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DIETARY TREATMENT IN UREMIA

Renal function, protein and lipid metabolism

by

Per-Ola Attman

Göteborg 1978

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Renal function,
protein and lipid metabolism

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen
med vederbörligt tillstånd av medicinska fakulteten vid Göteborgs universitet
kommer att offentligt försvaras i aulan, Sahlgrenska sjukhuset
fredagen den 8 december 1978 kl. 9.

av

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med. lic.

Göteborg 1978

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This study is based on the following papers, which will be referred to by their Roman numerals:

I. Jagenburg, R., Attman, P-O., Aurell, M. and Bucht, H.: Determination of glomerular filtration rate in advanced renal insufficiency.
Scand. J. Urol. Nephrol. 12: 133–137, 1978.

II. Attman, P-O., Bucht, H., Isaksson, B. and Uddebom, G.: Nitrogen balance studies with amino-acid supplemented low-protein diet in uremia.
Accepted for publication Am. J. Clin. Nutr.

III. Attman, P-O., Ewald, J. and Isaksson, B.: Body composition during long-term treatment of uremia with amino-acid supplemented low-protein diet.
Accepted for publication Am. J. Clin. Nutr.

IV. Attman, P-O. and Gustafson, A.: Lipid and carbohydrate metabolism in uremia.
Submitted for publication Eur. J. Clin. Invest.

V. Attman, P.-O. and Gustafson, A.: Lipid and carbohydrate metabolism in uremia. Influence of treatment with protein-reduced diet and essential amino-acids.
Submitted for publication Nutr. Metabol.

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ABBREVIATIONS

α–Lp	= alpha lipoprotein
apo–	= apolipoprotein
BCM	= body cell mass
BW	= body weight
EAA	= essential amino-acids
EAAH	= essential amino-acids and histidine in proportions according to Rose
FFA	= non-esterified fatty acids
GFR	= glomerular filtration rate
HDL	= high density lipoproteins
HLP	= hyperlipoproteinemia
HTG	= hepatic triglyceride lipase
ILDL	= intermediate low density lipoproteins
IVFTT	= intravenous fat tolerance test
IVGTT	= intravenous glucose tolerance test
LCAT	= lecithin: cholesterol acyl transferase
LDL	= low density lipoproteins
LPL	= lipoprotein lipase
PHLA	= post-heparin lipolytic activity
SW	= standard weight for height
TBK	= total body potassium
TBW	= total body water
TG	= triglycerides
UC	= ultracentrifugation analysis
VLDL	= very low density lipoproteins

INTRODUCTION

The uremic syndrome is the result of derangement of not only excretory but also homeostatic, endocrine and metabolic functions of the renal tissue. Furthermore, it is also influenced by the attempts of the organism to adapt to these abnormalities.

In renal failure various *substances accumulate* in body fluids, particularly nitrogen-containing metabolites, among which urea dominates. Patients with advanced uremic symptoms often have high serum urea levels and improvement of symptoms during treatment, whether dietary treatment or dialysis is often linked to the reduction of urea levels. However, several studies have clearly demonstrated that urea is not responsible for uremic toxicity in the levels that occurs in renal failure but can serve as a marker for the accumulation of other nitrogenous metabolites, possibly toxic and appearing in much lower concentrations[18, 20, 66, 74].

In renal failure there are *disturbances of fluid and electrolyte homeostasis*, with loss of the ability to adapt to changes in fluid and electrolyte intake or loss.

The importance of the kidneys as *metabolically active organs* becomes apparent in renal failure[61]. Impaired erythropoetin production or hydroxylation of 25-hydroxycholecalciferol leads to anemia or development of renal osteodystrophy. Furthermore, the metabolic clearance by the kidney of insulin, glucagon, cyclic AMP and other metabolically active substances is greatly reduced and may be important for the uremic toxicity[74].

Changes in the intracellular milieu have been demonstrated in renal failure in man, mainly in white blood cells[112, 127] and muscle biopsies[17, 28, 112]. The abnormal intracellular amino-acid pattern in the uremic muscle[24] may indicate defective transport of amino-acids over cell membranes and several abnormalities of intracellular enzyme activity have been found[18, 74, 150]. It is also likely that secondary consequences of uremia such as malnutrition and other permissive abnormalities, contribute to the development of clinical uremic toxicity.

Stageing of uremia

There is still no general agreement on the nomenclature for the different stages of renal failure but we have used the following principles. During the development of renal disease there is usually a period of varying length during which the metabolic consequences of impaired renal function can readily be demonstrated by laboratory methods although the patient remains virtually free from symptoms. This stage has often been described as *azotemia*, implying a rentention of nitrogenous metabolites in the blood.

When the glomerular filtration (GFR) has decreased below 15 – 20 ml/min the first clinical symptoms of renal failure usually appear, such as gastrointestinal symptoms, fatigue and itching. By this time the patient has reached the stage of *uremia*. This stage can further be divided according to severity. When GFR has decreased to 2 – 3 ml/min symptoms of severe uremic intoxication, such as metabolic acidosis, encephalopathy and peripheral neuropathy, hyperkalemia, oliguria with oedema and pericarditis, generally develop. This stage is called *terminal uremia*, implying that the patient will no longer survive unless immediate replacement therapy, dialysis or transplantation, is started.

The rate at which the patient will go through the different stages of renal failure depends above all on the rate of GFR decrease.

Conservative treatment of uremia

In many patients the time between first appearance of uremic symptoms and terminal uremia can be counted in months or even years. During this interval therapeutic measures can be taken that may drastically improve the patient's situation. These measures are often summarized under the heading *conservative treatment* of uremia, as opposed to *active treatment* i.e. dialysis and transplantation.

The aim of the treatment is to improve the general condition of the patient and prepare him for future dialysis and transplantation. The main objectives of the treatment are 1) *to correct fluid and electrolyte balance and acidosis;* 2) *to reduce accumulation of nitrogenous metabolites* in part reflected by serum urea; 3) *to improve nutrition* and counteract catabolism; and 4) *to treat complicating conditions* that aggravate renal failure, such as urinary tract infections or obstruction.

Points 2 and 3 can be achieved by dietary treatment. There is long-term evidence to show that reduction of protein intake leads to improvement of several symptoms of uremia and to reduced accumulation of urea. The dilemma is how to achieve a balance between the aim of reducing toxicity by strict protein reduction and the risk for development of protein malnutrition during prolonged treatment.

Modern dietary treatment of uremia was started in 1963, when Giordano[70] demonstrated that endogenous urea could be utilized for protein synthesis in uremic patients on low-protein diet. Based on these results Giovannetti and Maggiore[71] demonstrated that nitrogen balance could be achieved with a diet containing only 20 g protein of high biological value and the "Giordano-Giovannetti diet" was created for treatment of uremic patients[144]. Long-term difficulties were, however, encountered (bleeding tendency, polyneuropathy and pericarditis)[27, 100, 101].

It was realized not only that the requirements of essential amino-acids may be greater in uremia than can be met by the 20 g diet, but also that the total amount of nitrogen supplied was often too low[98, 100]. Thus the 20 g diet had to be supplemented with essential amino-acids[19, 23, 98, 100]. Moreover, histidine was shown to be an essential amino-acid in uremia[21].

An alternative supplementation has became available with the introduction of keto-analogues for five of the essential amino-acids[160], permitting a total nitrogen intake of only 4 g with maintained nitrogen balance.

With the supplemented 20 g protein diet and a total nitrogen intake of 4.5 – 6 g/day a positive nitrogen balance could be achieved and satisfactory long-term clinical results were demonstrated without development of malnutrition or neuropathy[23, 25, 121, 122]. However, it has been argued that this severe protein reduction might imply a risk for a continuous but slow development of protein malnutrition, and that patients with uremic symptoms should be treated with a more moderate reduction of protein[54, 97, 162, 169].

In short term studies, treatment with 40 g protein of high biological value has given equally good results with respect to nitrogen balance as the amino-acid-supplemented 20 g diet[96] but it has also been found that several patients on a 40 g protein diet had a negative nitrogen balance unless the diet was supplemented with essential amino-acids and histidine[69, 168].

The protein requirement of uremic patients is not known and is undoubtedly variable depending on pretreatment condition, age, energy need and also the amino-acid composition of the proteins and the supplementation.

The question of *recycling of non-protein nitrogen* has been the subject of considerable discussion since Giordano[70] and later Josephson[94] and Richards[133] demonstrated that labelled urea nitrogen was incorporated into body protein. Urea, degraded by bacterial urease to ammonia in the gut, can be reabsorbed. In healthy individuals this nitrogen recycling is presumably small in magnitude but it may be significant in uremia or during low protein intake[132]. Recycling of urea may even be less important than reutilization of nitrogen by increased transamination prior to urea formation[131, 132].

Principles of dietary treatment

The diet and the supplementation should provide sufficient amounts of non-essential and essential amino-acids and energy to maintain nitrogen balance without increase in the accumulation of nitrogenous metabolites thought to be of importance for uremic toxicity. In the absence of clinically applicable objective indices for assessing uremic toxicity, monitoring of urea levels and GFR seems to be the best guide.

A 20 g protein diet can be made varied, palatable and convenient. The protein sources are mainly meat, fish and eggs and the vegetable protein amounts to only a few grams. This means that the dietary protein has a high biological value. The energy requirement is met with protein-free bread and pasta products, together with a liberal use of fat, sucrose and hydrolyzed corn starch solutions. The diet has to be supplemented with vitamins and minerals.

The supplemental amino-acids have to be administered in coated tablets to conceal their obnoxious taste and the large amounts of tablets needed may sometimes cause practical problems for the patient.

Evaluation of long-term dietary treatment

The long-term results should be viewed not only isolated but also in the context of the results of dialysis treatment. In addition to the uremic neuropathy[25] and renal osteodystrophy[7], malnutrition and rapid development of atherosclerotic vascular disease may emerge as major problems and a suitable dietary treatment should not lead to development of such complications. As a background to the study to be presented, certain principles will be reviewed.

Assessment of nutritional status

Routine clinical methods[145] can not always be used in uremia. Body weight may fluctuate owing to variations in hydration. Low molecular weight proteins or metabolites may accumulate owing to impaired renal excretion. Tests of immunological function may be influenced by the uremia itself, without relation to nutrition. Among possible methods for assessment of nutritional status, the following three may be considered.

Nitrogen balance techniques: The validity of nitrogen balance studies is above all determined by the accuracy in determining nitrogen losses[91]. It is dependent on the patient's cooperation and requires hospitalization, which creates an artificial setting with for example, a reduction of the patient's physical activity in most cases. Furthermore, the nitrogen balance technique is not suitable for long-term studies.

Body composition determinations have advantages for long-term studies, particularly repeated determinations of body potassium and body water. However, calculations of body cell mass (BCM) from exchangeable potassium (^{42}K) involve uncertainities of isotope equilibration[36] which can be avoided by the use of a whole-body counter measuring the naturally occurring ^{40}K emission. Furthermore, the relationships between total body potassium and total body water and BCM, extracellular water and body fat may be different from normal, owing to the abnormalities of intracellular composition in uremia (paper III).

Taking these problems into account, estimation of BCM may nevertheless have great advantages for determination of nutritional status, since it can be carried out with good precision, is atraumatic and causes little inconvenience to the patient. However, only the net changes in BCM are determined, not the relative contribution of protein synthesis and breakdown.

Measurements of plasma proteins, plasma amino-acids and intracellular amino-acids:
Serum protein determinations are simple but the important problem is still to define which proteins that reflect nutritional status but are not influenced by a reduced GFR[99]. Several proteins with rather rapid turnover have been investigated e.g. transferrin, retinol-binding protein, prealbumin and complement factors[84, 139, 163, 168].

Transferrin levels are usually reduced in uremia and tend to improve with intensified nutrition[84, 121, 163]. However, the reduction of transferrin is present long before uremic symptoms appear with a rather moderate reduction of GFR[125], and is probably not related to nutrition. Furthermore, transferrin levels are influenced by the iron status of the patient.

Plasma amino-acid levels and especially relationships between certain essential and non-essential amino-acids, such as the valine/glycine ratio, may reflect acute nutritional deficiencies but are also influenced by the preceding meal[99]. The intracellular amino-acid pattern, as demonstrated in muscle biopsies, has been found to be abnormal in uremia, with a reduction of essential amino-acids not parallel to the changes in plasma[24]. Treatment with essential amino-acids has been found to improve the intracellular amino-acid pattern[24].

Development of atherosclerotic vascular disease

Treatment of uremia with dialysis and also with transplantation has been linked to an accelerated development of atherosclerotic vascular disease. Long-term observations[8, 107] have indicated cardiovascular disease to be the most important complication of treatment of uremia with respect to survival.

Several of the risk factors for development of atherosclerosis e.g. hypertension, platelet dysfunction, hyperuricemia, hyperparathyreoidism and particularly disturbances of lipid and carbohydrate metabolism, are present in uremia[74]. The dietary treatment could have negative influence on these disturbances of lipid and carbohydrate metabolism and assessment of such an influence therefore seems to be of significant interest.

Lipid and carbohydrate metabolism

Serum lipids are transported together with specific proteins (apolipoproteins) as *lipoproteins.* Based on differences in density, the lipoproteins can be described by a density spectrum which in turn is related to the relative amount of their components. They can be classified as high-density lipoproteins (HDL), low-density lipoproteins (LDL), very-low-density lipoproteins (VLDL) and chylomicrons (Fig. 1). Lipoproteins can also be classified according to their electrophoretic mobility into α-lipoproteins, pre-β-lipoproteins, β-lipoproteins and chylomicrons. The difference in mobility is determined by the presence of different apolipoproteins on the lipoprotein surface.

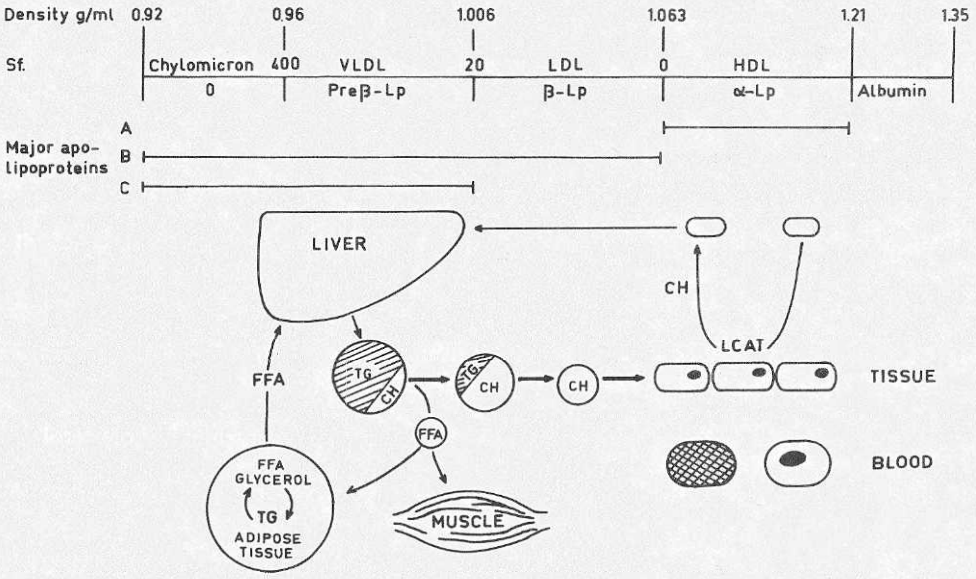

Fig. 1. Lipoprotein spectrum and metabolism with schematical presentation according to density classes, electrophoretic mobility and distribution of major apolipoproteins. (VLDL = very low density lipoproteins, LDL = low density lipoproteins, HDL = high density lipoproteins, Lp = lipoproteins, LCAT = lecithin: cholesterol acyl transferase, TG = triglycerides, CH = cholesterol, FFA = non-esterified fatty acids).

So far three major apolipoproteins have been identified – apolipoprotein A (apo–A), apolipoprotein B (apo–B) and apolipoprotein C (apo–C) – in addition to four minor apolipoproteins. These apolipoproteins are in turn characterized by their constitutive polypeptides, apo–A_I and apo–A_{II}, apo–B (presumably composed of two non-identical polypeptides) and apo–C_I, –C_{II} and –C_{III}.

Apart from having a function in lipid transport, these apolipoproteins also participate as activators or inhibitors of enzymes in lipoprotein catabolism[58, 73].

Lipoprotein metabolism

Dietary fat, primarily triglycerides (TG), is transported together with small amounts of cholesterol and protein in chylomicrons from the intestine to the general circulation. The nascent chylomicron contains apo–B and apo–A from the intestinal cell as its protein moiety and receives additional apo–C from HDL when entering the circulation[82]. The load of TG is hydrolysed by lipoprotein lipase to free fatty acids (FFA) and glycerol. FFA can be reassembled with locally synthesized glycerol to TG and stored in the adipose tissue or be utilized in peripheral tissues for energy production.

About 20 g of TG is formed daily in the liver and released to the circulation as VLDL–TG. TG production is regulated by availability of substrate – FFA preferentially released by lipolysis in adipose tissue and glycerol from glucose through the action of α-glycerophosphate and with insulin as a prerequisite.

VLDL are catabolized, in analogy with the chylomicrons, to glyceril and FFA by the action of lipoprotein lipase. This transformation is also accompanied by the return of apo-C and surface lipids to HDL where the lipids form the substrate for the lecithin: cholesterol acyl transferase (LCAT)-reaction[57, 58].

LDL, the major cholesterol-carrying lipoprotein, is catabolized after internalization in various peripheral cell systems. The liberated cholesterol is partly utilized for membrane synthesis. The internalization requires specific LDL receptors on the cell surface. Familial hypercholesterolemia is characterized by a defect in these LDL receptors[37]. The liberated intracellular cholesterol is subsequently taken up by HDL in a centripetal cholesterol transport[57, 104].

HDL is possibly formed in the intestine and liver[58, 82] and its major protein moiety is apo–A^{166}. HDL serves as a donator of the lipase-activating peptides, apo–C_I and –C_{II}, and as a platform for cholesterol esterification by LCAT. The major portion of HDL cholesterol esters presumably returns to the liver in the centripetal cholesterol transport to be catabolized to bile acids[104].

Fatty acids

Fatty acids are components for several lipids e.g. TG, cholesterol ester and phospholipids, and also appear as albumin-bound free fatty acids (FFA).

The fatty acids may be saturated or unsaturated, essential or non-essential (Table I). The primary essential fatty acid in humans has been found to be linoleic (18:2) acid in the (n – 6) series[89], from which arachidonic (20:4) acid can be synthesized. When lipolysis in adipose tissue is increased the excess FFA is utilized in the liver for glucose or TG production, which may cause an increase in VLDL production and release into the blood.

	Name	Carbon atoms and double bonds
Saturated	palmitic acid	16:0
	stearic acid	18:0
Unsaturated non-essential	palmitoleic	16:1 (n–7)
	oleic acid	18:1 (n–9)
Unsaturated essential	linolenic acid	18:3 (n–3)
	linoleic acid	18:2 (n–6)
	arachidonic acid	20:4 (n–6)

Table I. Some important naturally occurring fatty acids

Phospholipids

Phospholipids are present in serum lipoproteins and cell membranes and lecithin is the major (70 %) phospholipid in human serum. Lecithin is synthesized in the liver along two major pathways (Fig. 2). Pathway I (Kennedy pathway) is quantitatively most important, yielding palmitic (16:0) acid in the 1-position and linoleic (18:2) or oleic (18:1) acid in the 2-position[14]. A decrease of this synthesis pathway is seen in man after sucrose feeding[1] while an increase was observed after administration of linoleic acid[148]. Pathway II (Greenberg pathway) preferably gives stearic (18:0) acid in the 1-position and arachidonic (20:4) acid or other polyunsaturated fatty acids (e.g. 22:6) in the 2-position. This pathway appears to be more active in females and under the influence of estrogen[93]. Influences on one lecithin synthesis pathway appear to cause reciprocal changes in the other.

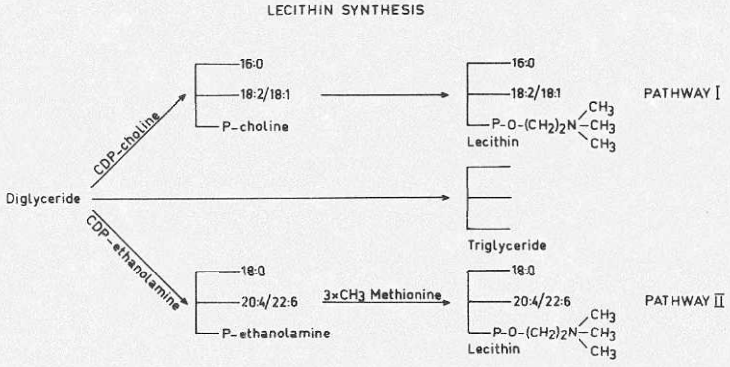

Fig. 2. Major pathways of liver lecithin synthesis. Pathway I (Kennedy pathway): Cytidine-diphosphate (CDP) – choline diglyceride pathway. Pathway II (Greenberg pathway): Cytidine-diphosphate (CDP) ethanolamine methylation pathway.

Lecithin cholesterol acyl transferase (LCAT)

In human serum about 70 per cent of cholesterol is in esterified form and 30 per cent is unesterified. Esterification of cholesterol in plasma is mediated by the enzyme LCAT, synthesized in the liver and carried with HDL[72, 158]. The fatty acid in the 2-position of lecithin is transferred to the 3-position of cholesterol (Fig. 3). This reaction is influenced by the fatty acid composition of lecithin, with linoleic (18:2) acid as a preferred substrate[143]. Decreased availability of the preferred substrate in LCAT might result in impaired removal of VLDL[148].

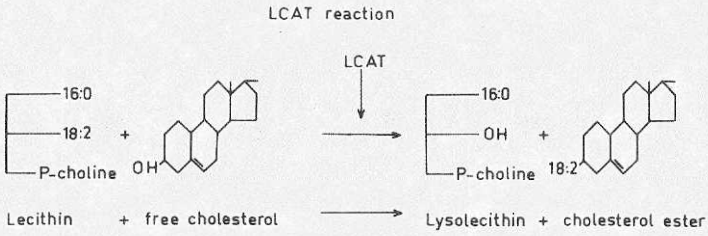

Fig. 3. LCAT-reaction. The enzyme lecithin: cholesterol acyl transferase (LCAT) catalyzes in serum the transfer of fatty acid in 2-position of lecithin into 3-position of cholesterol. 16:0 = palmitic acid, 18:2 = linoleic acid.

Lipoprotein lipase (LPL)

TG removal from VLDL and chylomicrons occurs in the capillary bed of most tissues by hydrolysis of TG through the action of the LPL system. Heparin and other polyvalent anions can liberate LPL from the tissues into the circulation and it can then be measured as postheparin lipolytic activity (PHLA). In postheparin plasma at least two[56, 76] or three[68] different lipase systems have been demonstrated. One (or two) of them is LPL activity and the other is called hepatic triglyceride lipase (HTG) or salt-resistant lipase. Apo–C_I and –C_{II} have been found to be essential activators for LPL hydrolysis of chylomicrons and VLDL–TG[68]. C_I and C_{II} are presumably recirculated from VLDL to HDL. LPL is stimulated by insulin and also by other hormonal influences and nutritional status[40]. Both low[40, 68, 76, 128] and normal[118] activity of PHLA or adipose tissue lipoprotein lipase have been found in hypertriglyceridemia.

The function of the HTG has been suggested to be in the further conversion of intermediate low density lipoproteins (ILDL) or remnant lipoproteins to LDL[76, 113]. Reduced activity of the HTG has been detected during estrogen administration[4] while androgens seems to increase the activity of this enzyme[55].

Intermediary metabolism

Protein, lipid and carbohydrate metabolism are closely linked to one another at several levels in the intermediary metabolism particularly in relation to gluconeogenesis and glucose utilization.

From glucose can be derived glycerol, via α-glycerophosphate, fatty acids, via acetyl-CoA and beta-oxidation and the carbon skeletons for amino-acids, via puruvate and lactate. Glucose can be formed from glycerol, the carbon skeletons of amino-acids, via puruvate and oxaloacetate and from lactate.

Transport of lipids is dependent on protein metabolism for synthesis of apolipoproteins, LCAT and LPL.

During metabolic stress from different causes, such as chronic disease, e.g. uremia, starvation or protein depletion, these interrelationships become more apparent[12, 44]. During low protein intake adaptive changes occur in the intermediate metabolism to preserve protein in the organism[147]. These adaptations are, however, dependent on the availability of carbohydrate and fat for energy production[44, 147]. Amino-acid-conserving enzymes increase their activity and nitrogen excretion is reduced[110, 133]. Insulin acts as an anabolic hormone in the preservation of the structural integrity of the cell. When, on the other hand, the energy supplies are insufficient, owing to e.g. anorexia, amino-acids are more readily catabolized for energy production and the formation of urea and other nitrogenous metabolites increases. With impaired renal function this catabolism causes a rapid accumulation of non-protein nitrogen, as indicated by elevated serum urea levels.

AIMS OF THE STUDY

In view of the problems outlined in the introduction it was thought to be of interest to investigate the effect of dietary treatment in uremia, with the following specific objectives:

1. To find a method for functional classification of patients by accurate determination of glomerular filtration rate (GFR) in renal insufficiency and to evaluate the limitations of GFR for successful dietary treatment.
2. To evaluate the amount of essential amino-acid supplementation to a 20 g protein diet necessary to maintain nitrogen balance.
3. To study to which extent the dietary treatment can maintain the body composition during long-term treatment.
4. To investigate lipid and carbohydrate metabolism in patients with uremia and the influence of dietary treatment.
5. To study the adherence to the diet during long-term treatment in uremic patients.

PATIENT SERIES AND MANAGEMENT OF PATIENTS

The different patient series were collected from among about 150 consecutive patients with uremia treated with protein-reduced diet at Sahlgrenska sjukhuset during 1970 – 1978.

Certain patients were included in more than one series, as demonstrated in Fig. 4. The total number of individuals studied was 80 (55 men and 25 women, aged 18 – 71 years). Selection of patients for study was determined by availability of investigation, ward and staff capacity and by the patient's willingness to participate. Patients with terminal uremia, oliguria, severe heart disease or other conditions rendering them inaccessible for long-term treatment were excluded.

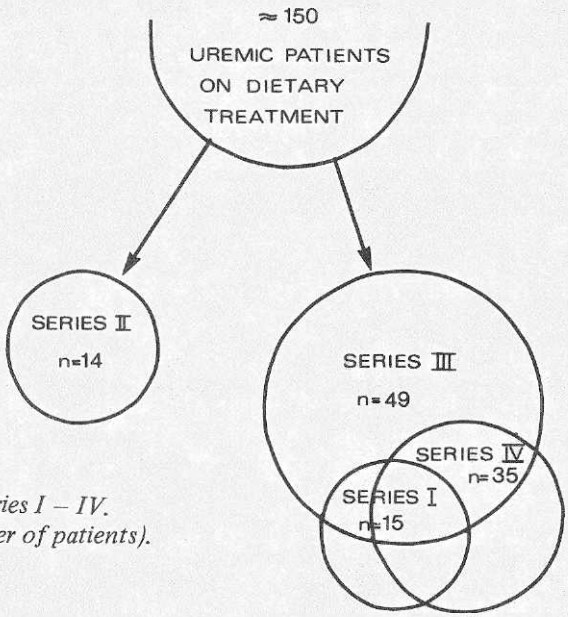

Fig. 4. Patient series I – IV.
(n = number of patients).

Patient series I (May 1975 to June 1976). Renal function studies (Paper I).

Fifteen patients (10 men and 5 women, mean age 46 years) were investigated before treatment and nine of these patients were examined at three-month intervals during treatment. In addition, two anephric women on dialysis were studied between two dialysis sessions.

Patient series II (February 1970 to November 1971). Nitrogen balance studies (Paper II).

Seventeen consecutive patients (10 men and 7 women, mean age 48 years) were selected for this study. Results from only 14 of these patients will be reported since three patients were started on nitrogen balance studies but had to be withdrawn because of rapidly developing terminal uremia, with severe gastrointestinal symptoms, overhydration and immediate need for dialysis. Clinical details of the 14 patients are shown in Table I, paper II.

Patient series III (September 1974 to March 1977). Body composition studies (Paper III).

Altogether 49 patients (37 men and 12 women, mean age 45 years) were studied, 38 of whom (30 men and 8 women) were investigated immediately before the start of dietary treatment. Twenty of the patients were followed during treatment. Thirteen of the remaining 18 patients had to be withdrawn for various reasons (dialysis 9, transplantation 2 and refusal to participate 2 patients), 3 patients died early and 2 patients had not participated for more than two months when the study was terminated. Eleven additional patients (7 men and 4 women), who started treatment before facilities for whole-body counting became available were studied during treatment alone. Thus 31 patients were examined at three-month intervals during treatment for 3 – 12 months (mean 9.2 months).

Patient series IV (June 1975 to March 1978). Lipid and carbohydrate metabolism (Paper IV – V).

Twenty-eight consecutive patients (20 men and 8 women, mean age 47 years) were investigated before the start of dietary treatment (Series IV A, paper IV).

In addition, 7 patients (5 men and 2 women) participated in certain investigations of lipid metabolism.

Six out of the 28 patients in series IV A did not complete three months' treatment, while 22 patients (16 men and 6 women, mean age 45 years) were investigated every three months during treatment for on average 9.1 months (series IV B, paper V).

Management of patients

All patients had the accepted *indications for treatment* with protein-reduced diet i.e. chronic irreversible renal failure with uremic symptoms. Most of the patients had mild to moderate clinical manifestations of uremia, such as gastrointestinal symptoms with anorexia, nausea and occasional vomiting, pruritus and fatigue. No patient had evidence of severe renal osteodystrophy or neuropathy.

The patients were admitted to the renal ward for initiation of dietary treatment and correction of disturbances in fluid and electrolyte balance and metabolic acidosis, as appropriate. Initially, the patients were given detoxification treatment with the 20 g protein diet only or in a very few cases, intravenous glucose and fat until oral nutrition could be started.

After 6 – 10 days an improvement of symptoms was generally observed together with a decrease of the serum urea level. Supplementation of the diet with the essential amino-acids and histidine (EAAH) was then started and the patients were discharged from the hospital shortly thereafter. The patients were then controlled at a special outpatient dietary clinic. The patients in series IV B were readmitted to the hospital for 3 – 4 days every three months for metabolic studies.

Duration of treatment (Fig. 5)

50 % of the patients were treated for 6 months and 25 % for 12 months. Forty-six of the 80 patients were put on hemodialysis treatment after on average 9.0 months, when the uremic symptoms could no longer be controlled by the dietary treatment. Thirteen patients were transplanted after on average 7.3 months without previous dialysis. Ten patients who were considered unsuitable for dialysis died from uremia after on average 7.1 months, while 2 patients died from myocardial infarction and septicemia respectively. Eight patients were still on dietary treatment (mean duration 8.1 months) at the end of the investigation (March 1978). In one patient with primary amyloidosis dietary treatment was withdrawn after 18 months owing to improvement of renal function.

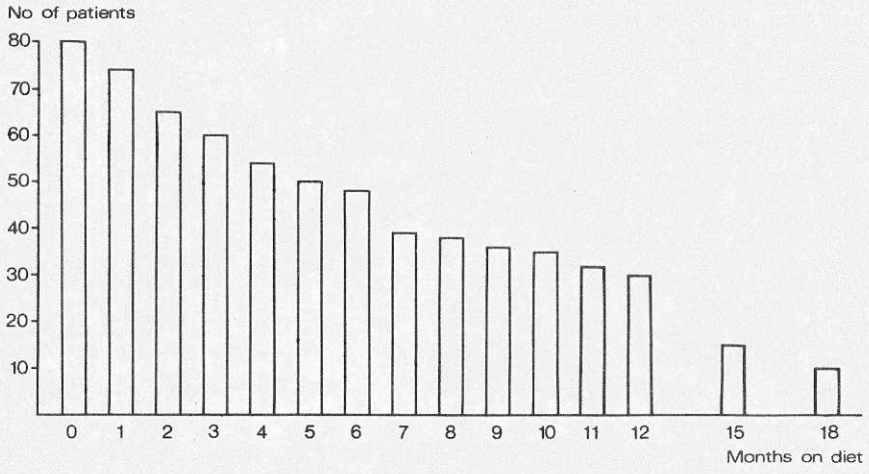

Fig. 5. Duration of dietary treatment in 80 patients with uremia (series I – IV).

Dietary treatment

Dietary counselling was given to all patients by the same dietitian. The patients were also given thorough information by the physician about the principles and aims of the treatment. After taking a dietary history, the dietitian gave individualized counselling to the patient and to his or her spouse in several sessions. The normal food habits of each patient and his family were as far as possible adapted to the dietary principles. The patients were also provided with a special cookery book for uremic patients[164].

After discharge from hospital the patients were seen at the outpatient dietary clinic by the author and the same dietitian, who collaborated to achieve the best possible dietary adherence and patient education.

Composition of the diet (Series III and IV)

The diet was based on a varied four-week menu[164], and an average daily protein intake of 20 g from varied sources was prescribed together with an energy content of 35 – 45 kcal (150 – 190 kJ)/kg BW. Energy was mainly supplied from dairy fat (butter, double cream) and protein-free bread, pasta products, sweet preserves etc. When necessary, a partially hydrolyzed corn starch solution (Trilgar®) was added.

Dietary adherence

In order to study the patient's adherence to the diet and to provide a basis for the study of the influence of diet on lipid and carbohydrate metabolism, four-day records of food intake were collected at three-month intervals from 19 patients in series IV B.

Since the dietary principles and counselling were identical for the patients in series III and IV, the results were considered generally representative for our patients on dietary treatment with the 20 g protein diet and EAAH.

The patients recorded, after receiving detailed instructions, their food intake during the four days (Wednesday – Saturday) preceding their follow-up admission to the hospital. The records were checked and completed by the dietitian with the help of the patient. Food tables were used for calculation of the daily intake of energy, protein, fat, carbohydrate and sucrose and for estimation of the ratio of polyunsaturated to saturated fatty acids (P/S). The records from two patients were incomplete and omitted while the records from 17 patients, covering in all 204 days could be evaluated (Table II).

The dietary content of essential amino-acids (EAA) could be calculated for on average 70 % of the protein intake. Assuming the same proportions of EAA in the remaining 30 % the mean calculated daily intake of EAA from the food was 8.0 g, corresponding to a mean E/T ratio of 2.3. The daily intake of individual amino-acids, expressed in per cent of the minimum requirement[134], is shown in Fig. 6.

	Energy kcal (kJ)	Protein g	Carbo-hydrate g	Sucrose g	Fat g	p/s-ratio
mean	2403 (10100)	20.3	291	99	125	0.34
SD	542 (2280)	2.8	93	44	25	0.09
per kg BW mean	35 (150)	0.3	4.2	1.4	1.8	

Table II. Calculated daily food intake in 17 patients with uremia on a protein-reduced diet. Mean values are derived from averages of 1 – 6 four-day records per patient. Sucrose content could be calculated for 92 % of dietary carbohydrate and ratio of polyunsaturated to saturated fat (p/s-ratio) for 88 % of dietary fat.

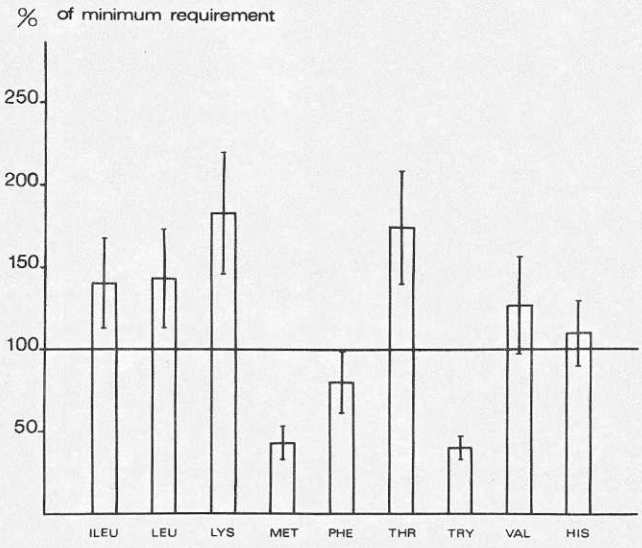

Fig. 6. Average daily content of the essential amino-acids and histidine in the 20 g protein diet. The content of amino-acids is expressed as per cent (mean ± SD) of minimum requirement[134].

In 13 patients records from two or more registration periods were available and the constancy of dietary adherence could be estimated. The mean difference in per cent (± SD) of registered intake between the first four-day record and a subsequent record was for:

Energy	10.1 ± 7.1 %	Carbohydrate	16.5 ± 13.0 %
Protein	9.7 ± 5.9 %	Sucrose	25.9 ± 27.7 %
Fat	12.7 ± 6.9 %	P/S ratio	26.3 ± 17.0 %

Supplementation with essential amino-acids and histidine (EAAH)

The EAAH were given in coated, tasteless tablets or (Series I) by i.v. infusion (Aminess®, AB Vitrum, Stockholm, Sweden).

Fifteen tablets or 200 ml of infusion solution contained:

	g	g N
L–isoleucine	1.15	0.12
L–leucine	1.65	0.16
L–lysine acetate	(1.70)	
eq. lysine	1.20	0.23
L–methionine	1.65	0.15
L–phenylalanine	1.65	0.14
L–threonine	0.75	0.09
L–tryptophan	0.38	0.05
L–valine	1.20	0.14
L–histidine	0.83	0.23
	10.46	1.31

The above dose corresponds to 1.5 times the minimum requirement for the essential amino-acids according to Rose[134].

Orally, 5 tablets were given three times a day at mealtimes, while the patients on i.v. substitution received 200 or 400 ml, as described in detail in paper II.

Medication

The diet was routinely supplemented by 1 – 2 iron and multivitamin tablets daily (Duroferon vitamin®, AB Hässle, Göteborg, Sweden). Sodium bicarbonate, 3 – 6 g/day, was prescribed for all but a few patients. Fluid retention, hypertension and cardiac insufficiency were treated when necessary with appropriate drugs (furosemide, hydralazine, propranolol, alprenolol, clonidine, digitoxin). No patient received corticosteroids or cytotoxic drugs except for two patients in series III, who were maintained on 5 mg of prednisolone daily.

Statistical methods

To describe data means and standard deviations have been used. To evaluate the effect of treatment or to compare groups of patients, Student's t-test was used either on original variables, assuming a normal distribution of the observations, or on transformed variables. Thus measurements of lipid variables have been subjected to a logarithmic transformation, due to apparent skewness observed in other materials. Non-parametric methods have been used in other parts of the study. Correlations have been computed with Spearman's rank correlation method. Values of $p \leqslant 0.05$ were considered significant.

METHODS AND RESULTS

Except where otherwise specified, the following methods were used in the different patient series. Creatinine was determined by an alkaline picrate method[85], without precipitation, adapted for automatic analysis (Vickers M300). The serum urea concentration was determined with a Technicon Autoanalyzer (method N1 C). The serum albumin concentration was measured by a bromocresol green-binding method using Albustrate® with measurement of absorbance 15 s after mixing reagent and serum. Serum iron and total iron binding capacity (TIBC), as a measure of transferrin, was determined according to Zak and Epstein[170]. From April 1977, however, ferrozine was used as chelator instead of bathophenantroline. The serum concentrations of creatinine, urea, albumin and TIBC were determined by the Department of Clinical Chemistry, University of Göteborg, at this hospital.

Determination of the glomerular filtration rate (GFR). Series I (Paper I).

Methods

Glomerular filtration rate (GFR) was determined by conventional clearance technique with collection of urine without indwelling catheters and measurement of plasma and urine concentration of the filtration markers (endogenous creatinine, inulin and ^{51}Cr–EDTA) – henceforth called *renal clearance* and calculated from the 160 min clearance of creatinine, inulin and ^{51}Cr–EDTA and 24 h clearance of creatinine and ^{51}Cr–EDTA. Clearance of ^{51}Cr–EDTA was also calculated by determination of the plasma elimination curve after single injection of the marker – henceforth called *plasma clearance.* The clearance value was obtained from the general formula Cl = D/A, where D is the dose of the marker injected and A the area under the plasma elimination curve.

160 min GFR determinations were done in the morning during four periods of 40 min duration. Collection of urine began 40 min after i.v. administration of inulin (Inutest® 0.2 ml/kg BW of 25 % solution) and ^{51}Cr–EDTA (50 – 200 μCi, Behringwerke AG). Blood samples were drawn 60, 100, 140 and 180 min after injection of the marker i.e. in the middle of each clearance period. The average diuresis was 2 – 5 ml/min i.e. about 50 % of GFR.

The **renal 24 h clearance (creatinine and ^{51}Cr–EDTA)** was determined during the 4 – 28 h following the injection of ^{51}Cr–EDTA. The clearance was determined from the plasma creatinine concentration at the beginning of the clearance period and from the mean ^{51}Cr–EDTA concentration.

Plasma clearance of ^{51}Cr–EDTA was determined 3 – 4 h and 4 – 28 h after injection with plasma sampling at 180, 200, 220 and 240 min and 24 h after injection of the marker. Clearance was calculated from the dose of ^{51}Cr–EDTA and the plasma elimination curve, as described by Brøchner-Mortensen[41].

Inulin was determined with blank correction as described in paper I. ^{51}Cr–EDTA was determined in a well scintillation counter with 3 ml sample volume and 30 min counting time.

GFR during dietary treatment: GFR was also determined from the 160 min ^{51}Cr–EDTA renal clearance every three months during dietary treatment in 24 patients from series III and IV.

Results

Determination of GFR: *Renal inulin clearance (160 min)* was chosen as the reference determination of GFR.

Renal ^{51}Cr–EDTA clearance (160 min) (Fig. 7) was closely correlated to inulin clearance, without systematic deviation.

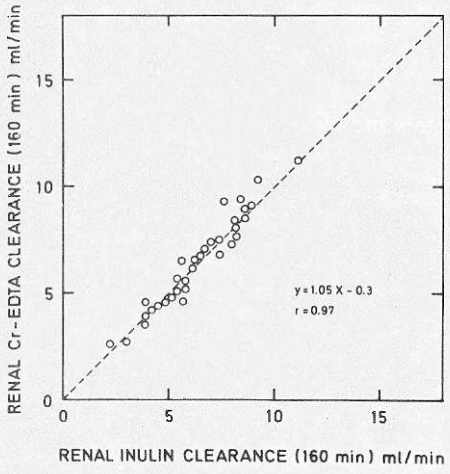

Fig. 7. Correlation between renal ^{51}Cr–EDTA clearance (160 min) and renal inulin clearance (160 min).

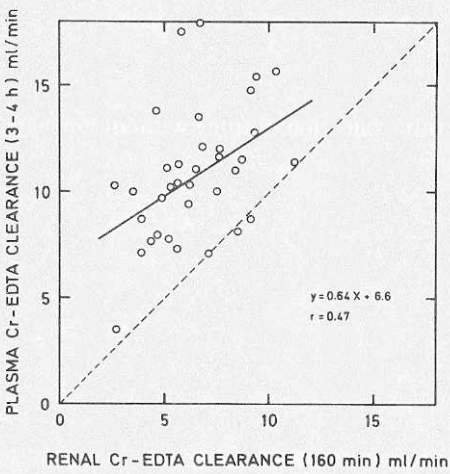

Fig. 8. Correlation between plasma clearance (3 – 4 h) and renal clearance (160 min) of ^{51}Cr–EDTA.

Plasma ^{51}Cr–EDTA clearance (2 – 4 h) (Fig. 8) overestimated GFR by on average 4.2 ml/min in the range 2.6 – 11.2 ml/min. Plasma clearance of ^{51}Cr–EDTA was, however, better correlated to the renal clearance when studied during the 4 – 28 h after injection of the marker (Fig. 7, paper I).

Serum creatinine was poorly correlated to GFR (Fig. 2, paper I) and *renal creatinine clearance (160 min)* overestimated GFR by on average 30 per cent (Fig. 3, paper I).

Renal clearances of creatinine and ^{51}Cr–EDTA (24 h) were poorly correlated to the corresponding 160 min renal clearances, giving on average 37 per cent lower values (Fig. 5, paper I).

Plasma ^{51}Cr–EDTA clearance in anephric patients (Table I, paper I). Calculations of the elimination of the marker 16 – 20 h after injection indicated an extrarenal clearance of approximately 2 ml/min.

GFR during dietary treatment

There was a continuous decrease of GFR during treatment (Fig. 9). The mean GFR was 7.8 ± 2.5 (SD) ml/min at the start of treatment and it is apparent that only a few patients with GFR < 4 ml/min remained on treatment for three months or longer. Furthermore, patients with GFR < 4 ml/min at the start did not complete 3 months' treatment.

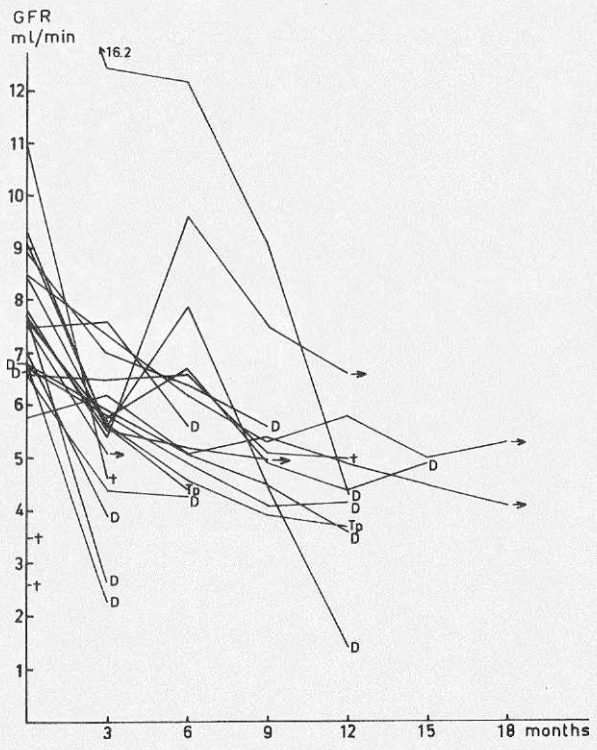

Fig. 9. Glomerular filtration rate (GFR), measured from the renal clearance (160 min) of ^{51}Cr–EDTA, in 24 patients with uremia during dietary treatment. Symbols (D = dialysis, Tp = transplantation, † = patient death, → = continued treatment) indicate status at the next three-month follow-up.

Nitrogen balance studies. Series II (Paper II).

Methods

After admission to the hospital, the patients received a 20 g protein diet (*basic diet*) only for 2 to 6 days, after which period supplementation with EAAH in different dosages was started, generally in periods of six days, as described in detail in paper II.

Nitrogen intake was measured by analysis of duplicate portions and left-overs during all but three balance studies, when the intake was calculated from food tables. Nitrogen in food and urine (24 h periods) was determined by micro-Kjeldahl technique using an auto-analyzer system (Technicon methods N–3b, N38).

Nitrogen balance was calculated as the *apparent nitrogen balance* i.e. the difference between nitrogen intake and nitrogen excretion in the urine. The mean daily apparent nitrogen balance was derived from the whole balance period.

The *corrected apparent nitrogen balance* was estimated taking into account changes in serum urea and estimations of total body water[140]. This correction of nitrogen balance is greatly influenced by the accuracy of the serum urea determinations. True and false day-to-day variations of only a few mmol/l are common and imply corrections by as much as 0.5 – 1 g N/day, which is highly significant within the nitrogen balance range studied. In order to minimize these possible errors, the mean daily change in total urea nitrogen for all patients during each balance period was used when estimating the mean corrected apparent nitrogen balance.

Patients with only two days on basic diet were apparently not always in steady state, while six days were judged to be sufficient from the observations in one patient who was studied for 14 days during one balance period and had reached a steady state within six days. The mean apparent nitrogen balance was therefore weighted according to the length of the balance period.

Accurate measurements of all extrarenal losses are important but very difficult to perform. Earlier studies[23] indicate that the fecal loss of nitrogen of about 1 g N/day in uremia is not influenced by diet or EAAH. Accurate determinations of dermal losses of nitrogen in uremia have not been published but there is no reason to assume that they are less than 1 g N/day estimated as a mean value in non-uremic subjects[91]. Extrarenal losses of nitrogen in uremia may thus be assumed to be about 2 g N/day.

Results (Table III, fig. 10)

a) Basic diet – 20 g protein (14 patients)

All patients had a negative apparent nitrogen balance, mean -2.1 ± 1.3 (SD) g/24h (men -2.1 ± 1.7, women -2.1 ± 0.9).

	Basic diet	Basic diet + i.v. EAAH		Basic diet + EAAH orally
	20 g/24 h	2.6 g N/24 h	1.3 g N/24 h	1.3 g N/24 h
No of patients	14	12	7	10
No of days	60	95	42	96
Apparent N-balance				
mean	−2.09 g N/24 h	+1.35 g N/24 h	+0.37 g N/24 h	+1.22 g N/24 h
SD	± 1.32 g N/24 h	± 1.75 g N/24 h	± 1.30 g N/24 h	± 1.71 g N/24 h
Corrected apparent N-balance				
mean	+0.04 g N/24 h	+1.31 g N/24 h	+0.52 g N/24 h	+1.96 g N/24 h

Table III. Apparent and corrected apparent nitrogen balance in uremic patients treated with protein-reduced diet. Effect of supplementation with essential amino-acids and histidine (EAAH). The mean values were derived from the average nitrogen balance per patient.

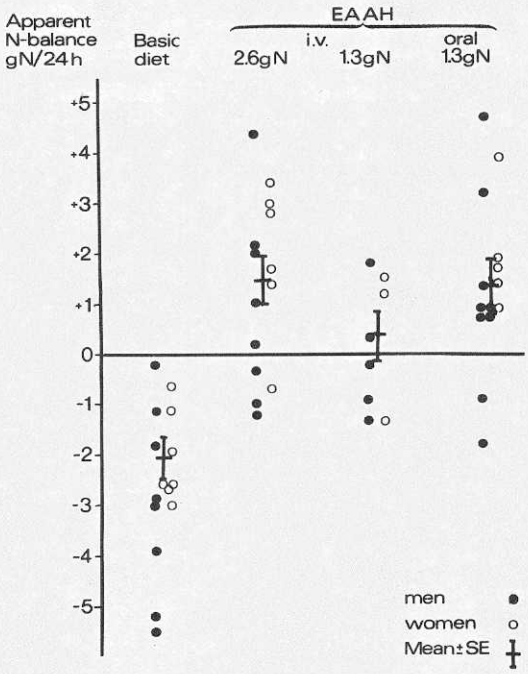

Fig. 10. Apparent nitrogen balance in 14 patients with uremia and mean ± SE treated with a 20 g protein diet (basic diet) and supplementation with essential amino-acids and histidine (EAAH) 2.6 g N and 1.3 g N i.v. and 1.3 g N by mouth (oral). Open symbols = women, closed symbols = men.

b) Intravenous supplementation of the basic diet with EAAH:

EAAH 2.6 g N (400 ml) i.v. (14 balance periods in 12 patients). The apparent nitrogen balance was improved in all patients and was positive in 10, mean +1.4 ± 1.8 (SD) g/24 h (men +0.9 ± 1.9, women +2.0 ± 1.5).

EAAH 1.3 g N (200 ml) i.v. (7 patients). The apparent nitrogen balance was improved in all patients and was positive in 3 patients, mean +0.4 ± 1.3 (SD) g/24 h (men +0.3 ± 1.3, women +0.5 ± 1.5).

c) Oral supplementation of the basic diet with EAAH

EAAH 1.3 g N, (16 balance periods in 10 patients). The apparent nitrogen balance was improved in all patients and was positive during 13 periods, mean +1.2 ± 1.7 (SD) g/24 h (men +0.9 ± 2.0, women +1.7 ± 0.8).

The apparent nitrogen balance improved significantly ($p = < 0.01$) during all periods with EAAH compared to the basic diet only. The mean paired difference ($n = 10$) between the apparent nitrogen balance with 2.6 g N i.v. and 1.3 g N by mouth was 0.59 g N/24 h ($p = 0.05$) while the opposite difference was found for corrected apparent nitrogen balance. With 1.3 g N i.v. the apparent nitrogen balance tended to be inferior to that with 2.6 g N i.v. (mean paired difference 2.4 g N, $n = 6$) and to that with 1.3 g N by mouth (mean paired difference 1.5 g N, $n = 5$) but the small number of pairs does not permit a valid statistical analysis of significance.

Body composition studies. Series III (Paper III).

Methods

Body composition was calculated from *body weight (BW), total body potassium (TBK)* and *total body water (TBW).* BW and body height (BH) were measured with standardized technique in connection with the whole-body counting procedure. TBK was calculated from ^{40}K measured in a sensitive 3π-whole-body counter[5]. TBW was determined after an oral load of 100 μCi tritiated water (THO) and determination of THO by liquid scintillation technique on sublimated samples of plasma after 2 h of equilibration.

The total expected SD of a single determination was 80 mmol for TBK[146] and 1.2 litres for TBW[38].

Control series

The findings were compared with those in a reference group of 489 apparently healthy men ($n = 134$) and women ($n = 355$), aged 20 – 70 years, consisting of participants in population studies and hospital staff[38].

Comparison was made for TBK and TBW for sex and BH.

Methodological considerations

The calculation of body cell mass (BCM) was based upon the assumption of an intracellular potassium concentration (ICK) of 150 mmol/l intracellular water and assuming that 3 mmol K = 1 g N = 25 g cell mass[114], which assumption is supported by analysis of biopsies from muscle tissue representing about 70 per cent of BCM[17].

However, in uremia there are clear difficulties in estimating BCM from TBK as both normal and low ICK and ratios of ICK to intracellular protein may occur (paper III).

In adults the reported abnormalities of intracellular composition have been less pronounced than in children and are also either associated with low exchangeable potassium or found in patients immediately before dialysis. Pending conclusive evidence of the relationship between TBK and BCM in uremic patients with normal TBK, the estimation of BCM was done as in the reference group. Marked overhydration was not evident in these patients.

Body composition before treatment (Table I, paper III)

Body weight (BW): 29 out of 37 men and 8 out of 12 women had BW within or above SW ± 10 %[106].

Total body potassium (TBK): 25 out of 30 men and 5 out of 8 women had TBK values within or above the mean ± 1 SD for height of the reference group (Fig. 11).

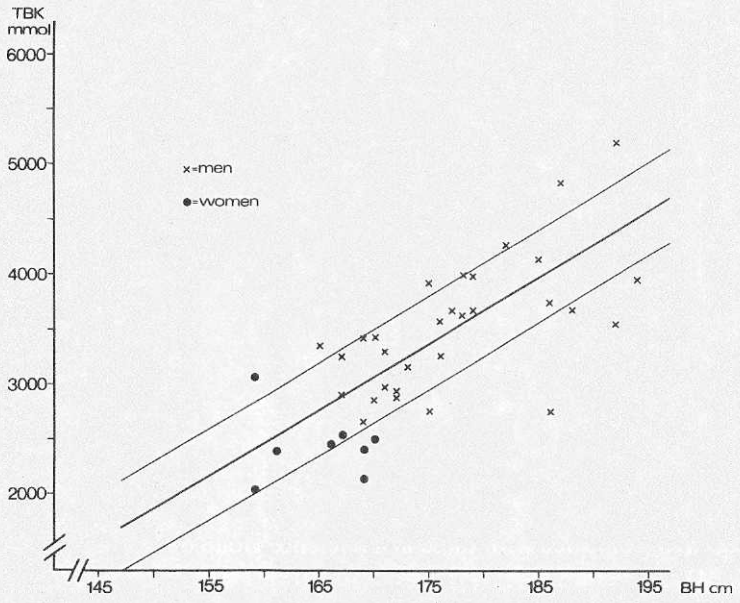

Fig. 11. Total body potassium (TBK) and body height (BH) in 30 male patients and 8 female patients with uremia before start of dietary treatment. Relation to mean ± SD for body height of control series.

Total body water (TBW): 19 out of 28 men and 3 out of 7 women had TBW values within the mean ± 1 SD for the reference group (Fig. 12).

In all patients TBK and TBW were highly correlated to BW ($r = 0.72$ and 0.89, $p < 0.001$). TBK and TBW were also correlated ($r = 0.83$, $p < 0.001$). Body composition variables were not correlated to serum creatinine or serum urea.

Body composition during treatment (Tables II and III, paper III)

The results were based on observations in 31 patients with a mean duration of observation of 9.3 months (range 3 – 12 months), (Fig. 13). There was no significant change of BH adjusted *mean BW* or *mean TBK* values during treatment.

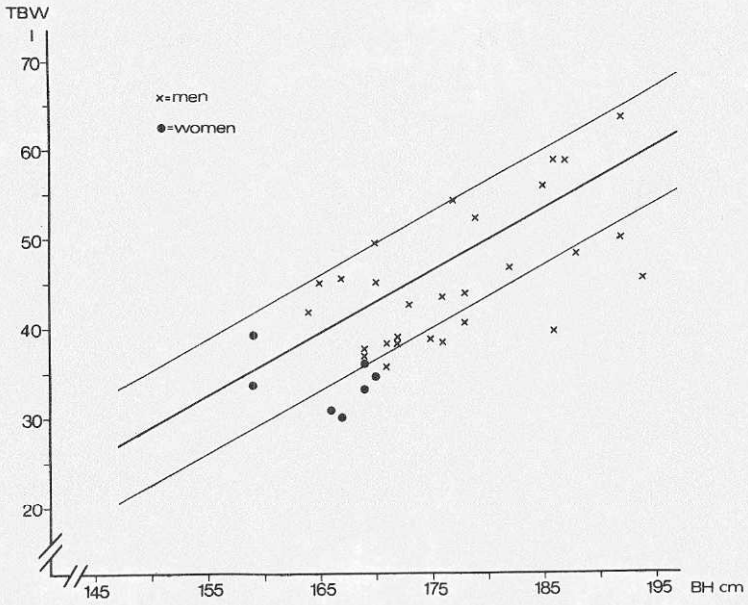

Fig. 12. Total body water (TBW) and body height (BH) in 28 male patients and 7 female patients with uremia before start of dietary treatment. Relation to mean ± SD for body height of control series.

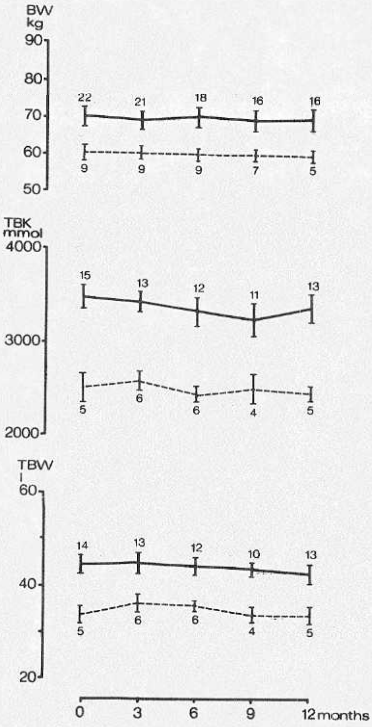

Fig. 13. Body weight (BW), total body potassium (TBK) and total body water (TBW) in 31 patients with uremia during treatment with protein-reduced diet and essential amino acids. Mean ± SE adjusted for differences in mean body height at different observation intervals. Figures refer to number of patients studied. Solid lines: men, broken lines: women.

In 10 out of 31 patients there was a decrease in TBK (mean 479 mmol, range 243 – 1651) between the first and last observation. Six out of 31 patients had an increase of TBK (mean 352 mmol, range 231 – 530).

Mean TBW did not change during treatment but in 10 out of 30 patients TBW decreased (mean 6.0 l, range 3 – 8.9) and in 4 out of 30 patients TBW increased (mean 6.5 l).

There was no correlation between the changes of BW, TBK and TBW or between the changes and the initial values.

Lipid and carbohydrate metabolism. Series IV (Paper IV – V).

Methods

Unless otherwise stated, venous blood samples were drawn in the morning, after an overnight fast, and centrifuged at low speed.

Serum lipids: Cholesterol was determined according to the method of Cramér and Isaksson[49], triglycerides (TG) according to the method of Carlson[46] and phospholipids as described by Bartlett et al.[15]. Non-esterified fatty acids (FFA) were determined with a modified Dole-procedure as used by Friedberg et al.[64].

Lipoprotein cholesterol: Very-low-density lipoproteins (VLDL) were isolated by preparative ultracentrifugation in the supernatant at density (D) 1.006 g/ml as described in paper IV.

Cholesterol content of α-lipoproteins (α–Lp) was determined in whole serum after the elimination of VLDL and LDL by precipitation with heparin and manganese chloride[43].

LDL cholesterol was estimated as the difference between cholesterol content of infranatant at density 1.006 g/ml and α-lipoprotein cholesterol.

The methodological errors were 3 % for serum cholesterol, 4 % for TG, 5 % for phospholipids and 4 % for α–Lp cholesterol.

Lipoprotein electrophoresis was performed on agarose gel as described by Gustafson et al.[79].

An intravenous fat tolerance test (IVFTT) with Intralipid® (AB Vitrum, Stockholm, Sweden) described by Carlson and Rössner[47], was performed in a subsample (n = 13), as described in paper IV. The disappearance rate (k_2) of Intralipid turbidity was expressed as the slope of the disappearance curve (per cent/min).

Lecithin: cholesterol acyl transferase activity (LCAT) was determined in 17 patients (14 men, 3 women) before treatment and at 3 (n = 10), 6 (n = 6) and T (n = 5) months.

LCAT was determined with a modified Stokke-Norum method[159, 166] and expressed as μmol esterified cholesterol (CE)l/h. The error of the method was 3 %.

Relative fatty acid composition of lecithin was determined by gas liquid chromatography (GLC) in 24 patients before the start of treatment and at 6 (n = 14), 12 (n = 9) and T (n = 16) months.

Preparation of lipids and lecithin fatty acid methyl esters: Separation of lipids was done by thin-layer chromatography on Silica gel. Preparation of fatty acid methyl esters was performed for lecithin according to the method of Olegård and Svennerholm[123].

Gas liquid chromatography (GLC) of methyl esters: The extracts containing the fatty acid methyl esters were analyzed in a Perkin Elmer Model 30 apparatus as described in paper V. Peaks were quantified by multiplying the height by the width at half height. 17:0 (heptadecanoic acid, Perkin Elmer) was used as an internal standard.

Quantification of serum lecithin: Serum lecithin was quantified from the fatty acid content (obtained by GLC) using a nomogram[93].

Ultracentrifugation (UC) analysis of lipoprotein cholesterol and TG content was performed on a subsample of 21 patients investigated before the start of treatment (n = 14) and during treatment (n = 14).

Preparative ultracentrifugation of serum was carried out at density (D) 1.006 g/ml and 1.063 g/ml.

After lipid extraction, the cholesterol content of supernatant (SUP) and infranatant (INF) at D 1.006 g/ml and D 1.063 g/ml and the TG content of SUP and INF D 1.006 g/ml and SUP D 1.063 g/ml was determined. The average recovery of cholesterol was 97 per cent (range 72 – 125) at D 1.006 g/ml and 102 per cent (range 69 – 149) at D 1.063 g/ml. TG recovery at D 1.006 g/ml was 76 per cent (range 55 – 133).

SUP D 1.006 g/ml was considered to represent the VLDL fraction while INF D 1.063 g/ml represented HDL. HDL–TG was calculated from serum–TG minus SUP D 1.063 g/ml. LDL cholesterol and LDL–TG were calculated as (SUP + INF D 1.006 g/ml) minus (VLDL + HDL). The relative distribution of cholesterol and TG in the VLDL, LDL and HDL was then applied to serum cholesterol and TG values to calculate the absolute amount of cholesterol and TG in the different density classes.

Apolipoprotein composition of lipoproteins: In a subsample of series IV the serum concentrations of apolipoprotein A (apo–A), B (apo–B), C_I (apo–C_I), C_{III} (apo–C_{III}) and E (apo–E) were determined by electro-immunoassay (EIA) before (n = 26) and after three (n = 6) and six months' treatment (n = 12).

EIA of apo–A was performed as described by Wiklund[166] while the EIA of apo–B, –C_I, –C_{III} and –E was performed at the Oklahoma Medical Research Foundation, Oklahoma City/Oklahoma, USA (Prof. P. Alaupovic) by methods previously described[2].

Carbohydrate metabolism: *Blood glucose* was determined in venous blood by a glucose oxidase method (Glox®, AB Kabi, Stockholm, Sweden) and *plasma insulin* by a solid phase antibody technique (Phadebase®, Pharmacia Fine Chemicals, Upsala, Sweden). An *intravenous glucose tolerance test (IVGTT)* was performed as described in paper IV and the fractional removal rate (k) of glucose was calculated as the slope of the log glucose values between 20 and 60 min and expressed as per cent disappearance per min.

Plasma insulin concentration was determined before and at 10, 30 and 90 min after injection of the glucose. *Serum lactate* concentration was determined in venous blood before the IVGTT (method Cat No 124.842, Boehringer Mannheim GmbH, FRG).

Control series

No control group of non-uremic subjects was investigated in parallel with the uremic patients. For serum lipids and lipoprotein cholesterol reference groups were found in population studies[16, 78, 79, 88, 149, 167] carried out at Sahlgrenska sjukhuset using the same methods as in the present investigation. Reference values for blood glucose and IVGTT in women were also obtained from a population study of 50-year-old women from the hospital[32], while corresponding values for men were obtained from a population study of 50-year-old men, using the same methods, performed in Upsala, Sweden[34, 83].

Reference data for the intravenous fat tolerance test were obtained from 31 healthy volunteers with normal serum lipid levels investigated at our laboratory[80]. The results were in close correspondence with those found by Carlson and Rössner in a similar group[47].

Reference data for UC analyses were obtained from 23 non-renal patients admitted for uncomplicated cholecystectomy.

Results for relative fatty acid composition of lecithin were compared with those of matched controls to young men with myocardial infarction[166] and healthy women aged 19 – 34 years[137]. For LCAT and apo–A, reference values were obtained from the above-mentioned controls to patients with myocardial infarction and for apo–B, apo–C_I, apo–C_{III} and apo–E from 50 healthy subjects investigated at the Oklahoma Medical Research Foundation[2].

Reference values for lactate were taken from the reference quoted in the kit.

Reference values for plasma insulin before and during IVGTT were obtained from a study of 12 hospitalized patients without renal disease (mean age 67 years) investigated in identical fashion at the same hospital[108]. The basal insulin values of the reference group were in close agreement with those obtained in an earlier, larger, healthy control series of 49 men and 23 women aged 50 – 55 years[30].

Methodological considerations

Laboratory investigations: All patients were investigated before and during treatment, on the same day of the week, after 2 – 4 days' hospitalization and the analyses were performed throughout the study by the same personnel at the Metabolic Laboratory. During treatment the changes in variables were evaluated at three-months intervals for twelve months and also at the last observation during treatment (T), reflecting the total time on diet. The mean observation time at T was 9.5 months.

Typing of hyperlipoproteinemia (HLP) was based on serum lipids, lipoprotein cholesterol content and visual inspection of the agarose electrophoresis (paper IV). Marked elevation of both cholesterol and TG, a broad β–band on electrophoresis and an increased ratio of VLDL cholesterol to serum TG (> 0.67)[62] was suggestive of HLP type III. Regular HLP-typing was possible to perform in 13 patients (series IV A) while in 15 patients the ratio of VLDL cholesterol to serum triglycerides fell well below the expected ratio of 1.0 to 2.2 (Fig. 3, paper IV), thus preventing the application of the above-mentioned criteria for HLP-typing.

Lipoprotein cholesterol determinations: A correlation ($r = 0.87$, $p < 0.001$) was found between the different methods of estimating LDL cholesterol. α–Lp cholesterol and HDL cholesterol were not correlated, however, and showed a mean difference of 0.45 mmol/l, HDL cholesterol being higher. This may be explained by the contamination of Lp–B in HDL but not in α–Lp[166].

Intravenous fat tolerance test: Reduction of the fractional removal rate (k_2) of Intralipid can be attributed either to a decreased TG clearance or merely an increased TG pool size. Rössner et al.[136] have demonstrated the correlation between endogenous TG turnover and the fractional elimination rate of Intralipid. However, in several studies the k_2 has been remarkably unaffected despite considerable changes in TG[117, 136], raising the question of the influence of TG pool size on k_2 and competition with TG removal sites. Intralipid k_2 does undoubtedly reflect chylomicron clearance but the interpretation of k_2 reflecting endogenous TG clearance may require caution.

Results before treatment (Paper IV)

Serum lipids and lipoproteins (Table IV): Serum TG were high in both series, with a concomitant increase of phospholipids in the male patients while serum cholesterol was not elevated. α–Lp cholesterol was low in both series. VLDL cholesterol was elevated but the estimated LDL cholesterol was not.

UC analyses of lipoprotein composition (Fig. 14) revealed that both the content and relative distribution of cholesterol was increased in VLDL, decreased in HDL and unchanged in LDL. TG was markedly increased in VLDL, as expected, but not in LDL or HDL.

	Series A	Series B	Control series
Serum cholesterol (mmol/l)	5.9 ± 0.3	6.0 ± 0.3	men: 6.2 ± 0.11 women: 7.1 ± 0.05
Serum triglycerides (mmol/l)	3.2 ± 0.4^{+++}	3.2 ± 0.3^{+++}	men: 1.3 ± 0.09 women: 1.3 ± 0.03
Serum phospholipids (mmol/l)	3.0 ± 0.1^{+}	2.9 ± 0.1	men: 2.7 ± 0.06 women: 3.0 ± 0.11
Lipoprotein cholesterol (mmol/l)			
VLDL	1.3 ± 0.3^{+++}	1.2 ± 0.2^{+++}	men: 0.5 ± 0.04 women: 0.3 ± 0.02
LDL	3.4 ± 0.3	3.6 ± 0.2	
α–Lp	1.2 ± 0.1^{++}	1.2 ± 0.1^{++}	men: 1.5 ± 0.03 women: 1.7 ± 0.04
FFA (μmol/l)	519 ± 38	544 ± 43	918 ± 115
IVFTT-k_2 (%/min)	4.2 ± 0.7^{+} (n = 10)	4.3 ± 0.5^{+} (n = 13)	men: 6.0 ± 0.4 women: 6.8 ± 0.6

Table IV. Serum lipids (cholesterol, triglycerides, phospholipids), lipoprotein cholesterol (VLDL, LDL, α-Lp), non-esterified fatty acids (FFA) and intravenous fat tolerance test in uremia. Mean (± SE) for men (series A, n = 20) and total series (series B, n = 28) in relation to appropriate control series (paper IV). $^{+} = p < 0.05$, $^{++} = p < 0.01$, $^{+++} = p < 0.001$.

Typing of hyperlipoproteinemia (HLP) was possible to perform in 13 out of 28 patients (cf. Methodological considerations). Five men and one woman had a normal lipoprotein pattern, one patient had type IIa, one patient type III and five patients had type IV HLP.

Intravenous fat tolerance test (Intralipid) (Table IV; fig. 5, paper IV) showed a reduced fractional removal rate (k_2) of Intralipid but the relationship between k_2 and TG was of the same magnitude in our series as in the normo- and hyperlipidemic non-renal control series.

Fatty acid composition of lecithin (Fig. 15): In the male uremic patients (n = 20) the fatty acid composition of lecithin was not different from that in control men except for a lower ($p = < 0.001$) content of arachidonic (20:4) acid, while stearic (18:0) acid was increased ($p < 0.01$). In females (n = 8) there was an increase in palmitic (16:0) ($p < 0.05$) and oleic (18:1) ($p < 0.01$) acids while linoleic (18:2) and arachidonic (20:4) acids were lower ($p < 0.05$) compared to the non-uremic controls.

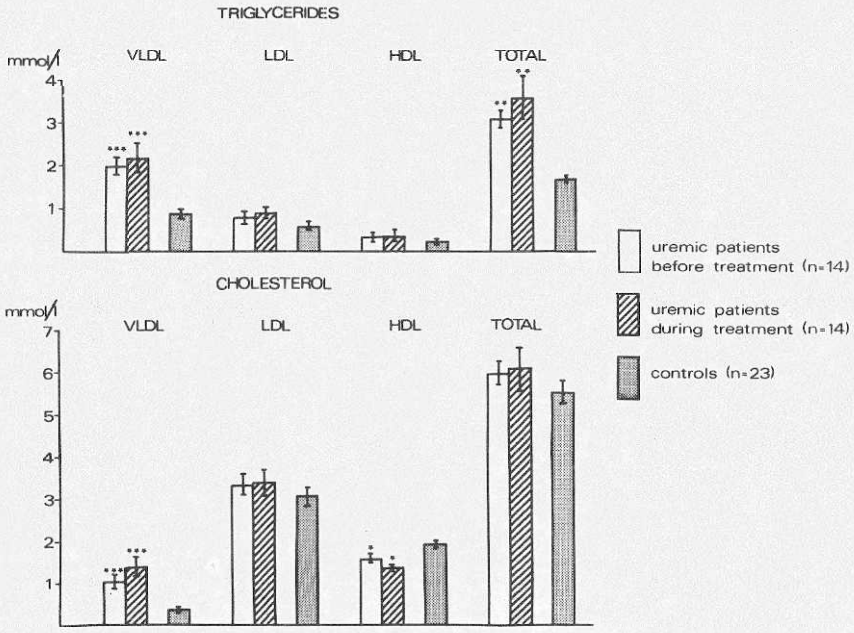

Fig. 14. Mean (± SE) cholesterol and triglyceride content of VLDL, LDL, HDL, determined by ultracentrifugation, and total serum in uremic patients (series IV) before (n = 14) and during treatment (n = 14) and in control series (n = 23). Differences between patient series and control series are indicated: $^{+} = p < 0.05$, $^{++} = p < 0.01$, $^{+++} = p < 0.001$.

LCAT (Fig. 16): The mean LCAT activity was 87.3 ± 25.1 (SD) μmol CE/l/h (n = 17), compared to 98.7 ± 24.9 in the control group (n = 40). These results would indicate lower LCAT in uremia, taking into account the higher mean triglyceride levels in the uremic patients, although the difference is not statistically significant ($p < 0.10$).

Apolipoproteins (Fig. 17): Apo–A, apo–B and apo–E levels were low while apo–C_I and especially apo–C_{III} were high compared to the controls.

Carbohydrate metabolism (Table V; fig. 2, paper IV): Mean fasting blood glucose levels were slightly elevated in the female patients, compared to the control series while the reverse was the case in the male series. The *IVGTT* revealed a low ($p < 0.01$) estimated k-value in both series.

Mean basal *plasma insulin* was not elevated nor were mean insulin levels during the IVGTT but *serum lactate* was low in both series. However, the increase in plasma insulin at 10 min was fourfold in the patient series, compared to a 2.7 fold increase in controls.

Correlations among lipid and carbohydrate variables:

Serum TG was well correlated to VLDL–TG ($r = 0.85$, $p < 0.001$, $n = 14$) and to VLDL cholesterol ($r = 0.71$, $p < 0.001$, $n = 33$) and also inversely correlated to α–Lp cholesterol ($r = -0.57$, $p < 0.001$, $n = 33$) but not to HDL cholesterol.

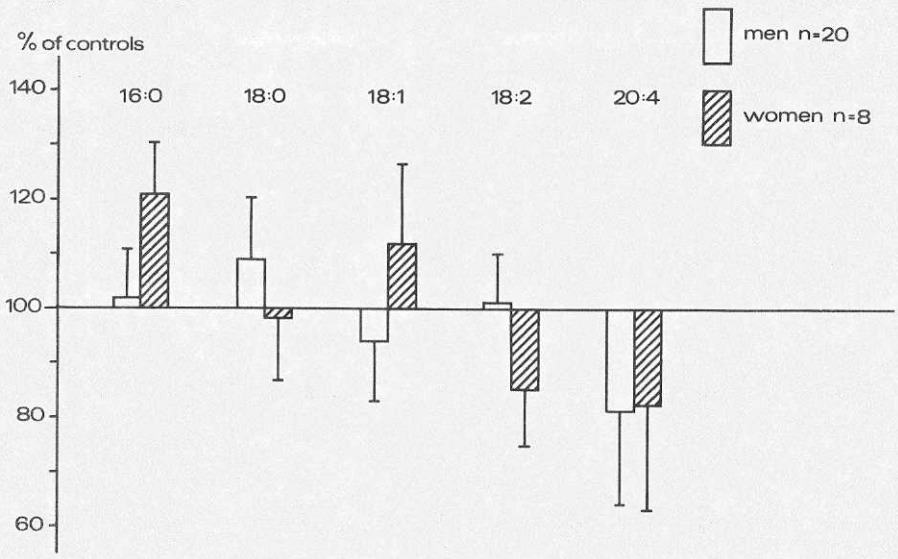

Fig. 15. Relative major fatty acid composition of lecithin in patients with uremia (n = 28) in per cent (mean ± SD) of means of control series. The relative contents of palmitic (16:0), oleic (18:1), linoleic (18:2) and arachidonic (20:4) acids in men and women are shown.

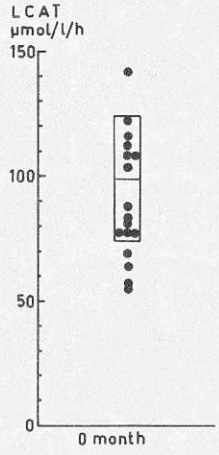

Fig. 16. LCAT activity in 17 patients with uremia before dietary treatment. The frame indicates mean ± SD of control series.

Serum TG was also correlated to basal plasma insulin ($r = 0.44$, $p < 0.05$, $n = 28$) and insulin during IVGTT ($r = 0.56$, $p < 0.05$, $n = 26$) (Fig. 4, paper IV) but not to BW or BW/SW.

Serum cholesterol was correlated both to VLDL ($r = 0.45$, $p < 0.01$, $n = 33$) and to LDL cholesterol ($r = 0.80$, $p < 0.001$, $n = 33$). In LDL, the cholesterol content was correlated to the TG content ($r = 0.76$, $p < 0.01$, $n = 14$).

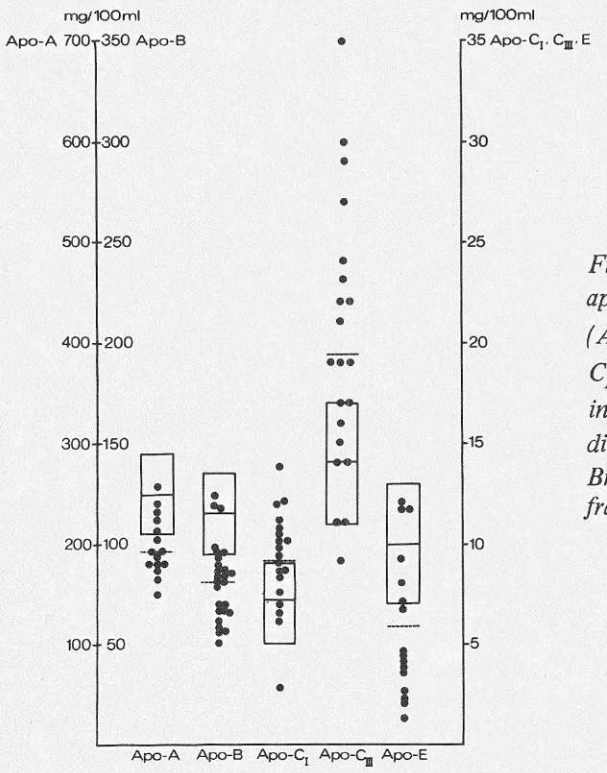

Fig. 17. Serum concentration of apolipoprotein A (Apo–A), B (Apo–B), C_I (Apo–C_I), C_{III} (Apo–C_{III}) and E (Apo–E) in patients with uremia before dietary treatment. Broken lines: mean values, frames: mean ± SD of control series.

	Series A	Series B	Control series
Blood glucose (mmol/l)	4.5 ± 0.1^{+}	4.6 ± 0.1^{+}	men: 4.9 ± 0.06 women: 3.9 ± 0.02
IVGTT-k	0.92 ± 0.06^{++}	0.92 ± 0.07^{++}	men: 1.62 ± 0.07 women: 1.88 ± 0.06
Plasma insulin (mU/l)			
0′	12.6 ± 1.5	13.5 ± 1.3	15 ± 3
10′	47 ± 5.7	50 ± 4.7	37 ± 5
Σ insulin	128 ± 11	129 ± 10	113 ± 12
Serum lactate (mmol/l)	0.6 ± 0.05^{++}	0.7 ± 0.04^{++}	1.4 ± 0.04

Table V. Blood glucose, intravenous glucose tolerance test (IVGTT), plasma insulin during IVGTT and serum lactate in uremia. Mean (± SE) for men (series A, n = 20) and total series (series B, n = 28) in relation to appropriate control series (paper IV). $^{+} = p < 0.05$, $^{++} = p < 0.01$.

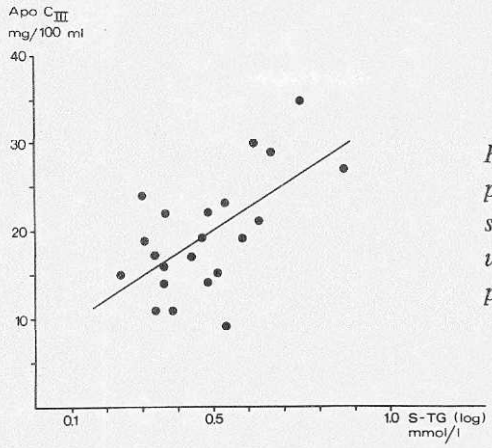

Fig. 18. Serum concentration of apolipoprotein C_{III} (Apo–C_{III}) versus (log) serum triglycerides (TG) in patients with uremia before dietary treatment ($r = 0.61$, $p < 0.01$, $n = 22$).

Plasma insulin was correlated to BW/SW ($r = 0.67$, $p < 0.001$, $n = 28$) but also to fasting blood glucose ($r = 0.43$, $p < 0.05$, $n = 28$).

Intralipid k_2 was inversely correlated to serum TG (Fig. 5, paper IV). There was also a tendency towards a negative correlation ($r = -0.55$, $p = 0.05$, $n = 13$) between k_2 and serum urea.

There was no correlation between *lecithin relative fatty acid composition* and the various lipid variables.

LCAT showed a weak correlation to serum TG ($r = 0.46$, $p = 0.05 < p < 0.10$, $n = 17$) and none to apo–A or α–Lp cholesterol.

Apolipoproteins: Apo–A was (as might be expected) correlated to α–Lp cholesterol ($r = 0.65$, $p < 0.05$, $n = 14$) but not to any other apolipoproteins.

Apo–B was closely correlated to serum cholesterol ($r = 0.51$, $p < 0.01$, $n = 26$) but also to apo–E ($r = 0.69$, $p < 0.01$, $n = 16$). Apo–C_{III} showed a correlation ($r = 0.61$, $p < 0.01$, $n = 22$) to serum TG (Fig. 18).

GFR was not correlated to lipid or carbohydrate variables.

Results during dietary treatment, mean 9.5 months (Table III, paper V)

GFR decreased continuously (Fig. 19) while *serum urea* decreased initially, after which there was a slow rise.

Mean *serum albumin* was low (but within the normal range) before the start of treatment and remained so during treatment.

The mean *serum transferrin* (TIBC) levels underwent on average a 12 per cent decrease during treatment ($p < 0.05$). However, transferrin remained within the reference limits (43 – 90 μmol/l) even at time T.

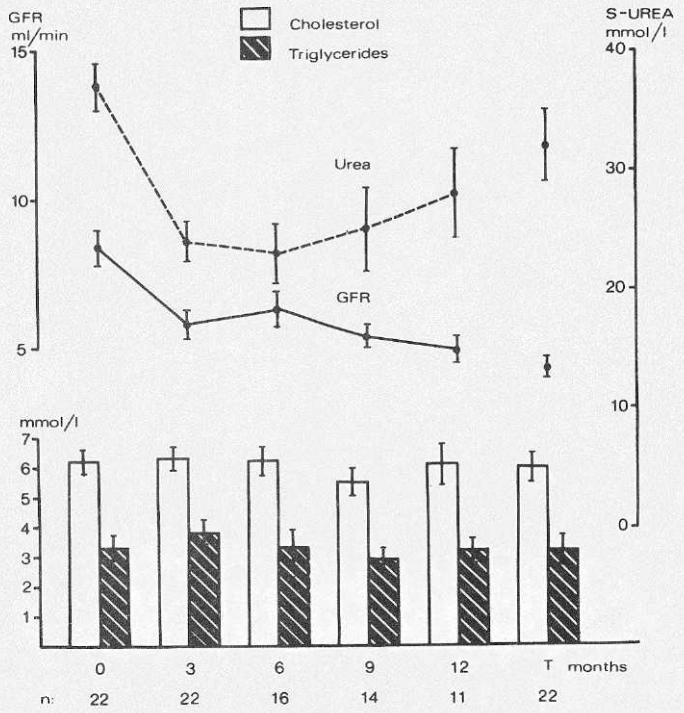

Fig. 19. Glomerular filtration rate (GFR, solid line), serum urea (S–UREA, broken line), serum cholesterol (open bars) and serum triglycerides (striped bars) during dietary treatment of uremia. Mean ± SE before (0 month) and at 3, 6, 9 and 12 months and at last observation (T) during treatment (n = number of patients).

Serum lipids and lipoproteins: There was no change in mean serum TG, cholesterol, phospholipids, lipoprotein cholesterol or FFA during treatment (Fig. 19). TG increased at time T by > 25 per cent in 7 out of 22 patients but there was an equally large decrease in 3 patients. *UC analyses* of TG and cholesterol in VLDL, LDL and HDL did not disclose any change from start of treatment to time T (n = 6).

Fatty acid composition of lecithin: Linoleic (18:2) acid and the sum of (n –6) acids increased during treatment, with a maximum at 6 months ($p < 0.05$), with inverse changes in palmitic (16:0) acid. *Plasma lecithin* was also increased ($p < 0.05$) at 6 months.

Mean **LCAT** activity and mean **apolipoprotein** values did not change during treatment.

Carbohydrate metabolism (Table VI): Mean fasting *blood glucose* levels remained unchanged as did mean *IVGTT–k*. Mean *plasma insulin* values were also unchanged. *Serum lactate* was low before dietary treatment and remained so during treatment.

	0 month		6 months		12 months		T	
	$\bar{X}$	± SD	$\bar{X}_\Delta$	n	$\bar{X}_\Delta$	n	$\bar{X}_\Delta$	n
Blood glucose (mmol/l)	4.6	0.5	−0.3	16	−0.6	10	−0.2	18
IVGTT-k (%/min)	0.99	0.34	−0.03	16	−0.12	9	−0.06	13
Plasma insulin (mU/l)								
0′	13.7	6.1	−1.7	16	−1.3	9	−2.4	13
10′	52.5	25.2	−3.5	15	−2.2	9	−8.8	13
Σ insulin	132.4	48.3	−12.7	15	+3.4	9	−15.2	13
Serum lactate (mmol/l)	0.7	0.2	−0.2	15	+0.4$^+$	9	+0.3	12

Table VI. Blood glucose, intravenous glucose tolerance test (IVGTT), plasma insulin during IVGTT and serum lactate during dietary treatment of uremia. Mean (± SD) before treatment (0 month) in total series (n = 22) and mean difference ($\bar{X}_\Delta$) from 0 month at 6 and 12 months and at last observation during treatment (T). $^+ = p < 0.05$, *n = number of patients.*

Correlations among changes in lipid and carbohydrate variables

The observed changes (Δ) in TG, cholesterol or α–Lp cholesterol were not correlated to changes in GFR or urea, to time on diet or to changes in BW. There were no correlations between Δ–TG and *composition of diet* (energy, fat, carbohydrate or sucrose content or P/S-ratio). However, the changes in serum cholesterol (Δ–cholesterol) were negatively correlated to the total amount of fat in the diet ($r = -0.51$, $p < 0.05$) and to the P/S-ratio ($r = -0.54$, $p < 0.05$) (Fig. 20). The changes in α–Lp cholesterol also tended to be correlated to the amount of fat in the diet ($r = 0.41$, $p = 0.11$, $n = 16$).

Fatty acid composition of lecithin: The changes in relative content of palmitic (16:0) acid tended to be negatively correlated to the P/S-ratio in the diet ($r = -0.60$, $p = 0.05$) and to time on diet ($r = -0.53$, $p = 0.05$). The relative content of linoleic (18:2) acid, on the other hand, increased with increasing P/S-ratio (Fig. 20).

Changes in 18:2 acid were negatively correlated to changes in 16:0 acid ($r = -0.76$, $p < 0.01$, $n = 14$) and to changes in 20:4 acid ($r = -0.63$, $p < 0.05$, $n = 14$). Changes in serum TG were not correlated to changes of relative fatty acid composition of lecithin.

Apolipoproteins: The changes in apo–B were correlated to those in apo–C_I ($r = 0.75$, $p < 0.01$, $n = 11$), in apo–C_{III} ($r = 0.61$, $p < 0.05$, $n = 12$) and in apo–E ($r = 0.72$, $p < 0.05$, $n = 8$) but also to the change in serum cholesterol ($r = 0.82$, $p < 0.001$, $n = 13$).

Changes in apo–C_{III} were also correlated to those in serum TG ($r = 0.59$, $p < 0.05$, $n = 12$).

Changes in apolipoprotein content were not correlated to those of other serum proteins e.g. albumin or TIBC, or to diet composition.

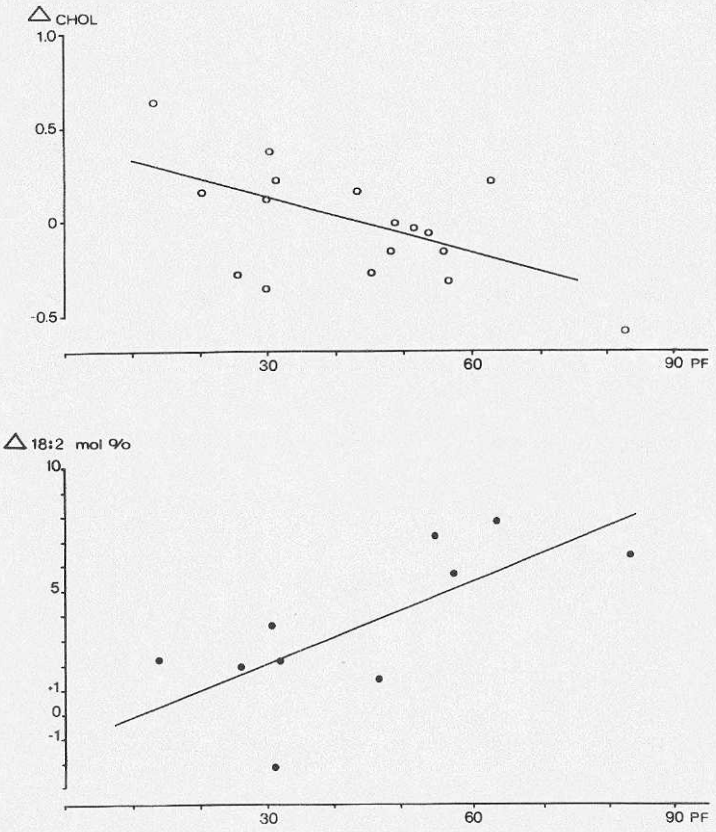

Fig. 20. Changes in serum cholesterol (ΔCHOL) and in relative content of linoleic (18:2) acid (Δ18:2) versus content of polyunsaturated fat (PF) in diet (P/S x dietary fat in grams) during dietary treatment of uremia. Changes in serum cholesterol are expressed as the logarithmically transformed ratio cholesterol at last observation (T)/cholesterol before treatment while changes in linoleic acid are expressed in mole per cent. (ΔCHOL vs PF: $r = -0.59$, $p < 0.05$, $n = 17$; Δ18:2 vs PF: $r = 0.77$, $p < 0.05$, $n = 10$).

DISCUSSION

Indications and contraindications for dietary treatment

The aim of the dietary treatment should be relief of uremic symptoms and maintenance of a good nutritional state without further deterioration of the disturbances in lipid and carbohydrate metabolism, preparing the patient for future treatment with dialysis or transplantation or in some cases to achieve good palliation. The treatment is, in a broad sense purely symptomatic and inflicts a definite strain on the daily life of the patient. Patient motivation is therefore the key to successful treatment and is created by amelioration of symptoms and increased wellbeing. Thus, only patients with *symptoms* of renal failure are suited for treatment. Patients with terminal uremia complicated by serositis, oliguria or severe neuropathy, on the other hand, do not benefit from dietary treatment.

The *rate of GFR decrease* is the most important single factor determining the duration of successful conservative management. Patients with uremic manifestations of similar degree show great differences in serum creatinine levels (paper I). The observed inaccuracy of serum creatinine and endogenous creatinine clearance for estimation of GFR (paper I) might be due to several factors, above all variations in tubular excretion of creatinine[86], fluctuations in creatinine production over the day and between days[126] and the methodological errors in the serum creatinine determinations. ^{51}Cr–EDTA was an equally good filtration marker as inulin, which was used as reference substance for the determination of GFR[86], but analysis of inulin is more cumbersome for clinical purposes. The plasma clearance of ^{51}Cr–EDTA (Fig. 8), the so-called *slope clearance*, (calculated from the plasma disappearance curve of the marker) did not provide an accurate measure at low GFR owing to the variable extrarenal clearance of the marker, as also found with normal or moderately reduced GFR[6, 42]. In addition, the slope of the disappearance curve was so small that determination of the area under the curve was difficult to perform with a reasonable degree of precision. Accurate determination of GFR in advanced renal insufficiency thus required an external filtration marker (inulin or ^{51}Cr–EDTA), with urine collection, and was routinely applied in the later patient series (IV).

Our data indicate that the therapeutic interval for dietary treatment seems to be from onset of uremic symptoms (GFR between 6.5 and 9.5 ml/min) until a GFR of about 4 ml/min (Fig. 9). Similar studies with accurate determination of GFR have not been published to our knowledge. Walser[161] has estimated the GFR limit to be about 3 per cent of normal. Pre-treatment determinations of GFR thus appear to be of practical value. It was also apparent (Fig. 9) that the rate of GFR decrease tended to be fairly uniform in the present series irrespective of the original disease.

Choice of diet

The dietary treatment of uremia in Gothenburg has been based on direct introduction of the 20 g protein diet when uremic symptoms appear. In a small group of patients with stable or unusually slowly progressing functional impairment treatment with a 40 g protein diet could be used. In most patients with progressive renal failure, however, the therapeutic period for the 40 g diet will be short. The patients would then have to learn two rather different diets within a short period of time.

Supplementation of the diet with essential amino-acids and histidine (EAAH)

The supplementation improved nitrogen balance in all patients. It is doubtful whether the difference in apparent nitrogen balance (0.57 g N/24 h) between 2.6 N i.v. and 1.3 g N by mouth has any biological significance. Furthermore, the corrected apparent nitrogen balance tended to be better with 1.3 g N by mouth than with 2.6 g N i.v. With 1.3 g N as EAAH, oral administration tended to be superior to infusion.

There is no readily available explanation for the finding in series II that the utilization of EAAH for protein synthesis was better when it was given orally than by the parenteral route. Similar conclusions have been made from studies in non-uremic individuals[156]. Intravenous infusion of amino-acids, particularly if done rapidly, may lead to immediate excretion in the urine or direct catabolism of the amino-acids. The hyperglucagonaemia of renal failure[29] might prevent full utilization of the infused amino-acids for protein synthesis. Oral amino-acids might also elicit a different insulin response than i.v. amino-acids[154], contributing to better amino-acid utilization. However, 2.6 g N as EAAH administered i.v. or by mouth in uremia has been found to give the same nitrogen balance but with a different amino-acid incorporation pattern in body proteins[22].

The effect of 2.6 g N by mouth was not investigated. It has, however, been shown by Bergström et al.[23] that a similar 20 g diet supplemented with 2.6 g N as EAAH by mouth gave an uncorrected mean nitrogen balance of +0.57 g N/24 h in close agreement with our results with only half that amount.

Our data would seem to indicate that routine supplementation with 1.3 g N orally as EAAH should be adequate for the stable adult uremic patient and that increase of EAAH above this level does not improve the nitrogen balance further to any great extent. It is of practical advantage for the patient to keep the EAAH supplementation at a lower level provided no deterioration of the nitrogen balance occurs. Together with the amino-acids in the diet, the calculated daily intake of essential amino-acids amounts to twice the minimum requirement[134] or more (Fig. 6).

It has been suggested that the composition of the amino-acid supplementation may be suboptimal since in uremia low intracellular concentrations of tryptophan, valine and ty-

rosine have been found together with an elevated phenylalanine/tyrosine ratio[24, 150]. The amino-acid mixtures could, on theoretical grounds, be altered accordingly, with addition of tyrosine and increase of the tryptophan and methionine content, being low in the supplemented diet.

Adherence to diet

Previously reported data on dietary adherence in uremic patients have been obtained during hospital conditions or in the metabolic ward and are not directly comparable with those obtained in the present study during routine outpatient treatment over a longer period of time.

Our data indicate that the adherence to the prescribed diet was good (Table II) even when checked on repeated occasions, with less than 15 per cent mean variation of energy and protein intake. Although the fat and sucrose contents were well above recommended levels for healthy subjects the diet was sufficiently varied and palatable to be tolerable for the patients. Furthermore the large amounts of fat and sucrose gave cause for investigation of the influence of the diet on lipid and carbohydrate metabolism.

During the study we have gained the experience, that adherence to diet depends on the skill of the dietitian in adapting the diet, as far as possible, to the ordinary food habits of the patient and his family. Together with the culinary demands, these circumstances exclude the use of special formula diets etc.

Long-term aspects of protein metabolism during dietary treatment of uremia

The average patient with chronic renal failure and early uremic symptoms had a relatively maintained body composition also during dietary treatment unless the situation was complicated by catabolic stress, intercurrent illness or major nutritional difficulties.

The nitrogen balance data indicated that a low-protein diet supplemented with EAAH improved the nitrogen balance but that several patients were still in a slightly negative balance when all losses were taken into account. These data, together with body composition data, therefore indicate that in many cases a further adaptation to reduced nitrogen intake must have taken place over a longer period of time in accordance with the findings in non-uremic patients[147].

A slight decrease of BCM without increase of TBW, however, was found in a few male patients despite very good clinical results and maintained BW over 12 months. They all had markedly low serum urea levels during treatment and it is conceivable that the amount of endogenous nitrogen was insufficient and their total nitrogen intake consequently slightly too low. Apart from the finding of decreasing TBK values these patients were not clinically distinguishable from the patients with the most promising clinical results.

Data on body composition validated the assumption that long-term dietary treatment with the EAAH supplementation used in this study is sufficient to prevent a clinically important protein malnutrition, unless the further course (until terminal uremia) is complicated by intercurrent disease. Our clinical results with this long-term treatment were in accordance with those in a comparable series of patients with chronic renal failure, treated with a similar diet, but with a larger amino-acid supplementation[121]. No clinical evidence of malnutrition, serum protein depletion or progress of neuropathy was found in that series either.

Serial determinations of body composition during treatment with EAAH-supplemented diet have to our knowledge not been reported before, but in a number of series of patients treated for varying periods of time with a protein-reduced diet BCM has been found to be maintained[26, 31, 35, 60, 103, 115].

Since mean BW, BCM, and TBW remained unchanged, this also applies to body fat, thereby indicating that the energy stores were generally maintained and that sufficient energy was supplied by the diet. The lack of correlation between changes in BW and TBK or TBW, might also indicate that in renal failure, changes in BW do not necessarily reflect the nutritional state.

Data on serum albumin and transferrin support the interpretation of maintained nutritional status and the slight decrease (12 %) of transferrin could have been influenced by the iron supplementation and also a systematic trend towards lower values over the years covered by the investigation.

Lipid and carbohydrate metabolism in chronic renal failure

Carbohydrate metabolism

In the present series of uremic patients, the glucose utilization rate was reduced, with a low k-value at IVGTT (< 1.1 in 23 out of 28 patients), while blood glucose levels were within normal limits, as was plasma insulin, both in the fasting state and after a glucose load. This would indicate a resistance to the glucose assimilation by insulin in uremia as has earlier been demonstrated in man[52, 53, 165] as well as in experimental uremia in the dog[151].

Furthermore, the metabolic clearance of insulin is delayed in renal failure, with increase of both plasma insulin, proinsulin and C-peptide[52, 53, 92, 111, 130]. In the present study, lack of hyperinsulinaemia (in spite of prolonged elevation of blood glucose during a glucose load) might therefore be related to a defect in insulin release from the uremic pancreas[52, 130].

In the context of the suggested insulin resistance the recent finding by Lutz[109] of an insulin "insensitivity" owing to formation of a complex between insulin and a basic polyamine-containing polypeptide accumulating in uremia, is of great interest. It should be

pointed out, however, that in the present series no correlation was found between serum urea or GFR and plasma insulin or the k-value in the IVGTT.

The unexpected finding in this series of low lactate levels may also be related to this suggested insulin resistance, causing higher utilization of lactate in gluconeogenesis.

Lipid and lipoprotein metabolism

Increased serum triglycerides (TG) (hypertriglyceridemia) as found in the present study (paper IV, V), has been a regular finding in uremia[10, 11, 51, 90, 120]. In the present study serum phospholipids were also elevated simultaneously with the increase in TG while serum cholesterol was within the "normal" range. The cause of the hypertriglyceridemia was chiefly an increase in very-low-density lipoproteins (VLDL), but the VLDL cholesterol was not always increased in proportion to serum TG, thereby indicating that lipoproteins of higher density, i.e. low-density lipoproteins (LDL) and high-density lipoproteins (HDL), could be enriched in TG. This was not verified in the lipid analyses on ultracentrifugal fractions (UC), however. However the cholesterol content in HDL, as well as in α–Lp, was lower than in control series, and also lower than might be expected in relation to the hypertriglyceridemia[78]. That is, the HDL composition was characterized by an increased TG to cholesterol ratio (fig. 14). In agreement with these findings, the apolipoprotein content, apo–A, was also low (fig. 17). Low HDL cholesterol[9, 90, 120] as well as low α–Lp[105] has been found in uremia, while, in an earlier study, where apo–A was measured in whole serum[39], this protein moiety appeared to be normal in uremic patients on dialysis.

Hypertriglyceridemia (in the post-absorbtive state) is due either to an increased production or to a decreased clearance of TG-rich lipoproteins, VLDL or chylomicrons, or to a combination of both these mechanisms.

An increased production of VLDL (from the liver) could be enhanced by increased availability of TG substrates i.e. fatty acids as FFA, liberated by lipolysis from adipose tissue, and of glycerol from glucose via α-glycerophosphate (cf. Introduction).

In the present study the level of FFA was initially, if anything, low, which is surprising in view of the fact that these patients could be expected to be in a catabolic state. Furthermore, increased levels of circulating catecholamines and cAMP, as found in uremia[81, 142] would be expected to enhance adipose tissue lipolysis. However, a normal or even a low level of FFA could still be compatible with an increased turnover of FFA, and with substrate excess for TG synthesis. An increased glycerol release from adipose tissue indicating enhanced lipolysis has been found in uremia[157]. A high utilization of FFA for TG synthesis in the liver could be further promoted by a lower fatty acid incorporation in adipose tissue (FIAT) in the uremic patients[157].

In relation to TG substrate, blood-glucose levels were not elevated in most patients, and neither was fasting plasma insulin, as judged from mean values (paper IV). However, there was a relationship between plasma insulin and serum TG, which has often been interpreted as indicating an influence by insulin on TG synthesis and VLDL secretion[11, 124].

TG production and VLDL secretion from the liver has also been suggested to be enhanced by certain fatty acids[95]. In our female patients the relative fatty acid composition of plasma lecithin indicated a small increase of palmitic (16:0) acid and decrease of linoleic (18:2) acid, but it is doubtful whether these mariginal changes could influence on liver TG synthesis and were furthermore corrected during dietary treatment.

In connection with the recently launched lipoprotein family concept, it has been suggested[2] that the relative distribution of lipoprotein families in serum might also be used as a guidance to the genesis of hypertriglyceridemia. Thus, an increased relative content of polypeptide C_{III} has been taken as an expression of increased hepatic TG synthesis and VLDL secretion[2]. There was a strikingly high level of C_{III} in our uremic patients (Fig. 17), and furthermore a correlation between serum TG and the C_{III} levels (Fig. 18).

The alternative cause of hypertriglyceridemia is *a decreased clearance of VLDL and chylomicrons*. The TG distribution among lipoprotein fractions would favour this mechanism as a major cause of hypertriglyceridemia in our series of uremic patients. The appearance of a typical HLP type-III pattern in one patient as well as of a type-III index[155] > 1.2 in 7 out of 14 uremic patients in the present series would indicate the presence of intermediate low density lipoproteins (ILDL) (cf. introduction) and thus would most likely be an expression of a reduced catabolic rate of VLDL to LDL. This would be in agreement with earlier findings in uremia[10, 90, 120]. Furthermore, in the present study the TG clearance rate, studied as fractional removal of TG as Intralipid particles, was reduced. If it can be assumed[135, 136] that the fractional turnover of Intralipid particles is an expression of the turnover of VLDL, this finding would imply further evidence for a reduced catabolic rate of VLDL in uremia.

In uremia lipoprotein lipase (LPL) activity is generally reduced, both in post-heparin plasma[11, 51, 113, 116] and in adipose tissue[40, 129], even though the enzyme content *per se* may be unchanged[13]. Preliminary data on a subsample of the present patient series would indicate, in accordance with recently published data on LPL activity in uremia[113], that a reduction of the hepatic triglyceride lipase (HTG) accounts for a major part of the decrease of the post-heparin lipolytic activity in plasma. This deficiency could contribute to the appearance of ILDL and hypertriglyceridemia.

Furthermore, analysis of lecithin:cholesterol acyl transferase (LCAT) activity may be of value in the evaluation of the mechanism for hypertriglyceridemia[158]. Our data indicate a reduced LCAT activity in relation to serum TG, which again would be expected to be related to a reduced conversion of VLDL to LDL[72] (cf. introduction). On the other hand, a reduced LCAT might also be secondary to a general impairment of liver protein synthesis in uremia[33] (cf. intermediary metabolism).

In the context of the lipoprotein family concept and in the light of recent findings[2, 67], and also in view of our findings of strong indication of a reduced catabolic rate of VLDL the low content of apo–E is surprising. One characteristic finding in non-uremic hypertriglyceridemic patients with marked increase in serum TG (> 5.0 mmol/l) is a simultaneous marked increase of apo–E as also found in type III hyperlipoproteinemia[67]. Again, however, in the catabolic state of the uremic patient, a depression of apo–E synthesis[59] could still be a possible explanation for the lack of a characteristic apo–E increase (cf. intermediary metabolism). In this context, it should be remembered that also the liver-synthesized apolipoprotein B tended to be low, possibly also as an expression of a reduced protein synthesis.

These studies in uremic patients indicate a reduced catabolic rate of VLDL–TG as the main cause of hypertriglyceridemia, and this would agree with recent experience from TG turnover studies, demonstrating a low catabolic rate of TG-carrying lipoproteins in uremia in man[48] and in the rat[13, 75]. It should be emphasized, however, that in uremia the validity of the present approach to TG turnover studies may still be questioned[119].

Furthermore, our studies with dietary management as a means of correcting the metabolic situation in uremic patients showed that a protein-reduced diet with increased carbohydrates (as sucrose), and fat did not unfavourably influence the serum TG level and thereby serum TG formation in the liver, as would have been expected if an increased TG synthesis had been a basic metabolic defect in uremia.

Influence by drug treatment on lipid and carbohydrate metabolism

Although the number of patients not on beta-blocking agents (alprenolol, propranolol) was low (n = 5) in the present series (IV), the difference in serum TG values could be of interest. In the 23 patients treated for hypertension with beta-blocking agents the mean serum TG value was slightly higher (3.4 vs 2.5) than in the five untreated patients. This finding is interesting in view of similar findings recently obtained in uremic patients on dialysis[39], where beta-blockade was linked to a higher serum TG value. Therefore, the possibility that the use of beta-blocking agents in uremia contributes to derangements in lipid metabolism, cannot be ruled out.

Influence of dietary treatment on lipid and carbohydrate metabolism

The reduction in clinical manifestations of uremia during dietary treatment was possibly due to beneficial influences on certain fundamental factors also contributing to the abnormalities in lipid and carbohydrate metabolism[63, 141]. This general beneficial influence of the diet might partly be related to the avoidance of excessive energy supply by the diet[141] but may also be due to an increased physical activity and energy expenditure. On the other hand, if an energy deficiency had been present as an explanation for maintained lipid levels during treatment, the constancy in body composition would not have been expected.

Furthermore, the dietary treatment did not influence the glucose utilization rate or plasma insulin values, as has been observed after dialysis[50, 52, 53, 153] in spite of a substantial decrease in serum urea. Interestingly enough, the earlier verified relationship between plasma insulin and serum TG was lost during dietary treatment.

Serum lipids and serum lipoproteins, as well as variables relating to glucose metabolism, did not change during dietary treatment, although the sucrose intake was high. Individual TG changes (including 7 out of 22 patients with a marked increase) were not correlated to either carbohydrate or total fat and total energy intake or the content of polyunsaturated fat in the diet. This lack of influence of the high sucrose content in the diet should be compared with corresponding results in short-term studies in non-uremic patients[45], where the serum TG was increased concomitant with a reduced elimination rate of exogenous fat (Intralipid) and a reduction in the relative content of plasma lecithin linoleic (18:2) acid.

The high fat content in the protein-reduced diet did not appear to have an unfavourable effect on serum lipids or lipoproteins, which was possibly related to the relatively high content of polyunsaturated fatty acids in the diet. In short term studies in uremia, a marked increase of polyunsaturated fat (concomitant with a reduction in carbohydrates) has been shown to decrease TG production and serum TG values[138]. This favourable effect on serum TG of polyunsaturated fat in the diet may in addition be related to an increased TG elimination rate[117].

In contrast to the lack of influence on serum TG, serum cholesterol changes were related to dietary fat intake in the diet, with a decrease in relation to a higher polyunsaturated fat intake, in accordance with findings in non-uremic subjects. An additional interesting finding was that the α-lipoprotein cholesterol changes appeared to parallel those of serum cholesterol in relation to total fat and polyunsaturated fat intake.

Finally, the apolipoprotein content in serum was not to any great extent influenced by the protein-reduced diet, possibly indicating that low pretreatment values of apo-B, D and E were not related to an inadequate intake of protein or essential amino-acids (cf. Intermediary metabolism).

Lipid and carbohydrate metabolism and atherosclerosis in uremia

Certain of the abnormalities of lipid and carbohydrate metabolism found in the present study, could be associated with the development of atherosclerosis in uremia.

Lipoprotein content and composition: Increased VLDL content of cholesterol and triglyceride as well as abnormal HDL composition with increased relative content of triglyceride and reduced content of apolipoprotein A were characteristic findings in male patients with myocardial infarction[166] and those were also our findings in uremia.

An isolated increase of apolipoprotein C_{III} together with elevated triglyceride levels was a second characteristic of the lipoprotein pattern in uremia but the possible association with atherosclerotic vascular disease needs further study.

Insulin resistance with normal or elevated plasma insulin levels are additional apparent features in patients with myocardial infarction[16a] particularly in connection with hypertriglyceridemia and moderate obesity. Moreover a correlation has been demonstrated between plasma insulin and the cholesterol content of the arterial wall[36a]. Insulin resistance appears to be an important characteristic of uremia, although the plasma insulin levels were not elevated in the present study, and could be of importance for development of atherosclerosis.

The *dietary treatment* did not influence on the above mentioned abnormalities and should in this respect not excert a further negative influence on the development of vascular disease. However, it appears to be of considerable interest to further investigate these abnormalities of lipid and carbohydrate metabolism in uremia and also in connection with the rapid improvement in renal function after a successful renal transplantation.

Intermediary metabolism in uremia and the influence of dietary treatment

In uremia derangements of protein as well as of lipid and carbohydrate metabolism occur and the similarities to the metabolic situation in malnutrition have often been pointed out[61].

Although the genesis to these derangements is multifactorial, one important basic abnormality appears to be a disturbance in insulin action. This is characterized by an *insulin resistance* together with a relatively decreased pancreatic insulin response to hyperglycemia (Fig. 21).

The insulin resistance has been related to several factors like:

- accumulation of inhibitory metabolites[109],
- increased levels of insulin antagonists e.g. catecholamines[142], glucagon[29] and growth hormone (particularly when uremia is accompanied by malnutrition)[12, 50, 52, 87],
- potassium depletion or disturbances of calcium homeostasis due to secondary hyperparathyreoidism[3, 28, 52] and
- adaptation to transient hyperinsulinemia, due to decreased metabolic clearance of insulin, during the earlier course of renal insufficiency[52, 61, 130].

In uremia there is thus a combination of insulin resistance and a normal or only slightly elevated plasma insulin while fasting blood glucose is usually not elevated. This metabolic situation differs from that in adult onset diabetes and in hypertriglyceridemia associated with obesity, where insulin resistance is usually accompanied by elevated plasma insulin levels[102, 124]. This *relative insulin deficiency* in uremia may be expected to be accompa-

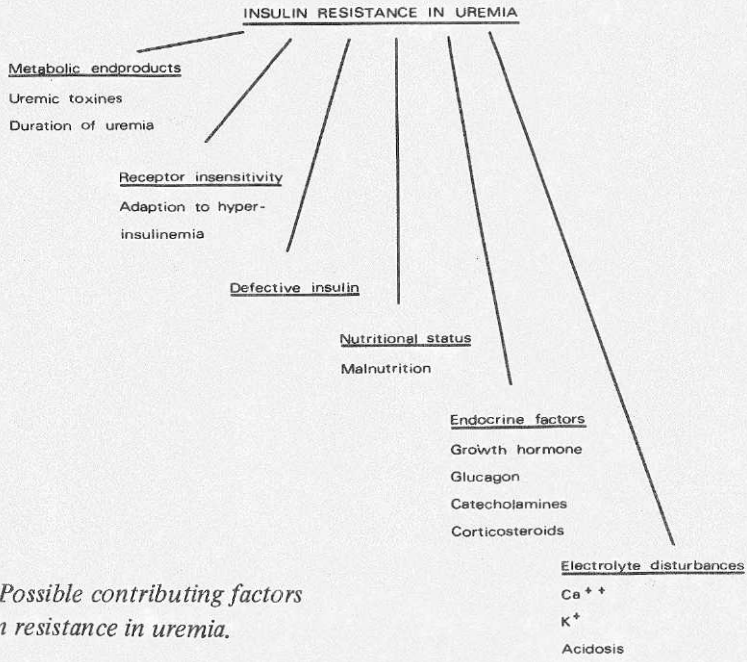

Fig. 21. Possible contributing factors to insulin resistance in uremia.

nied by several basic defects in the intermediary metabolism and also associated with the dietary treatment (Fig. 22).

A decreased peripheral glucose utilization primarily affecting muscle tissue. This defect may be partially overcome by increased physical activity concomitant with relief of uremic symptoms during dietary treatment (cf. Introduction).

An *increased formation of glucose*[65] from lactate, amino-acids and glycerol (Fig. 22). The low plasma lactate levels found in the present series could be an expression for a compensatory overutilization of lactate in gluconeogenesis (cf. Introduction, Intermediary metabolism). On the other hand a low protein intake could limit the utilization of amino-acids in gluconeogenesis.

A decreased amino-acid utilization in protein synthesis. This decrease of protein synthesis could affect not only structural proteins (like cellular proteins in e.g. muscle tissue) and transport proteins (apolipoproteins, serum proteins) but also synthesis of specific enzymes e.g. LCAT[33, 77] (cf. Lipid metabolism). Decreased synthesis of protein in the liver may be reflected by the low plasma albumin and in the present study, also a low content of specific apolipoproteins. Apo–B and apo–E (presumably formed in the liver) were unexpectedly low in relation to other metabolic findings (cf. Lipid metabolism). Low plasma LCAT activity (cf. Lipid metabolism) might possibly also be an expression of a low level of protein synthesis (and enzyme availability) in relation to uremic insulin deficiency. LCAT is known to be a sensitive protein enzyme, easily influenced by liver disease and disturbances of protein synthesis[33]. Other enzymes of considerable interest in

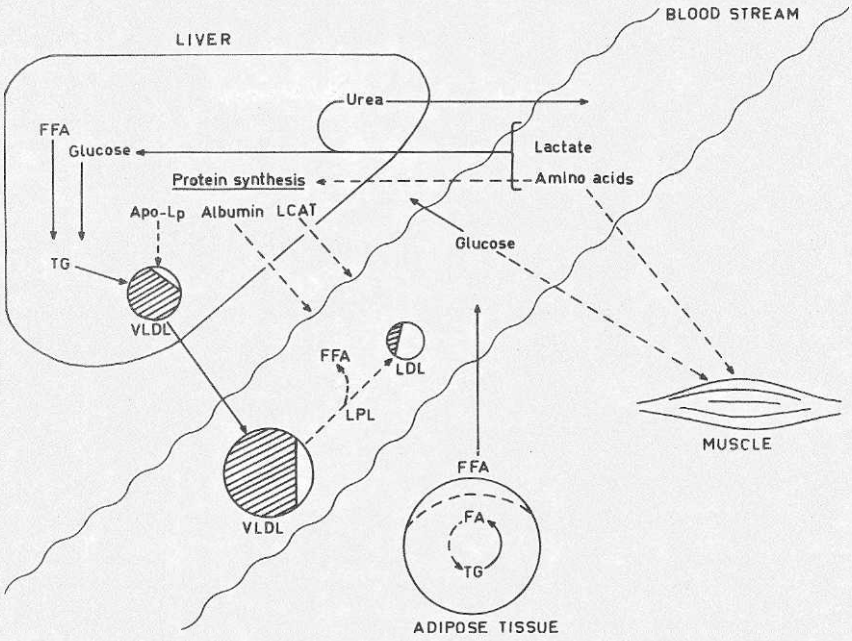

Fig. 22. Possible metabolic consequences of insulin resistance in uremia. Solid lines indicate increase and broken lines decrease of metabolic pathways. (FA = fatty acids, Apo–Lp = apolipoproteins, LDL = low density lipoproteins, LPL = lipoprotein lipase, TG = triglycerides, VLDL = very low density lipoproteins).

relation to hypertriglyceridemia are the lipoprotein lipases, particularly the hepatic TG lipase (HTG) (cf. Lipid metabolism) which has been found to be reduced in uremia and linked to the appearance of intermediate density lipoprotein particles (ILDL).

In uremia, protein synthesis may be impaired through a decreased amino-acid utilization and treatment with a low-protein diet could further accentuate the unfavourable influence on protein metabolism (cf. Introduction).

However, the *treatment with the amino-acid supplemented diet* led to an improvement of not only the uremic symptoms but also of nitrogen balance with a *reduced accumulation of nitrogenous metabolites* (paper II). The maintained low serum urea levels and the maintained body cell mass and serum proteins would furthermore indicate a lasting *anabolic effect* of the treatment (paper III).

The reduced accumulation of metabolites during treatment would also contribute to the improvement of uremic toxicity. As shown in the present study, this could in turn imply a *favourable effect on the intermediary metabolism*, so that the large amounts of fat and sucrose in the diet could be assimilated without further negative influence on neither serum lipid nor carbohydrate metabolism (paper IV, V). From these results it may also be concluded that these last-mentioned derangements of metabolism in uremia would rather be related to loss of renal function *per se* than to malnutrition.

SUMMARY

Treatment with an essential amino-acid supplemented low-protein diet is effective for relief of uremic symptoms. Long-term dietary treatment is, however, associated with several problems:

- recognition of the functional limits for successful treatment
- a risk of protein depletion and malnutrition
- aggravation of the disturbances in lipid and carbohydrate metabolism
- possibilities of dietary adherence

The aim of this investigation was to study these problems in a clinically homogeneous series of patients who could be treated under strict surveillance by the same team for a longish period of time.

From the results of the investigation, the following conclusions are drawn:

1. Accurate measurement of the glomerular filtration rate (GFR) in advanced renal insufficiency should be performed with an external marker (^{51}Cr–EDTA or inulin) and with collection of urine in the clearance determination.
2. The lower limit of GFR for successful dietary treatment was about 4 ml/min.
3. A 20 g protein diet should be supplemented with the essential amino-acids and histidine (EAAH) to achieve nitrogen balance. In routine treatment supplementation with 1.3 g N as EAAH, i.e. 9.6 g essential amino-acid mixture according to Rose and 0.8 g histidine, is sufficient for most patients.
4. Measurements of body composition are convenient for long-term observation during treatment. In most patients with early uremic symptoms the relatively normal body composition was maintained during long-term treatment up to 12 months, unless complications of catabolic nature, e.g. intercurrent disease or nutritional difficulties, occurred. Repeated determination of body cell mass appears to be a sensitive method for early detection of malnutrition.
5. Adherence to the diet used appeared to have been good in a routine outpatient population, indicating that dietary counselling was adequate and that the diet was sufficiently palatable and varied even during prolonged treatment.
6. Lipid and carbohydrate metabolism was deranged in uremia. Impaired glucose tolerance, hypertriglyceridemia, a low elimination rate of exogenous fat and a low content of apolipoprotein and cholesterol in high-density lipoproteins (HDL) were the principal findings. Reduced catabolism of triglyceride-rich lipoproteins appeared to be the dominating cause of hypertriglyceridemia in renal failure. The abnormalities may at least partly be linked to disturbances in the different effects of insulin.
7. Dietary treatment with a high energy intake from fat and sucrose did not have an unfavourable influence on the deranged lipid and carbohydrate metabolism.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to:

Professor Härje Bucht, my teacher in nephrology, who stimulated me to start the work on dietary treatment and who has guided me with his kind and never failing support.

Professor Björn Isaksson, who has taught me clinical nutrition and has provided excellent working conditions, valuable support and constructive criticism.

Professor Anders Gustafson, who introduced me to the field of lipid metabolism. His enthusiasm, knowledge and constant guidance, together with his generous hospitality, have been greatly appreciated.

Associate Professors Rudolf Jagenburg and Mattias Aurell and Drs Jürgen Ewald and Olle Wiklund for their stimulating collaboration and helpful advice.

Professor Per Björntorp, for generously providing laboratory facilities.

Associate Professor Göran Lindstedt and the staff of the Department of Clinical Chemistry for valuable help with certain analyses.

Professor Petar Alaupovic at the Oklahoma Medical Research Foundation for performing the apolipoprotein analysis and for valuable discussion.

Mrs Gunilla Uddebom, who has been responsible for the dietary counselling, which she has tackled with great enthusiasm and skill.

Nurse Anna-Lisa Leijon for taking such excellent care of the patients on the renal ward.

Bertil Arvidsson, M.Sc. and the Department of Radiation Physics for the whole body counting examinations and head technician Margareta Andersson and her staff for valuable technical assistance with the total body water determinations and preparation of figures.

The staff of the Clinical Metabolic Laboratory, in particular Ms Inga Hvass, Aira Lidell and Margit Wettesten, for performing the lipid analyses and Mrs Margareta Medberg for performing the fatty acid analyses.

Miss Margareta Hermansson for painstaking analysis of the dietary records.

Bo Eriksson, Fil lic, for performing the statistical work and for fruitful discussion.

Ms Ingrid Einvall, Marie-Louise Johansson and Gunilla Nilsson for excellent secretarial assistance, Mrs Barbro Johansson for typing of the final manuscript and Mr Karl Sandell for skilful photographic work.

Mr John Gulliver, for revising the English text.

This work was supported by the Swedish National Association against Heart and Chest Diseases, the Swedish Medical Research Council (19X–570 and 19X–05231–01, –2), AB Astra and AB Vitrum.

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Printed in Sweden, Gotab, Kungälv, 1978.25560