1	38.0	50.0	10.0	58.0	69.3	11.3
H	50.0	63.5	13.5	48.0	61.3	13.3	38.0	48.0	10.7	24.6	33.6	9.0	50.0	62.9	12.9
Mean	50.5	65.6	15.0	48.6	62.8	14.1	41.4	51.9	10.6	32.1	43.7	11.4	54.2	69.0	14.9
SD	9.5	10.0	2.4	8.9	9.0	2.5	5.0	5.6	1.3	6.5	7.4	1.7	7.3	6.7	2.9

Appendix L

SERUM TOTAL PROTEIN AND ALBUMIN CONCENTRATIONS IN THE WHITE POPULATION GROUP

	PROTEINS (g/l)	
WHITE ADULTS	TOTAL	ALBUMIN
1	65	44
2	65	40
3	73	37
4	63	41
5	76	40
6	61	46
Mean	67.2	41.3
SD	5.9	3.2
7	70	46
8	69	37
9	62	44
10	63	42
11	62	38
12	63	38
Mean	64.8	40.8
SD	3.7	3.7
Overall Mean	66.0	41.1
SD	4.9	3.3

SAMPLES 1-6: MALES
SAMPLES 7-12: FEMALES

Appendix M

SERUM TOTAL PROTEIN AND ALBUMIN CONCENTRATIONS FOR BLACK URBAN AND RURAL POPULATION GROUPS

URBAN GROUP		
SAMPLE NO	TOTAL PROTEIN (g/l)	ALBUMIN (g/l)
1	72	40
2	66	43
3	70	46
4	63	43
5	68	41
6	67	39
7	72	46
8	66	47
9	70	49
10	75	40
11	65	42
12	68	44
13	69	47
14	76	50
15	73	48
16	74	39
17	69	46
18	66	44
19	75	40
20	76	44
Mean	69.9	43.9
SD	3.8	3.4
21	66	47
22	73	47
23	74	46
24	60	44
25	68	42
26	57	43
27	65	44
28	80	46
Mean	69.1	44.9
SD	6.2	1.9
Overall Mean	69.7	44.2
SD	4.5	3.0

SAMPLES 1-20: MALES
SAMPLES 21-28: FEMALES

RURAL GROUP		
SAMPLE NO	TOTAL PROTEIN (g/l)	ALBUMIN (g/l)
1	70	39
2	66	38
3	64	40
4	63	44
5	65	44
6	62	42
7	63	39
8	64	41
9	66	66
10	68	42
Mean	65.1	45.1
SD	2.4	2.6

All males

Appendix N

SERUM TOTAL PROTEIN (TOTAL) AND ALBUMIN (ALB) CONCENTRATIONS IN CHILDREN WITH KWASHIORKOR ON ADMISSION AND DURING TREATMENT

	PROTEINS (g/l)					
	ON ADMISSION		TREATMENT 1ST WEEK		2ND WEEK	
SAMPLE NO	TOTAL	ALB	TOTAL	ALB	TOTAL	ALB
1	48.3	20.5	62.6	31.6	60.6	38.0
2	38.2	21.4				
3	52.2	28.9				
4	34.7	19.0				
5	56.1	35.9				
6	39.5	23.8				
7	52.1	26.4				
8	51.6	30.0	63.6	34.2	64.3	36.4
9	39.8	24.0	52.4	28.2	61.4	36.2
10	34.7	24.6	58.6	30.2		
11	38.4	22.2	63.6	32.8		
12	49.3	21.5				
13	52.2	27.5				
Mean	45.2	25.1				
SD	6.7	4.6				
14	52.1	27.3				
15	49.3	26.0				
16	40.4	22.6				
17	39.8	24.4				
18	39.5	23.8				
19	46.3	22.2	56.4	30.5		
20	56.3	21.1				
21	38.6	22.8				
22	38.4	21.8				
Mean	44.5	23.2				
SD	6.7	1.9				
Overall Mean	44.8	24.3	59.5	31.4	63.1	36.9
SD	6.9	3.8	4.5	2.1	2.3	1.0

SAMPLES 1-3: MALES
SAMPLES 14-22: FEMALES

Appendix O

SERUM TOTAL PROTEIN AND ALBUMIN CONCENTRATIONS IN WELL-NOURISHED CHILDREN (CONTROLS)

	PROTEINS (g/l)	
SAMPLE NO	TOTAL	ALBUMIN
1	70	50
2	66	48
3	68	38
4	65	46
5	72	42
6	72	44
7	68	42
8	69	44
Mean	68.8	44.3
SD	2.5	3.7
9	68	43
10	72	39
11	71	45
12	69	46
13	75	49
14	72	48
15	67	39
Mean	70.5	44.1
SD	2.8	4.8
Overall Mean	69.6	44.2
SD	2.7	3.7

SAMPLES 1-8: MALES
SAMPLES 9-15: FEMALES

Appendix P

SERUM TOTAL PROTEIN (TOTAL) AND ALBUMIN (ALB) CONCENTRATIONS IN EIGHT SUBJECTS (A-H) MAINTAINED ON A LOW-PROTEIN DIET

	PROTEINS (g/l)									
	INITIAL		1ST WEEK		2ND WEEK		3RD WEEK		2 MONTHS AFTER RESUMING NORMAL DIET	
	TOTAL	ALB	TOTAL	ALB	TOTAL	ALB	TOTAL	ALB	TOTAL	ALB
A	67	46	65	37	58	32	56	29	70	41
B	66	43	60	41	59	34	50	33	67	44
C	68	36	60	37	61	30	52	32	68	38
D	67	46	61	38	62	31	53	32	71	46
E	66	39	62	38	58	32	55	29	66	34
F	64	40	61	36	60	32	51	32	68	48
G	70	38	62	37	59	34	50	30	69	37
H	63	35	59	35	61	35	57	26	72	37
Mean	66.4	40.4	61.3	37.4	59.8	32.5	53.0	30.4	68.9	40.6
SD	2.2	4.2	1.8	1.5	1.5	1.7	2.7	2.3	2.7	4.9

CONCENTRATIONS OF SERUM PROTEIN FRACTIONS IN THE ADULT WHITE POPULATION GROUP

SAMPLE NO	PROTEINS (g/l) ALBUMIN	GLOBULINS			
		α_1	α_2	β	γ
1	39.0	1.3	6.5	9.1	9.1
2	39.0	1.3	5.2	9.1	10.4
3	41.6	2.2	5.8	10.2	13.1
4	39.7	1.3	5.0	6.9	10.1
5	5[illegible].4	0.8	7.6	7.6	7.6
6	36.6	1.8	6.7	9.8	6.1
Mean	41.4	1.5	6.1	8.8	9.4
SD	5.6	0.5	1.0	1.3	2.4
7	46.2	1.4	4.2	7.0	11.2
8	40.0	2.1	8.3	[illegible].0	9.7
9	42.2	1.2	5.6	6.2	6.8
10	37.2	1.9	5.7	7.6	10.7
11	39.1	0.6	3.7	7.4	11.2
12	42.2	1.9	3.2	7.6	8.2
Mean	41.2	1.5	5.1	7.5	9.6
SD	3.1	0.6	1.9	0.9	1.8
Mean	41.3	1.5	5.6	8.1	9.5
SD	4.3	0.5	1.5	1.3	2.0

SAMPLES 1-6: MALES
SAMPLES 7-12: FEMALES

Appendix R

CONCENTRATIONS OF SERUM PROTEIN FRACTIONS IN THE ADULT BLACK URBAN POPULATION GROUP

SAMPLE NO	PROTEINS (g/l)				
	ALBUMIN	GLOBULINS			
		α_1	α_2	β	γ
1	43.2	1.4	5.8	8.6	13.0
2	38.3	1.3	8.0	7.3	11.2
3	44.1	2.1	7.0	5.6	11.2
4	40.0	1.3	6.9	5.0	10.1
5	40.1	1.3	6.8	8.8	10.1
6	40.9	1.5	6.1	7.4	11.4
7	45.4	2.2	4.3	6.5	13.7
8	39.6	1.3	5.3	7.9	11.9
9	44.1	2.1	7.0	8.4	8.4
10	45.8	2.3	6.8	7.5	12.8
11	37.7	2.0	5.9	9.1	9.8
12	39.4	1.4	5.4	8.8	12.9
13	39.3	2.1	8.2	9.0	10.4
14	42.6	3.0	6.0	8.4	15.2
15	43.1	2.2	6.6	6.6	14.6
16	44.4	2.2	5.2	7.4	14.1
17	43.5	2.1	6.9	6.2	9.7
18	35.0	1.3	7.3	9.9	11.2
19	41.6	1.5	6.6	8.8	13.1
20	47.9	1.5	5.3	7.6	13.7
Mean	41.8	1.9	6.4	7.7	11.9
SD	3.2	0.6	1.0	1.3	1.9
21	44.2	1.3	5.9	5.9	8.6
22	42.3	0.7	7.3	8.8	13.9
23	42.9	1.5	5.9	10.4	13.3
24	35.4	1.2	5.4	7.2	10.8
25	42.8	1.4	5.4	8.2	10.2
26	39.5	2.0	7.4	8.0	10.1
27	40.9	1.3	1.3	6.5	8.5
28	49.6	2.4	5.6	9.6	12.8
Mean	42.2	1.5	5.5	8.1	11.0
SD	4.1	0.5	1.9	1.5	2.1
Mean	42.0	1.8	6.1	7.8	11.7
SD	3.4	0.6	1.3	1.3	1.9

SAMPLES 1-20: MALES
SAMPLES 21-28: FEMALES

Appendix 6

CONCENTRATIONS OF SERUM PROTEIN FRACTIONS IN THE RURAL BLACK POPULATION GROUP

SAMPLE NO	PROTEINS (g/l)				
	ALBUMIN	GLOBULINS			
		α_1	α_2	β	γ
1	32.5	2.8	7.0	9.1	11.2
2	42.2	2.0	6.0	6.6	9.2
3	37.8	0.4	6.4	9.0	10.2
4	39.7	3.2	6.3	7.6	8.2
5	34.3	3.3	7.2	9.8	11.1
6	40.9	1.2	5.0	5.6	9.3
7	37.2	2.5	6.3	7.6	9.5
8	38.4	0.6	6.4	8.3	10.2
9	44.9	1.3	4.6	5.9	5.9
10	43.5	0.7	6.1	8.2	9.5
Mean	39.2	1.8	6.1	7.8	9.4
SD	3.9	1.1	0.8	1.4	1.5

ALL MALES

Appendix T

CONCENTRATION OF SERUM PROTEIN FRACTIONS IN THE CONTROL GROUP (CHILDREN)

SAMPLE NO	PROTEINS (g/l)				
	ALBUMIN	GLOBULINS			
		α_1	α_2	β	γ
1	42.0	0.7	7.0	9.1	11.2
2	37.6	2.6	6.6	8.6	10.6
3	39.7	1.3	5.9	7.2	11.1
4	39.0	1.3	3.9	7.8	13.0
5	44.6	1.4	7.2	7.9	10.8
6	46.1	1.4	6.5	9.4	8.6
7	40.1	1.3	6.8	8.8	10.9
8	42.1	2.1	6.2	6.2	12.4
Mean	41.4	1.5	6.3	8.1	11.1
SD	2.9	0.6	1.0	1.1	1.3
9	40.1	2.7	6.8	8.8	10.2
10	42.5	2.9	7.2	9.4	11.5
11	43.3	2.8	7.1	8.5	9.2
12	40.0	2.1	6.9	9.0	13.1
13	44.3	1.5	7.5	9.8	12.0
14	46.1	2.2	6.5	7.2	10.1
15	40.9	2.0	6.0	6.7	11.4
Mean	42.5	2.3	6.9	8.5	11.1
SD	2.3	0.5	0.5	1.1	1.3
Mean	41.9	1.9	6.6	8.3	11.1
SD	2.6	0.7	0.9	1.1	1.2

SAMPLES 1-8: MALES

SAMPLES 9-15: FEMALES

Appendix U

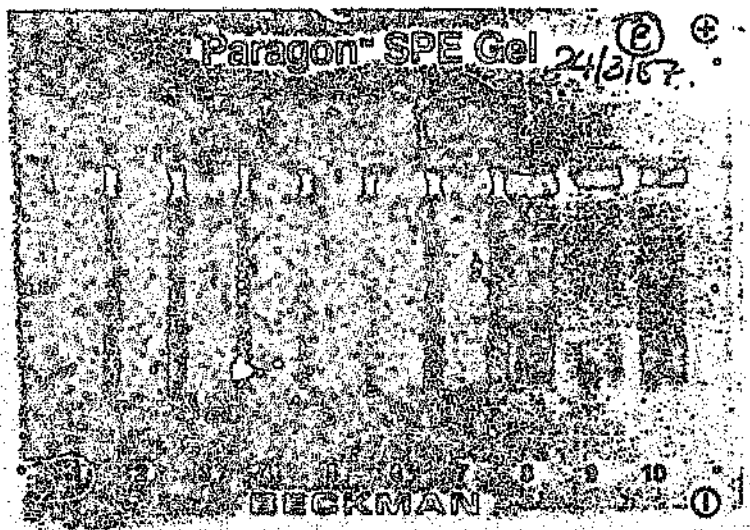
CONCENTRATIONS OF SERUM PROTEIN FRACTIONS IN CHILDREN WITH KWASHIORKOR ON ADMISSION INTO HOSPITAL AND DURING TREATMENT

	PROTEINS (g/l)														
	ON ADMISSION					TREATMENT 1ST WEEK					2ND WEEK				
	ALBUMIN	GLOBULINS				ALBUMIN	GLOBULINS				ALBUMIN	GLOBULINS			
		α_1	α_2	β	γ		α_1	α_2	β	γ		α_1	α_2	β	γ
1	21.7	5.3	7.7	2.9	10.6	27.5	3.8	12.5	8.8	10.0	38.3	2.0	6.6	8.6	12.5
2	15.3	2.7	5.7	2.3	12.2										
3	19.3	3.1	9.4	3.1	17.2										
4	14.9	1.7	6.2	2.1	9.7										
5	23.6	5.0	9.0	6.7	11.8										
6	19.0	2.8	5.1	3.6	9.1										
7	25.0	5.7	8.9	3.6	8.9										
8	23.7	3.6	7.2	5.2	11.9	42.0	1.3	9.5	8.3	8.9	37.7	2.0	6.5	8.5	10.5
9	18.3	2.4	5.6	2.4	11.1	27.2	2.6	8.9	5.2	8.3	35.0	2.5	6.1	8.6	9.2
10	14.9	2.4	7.3	2.8	7.3	27.0	4.7	10.5	5.3	11.1					
11	19.7	1.9	6.2	4.6	6.2	34.3	5.1	6.4	8.9	8.9					
12	24.7	3.0	6.9	4.9	9.9										
13	18.8	3.2	11.5	4.2	12.5										
Mean	19.9	3.2	7.4	3.7	10.6										
SD	3.6	1.3	1.8	1.3	2.7										
14	21.9	4.2	8.3	4.7	13.0										
15	23.2	3.5	7.9	3.9	10.8										
16	16.2	3.6	6.9	3.6	10.1										
17	15.1	2.6	7.2	4.2	10.3										
18	18.2	2.0	5.9	5.1	8.3										
19	13.4	3.7	8.8	3.7	16.2	22.0	3.4	9.0	9.0	13.0					
20	24.5	2.1	8.0	4.8	13.9										
21	13.5	3.9	2.2	3.9	11.2										
22	16.9	2.7	5.7	3.5	9.6										
Mean	18.1	3.1	6.8	4.2	11.5										
SD	4.2	0.8	2.0	0.6	2.4										
Overall Mean	19.2	3.3	7.2	3.9	11.0	30.3	3.5	9.5	7.6	10.0	37.0	2.2	6.4	8.6	10.7
SD	3.9	1.2	1.9	1.1	2.6	7.0	1.4	2.0	1.8	1.8	1.8	0.3	0.3	0.1	1.6

SAMPLES 1-13: MALES
SAMPLES 14-22: FEMALES

Appendix U_1

ELECTROPHORETIC RUN OF SERUM PROTEINS ON AGAROSE GEL



1-2 ADULT WHITES
3-4 ADULT RURAL BLACKS
5-6 ADULT URBAN BLACKS
7-8 WELL-NOURISHED CHILDREN
9-10 KWASHIORKOR

*Note the diminished albumin concentrations in kwashiorkor patients.

Appendix V

VALUES FOR SERUM PROTEIN FRACTIONS IN EIGHT SUBJECTS (A-H) MAINTAINED ON A LOW-PROTEIN DIET FOR THREE WEEKS

	PROTEINS(g/l)																					
	INITIAL					1ST WEEK					2ND WEEK					3RD WEEK					2 MONTHS AFTER RESUMING NORMAL DIET				
	ALBUMIN	GLOBULINS				ALBUMIN	GLOBULINS				ALBUMIN	GLOBULINS				ALBUMIN	GLOBULINS				ALBUMIN	GLOBULINS			
		α_1	α_2	β	γ		α_1	α_2	β	γ		α_1	α_2	β	γ		α_1	α_2	β	γ		α_1	α_2	β	γ
A	37.5	2.1	8.7	7.4	11.4	42.9	1.3	5.2	6.5	9.1	36.0	0.6	4.6	7.0	9.9	27.4	2.2	6.7	10.1	10.1	44.1	1.4	7.0	7.7	9.8
B	36.3	2.0	6.6	9.2	11.9	39.0	0.6	4.8	6.6	9.0	35.4	1.2	5.3	8.9	8.3	23.5	2.0	4.0	9.5	10.5	42.9	1.3	6.7	8.0	8.0
C	38.8	3.4	6.8	9.5	9.5	39.0	1.2	4.8	6.6	8.4	36.6	1.2	6.7	8.5	7.9	29.1	2.8	5.2	7.8	7.3	40.8	1.4	6.8	9.5	9.5
D	35.5	2.0	8.0	8.7	12.7	39.7	0.6	4.9	6.7	9.2	37.2	0.6	5.0	7.4	11.8	30.7	1.6	4.2	6.4	10.1	41.2	1.4	7.1	9.2	12.1
E	45.5	2.0	5.9	5.3	7.3	40.9	0.6	4.3	7.4	8.7	31.9	1.2	4.6	8.1	12.2	27.0	1.7	5.5	10.5	11.6	45.0	2.0	5.9	8.6	5.9
F	35.8	2.6	8.3	8.9	8.9	39.7	1.2	6.7	7.9	5.5	34.8	1.2	6.0	7.8	10.2	26.0	1.5	5.1	8.7	10.2	40.8	2.0	7.5	8.8	8.8
G	42.7	2.1	7.0	9.1	9.1	43.4	0.6	4.9	6.2	6.8	36.0	1.2	3.5	6.5	10.8	28.5	2.5	5.0	5.0	6.5	44.9	1.4	6.9	9.0	6.9
H	39.7	1.9	6.3	8.8	6.3	41.3	1.2	4.7	5.3	6.5	35.4	1.8	6.1	7.3	10.4	26.7	2.3	5.7	10.3	13.1	42.5	2.2	7.2	8.6	11.5
Mean	39.0	2.3	7.2	8.5	9.6	50.7	0.9	5.0	6.7	7.9	35.4	1.1	5.2	7.7	10.1	27.4	2.1	5.2	8.5	9.9	42.8	1.6	6.9	8.7	9.1
SD	3.6	0.5	1.0	1.5	2.2	1.7	0.3	0.7	0.8	1.4	1.6	0.4	1.0	0.8	1.5	2.2	0.5	0.9	2.0	2.1	1.8	0.4	0.5	0.6	2.1

Appendix V

VALUES FOR SERUM PROTEIN FRACTIONS IN EIGHT SUBJECTS (A-H) MAINTAINED ON A LOW-PROTEIN DIET FOR THREE WEEKS

	PROTEINS(g/l)																					
	INITIAL					1ST WEEK					2ND WEEK					3RD WEEK					2 MONTHS AFTER RESUMING NORMAL DIET				
	ALBUMIN	GLOBULINS				ALBUMIN	GLOBULINS				ALBUMIN	GLOBULINS				ALBUMIN	GLOBULINS				ALBUMIN	GLOBULINS			
		α_1	α_2	β	γ		α_1	α_2	β	γ		α_1	α_2	β	γ		α_1	α_2	β	γ		α_1	α_2	β	γ
A	37.5	2.1	8.7	7.4	11.4	42.9	1.3	5.2	6.5	9.1	36.0	0.6	4.6	7.0	9.9	27.4	2.2	6.7	10.1	10.1	44.1	1.4	7.0	7.7	9.8
B	36.3	2.0	6.6	9.2	11.9	39.0	0.6	4.8	6.6	9.0	35.4	1.2	5.3	8.9	8.3	23.5	2.0	4.0	9.5	16.5	42.9	1.3	6.7	8.0	8.0
C	38.8	3.4	6.8	9.5	9.5	39.6	1.2	4.8	6.6	8.4	36.6	1.2	6.7	8.5	7.9	29.1	1.3	5.2	7.8	7.3	40.8	1.4	6.6	9.5	9.5
D	35.5	2.0	8.0	8.7	12.7	39.7	0.6	4.9	6.7	9.2	37.2	0.6	5.0	7.4	11.8	30.7	1.6	4.2	6.4	10.1	41.2	1.4	7.1	9.2	12.1
E	45.5	2.0	5.9	5.3	7.3	40.9	0.6	4.3	7.4	8.7	31.9	1.2	4.6	8.1	12.2	27.0	1.7	5.5	10.5	11.6	45.0	2.0	5.9	8.6	5.9
F	35.8	2.6	8.3	8.9	8.9	39.7	1.2	6.7	7.9	5.5	34.8	1.2	6.0	7.8	10.2	26.0	1.5	5.1	8.7	10.2	40.8	2.0	7.5	8.8	8.8
G	42.7	2.1	7.0	9.1	9.1	43.4	0.6	4.9	6.2	6.8	36.0	1.2	3.5	6.5	10.0	28.5	2.5	5.0	5.0	6.5	44.9	1.4	6.9	9.0	6.9
H	39.7	1.9	6.3	8.8	6.3	41.3	1.2	4.7	5.3	6.5	35.4	1.8	6.1	7.3	10.4	26.7	2.3	5.7	10.3	13.1	42.5	2.2	7.2	8.6	11.5
Mean	39.0	2.3	7.2	8.5	9.6	50.7	0.9	5.0	6.7	7.9	35.4	1.1	5.2	7.7	10.1	27.4	2.1	5.2	8.5	9.9	42.8	1.6	6.9	8.7	9.1
SD	3.6	0.5	1.0	1.5	2.2	1.7	0.3	0.7	0.8	1.4	1.6	0.4	1.0	0.8	1.5	2.2	0.5	0.9	2.0	2.1	1.8	0.4	0.5	0.6	2.1

Appendix W

SERUM LIPOPROTEINS IN THE WHITE ADULT POPULATION GROUP

	LIPOPROTEINS (%)			
SAMPLE NO	CHYLOMICRONS	LDL	VLDL	HDL
1	0.8	56	19.2	24.0
2	0.6	58	19.4	22.0
3	-	61	19.0	20.0
4	0.2	54	23.8	22.0
5	-	58	21.0	21.0
6	-	60	22.0	18.0
Mean	0.3	57.8	20.7	21.2
SD	0.4	2.6	1.9	2.0
7	0.6	61	17.4	21.0
8	0.4	66	15.6	18.0
9	0.4	50	20.6	29.0
10	0.6	57	18.4	24.0
11	0.4	52	21.6	26.0
12	0.6	55	22.0	22.4
Mean	0.5	56.3	19.3	23.4
SD	0.1	5.9	2.5	3.9
Overall Mean	0.4	57.3	20.0	22.3
SD	0.3	4.4	2.3	3.2

SAMPLES 1-6: MALES

SAMPLES 7-12: FEMALES

Appendix FF_1

THE RELATIVE PERCENTAGES OF LECITHIN FATTY ACIDS IN THE ADULT BLACK MALE (URBAN) POPULATION GROUP

FATTY ACIDS	16:0	16:1	18:0	18:1	18:2	18:3ω6	18:3ω3	20:3	20:4	20:5	22:5	22:6
1	29.3	0.15	21.2	13.0	23.6	nd	0.17	1.52	5.66	0.80	2.92	1.47
2	27.4	0.09	17.7	13.3	28.9	nd	0.11	1.74	7.94	0.75	1.08	1.12
3	31.4	1.16	20.4	10.6	24.7	nd	0.08	2.85	5.14	0.40	1.54	1.55
4	27.4	0.32	17.8	13.2	27.8	nd	0.09	1.79	7.92	0.56	1.37	1.61
5	28.4	0.26	20.8	17.0	22.2	nd	0.09	1.91	5.57	0.40	1.44	1.66
6	29.3	0.99	19.4	12.9	25.6	nd	0.36	1.40	7.22	0.44	0.84	1.41
7	28.6	1.29	18.5	17.2	19.7	nd	0.16	2.78	8.86	0.27	1.03	1.48
8	30.1	0.33	15.7	12.4	25.7	nd	0.14	2.54	8.85	0.80	1.63	1.68
9	28.9	0.82	16.5	14.0	24.3	nd	0.10	2.57	8.89	0.81	1.58	1.28
10	26.3	0.60	17.3	20.2	29.5	nd	0.15	2.40	8.43	0.80	0.60	1.51
11	27.0	0.82	15.5	12.0	30.3	nd	0.40	2.52	8.83	0.81	0.64	0.99
12	29.2	0.33	14.9	12.3	29.6	nd	0.21	2.28	8.67	0.73	0.58	1.07
13	26.6	0.22	18.7	10.0	31.5	nd	0.16	2.42	7.03	0.78	0.92	1.42
14	28.9	0.32	16.2	11.0	30.4	nd	0.18	2.34	7.92	0.38	0.78	1.18
15	31.3	0.42	14.8	11.6	28.6	nd	0.16	2.31	7.67	0.52	0.98	1.51
16	27.3	0.31	14.2	11.2	31.2	nd	0.19	2.81	8.74	0.68	1.32	1.86
17	29.8	0.15	16.2	11.2	29.4	nd	0.19	1.94	7.71	0.51	1.42	1.21
18	27.8	0.15	15.3	12.7	30.1	nd	0.17	2.61	7.98	0.51	1.07	1.52
19	30.0	0.31	15.9	11.4	26.6	nd	0.21	2.29	8.94	0.61	1.59	2.04
20	29.3	0.12	16.5	12.2	26.6	nd	0.19	2.39	8.01	0.60	1.50	2.36
Mean	28.7	0.46	17.2	12.6	27.1	nd	0.18	2.27	7.81	0.62	1.21	1.50
SD	1.43	0.36	2.09	1.83	3.12		0.08	0.42	1.17	0.16	0.43	0.33

nd: not detectable; for statistical analysis values were taken as zero.

Appendix DD

INDIVIDUAL VALUES FOR SERUM TOTAL PHOSPHOLIPID (mg%) IN EIGHT SUBJECTS MAINTAINED ON A LOW-PROTEIN DIET FOR THREE WEEKS

	PHOSPHOLIPIDS				
	INITIAL	1ST WEEK	2ND WEEK	3RD WEEK	2 MONTHS AFTER RESUMING NORMAL DIET
A	150.2	49.2	132.6	103.2	153.3
B	143.8	144.0	136.8	122.2	140.2
C	121.1	120.2	118.2	111.2	124.6
D	152.6	148.2	140.3	133.1	152.6
E	154.8	153.6	146.3	140.2	153.7
F	142.9	138.3	130.2	121.4	141.7
G	122.6	121.3	116.3	111.4	130.6
H	124.8	120.6	118.3	112.3	128.6
MEAN	139.1	136.9	129.9	122.8	141.4
SD	14.0	14.1	11.3	11.0	12.8

Appendix X

SERUM LIPOPROTEINS IN THE BLACK URBAN ADULT POPULATION GROUP

SAMPLE NO	LIPOPROTEINS (%) CHYLOMICRONS	LDL	VLDL	HDL
1	-	59	21.0	20.0
2	-	54	24.0	22.0
3	-	61	14.0	25.0
4	-	65	12.0	23.0
5	-	57	20.0	23.0
6	-	54	25.0	21.0
7	-	55	23.0	22.0
8	-	59	20.0	21.0
9	-	59	20.0	21.0
10	-	54	23.0	23.0
11	-	54	22.0	24.0
12	-	58	24.0	18.0
13	-	61	20.0	19.0
14	-	62	20.0	18.0
15	-	58	19.0	23.0
16	-	56	20.0	24.0
17	-	63	13.0	24.0
18	-	60	15.0	25.0
19	-	58	16.0	26.0
20	-	57	24.0	19.0
Mean	0.0	58.2	19.9	21.9
SD	0.0	3.2	4.0	2.5
21	-	60	10.6	29.4
22	0.3	62	13.3	24.4
23	0.1	59	18.9	22.0
24	0.8	65	13.2	21.0
25	0.2	60	19.8	20.0
26	0.4	59	20.8	19.8
27	0.6	57	19.6	23.0
28	1.2	62	16.8	20.0
Mean	0.4	60.5	16.6	22.5
SD	0.4	2.4	3.8	3.2
Overall				
Mean	0.1	58.9	19.0	22.1
SD	0.3	3.1	4.1	2.7

SAMPLES 1-20: MALES
SAMPLES 21-28: FEMALES

Appendix Y

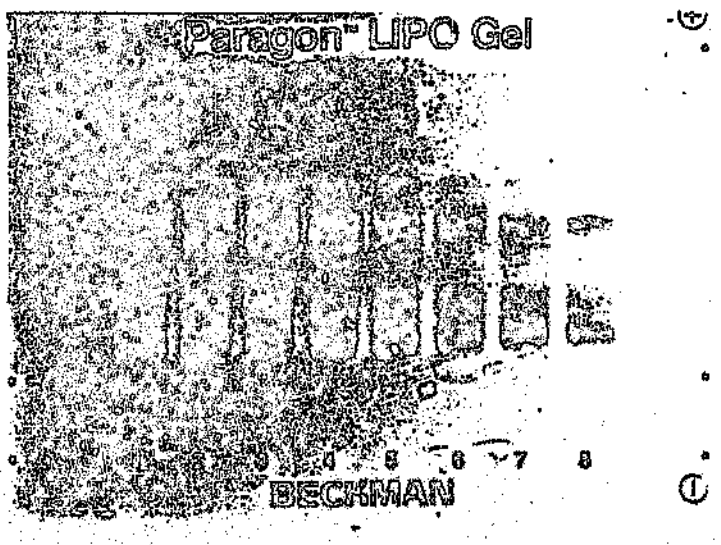
SERUM LIPOPROTEINS IN THE RURAL BLACK POPULATION GROUP

SAMPLE NO	CHYLOMICRONS	LIPOPROTEINS (%) LDL	VLDL	HDL
1	-	50	26	24
2	-	49	25	26
3	-	54	22	24
4	-	51	22	27
5	-	60	22	18
6	-	56	25	19
7	-	58	16	26
8	-	52	18	30
9	-	57	19	24
10	-	51	20	29
Mean	-	53.8	21.5	24.7
SD	-	3.8	3.3	3.9

ALL MALES

Appendix Y_1

ELECTROPHORETIC RUN OF SERUM LIPOPROTEINS ON AGAROSE GEL



1-5 ADULT WHITES
6-8 ADULT BLACKS (one rural and two urbans)

Appendix Z

SERUM LIPOPROTEINS IN WELL-NOURISHED CHILDREN (CONTROL GROUP)

	LIPOPROTEINS (%)			
SAMPLE NO	CHYLOMICRONS	LDL	VLDL	HDL
1	-	63.0	20.0	17.0
2	-	65.0	18.0	17.0
3	0.6	60.2	16.0	23.2
4	0.4	60.0	15.6	24.0
5	-	62.0	21.0	17.0
6	-	63.0	21.0	16.0
7	0.8	61.5	16.4	21.3
8	0.8	58.4	15.8	25.0
Mean	0.3	61.6	18.0	20.1
SD	0.3	2.0	2.4	3.8
9	-	39.0	21.0	20.0
10	0.4	60.0	17.6	22.0
11	1.1	61.0	20.0	17.9
12	0.6	56.0	15.6	27.8
13	-	61.0	21.0	18.0
14	-	59.0	22.0	19.0
15	0.8	56.4	12.8	30.0
Mean	0.4	58.9	18.6	22.1
SD	0.4	2.0	3.3	4.8
Overall Mean	0.4	60.4	18.3	21.0
SD	0.4	2.4	2.8	4.3

SAMPLES 1-8: MALES
SAMPLES 9-15: FEMALES

Appendix AA

SERUM LIPOPROTEINS IN EIGHT SUBJECTS (A-H) MAINTAINED ON A LOW-PROTEIN DIET

	LIPOPROTEINS %																			
	INITIAL				1ST WEEK				2ND WEEK				3RD WEEK				2 MONTHS AFTER RESUMING NORMAL DIET			
	CHYLO	LDL	VLDL	HDL	CHYLO	HDL	VLDL	HDL	CHYLO	LDL	VLDL	HDL	CHYLO	LDL	VLDL	HDL	CHYLO	LDL	VLDL	HDL
A	1.0	56.4	11.3	31.3	nd	55.0	34.5	10.5	nd	55.6	26.2	18.2	0.2	57.7	14.4	27.7	0.9	57.4	11.3	30.4
B	1.2	63.5	14.0	21.3	nd	55.1	40.6	4.2	0.2	55.2	28.9	15.7	0.3	56.1	14.6	28.9	1.6	62.5	13.0	22.9
C	nd	64.6	6.0	29.4	nd	58.4	33.8	7.8	nd	54.6	21.2	24.2	0.1	51.5	19.5	28.9	0.6	63.6	10.0	25.8
D	1.6	60.2	15.0	23.2	nd	58.6	33.0	8.1	nd	57.2	28.0	14.8	nd	57.8	14.1	28.1	1.3	58.6	13.1	27.0
E	1.9	63.3	15.0	33.2	nd	59.7	35.0	5.3	nd	56.4	25.2	18.4	nd	55.4	19.0	25.6	1.8	65.2	12.0	22.0
F	nd	56.5	15.7	27.8	nd	57.3	35.1	7.6	nd	56.3	26.2	17.5	0.1	57.3	17.6	25.0	nd	57.6	15.7	26.7
G	1.0	51.2	23.1	24.6	0.2	63.7	29.6	5.5	0.1	60.1	20.6	19.2	nd	59.0	13.0	28.0	0.8	50.2	19.6	29.4
H	0.0	50.5	25.2	24.3	0.6	51.3	33.0	15.1	0.3	52.6	22.3	24.8	0.1	54.4	17.1	28.4	nd	52.5	21.6	25.9
Mean	0.8	58.3	15.7	26.9	0.1	57.4	34.3	8.0	0.08	56.0	24.8	19.1	0.1	56.2	16.2	27.6	0.9	58.5	14.5	26.3
SD	0.8	5.5	6.1	4.2	0.2	3.7	3.1	3.5	0.1	2.2	3.1	3.6	0.1	2.4	2.4	1.5	0.7	5.3	4.1	2.9

Appendix BB

SERUM TOTAL PHOSPHOLIPID CONCENTRATION IN THE DIFFERENT ADULT POPULATION GROUPS

	PHOSPHOLIPIDS (mg%)		
SAMPLE	URBAN BLACKS	RURAL BLACKS	WHITES
1	129.4	162.8	142.9
2	151.1	151.4	154.6
3	161.1	147.6	153.8
4	162.8	129.2	150.1
5	133.3	161.1	165.0
6	141.4	162.8	143.6
7	121.8	153.6	152.8
8	147.6	148.5	111.1
9	148.5	121.8	122.1
10	152.5	147.6	124.2
11	154.2		128.6
12	140.6		141.1
13	138.2		
14	141.1		
15	156.4		
16	151.3		
17	142.4		
18	153.1		
19	161.1		
20	132.4		
21	147.1		
22	153.2		
23	161.4		
24	139.8		
25	162.2		
26	166.7		
27	137.2		
28	134.9		
Overall Mean	147.2	148.6	140.8
SD	11.5	13.7	16.1

URBAN BLACKS
SAMPLES 1-20: MALES
SAMPLES 21-28: FEMALES

WHITES
SAMPLES 1-6: MALES
SAMPLES 7-12: FEMALES

SEX DIFFERENCES

WHITES		
Males	mean	151.7
	SD	8.2
Females	mean	130.0
	SD	14.8
URBAN BLACKS		
Males	mean	146.0
	SD	11.3
Females	mean	150.3
	SD	12.4
RURAL BLACKS (all males)		

Appendix CC

SERUM TOTAL PHOSPHOLIPID CONCENTRATION IN PATIENTS WITH KWASHIORKOR AND NORMAL CONTROLS

	PHOSPHOLIPIDS (mg%)	
SAMPLE NO	KWASHIORKOR	CONTROLS
1	108.7	162.1
2	98.9	130.2
3	103.1	158.6
4	104.0	149.6
5	80.2	152.9
6	115.1	154.8
7	96.3	152.1
8	92.0	151.3
9	112.8	148.8
10	89.4	142.3
11	91.1	165.2
12	96.9	156.4
13	107.2	161.1
14	92.0	156.2
15	[illegible]	148.2
16	87.5	
17	133.3	
18	121.0	
19	110.4	
20	98.9	
21	103.1	
22	114.3	
Mean	102.1	152.7
SD	12.6	8.6

KWASHIORKOR
SAMPLES 1-13: MALES
SAMPLES 14-22: FEMALES

CONTROLS
SAMPLES 1-8: MALES
SAMPLES 9-15: [illegible]LES

SEX DIFFERENCES

KWASHIORKOR		
Males	Mean	99.7
	SD	10.1
Females	Mean	105.5
	SD	15.5
CONTROLS		
Males	Mean	151.5
	SD	9.5
Females	Mean	154.0
	SD	8.0

Appendix EE

THE RELATIVE PERCENTAGES OF LECITHIN FATTY ACIDS IN THE ADULT WHITE POPULATION GROUP

FATTY ACIDS	16:1	16:1	18:0	18:1	18:2	18:3ω6	18:3ω3	20:3	20:4	20:5	22:5	22:6
1	32.3	0.12	16.1	13.3	21.6	0.29	0.13	3.08	6.86	0.35	2.99	2.75
2	30.6	0.13	17.6	13.8	21.8	0.20	0.60	3.13	6.97	0.60	2.06	2.45
3	34.6	0.21	15.4	13.3	22.8	0.18	0.12	2.63	5.88	0.32	2.64	1.63
4	34.2	0.33	14.7	15.3	24.5	nd	0.14	1.65	5.01	0.18	1.86	1.98
5	36.2	0.06	13.5	13.2	22.0	0.07	0.23	2.94	8.31	0.24	1.03	2.11
6	30.9	0.16	15.9	12.8	24.1	0.07	0.32	3.19	7.55	0.29	1.90	2.69
Mean	33.2	0.17	15.5	13.6	22.8	0.14	0.26	2.77	7.67	0.33	2.08	2.27
SD	2.24	0.09	1.39	0.87	1.25	0.11	0.18	0.58	1.18	0.15	0.68	0.44
7	31.7	0.32	16.2	13.5	24.2	0.05	0.13	3.61	6.87	0.32	1.39	1.56
8	31.8	0.21	14.4	16.7	24.9	0.05	0.21	2.82	5.19	0.27	1.47	1.89
9	31.1	0.23	12.9	12.9	27.6	0.14	0.25	2.86	6.67	0.20	2.01	3.01
10	29.6	0.27	16.1	13.0	28.6	0.10	0.23	1.86	5.28	0.32	0.88	2.85
11	29.7	0.25	15.5	12.7	28.7	0.03	0.30	2.78	7.03	0.16	0.85	1.78
12	31.6	0.22	13.3	13.6	28.6	0.09	0.33	2.75	6.74	0.27	0.80	1.94
Mean	30.9	0.25	14.7	13.6	27.1	0.08	0.24	2.78	6.30	0.26	1.23	2.17
SD	1.00	0.04	1.61	1.85	2.02	0.04	0.07	0.56	0.83	0.06	0.48	0.60
Overall												
Mean	32.0	0.21	15.1	13.7	25.0	0.11	0.25	2.78	6.53	0.29	1.66	2.22
SD	2.03	0.08	1.39	1.16	2.76	0.08	0.13	0.54	1.00	0.11	0.71	0.51

SAMPLES: 1-6: MALES
SAMPLES: 7-12: FEMALES

Appendix FF_2

THE RELATIVE PERCENTAGES OF LECITHIN FATTY ACIDS IN THE ADULT BLACK FEMALE (URBAN) POPULATION GROUP

FATTY ACIDS	16:0	16:1	18:0	18:1	18:2	18:3ω6	18:3ω3	20:3	20:4	20:5	22:5	22:6
1	33.2	0.26	15.7	13.3	27.1	nd	0.42	2.70	4.95	0.22	0.52	1.54
2	34.0	0.26	15.8	13.3	26.0	nd	0.41	2.21	4.70	0.68	0.85	1.64
3	37.4	0.21	15.9	12.9	22.0	nd	0.48	2.27	4.94	0.18	0.52	2.97
4	32.6	0.27	16.4	14.9	24.6	0.10	0.47	2.18	4.58	0.16	0.72	3.05
5	34.8	0.26	18.3	12.6	21.2	nd	0.31	3.55	5.22	0.59	0.67	2.32
6	37.2	0.21	18.7	13.9	20.4	0.09	0.46	1.59	3.65	0.66	0.57	2.26
7	36.3	0.23	17.7	13.1	23.0	0.09	0.16	2.01	4.89	0.10	0.83	1.41
8	34.8	0.28	18.5	12.9	22.1	0.10	0.28	2.63	5.01	0.43	0.68	2.15
Mean	35.0	0.25	17.1	13.4	23.3	0.05	0.37	2.39	4.74	0.38	0.67	2.17
SD	1.79	0.03	1.32	0.73	2.36	0.05	0.11	0.58	0.68	0.24	0.13	0.62
TOTAL = MALES + FEMALES												
MEAN	30.6	0.40	17.2	12.8	26.2	0.01	0.23	2.31	6.93	0.55	1.08	1.65
SD	3.19	0.32	1.88	1.62	3.49	0.03	0.13	0.46	1.73	0.22	0.52	0.56

nd = not detectable; for statistical analysis values were taken as zero.

Appendix GG

THE RELATIVE PERCENTAGES OF LECITHIN FATTY ACIDS IN THE MALE RURAL ADULT BLACK POPULATION GROUP

FATTY ACIDS	16:0	16:1	18:0	18:1	18:2	18:3ω6	18:3ω3	20:3	20:4	20:5	22:5	22:6
1	30.5	0.92	16.0	10.4	28.1	nd	0.82	2.61	8.97	0.53	0.62	1.03
2	26.9	1.32	16.4	11.8	30.4	nd	0.12	1.96	8.01	0.58	1.04	1.21
3	29.8	0.32	16.0	9.9	30.0	nd	0.47	2.64	9.34	0.36	0.32	0.83
4	31.7	0.26	14.5	10.4	30.6	nd	0.22	1.92	8.17	0.59	0.46	1.07
5	29.0	1.03	15.7	12.7	25.4	nd	0.76	2.89	9.10	0.62	0.64	1.02
6	32.2	0.86	14.4	.3	26.9	nd	0.29	3.07	10.4	0.86	0.76	0.97
7	31.8	0.89	16.1	10.4	22.3	nd	1.01	3.70	10.0	0.53	1.20	1.70
8	27.8	0.63	15.2	8.97	31.6	nd	0.09	2.60	8.81	0.86	1.22	2.01
9	25.8	1.02	15.1	14.3	24.6	nd	0.42	2.95	10.7	0.99	1.61	2.31
10	29.9	0.62	15.5	13.7	25.6	nd	0.23	2.57	8.92	0.76	0.57	1.01
Mean	29.5	0.79	15.5	11.2	27.8	nd	0.44	2.69	9.26	0.67	0.84	1.32
SD	2.15	0.33	0.70	1.86	3.02	-	0.32	0.52	0.92	0.19	0.41	0.51

ALL MALES
nd = not detectable; for statistical analysis values were taken as zero

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Appendix HH

THE RELATIVE PERCENTAGES OF LECITHIN FATTY ACIDS IN WELL-NOURISHED CHILDREN

FATTY ACIDS	16:0	16:1	18:0	18:1	18:2	18:3ω6	18:3ω3	20:3	20:4	20:5	22:5ω3	22:6
1	29.0	0.90	15.2	10.4	28.0	0.11	0.18	2.66	8.72	0.37	1.93	2.34
2	32.9	0.85	14.8	10.9	25.3	0.11	0.20	2.04	6.11	0.81	1.08	1.69
3	33.3	1.82	15.4	7.68	26.0	0.06	0.20	3.82	8.27	0.56	0.09	1.78
4	34.3	0.52	16.8	14.0	22.6	nd	0.20	2.01	4.91	0.43	1.86	2.30
5	32.4	0.60	15.8	13.7	27.9	0.08	0.16	2.01	6.62	0.46	0.81	1.31
6	31.8	0.21	16.4	13.7	25.9	nd	0.18	2.86	5.19	0.60	1.03	1.94
7	29.1	0.71	17.2	11.3	24.8	0.03	0.68	3.62	8.77	0.51	1.11	2.01
8	31.0	1.83	16.2	8.85	26.4	0.08	0.12	4.20	8.68	0.44	0.91	1.11
Mean	31.7	0.93	16.0	11.32	25.9	0.06	0.24	2.90	6.91	0.52	1.10	1.81
SD	1.92	0.59	0.82	2.36	1.74	0.04	0.18	0.88	1.87	0.14	0.59	0.44
9	30.2	1.12	16.0	10.2	29.3	0.08	0.20	2.65	9.13	0.68	0.89	3.10
10	29.7	0.58	14.6	10.7	28.3	0.10	0.18	3.69	7.88	0.39	1.10	2.77
11	24.7	1.08	16.8	13.9	24.2	0.08	1.10	3.90	7.89	0.61	1.12	2.34
12	28.7	0.86	17.6	13.4	24.1	0.08	0.21	3.60	7.81	0.50	0.88	2.05
13	32.0	0.61	15.8	11.2	26.0	nd	0.09	3.61	7.03	0.36	0.71	1.81
14	29.9	0.58	16.5	12.6	27.6	nd	0.18	3.61	6.33	0.31	0.80	1.48
15	33.6	0.46	15.2	11.2	28.9	0.06	0.11	2.26	4.23	0.40	1.61	1.81
Mean	30.1	0.73	16.1	11.89	27.0	0.06	0.20	3.35	7.19	0.46	1.02	2.19
SD	2.22	0.30	1.00	1.42	2.14	0.04	0.36	0.50	1.56	0.14	0.30	0.58
Overall												
Mean	31.0	0.84	16.0	11.6	26.3	0.06	0.27	3.11	7.04	0.50	1.12	2.00
SD	2.15	0.47	0.88	1.93	1.88	0.04	0.27	0.77	1.68	0.14	0.38	0.53

SAMPLES: 1-8: MALES
SAMPLES: 9-15: FEMALES
nd: not detectable; for statistical analysis values were taken as zero

Appendix II

THE RELATIVE PERCENTAGES OF LECITHIN FATTY ACID IN CHILDREN WITH KWASHIORKOR

FATTY ACIDS	16:0	16:1	18:0	18:1	18:2	18:3ω6	18:3ω3	20:3	20:4	20:5	22:4	22:5ω6	22:5ω3	22:6
1	29.7	1.52	10.4	10.4	15.8	0.18	0.71	4.13	10.6	1.10	13.1	0.26	1.65	0.37
2	34.1	2.55	6.19	18.8	21.8	0.12	0.15	0.79	6.72	0.69	3.42	0.36	2.42	1.18
3	27.7	2.78	7.43	22.3	13.0	0.38	1.00	1.84	6.18	0.98	11.7	nd	2.67	0.91
4	33.6	2.23	7.32	14.9	21.1	0.26	0.18	0.83	8.62	0.23	5.92	0.80	2.95	0.95
5	30.4	4.57	8.36	20.3	18.8	0.29	0.58	1.50	5.10	0.15	7.30	0.79	1.13	0.55
6	30.1	5.18	9.21	12.9	18.8	0.33	0.85	0.99	9.14	0.62	9.25	0.61	0.94	0.77
7	31.5	7.19	7.52	17.2	13.4	0.20	0.51	0.64	4.63	0.24	10.8	1.40	3.94	0.52
8	29.7	3.31	9.78	10.5	20.6	0.40	0.65	1.44	5.10	0.50	6.80	0.74	1.25	0.77
9	28.6	3.06	7.14	15.9	18.8	0.26	0.61	2.76	8.72	0.66	7.92	0.80	2.01	0.76
10	29.2	3.05	8.10	16.6	19.7	nd	0.55	1.67	8.89	0.45	9.46	0.63	0.87	0.69
11	35.4	2.63	6.00	19.2	22.1	0.14	0.29	0.76	6.50	0.66	2.52	0.25	2.45	1.01
12	31.8	2.72	8.51	16.2	20.7	0.21	0.27	1.68	8.57	0.42	6.10	0.96	1.18	0.64
13	32.6	2.06	8.22	13.4	16.0	0.33	0.39	2.01	6.89	0.90	13.6	0.61	2.06	0.87
Mean	31.1	3.30	8.01	16.7	18.5	0.25	0.50	1.62	7.36	0.58	8.45	0.63	1.96	0.77
SD	2.31	1.52	1.29	3.26	3.05	0.14	0.26	0.97	1.85	0.25	3.47	0.36	0.91	0.22
14	29.5	2.60	6.62	10.6	16.2	1.03	0.36	1.94	13.2	1.72	12.8	0.99	1.26	0.94
15	29.5	2.43	6.64	10.9	16.5	0.60	1.03	2.29	12.6	1.96	12.8	0.58	0.69	1.12
16	37.0	2.03	7.26	15.9	12.9	0.27	0.53	2.10	8.43	0.40	9.27	0.97	1.80	0.99
17	29.6	1.61	10.2	9.93	15.9	0.20	0.51	3.93	10.3	0.92	14.0	nd	1.54	0.68
18	30.6	2.61	8.26	15.6	16.8	0.32	0.66	1.52	8.62	0.88	10.7	0.91	1.61	0.91
19	28.6	3.03	9.63	18.2	20.4	nd	0.62	1.93	5.30	0.63	6.01	1.65	3.38	0.62
20	31.1	2.58	8.30	15.9	20.3	0.39	0.71	2.41	8.73	0.43	6.15	0.90	1.26	0.74
21	34.4	3.65	7.82	19.4	19.2	0.27	0.69	1.34	4.82	0.85	5.65	0.66	0.45	0.74
22	30.9	7.24	7.17	16.8	13.3	0.12	0.35	0.71	5.41	1.26	10.2	1.30	4.02	1.04
Mean	31.2	3.09	7.99	14.8	16.8	0.40	0.61	2.02	8.62	1.00	9.73	0.88	1.78	0.86
SD	2.73	1.66	1.26	3.47	2.73	0.30	0.21	0.88	3.09	0.56	3.20	0.46	1.18	0.17
Overall Mean	31.6	3.21	8.00	15.9	17.8	0.29	0.55	1.78	7.88	0.76	8.98	0.73	1.89	0.81
SD	2.82	1.54	1.25	3.40	2.98	0.22	0.24	0.94	2.45	0.45	3.35	0.41	1.01	0.20

SAMPLES 1-13: MALES
SAMPLES 14-22: FEMALES
nd = Not detectable; for statistical analysis values were taken as zero

Appendix JJ

THE RELATIVE PERCENTAGES OF LECITHIN FATTY ACIDS IN EIGHT SUBJECTS MAINTAINED ON A LOW-PROTEIN DIET FOR THE TIME PERIODS INDICATED

FATTY ACIDS		16:0	16:1	18:0	18:1	18:2	18:3ω6	18:3ω3	20:3	20:4	20:5	22:5	22:6
Basal	A	13.5	0.73	17.7	10.9	27.1	0.33	0.40	3.29	16.2	1.64	2.00	5.98
	B	21.1	0.54	17.8	14.2	25.3	nd	0.24	3.25	10.3	0.49	1.87	4.02
	C	27.8	0.31	17.2	14.0	23.0	nd	0.44	3.82	9.35	0.50	1.51	1.71
	D	18.9	0.43	17.2	10.8	30.2	0.06	0.32	3.52	12.8	0.84	0.87	4.30
	E	15.0	0.20	20.6	11.9	30.2	0.05	0.32	3.84	11.8	0.97	1.00	4.03
	F	20.4	nd	20.2	14.7	30.3	0.37	0.48	2.97	6.84	0.41	0.73	2.06
	G	27.1	nd	16.1	12.4	28.6	0.41	0.50	3.82	6.55	0.69	0.80	4.46
	H	22.8	0.89	17.5	14.5	28.7	0.30	0.44	3.13	6.86	0.81	0.91	2.91
Mean		20.8	0.39	18.1	12.9	27.9	0.19	0.39	3.46	10.1	0.79	1.21	3.68
SD		5.13	0.32	1.54	1.61	2.62	0.18	0.05	0.34	3.44	0.39	0.51	1.39
1st Week	A	29.7	nd	12.9	10.7	30.4	2.13	0.18	2.53	8.30	0.34	0.38	1.95
	B	30.2	nd	10.8	9.76	23.9	13.3	0.16	2.25	6.91	0.15	0.26	2.16
	C	29.3	nd	14.4	11.8	26.2	2.61	nd	2.38	9.17	0.51	0.65	2.70
	D	28.1	nd	14.2	10.0	29.9	2.21	0.12	2.36	9.33	0.22	0.49	2.69
	E	24.2	nd	12.6	8.21	30.9	10.9	0.22	2.58	7.83	0.29	0.28	1.90
	F	31.1	nd	11.6	11.1	9.3	4.05	0.03	2.38	7.00	0.14	0.72	2.54
	G	31.4	nd	14.2	12.7	28.6	4.18	nd	3.39	7.00	0.51	0.75	2.89
	H	25.7	nd	14.4	9.22	20.4	21.1	0.09	1.97	5.91	0.47	0.66	2.91
Mean		28.7	nd	13.2	10.4	27.5	7.57	0.10	2.48	7.68	0.33	0.52	2.47
SD		2.56	-	1.39	1.46	3.67	6.92	0.08	0.41	1.20	0.15	0.20	0.41
2nd Week	A	31.2	nd	16.6	10.9	28.4	0.52	0.19	2.35	7.74	0.25	0.26	1.47
	B	37.6	nd	18.4	9.54	19.6	4.61	0.86	2.22	5.46	0.08	0.10	1.43
	C	34.1	nd	18.4	11.8	23.2	0.97	0.47	1.60	6.95	0.21	0.30	1.88
	D	29.3	nd	15.6	10.9	27.7	1.00	0.36	2.51	9.73	0.06	0.11	2.21
	E	32.3	nd	17.4	11.3	25.5	1.92	0.07	3.10	7.44	0.18	0.17	0.55
	F	33.2	nd	13.3	11.3	31.7	2.40	0.27	1.85	4.65	0.06	0.13	1.00
	G	36.5	nd	18.0	11.2	23.0	2.14	0.42	2.41	5.11	0.26	0.17	0.81
	H	40.3	nd	16.7	10.3	20.9	5.54	0.11	1.53	3.66	0.11	0.17	0.53
Mean		34.4	nd	16.8	10.9	25.0	2.39	0.34	2.20	6.34	0.15	0.18	1.24
SD		3.63	-	1 70	0.71	4.09	1.80	0.25	0.52	2.00	0.08	0.07	0.62
3rd Week	A	26.3	nd	13.1	8.94	19.8	21.4	0.10	2.38	5.38	0.19	nd	1.67
	B	27.4	nd	11.4	8.48	15.1	31.4	nd	0.86	3.88	nd	nd	1.35
	C	32.9	nd	16.2	11.8	18.2	9.28	0.08	1.49	5.13	0.20	nd	4.53
	D	27.3	nd	16.8	11.5	20.9	14.3	nd	1.88	5.42	0.24	0.21	1.18
	E	29.0	nd	17.9	10.5	26.3	4.82	nd	2.74	6.70	0.34	nd	1.65
	F	33.1	nd	11.8	10.2	27.4	9.63	0.22	1.54	4.34	0.20	nd	1.22
	G	27.7	nd	11.6	8.39	22.1	0.07	1.62	4.96	0.32		nd	0.99
	H	31.0	nd	13.7	9.42	20.2	17.6	nd	1.80	4.39	0.36	0.54	0.87
Mean		29.4	nd	14.0	9.92	21.3	16.3	0.06	1.79	5.03	0.23	0.09	1.68
SD		2.65	-	2.47	1.34	4.05	8.62	0.08	0.57	0.08	0.12	0.19	1.20
2 Months after resuming normal diet	A	14.1	0.71	17.7	11.1	28.0	0.42	0.41	3.19	14.1	1.70	2.02	6.33
	B	21.3	0.65	18.0	14.5	25.1	0.06	0.30	3.27	10.2	0.51	1.86	4.04
	C	27.7	0.34	17.3	14.2	21.5	0.10	0.50	3.9	10.6	0.56	1.37	1.72
	D	19.8	0.47	16.8	10.5	29.3	0.11	0.34	3.62	13.0	0.91	0.92	3.94
	E	15.2	0.24	20.4	11.9	29.3	1.16	0.40	4.09	11.9	1.00	1.07	4.07
	F	21.7	nd	20.8	14.6	30.2	0.51	0.49	3.03	5.73	0.48	0.67	2.44
	G	27.6	nd	16.1	12.1	28.5	0.43	0.52	3.95	9.50	0.71	0.80	0.82
	H	23.0	0.97	17.7	14.5	29.1	0.33	0.48	3.03	7.00	0.88	1.01	1.92
Mean		21.3	0.42	18.1	12.9	27.6	0.39	0.38	3.52	10.3	0.84	1.22	1.16
SD		5.00	0.35	1.66	1.70	2.91	0.36	0.17	0.44	2.91	0.40	0.50	1.77

nd = not detectable; for statistical analysis values were taken as zero

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