FACTORS PREDISPOSING TO ARTERIAL DISEASE IN CHILDREN

WITH CHRONIC RENAL FAILURE

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THESIS

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TO MY PARENTS FATMA AND ABDULAZIZ,

FOR ALL THEIR SUPPORT AND ENCOURAGEMENT

THROUGHOUT MY LIFE

ABSTRACT:

Premature atherosclerotic disease is a major cause of morbidity and mortality in chronic renal failure (CRF). Hypertension, hyperlipidaemia, abnormalities of nitric oxide (NO) biochemistry, drugs and the uraemic milieu are possible causes. Endothelial dysfunction is a key early event in atherogenesis. In this thesis, a non-invasive technique of high-resolution ultrasound has been used to assess the physiology of endothelial function in the brachial artery of children with stable CRF but without other risk factors of atherosclerosis. Lipid subfractions and nitric oxide (NO) biochemistry were also studied in the children.

NO metabolites and endogenous NO synthetase (eNOS) inhibitors were measured as an assessment of endothelial NO metabolism.

Brachial artery dilatation to flow (FMD) was reduced in CRF children when compared to matched controls for age and vessel diameter. In contrast, response to glyceryltrinitrate (GTN) was similar in both groups. Antibodies against oxidised LDL (ox-LDL) were high in CRF. Endogenous NOS inhibitors were high in CRF, and intermediate NO metabolites were low.

This study shows that endothelium dependent dilatation of the brachial artery is impaired in children with CRF who do not have co-existing risk

factors for atherosclerosis. This may represent early evidence of atherogenic vascular disease.

In the second half of the thesis, the effect of enteral feeds on lipid levels in children with CRF were studied. This is important because the anorexia of CRF is frequently managed with high carbhydrate and high fat enteral feeds, which might predispose to atherogenic blood lipid profiles. Plasma lipid sub-fractions were, therefore, measured in children with CRF whose diet was either managed conservatively or by enteral feeding.

Overall, TGs were high, TC was at the upper limit of normal, and LDL, HDL, apoprotein A1 (apo A1), A2 (apo A2) and B (apo B), and lipoprotein (a) (Lp (a)) were within the normal range. There was an inverse correlation between TGs and GFR.

There were no differences in the levels of TC, TGs, LDL, HDL, apo A1, apo A2 or Lp (a) between tube fed and non-tube fed children. We conclude that enteral feeding does not enhance hyperlipidaemia.

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ADMA	asymmetric dimethylarginine
AGE	advanced glycation end products
ANCOVA	analysis of covariance
Apo A1	apoprotein A1
Apo A2	apoprotein A2
Apo B	apoprotein B
CAD	coronary artery disease
CAPD	continuous ambulatory peritoneal dialysis
CETP	cholesterol ester transfer protein
cGMP	cyclic GMP
CHD	coronary heart disease
CHO	carbohydrate
CHOD-PAP	cholesterol C system high performance
	cholesterol oxidase 4-aminophenazone
CRF	chronic renal failure

CVD	cardiovascular disease
EDRF	endothelium derived relaxing factor
EF	enteral feeds
ELISA	enzyme-linked immunosorbent assay
eNOS	endogenous NO synthetase
ESRD	end-stage renal failure
FA	fatty acids
FMD	flow mediated dilatation
GFR	glomerular filtration rate
GPO-PAP	glyceryl phosphate oxidase 4-aminophenazone high performance enzymatic colorimetric test
GTN	glyceryl/trinitrate
HD	haemodialysis
HDL	high density lipoprotein
HPL	hyperlipidaemia

HTGL	hepatic triglyceride lipase
IDL	Intermediate density lipoprotein
iNOS	inducible NO synthetase
LCAT	Lecithin cholesterol acyltransferase
Lp(a)	lipoprotein (a)
Lp-Bc	lipoprotein B particles
LPL	lipoprotein lipase
Monos	mono-saturated fatty acids
nNOS	neuronal NO synthetase
NO	nitric oxide
NOS	NO synthetase
ox-LDL	oxidised LDL
PD	peritoneal dialysis
PGI2	prostacyclin
PUFAs	poly-unsaturated fatty acids
PUV	posterior urethral valve
PWV	pulse wave velocity

RRT	renal replacement therapy
SD	standard deviation
SDMA	symmetric dimethylarginie
SDS	standard deviation score
SEM	standard error of mean
SFAs	saturated fatty acids
TC	total cholesterol
TGs	triglycerides
VLDL	very low density lipoprotein
VUR	vesico-ureteric reflux

ACKNOWLEDGMENTS

CHAPTER (1)

INTRODUCTION

INTRODUCTION

1.1 Occurrence and Pattern of Cardiovascular Disease in Chronic Renal Failure

1.1.1   Occurrence

Cardiovascular complications are the principal cause of morbidity and mortality in patients with end-stage renal disease (ESRD) (1), and account for almost half of the total mortality in ESRD (2,3). The association between renal disorders and premature vascular complications was shown in pioneer studies more than 2 decades ago (4-6) and an increased mortality from cardiovascular disease (CVD) amongst patients with pre-dialysis chronic renal failure (CRF), on dialysis and following transplantation has been well documented (7-9). Despite advances in dialysis technology, there is no evidence for a decrease in the prevalence of atherosclerosis during the last decade, as shown in a recent study by the European Renal Association - European Dialysis and Transplant Association (ERA-EDTA) Registry (1). This

report showed that the cardiovascular mortality rate of chronic dialysis patients, who were stratified into various sub-categories according to age, gender, and geographic origin, was approximately 20 times higher than that of the general population. This is particularly true in young patients: for example, in subjects on maintenance dialysis aged 20 to 44 years, the incidence of cardiovascular death is approximately 150 times that of age-matched controls (2,3). The relative risk of death from myocardial infarction in patients receiving any form of renal replacement therapy (RRT) in the United Kingdom (UK) has been reported to be 5-10 times that of the general population (7). There is a different prevalence of CVD amongst patients with CRF between different countries. EDTA registry data (4) showed a greater mortality from myocardial ischaemia and infarction amongst patients on RRT from Northern Europe compared to those in Southern Europe (1). CVD mortality rate for patients from the UK was 3-4 times higher than that of patients from Italy; however a similar increase relative to the general population was observed in both countries. Either the age distribution of the patients or the frequency of diabetes could not explain the difference. Therefore the differing prevalence of CVD amongst patients with CRF between individual countries may just reflect the differing prevalence of CVD in the general population.

1.1.2   Pattern

1.1.2.1 Cardiac arrest and cardiac failure

Cardiac arrest and cardiac failure are quite frequent causes of death,
particularly in the younger patient groups. During the first year of RRT,
cardiac arrest occurred in paediatric patients at a higher rate than in
adults up to 55 years of age (10). The death rates due to cardiac causes
are high during the first year, but decrease during years 2-5 on RRT in
the younger patient group. This could be explained by transplantation,
which is more frequently performed in the young, and effectively
prevents many cardiac deaths such those due to hyperkalemia and
over-hydration. Other coronary artery-independent mechanisms, which
have important roles in the pathogenesis of cardiac failure, include
myocardial hypertrophy, valvular disease and anaemia (11). Sudden
cardiac death or severe left ventricular dysfunction accounts for 30% of
the mortality of ESRD (2).

1.1.2.2 Myocardial ischaemia and infarction

Myocardial ischaemia and infarction contribute significantly to causes of death in ESRD (10,11). The major underlying factor leading to myocardial infarction is arterial disease (4). Myocardial infarction accounts for approximately 15% of the total number of deaths (2). In males with standard primary renal diseases, coronary deaths occur at almost twice the rate in females (10) . Coronary deaths in females in the general population have been recorded at only about a quarter of the rate in males of any age group. The death rate due to coronary artery disease is determined mainly by age and not by the time spent on RRT. The younger the patient on RRT, the higher the rate of myocardial ischaemia and infarction in comparison to the normal population, as shown from statistics from the population of the Federal Republic of Germany (FRG) (12). Male patients with standard primary renal diseases had death rates from myocardial infarction which, when compared with the general population of the FRG, were over 150 times higher in the age group 15-24 years, 75 times in those aged 25-34, 25 times in those aged 35-44, 10 times higher in those aged 45-54, and over 5 times higher in those aged 55-64 years. These relationships were

at least doubled in females on RRT. The high incidence of CVD in young patients has been shown in other studies (1,3). Previous studies showed that there is a little difference in the incidence of coronary artery disease between grafted patients and all patients on RRT, with death rates higher during the 1st year and lower during 2nd and 3rd year after transplantation (10). This is not a consistent finding however, as other studies showed that following transplantation there is a significantly lower risk of death from CVD (7).

1.2 Arterial Disease in CRF

Arterial disease is the major underlying factor leading to myocardial infarction and cerebrovascular events (4) which occupy an important place in the mortality of patients with ESRD. The arterial disease in CRF is usually due to growth of a thrombus at the site of an atherosclerotic plaque. The arteries act both as conduits and as cushions (13) they deliver blood with minimal loss of mean pressure to body tissues and organs, and at the same time smooth the pulsation resulting from intermittent cardiac ejection.

1.2.1 Conduit Function of Arteries

The efficiency of conduit function is related to the width of the arteries

and the near constancy of mean blood pressure along the arterial tree.

In the supine position, the mean pressure drop between the ascending

aorta and arteries in the forearm or leg is only 2 to 3 mm Hg (13,14). In

the resting state, conduit function is maintained until an artery is

narrowed to 20% or less of its original diameter (11). Disorders of

conduit function that occurs by narrowing the vessel lumen, affect the

tissues and organs downstream by ischaemia or infarction (13).

Atherosclerosis is the most common disease that disturbs conduit

function. Atherosclerosis is primarily an intimal disease, focal and patchy

in its distribution, and characterised by the presence of plaques in the

carotid bifurcation, coronary arteries, renal arteries, infrarenal aorta, and

femoral arteries. The hypothesis that atherosclerosis is accelerated in

patients with ESRD was suggested by Lindner et al in 1974.

Subsequent post-mortem (15) and angiographic (16) studies confirmed

that the prevalence of atherosclerotic coronary artery disease (CAD) is

increased in patients on dialysis when compared with subjects of similar age without renal impairment. Recently, a similar finding has been reported in a large study from Japan, using an ultrasonographic technique, demonstrating an increased intima/media thickness of the carotid artery in chronic haemodialysis patients, compared with age-matched healthy control subjects (17). The high prevalence of CAD among CRF patients appears to result from multiple risk factors, including a positive family history for atherosclerosis, systemic arterial hypertension, alteration in shear stress, smoking, lipid disturbances, hyperhomocysteinemia, and growth factors and mediators of inflammation.

1.1.2   Cushioning Function of Arteries

The arteries transform pulsatile flow in central arteries to almost steady flow in the tissue (13). During systole around 50% of the stroke volume is directly forwarded into the peripheral circulation, and the other 50% is stored in the aorta and major arteries, distending their walls and storing part of the energy then available in diastole. During diastole the stored energy passively recoils the aorta, pressing the stored blood volume into the peripheral circulation. This 'Windkessel function', in the form of distensibility and compliance, is very efficient in young and healthy

humans, with minimal extra energy lost (10-15%) on account of intermittent ventricular ejection than if the heart's output were non-pulsatile and continuous (18). The physical properties of arterial walls determine the amplitude of pressure waves, and their propagation and reflection along the arteries. Ejection of blood from the heart into the aorta generates a primary pressure wave (incident), which is propelled forward to other arteries throughout the body at a given velocity (pulse wave velocity-PWV) (19). PWV increases with arterial stiffening and therefore is higher in the distal parts of the arterial tree. The incident wave is reflected at any point of structural or geometric discontinuity of the arterial tree, generating a natural counter-pulsation (reflected wave) travelling backward from peripheral reflecting sites towards the ascending aorta (19,20). Incident and reflected waves sum up to determine the amplitude and shape of the measured pulse pressure waves, which depend on the amplitude and phase of relationship between the component waves (11). Arterial stiffening increases systolic and pulse pressure, both directly by increasing the amplitude of the pressure wave and indirectly by increasing PWV, causing an early return of the reflected wave from the periphery to the aorta. The direct mechanism is responsible for an increased pulse or systolic pressure in the entire arterial system, while the indirect mechanism predominantly increases aortic and left ventricular pressure in late systole at the

expense of mean diastolic pressure, which is decreased. This result in

an increased hydraulic load imposed on the left ventricle and a reduction

in sub-endocardial coronary blood supply during diastole.

Arteriosclerosis (which is a different process from atherosclerosis but

frequently coexists with it) is the principal cause of altered cushioning

function (18). Arteriosclerosis results in diffuse fibroelastic intimal

thickening, an increase in medial ground substance and collagen, and

fragmentation of the elastic lamella, with secondary fibrosis and

calcification of the media. The changes are more pronounced in the

aorta and central arteries than in limb arteries. Arteriosclerosis is

considered as a physiological ageing phenomenon that is accelerated

by hypertension.

In ESRD, arterial structural changes similar to those of the ageing

process occur, characterised by diffuse dilation, hypertrophy and

stiffening of the aorta and major arteries. Arterial remodelling usually

occurs in response to long-term changes in haemodynamic conditions,

and interaction with locally generated growth factors, vasoactive

substances, and inflammatory mediators (21) The endothelium seems to

play an important role in the remodelling process (11).

Arteriosclerosis does not affect conduit function, in contrast to

1.3 Risk Factors for Atherogenesis In Chronic Renal Failure

1.3.1 Arterial Hypertension

Hypertension is common in subjects with ESRD, occurring in 80% of adult patients with ESRD before starting dialysis therapy. After dialysis treatment hypertension persists in a large proportion of patients. In particular, the physiologic blood pressure nocturnal dip is frequently absent (26). There is an association between high blood pressure and occlusive vessel wall changes. The frequency of atheromatous plaques is increased in-patients with arterial hypertension. This can be inferred from the fact that atherosclerotic plaques are virtually confined to systemic arteries where the tensile stress is high; in aortic coarctation, atherosclerosis is accelerated in upper body arteries where the pressure is high, whereas it is decreased in severity in lower body arteries where the pressure is lower (11). Further evidence is that the development of atherosclerosis in autogenous vein by-pass grafts can be experimentally prevented by a rigid external support that counteracts the increase in transmural pressure of the graft (27). Hypertension seems to enhance

the endothelial permeability to macromolecules thereby directly

activating stress-sensitive ion channels and predisposing to

atherosclerosis (11).

Hypertension is also associated with arteriosclerosis. It induces vascular

hypertrophy and causes an increase in the thickness of the arterial

intima-media layer and a decrease in lumen diameter, resulting in an

increase in media/lumen ratio. In the non-uraemic population, arterial

hypertrophy compensates for the increase in blood pressure or radius to

maintain normal stress, resulting in an increase in the wall to lumen ratio

(28). This does not occur in ESRD, where decreased arterial

distensibility results from arterial wall hypertrophy and the incremental

modulus of elasticity is increased in comparison with age and pressure

matched non-uraemic controls (23). This results in fibroelastic intimal

thickening, calcification of elastic lamellae and ground substance

deposition (29), causing arteriosclerosis.

Hypertension therefore plays an important role in the pathogenesis of

atherosclerosis, which is associated with ischaemia and haemorrhage

(30). A significant reduction in cerebro-vascularmorbidity and mortality

can be achieved with satisfactory control of hypertension (31). The

relationship between hypertension and ischaemic heart disease is less

well established, as some studies have shown that the control of hypertension is associated with an improvement of ischaemic cardiac events, while other studies have shown only a borderline or absent effect (31). On the other hand, there is evidence that drug treatment may increase coronary risk in certain subgroups, as observed in the Multiple Risk Factor Intervention Trial (32). In that study, men with a diastolic BP less than 100 mmHg who received drug treatment had a higher mortality rate than those in the usual care group. Similar results were found in other studies (33). There are many possible explanations for these findings (34):

Firstly, it may be that hypertensive patients may have an increased level of unidentified factors implying higher coronary heart disease (CHD) risk, not corrected by anti-hypertensive drugs. BP could be a marker of a metabolic, atherogenic or thrombogenic state (35). Hypertensive patients have higher serum total cholesterol (TC) and triglycerides (TG) levels and lower high density lipoprotein (HDL) cholesterol .

Secondly, there may be an optimal low BP level below which it is harmful to further decrease BP.

Thirdly, the achieved BP reductions were too small to result in a decline of CHD risk.

Fourthly, the drugs may have harmful effects that are not yet fully recognised.

Fifthly, The trials were too short and did not include the appropriate population group, as drug trials have mainly been undertaken in middle-aged men and women. In addition, exposure time to an increased BP may be too long for a reversal of the CHD risk by a relatively short period of drug intervention (36).

In CRF, an association between hypertension and occlusive wall changes was found in chronic haemodialysis patients (30) and rigorous control of high blood pressure at the time of incipient renal failure led to a significant decrease of the incidence of myocardial ischaemia after initiation of dialysis therapy (37). There is a high prevalence of isolated systolic hypertension due to arterial stiffening in ESRD patients (38). Increased pulse pressure, which is associated with systolic hypertension, has been found to be an independent and significant predictor of myocardial infarction and coronary death (39,40)

1.3.2 Disturbances of Lipid Metabolism

Hyperlipidaemia (HLP) is one of the factors believed to be responsible for the high incidence of atherosclerosis in CRF (41). Not all uraemic patients have HLP: prevalence figure of hypertriglyceridaemia is around 70% in adults (greater than or equal to 2.2 mmol/l)(42). The prevalence in uraemic children has not been firmly established, but paediatric nephrologists are often confronted with high blood lipid levels in children with CRF (43)

1.3.2.1 The most important abnormalities are:

1. An increase in the serum level of triglyceride (TG), intermediates density lipoprotein (IDL), cholesterol rich very low density lipoprotein (VLDL) and apolipoprotein (apo) B, and apo Bcontaining lipoproteins consisting of cholesterol rich lipoprotein B particles (Lp-Bc) (11).

2. A decrease in high density lipoprotein (HDL)-cholesterol, a decrease of the apoAI/apoCIII ratio and a reduction of apoCII/apoCIII (11).

All of these lipid changes, which have been found in adults, are believed to predispose to atherosclerosis. Studies in children have demonstrated similar findings, but with a higher incidence of hypercholesterolaemia (43). Uraemia also disturbs the dynamics of cholesterol transport, causing a diminished cholesterol transfer rate in serum from HDL to VLDL and an inhibition of reverse cholesterol transport from peripheral cells to the circulation (44). These changes result from a decrease in lecithin cholesterol acyltransferase (LCAT) enzymatic activity (45) and the accumulation of an inhibitor of the cholesterol ester transfer protein (CETP) (46). A decrease of the LDL receptor number at the cell surface probably contributes to the abnormal lipoprotein as well (47).

Most authors agree that the main abnormality is decreased lipoprotein catabolism, resulting in incompletely cleared intermediate particles and diminished formation of HDL (48). Lipoprotein lipase (LPL) activity has been found to be reduced in uraemic adults (49) and children (50). Diminished activity of hepatic triglyceride lipase (HTGL) has also been described (43). The remodelling of lipoproteins is associated with the exchange of apolipoproteins, resulting in abnormal apolipoprotein patterns within the different subclasses of lipoproteins (43):

• Decreased apo AI, apoAII

- Increased apo A IV

- Increased apo B48

- Decreased apo CII, increased apo CIII in HDL and VLDL: abnormal distribution of apoE

Diminished LPL / HTGL activities could be explained by insulin deficiency or resistance (51), increased levels of parathyroid hormone (2), the presence of LPL inhibitors in uraemic plasma (52), reduced apoCII/apoCIII ratio, and a low rate of fatty acid incorporation into adipose tissue (53)

Lipoprotein (a) (Lp(a)) is frequently increased in patients with CRF(43). Lp(a) is a cholesterol rich protein resembling LDL and an independent risk factor for the development of atherosclerosis and thrombosis (54). The phenotype of the protein moiety apo(a) which is genetically determined, controls Lp(a) levels (55). Increased Lp(a) levels might prove to be a marker for increased risk of atherosclerotic and thrombotic complications in CRF patients (43).

Uraemic patients have increased lipoprotein oxidation (56) and transformation by advanced glycation end-products (AGEs) (57). Both

processes enhance the occurrence of atherosclerosis (11). Glucose derived AGEs such as pyrraline and pentosidine cross- link proteins and cause monocyte activation (58) and tissue damage (59) .

The relationship between the abnormalities of lipoprotein metabolism and atheromatous vessel disease remains controversial (11,43). Some studies show that dialysis patients with vascular disease have more unfavourable lipoprotein profiles (increased TG, TC, VLDL) compared with patients who have no evidence of vascular disease (43,60). The same finding was found in patients with advanced CRF before they started dialysis (11). In contrast, numerous studies have shown that serum lipid levels are not significantly related to atherosclerotic lesions or to the development of ischaemic heart disease in adults treated with haemodialysis (43). The same controversy surrounds the association between lipid abnormalities and carotid artery ultrasonographic changes (intima / media thickness and atheromatous plaques) (11).

1.3.3 Endothelial Factors (Abnormal Nitric Oxide Metabolism)

Endothelial dysfunction is a key event that occurs early in the course of atherogenesis (61), preceding the formation of atherosclerotic plaque (62), and resulting in the inability to release endothelium-derived relaxing factor (EDRF) (63). EDRF is thought to be identical or closely related to nitric oxide (NO) (64), which is a major regulator of arterial smooth muscle tone and blood flow. NO not only protects against atherogenesis by relaxing vascular smooth muscle, but also by inhibiting the interaction of platelets and white blood cells with the endothelium (65). Impairment of the arginine-NO pathway in CRF (66) could be caused by dyslipidaemia. This may be secondary to decreased synthesis of nitric oxide or increased degradation of nitric oxide due to superoxide anions (67). Synthesis of NO is inhibited by asymmetric dimethylarginine (ADMA), which has been shown to accumulate in adults with CRF (66,68). When present in high concentration, ADMA increases vascular tone (69) but may also enhance the atherogenic process in low concentrations which have no direct effect on vascular tone (70,71). NO could be more rapidly inactivated in dialysis patients,

due to the excessive generation of oxygen derived free radicals (72).

The endothelium also produces other vasoconstrictor substances such as endothelin-1, which is increased in uraemic patients (73) A variety of other factors reflect an activation and/or dysfunction of the vascular endothelium in CRF, such as increased plasma levels of von Willebrand factor, plasminogen activators, thrombomodulin, fibrinogen, proconvertin and the inhibitor of type-1 plasminogen activator (11).

1.3.4   Shear Stress and Alteration In Blood Flow

Both low and high shear stress could affect the development of atherosclerotic lesion (11). Increased blood flow velocity and wall shear stress has been shown to enhance the permeability of the endothelial layers to macromolecules, causing erosions of the endothelium and vessel wall injury (74). On the other hand, sites with low shear stress, like the bifurcation of the aorta, could develop atherosclerotic plaques as result of its adverse effect on the mass transport of lipids across the endothelial layer (75). It seems fluctuating shear stress related to secondary non-laminar and pulsatile flow is the major etiologic factor. Its

role in the development of atherosclerosis in ESRD patients has not

been specifically investigated due to the difficulty of measuring the level

of shear stress in humans (11).

1.3.5   Disturbances of Glucose Metabolism

Insulin resistance is usually present in patient with CRF (11). In

experimental uraemia, insulin-dependent glucose uptake of cardiac

tissue is diminished (76). In addition, insulin resistance contributes to the

disturbed activation of lipoprotein lipase and the accumulation of VLDL

and IDL in renal failure (77). Haemodialysis or the administration of

exogenous insulin may improve lipid disturbances. Patients on

continuous ambulatory peritoneal dialysis (CAPD) receive a continuous

load of glucose from the dialysate. CAPD may, therefore, have more

atherogenic potential because of stimulation of different lipid

disturbances, such as an increase in the production of VLDL with

hypertriglyceridemia and a decrease in HDL cholesterol, by the high

glucose intake (78).

Diabetic patients with CRF are more prone to vascular complications

because of slow irreversible changes in extracellular molecules due to

hyperglycemia induced covalent modification, such as advanced

glycation end products (AGEs) (11).

1.3.6   Hyperhomocysteinemia

Hyperhomocysteinemia is present in CRF patients; pre-dialysis, dialysis

and post- transplantation (11). Hyperhomocysteinemia has been

identified as a vascular risk factor in the general population (80), as well

as in dialysis patients (79). Homocysteine accelerates LDL auto-

oxidation, favours vascular thrombosis, and enhances vascular smooth

muscle proliferation (11). Folic acid deficiency had been blamed as the

main cause of homocysteine accumulation (80), which could be

normalised, with supra-physiologic doses of folic acid, vitamin B6 and

vitamin B12 (11).

1.3.7   Other Potential Risk Factors

1.3.7.1   Disturbance of Calcium and Phosphate Metabolism

Vascular calcification is more common in uraemic than non-uraemic

patients (11). It is favoured by an increased calcium-phosphate product which occurs because of phosphate retention. In dialysis patients, a significant relation was found between the degree of carotid intima/media thickness and the plasma levels of phosphate and parathyroid hormone (17).

1.3.7.2 Vitamin E Deficiency

Vitamin E deficiency may enhance the occurrence of coronary heart disease in the general population (80,81). Vitamin E protects against cardiovascular disease by several mechanisms, including its anti-oxidative action to limit lipoprotein oxidation; its inhibitory effects on platelet adhesion and aggregation, and on monocyte adhesion to endothelial cells; its antiproliferative effects on vascular smooth muscle; and its intracellular effects on the monocyte leading to a decreased ability to release oxygen radicals and cytokines (11). The role of vitamin E administration in the reduction of cardio- or cerebrovascular mortality and morbidity is a still controversial (11). Prolonged dialysis is associated with a decrease of the vitamin E content in LDL and dialysis patients may benefits from relatively high doses of vitamin E to protect against LDL oxidation (11).

1.3.7.3 Cigarette Smoking

Tobacco consumption in uraemic patients has deleterious effects on the vascular system, as it does in the normal population (82).

1.3.7.4 Relative Estrogen Deficiency

Estrogen inhibits LDL oxidation and protects against atherosclerotic disease (11). Uraemic patients frequently have relative estrogen deficiency secondary to ovarian dysfunction as result of an abnormal hypothalamic- pituitary ovarian axis (83,84).

1.3.7.5 Vitamin B6 Deficiency

Vitamin B6 deficiency frequently occurs in dialysis patients and could contribute to the high incidence of vascular disease (11), as in non-renal patients in whom a negative relation has been found between the plasma level of pyridoxal-5-phosphate (the co-enzyme of vitamin B6) and the prevalence of extracranial carotid stenosis (79)

1.3.7.6 Cytokines and other Proatherogenic Substances

Uraemic patients have enhanced generation of a variety of cytokines with increased activation of monocytes and platelets through the contact with bioincompatible dialysis membranes. This could enhance atherosclerosis as some cytokines such as interleukin-1 have been described as pro-atherogenic (11).

Uraemia is also associated with excessive generation of other pro-atherogenic factors, such as endothelin-1, angiotensinogen and thromboxane B.

1.3.8 Viral Infection

Viral infections such as cytomegalovirus or adenovirus occur more frequently in CRF patients and could enhance atherosclerosis via the induction of vascular smooth muscle proliferation (11).

1.3.9 Drugs

1.3.9.1 Immunosuppressive Drugs

Corticosteroids and cyclosporine may be atherogenic via their negative effect on lipoprotein metabolism causing an increase in TC and TGs (85). In addition cyclosporine stimulates Lp(a) formation (86) and could accelerate the development of atherosclerosis via its suppression of cell-mediated immunity (87)

1.3.9.2 Diuretics and Antihypertensive Drugs

Drugs such as thiazides and beta blockers may cause an increase in serum TGs and LDL cholesterol and decrease LPL activity and therefore may be atherogenic.

1.3.9.3 Heparin

The regular administration of heparin has a negative effect on the lipopolytic enzymes LPL, HTGL and LCAT. Low molecular weight heparin does not have this negative effect .

The current view is that ESRD per se does not appear to induce an

acceleration of the atherosclerotic process. The presence of

numerous risk factors for atherosclerosis in these subjects

appears to be responsible for their increased prevalence of

coronary heart disease.

1.4 Nature of the Study

The outlook for children with CRF has improved so that now even

infants can be dialysed and transplanted successfully. However, their

long-term morbidity and mortality are uncertain, but they might be

expected to develop all the above-described complications at an early

age. Infants and young children (< 5 years old) make up a considerable

part of any paediatric ESRF program. In the North American Paediatric

Renal Transplant Co-operative Study (NAPRTCS) 11.1 % of the dialysis

population were infants and 22 % were less than 6 years old, 6.1 % of

the transplanted patients were less than2 years of age and 16.4%

between the ages of 2 and 5 years (88). In Europe, the EDTA registry

showed that less than 10% of children on RRT were in the younger age

group(0.5 - 4 years) (89). Feeding is a major problem in those young

children and poor appetite and vomiting results in growth retardation.
Enteral feeds (EF) are used in children with declining growth velocity,
with the best results for height in children who started enteral feeding
aged <2 years (90) Long-term overnight EF promotes rapid weight gain
with sustained catch-up growth (91) and does not prevent the
development of normal eating habits (92), but it might have an adverse
effect on the blood lipid profiles with an increased risk of coronary heart
disease. Therefore there is a need to determine whether the atherogenic
process starts in childhood in children with CRF and if any recognized
treatments such as EF predispose to arterial disease.

The purposes of this study therefore are as follows:

1. To study lipid sub-fractions in children with CRF, as hyperlipidaemia
   is known risk factor for vascular disease.

2. To determine whether enteral feeding adversely affects plasma lipids
   in children with CRF.

3. To see if the process of atherogenesis has begun in children with
   CRF, even in the absence of other risk factors for CHD.

4. To examine if CRF affects NO metabolism believed to be an
   important antiatherogenic factor.

1.4.1 The hypotheses are as follows:

1.4.1.1 Endothelial Function In Children With CRF

We hypothesised that CRF in the absence of other risk factors
predisposes to coronary heart disease. Therefore we examined
endothelial function which is the key early event in the process of
atherogenesis, in children with CRF but without other recognised risks
for CHD. In order to avoid confounding variables, we purposefully
selected subjects with CRF who were neither smokers, nor
hypertensive, hypercholesterolaemic nor diabetic, and were not taking
vasoactive drug therapy. A non-invasive technique using high resolution
ultrasound was used to assess the vascular response of brachial artery
to endothelial dependent and independent stimuli.

1.4.1.2 Nitric Oxide Metabolites Study

The metabolism of nitric oxide (NO), which is an endothelium derived
relaxing factor (EDRF), is disturbed in adults with CRF (66,68). We
examined different metabolites of NO in CRF children, who also
underwent studies of endothelial function. The hypothesis we tested is

that endothelial dysfunction caused by CRF could result in abnormal NO

release and therefore predisposes those children to atherogenesis by

both mechanisms.

1.4.1.3 Lipid Sub-fractions IN CRF patients and the Effect of Enteral Feeding

We examined lipid sub-fractions in CRF children, as hyperlipidaemia is a

known complication of CRF. The effect of enteral feeding, which is

frequently used to manage the anorexia of CRF, on lipid sub-fractions

was studied as well. Theoretically, enteral feeding could enhance

hyperlipidaemia and therefore increase the incidence of CHD.

CHAPTER (2)
PATIENTS

PATIENTS

2.1 Introduction

This chapter provides an overall summary of the principal epidemiological and clinical features of the children included in all sections of the study. It is on these patients that the clinical and laboratory studies described in later chapters in this thesis were undertaken.

2.2 Endothelial Function and Nitric Oxide Study

2.2.1 Patients (Appendix 2.1)

2.2.1.1 Age and sex

Twenty three children (18 boys), aged 7.8 - 17.0 years (median 12.0), with CRF were studied.

2.2.1.2 Primary diagnosis

Their diagnoses were renal dysplasia (19), reflux nephropathy (2), Alport's syndrome (1), and focal glomerulosclerosis (1).

2.2.1.3 Renal function

They were all conservatively managed, median (range) GFR 14.4 (8.8-34.5) ml/min/1.73m^2.

2.2.1.4 Selection

Patients were selected from an outpatient population because they were:

♦ Normotensive (mean (SEM) systolic blood pressure standard deviation score (BPSDS) for age -0.04 (0.15), diastolic -0.11 (0.18)).

♦ Plasma total cholesterol (TC) <5.2mmol/l and low density lipoprotein (LDL) <3.3 mmol/l, both of which are the upper range of normal values.

♦ Not diabetic.

♦ Not nephrotic (defined as serum albumin ≤ 30 g/l or proteinuria >40mg/m^2/hour), mean(SEM) serum albumin 42.9 (0.6) g/l, 24 hour protein excretion 0.85 (0.2) g).

♦ Not taking vaso-active or lipid-lowering medications.

2.2.2 Controls

2.2.2.1 Endothelial study

Patients were matched with 23 control subjects (friends or relatives of hospital staff) for age and brachial artery diameter (Appendix 2.2). Twenty of the controls were matched for gender with the CRF patients (87%). Ten boys and 3 girls in CRF group, compared to 9 boys and 4 girls in the controls, were over the normal age for onset of puberty (over 11 years in boys and 10 years in girls).

2.2.2.2 Nitric oxide study

Nitric oxide metabolites in the CRF patients were compared to values 6 control children, aged 6 - 16 (median 11 years), who were having blood taken for family genetic screening of non-cardiac abnormalities

2.3 Lipid Study

2.3.1 Age and sex

Forty seven children (32 boys) aged 1-17 years (mean 9.3 ± 5.2 (SD), median 10.4) with CRF (defined for the purposes of this study as a plasma creatinine concentration >150 μmol/l (1.7mg/dl)) were studied (Appendix 2.3). Sixteen of these children were included in both the endothelial function study and the lipid study as shown in the appendices.

2.3.2 Primary diagnosis

The distribution of causes of CRF in the patients was typical of that found in any paediatric renal failure programme: the principal cause was renal dysplasia, either alone (22 children), or in association with posterior urethral valve (2) or vesico-ureteric reflux (9). Other diagnoses were focal segmental glomerulosclerosis (4), cryptogenic pyelonephritis (3), Alpor'ts syndrome (1), prune belly syndrome (1), acute cortical necrosis (1), haemolytic uraemic syndrome (1), Wilms' tumour (1), a child nephrectomised as treatment of congenital nephrotic syndrome(2). Children with active nephrotic syndrome (serum albumin ≤ 30g/l or proteinuria >40mg/m^2/hour) were excluded because of its effect on lipid metabolism (92).

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2.3.3 Renal function

Ten of the children were receiving peritoneal dialysis (PD). The median (range) glomerular filtration rate (GFR) of the other 37 children was 15.3 (4.6 - 34.5) ml/min/$1.73m^2$SA. The mean (SD) creatinine of all children was 416 (211) µmol / l , those on conservative mangament 355 (151) µmol/l and those on PD 647 (246) µmol/L.

2.3.4 Peritoneal dialysis and enteral feeding

Five of the 37 children who were managed medically and 5 of those receiving PD were enterally fed (Appendix 2.3.A-D).

CHAPTER (3)

ENDOTHELIAL FUNCTION IN CHILDREN

WITH CHRONIC RENAL FAILURE

ENDOTHELIAL FUNCTION IN CHILDREN WITH CHRONIC RENAL FAILURE

3.1 Introduction

Premature atherosclerosis remains the major cause of morbidity and mortality in adults with CRF (41). An increasing number of children with CRF are surviving to adulthood, both because of advances in dialysis and transplantation, and because treatment is being extended to younger patients. Their long-term morbidity and mortality are uncertain, but they might be expected to have similar vascular complications to adults with CRF, at an even earlier age.

Although the clinical manifestations of atherosclerosis do not usually occur before adulthood, the process begins in childhood (93). Endothelial dysfunction is a key early event that precedes the formation of atherosclerotic plaques, and results in reduced bioavailability of NO which may be an important anti-atherogenic agent (65). In CRF,

abnormal endothelial function and NO activity may result from both the metabolic consequences of CRF, such as reduced clearance of endogenous NO synthetase inhibitors (66,68) and increased oxidative stress (94), as well as from the presence of other classical risk factors such as hyperlipidaemia and hypertension (41)

Recently a new non-invasive technique using high-resolution ultrasound to assess vascular reactivity in the conduit arteries of the systemic circulation had been developed. It can be used to study endothelial function from as early as the first decade of life (95). It had been shown previously that endothelial dysfunction may occur before clinical evidence of vascular disease in subjects with hypercholesterolaemia (96), diabetes (97) and in cigarette smokers (98).

In the current study we have examined the influence of CRF on endothelial function in young subjects. In order to avoid confounding variables, we purposefully selected subjects with CRF who were neither smokers, nor hypertensive, hypercholesterolaemic nor diabetic, and were not taking vasoactive drug therapy.

Our findings suggest that CRF has a direct adverse effect on endothelial function in this young patient group. This may influence later morbidity and mortality from large vessel atherosclerotic disease independently of other risk factors.

3.2 Methods

Endothelial function was measured in all CRF children and controls. Plasma TC, TGs, LDL and HDL were measured in the CRF group and in 12 of the controls who were willing to have blood tests. Other lipid subfractions (Apo A1, ApoB, lipoprotein(a) [Lp(a)], antibodies against oxidised LDL [ox-LDL]) and blood glucose were measured in the CRF group. All blood samples were taken after an overnight fast (although tap water was allowed), were immediately centrifuged, and the plasma was stored at -70°C. Each subject and/or their parents gave informed consent to the study, which was approved by the Local Committee on Ethical Practice.

3.3.1 Endothelial function study (figure 3.1)

Endothelial and smooth muscle function were studied non-invasively by examining brachial artery responses to endothelial dependent and independent stimuli (95). Serial diameter changes were measured at rest; in response to reactive hyperaemia (with increased flow producing endothelial dependent vasodilatation); again at rest; and finally after sublingual glyceryl trinitrate (GTN), an endothelial independent

vasodilator. All subjects were scanned in a supine position following a 10 minute rest period. A high resolution B-mode ultrasound image of the brachial artery was obtained in longitudinal section, 5 - 10 cms above the antecubital fossa, using a 7MHz linear array transducer and Acuson 128XP/10 system (Acuson, Mountain View, California), connected to a wall-tracking System (Ingenious Systems, Netherlands) allowing accurate on line diameter measurements. The center of the artery was identified when the clearest picture of the anterior and posterior vessel wall layers was obtained. Depth and gain settings were set to optimize the lumen/arterial wall interface, and machine-operating parameters were not changed throughout the study. The arm remained in the same position and a satisfactory transducer position was maintained using a stereotactic clamp. All the ultrasound scan data was recorded on superVHS video for later flow analysis and all scans were performed by the same operator. To measure the brachial artery diameter an M-line was placed perpendicular to the vessel walls on the B-mode image, the radio-frequency signals from the M-mode output were then relayed to the wall-tracking System. On completion of 5 seconds of data acquisition, the first radio-frequency signal was displayed and electronic markers placed at the vessel wall/lumen interface and a beat by beat computation of the end diastolic diameter and mean over 5 - 10 cardiac cycles was obtained. Reproducibility and

repeatability of this method have been previously reported (99,100). A resting scan was recorded and arterial flow velocity was measured using a pulsed Doppler signal at a 70° angle to the vessel with the range gate (1.5mm) in the center of the artery. Volume blood flow was calculated by multiplying the velocity time integral of the Doppler flow signal (corrected for angle) by the heart rate and the vessel cross-sectional area (πr^2). A pneumatic tourniquet placed around the forearm was then inflated to 300mmHg for 4.5 minutes followed by rapid release, inducing increased flow. The post hyperaemic diameter was measured between 55 and 65 seconds after cuff deflation. Peak reactive hyperaemia was calculated as the maximal flow change within 15 seconds of cuff deflation divided by the flow during the resting (baseline) scan reported as percentage increase in flow. A further reactive hyperaemia was calculated in the same way 15 seconds after cuff release. Since the velocity is taken from the centre of the artery, absolute values may be over-estimated, but the relative values before and after cuff inflation are accurate. A further 10 minutes was allowed for vessel recovery after which a second resting scan was recorded. A 400µg sublingual dose of GTN was then administered and a final scan was recorded three minutes later. Flow mediated dilatation (FMD) in the brachial artery following reactive hyperaemia and endothelium independent dilatation

following GTN administration were expressed as percentage diameter change relative to the first base line scan.

To assess the reproducibility of the ultrasound technique in CRF, seven of the children were seen on three occasions, all within four months of the first study.

The ultrasound study was done by Ann Donald, I have reviewed the video tapes and calculated peak reactive hyperaemia and hyperaemia after 15 seconds from cuff release

3.3.2 Lipid analysis

Antibodies against ox-LDL were measured by ELISA with a 450 nm filter, based on a set of standardised serum and controls obtained from a O-lab-ELISA kit (Biomedica Gruppe, Austria)(101). TC was measured using the cholesterol C system high performance cholesterol oxidase 4-aminophenazone (CHOD-PAP) method and TG by the glyceryl phosphate oxidase 4-aminophenazone (GPO-PAP) high performance enzymatic colorimetric test (both Boehringer Mannheim Diagnostica GmbH)(102). HDL was measured following precipitation of Apo B containing lipoproteins a phosphotungstic acid method and the CHOD-

following GTN administration were expressed as percentage diameter change relative to the first base line scan.

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enzyme-linked immunosorbent assay (ELISA) (Immuno ltd)(105). All assays were validated by the national external quality assessment scheme.

3.3.3 Statistics

Descriptive statistics are expressed as mean ± standard error of the mean (SEM). The CRF and control groups were compared using two-sample t-tests. Univariate regression analysis was used to assess the relationship between the two dependent variables, flow mediated and GTN induced dilatation, and sex, age, vessel size, and TC in all the children, and with TG, HDL, LDL, ApoA1, ApoB, Lp(a), antibodies against ox-LDL, GFR and NO metabolites in the 23 children with CRF. Variability between results of the high resolution ultrasound technique (repeatability) for the seven patients who had been studied on more than one occasion was calculated as the ratio of the within subject standard deviation (SD - the square root of the residual mean square taken from analysis of variance for repeated measures) to the overall mean. This gives the estimated coefficient of variation (CV%). Statistical significance was inferred at a p value of <0.05.

3.3 Results

3.3.1 Endothelial physiology (figure 3.2, table 3.1)

There was no difference in resting vessel size, peak reactive

hyperaemia, or reactive hyperaemia at 15 seconds after cuff release

between CRF and controls (table4.1)(appendix 4.1). However FMD (%)

in CRF was significantly impaired in comparison to the controls [4.9(0.6)

vs. 8.6(0.6), p<0.0001]. In contrast, dilatation with GTN did not differ

from the controls [25.1(1.6) vs. 23.3(1.2), p=0.31].

On univariate analysis, there was no correlation between endothelium

dependent (FMD) (table 3.2) or independent brachial artery dilatation

(table 3.3)and measures of renal function, lipid subfractions, or NO

biochemistry.

3.3.2 Study of repeatability

For each child, there was a directionally similar response to increased flow and to GTN with reproducible failure to dilate to increased flow on the 3 study occasions. The mean (range) across visits of observed FMD was 5.0% (1.5-7.3). Table 3.4 shows the results for each child at each study. From analysis of variance for repeated measures, the estimated coefficient of variance between visits was 4.3%.

3.3.3 Lipid Studies (table 3.5)

There was no significant difference between TC, HDL and LDL levels in the patients and the controls. However TG levels were higher in the CRF patients and antibodies against ox-LDL were elevated (appendix 4.4). GFR correlated with TGs (r=- 0.56, p=0.005) (figure 3.5),log TGs (r= - 0.64, p=0.001) and TC (r= - 0.50, p=0.026) (figure 3.4) levels even when age was taken into consideration (for TC, p=0.01 and for Tgs, p=0.003) , but not with other lipid subfractions.

3.4 Discussion

Our results show that reduced flow-mediated dilatation is already present, possibly due to impaired endothelial function, in the conduit arteries of children with CRF by the first decade of life. It is likely that this predisposes to the development of atheroclerosis,

which causes important morbidity and mortality in CRF patients in later life (41).

A number of factors may contribute to endothelial dysfunction in CRF, including dyslipidaemia(41), drug therapy, increased oxidative stress (94) and the metabolic consequences of CRF themselves. In this study, we set out to determine the influence of CRF on endothelial function as directly as possible by excluding patients with hypertension, high plasma cholesterol levels and those receiving vaso-active drugs. We were able to study endothelial function from a very early stage before acquired risk factors are likely to play a major role, because of the availability of a non-invasive technique to examine vascular physiology in conduit arteries of the systemic circulation. Vasodilatation to increased flow (an endothelium dependent stimulus) is contrasted with response to GTN (which acts independently of the endothelium). This technique, both in earlier studies and in the CRF patients, has been shown to be accurate and reproducible (99-100). As FMD in the brachial artery can be

attenuated by intra-arterial infusion of L-NMMA, it is likely that this

method assesses the integrity of the L-arginine/NO pathway in conduit

arteries (106). Furthermore, a close correlation has been demonstrated

between endothelial function in the brachial artery, assessed using our

method, and endothelial function in the coronary arteries assessed

invasively using acetylcholine (107).

Our findings of markedly reduced FMD in young subjects from as early

as the first and second decades of life indicate that CRF may be

contributing to endothelial abnormalities in addition to the influences of

other vascular risk factors.

Table 3.1. Vascular study results in children with CRF (23 patients) and controls (23 children)

	CRF	CONTROLS	P
Peak reactive hyperaemia (%)	284.4(23.7)	357.6(29.3)	0.06
Reactive hyperaemia at 15 seconds(%)	262.3(27.2)	241(24.5)	0.61
Vessel size (mm)	2.9 (0.1)	2.9 (0.1)	0.72
FMD (%)	4.9(0.6)	8.6(0.6)	<0.0001
GTN (%)	25.1(1.6)	23.3(1.2)	0.31

Results are expressed as mean $\pm$ SEM

Table 3.2. Correlations between endothelium dependent dilatation (FMD) and GFR, lipid subfractions and NO biochemistry

	R Value	P Value
GFR	0.045	0.84
Total cholesterol (mmol/l)	0.14	0.51
Low density lipoprotein (mmol/L)	0.087	0.69
High density lipoprotein(mmol/l)	0.13	0.55
Log Triglycerides(mmol/l)	0.056	0.8
Antibodies against OX-LDL(mu/ml)	0.34	0.17
ApoA1(g/L)	0.24	0.27
ApoB(g/L)	0.16	0.48
Log Lp(a)(g/L)	0.25	0.25
nitrate(uM)	0.08	0.75
nitrite(uM)	0.19	0.44
ADMA(uM)	0.21	0.36
SDMA(uM)	0.003	0.99
low mol.wt. nitrosothiol(uM)	0.18	0.46
high mol.wt. nitrosothiol(uM)	0.14	0.55

Table 3.3. Correlation between endothelium independent dilatation and GFR, lipid subfractions and NO biochemistry

	R Value	P Value
GFR	0.27	0.20
Total cholesterol (mmol/l)	0.06	0.79
Low density lipoprotein (mmol/L)	0.14	0.54
High density lipoprotein(mmol/l)	0.24	0.28
Log Triglycerides(mmol/l)	0.25	0.25
Antibodies against OX-LDL(mu/ml)	0.16	0.95
ApoA1(g/L)	0.14	0.53
ApoB(g/L)	0.02	0.91
Log Lp(a)(g/L)	0.09	0.67
nitrate(uM)	0.14	0.57
nitrite(uM)	0.40	0.09
ADMA(uM)	0.19	0.41
SDMA(uM)	0.23	0.34
low mol.wt. nitrosothiol(uM)	0.12	0.61
high mol.wt. nitrosothiol(uM)	0.11	0.64

Table 3.4. Vascular study results in children with CRF who had multiple tests

Patients	Visit (1)		Visit (2)		Visit (3)		Mean of the 3 visits	
	FMD(%)	GTN(%)	FMD(%)	GTN(%)	FMD(%)	GTN(%)	FMD%	GTN%
1	4	23	5	16	8	21	5.7	20
2	7	17	4	14	3	20	4.7	17
3	7	20	10	27	7	25	8	24
4	4	24	3	33	5	17	4	24.7
5	5	14	4	30	2	16	3.7	20
6	4	25	10	29	8	19	7.3	24.3
7	0	23	3	26			1.5	24.5
Mean(SD)	5.17(1.47)	20.5 (4.3)	6.0(3.16)	24.83(7.9)	5.5(2.6)	19.7(3.2)	5.0 (2.2)	22.1(3.05)

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Table 3.5. Lipid subfractions

	CRF (23)	Controls (23)	Normal Values	P
Age (years)	12.1(0.5)	12.6(0.5)		0.53
Total cholesterol (mmol/l)	5 (0.1)	4.8 (0.3)	<5.2	0.52
Low density lipoprotein (mmol/l)	3.0 (0.1)	3.0 (0.2)	<3.3	0.97
High density lipoprotein(mmol/l)	1.2 (0.04)	1.2 (0.04)	1.0-2.0	0.92
Triglycerides(mmol/l)	1.6 (0.15)	0.9(0.15)	<1.7	0.003
Antibodies against OX-LDL(mu/ml)	260(37.5)		<250	
ApoA1(g/l)	1.6(0.03)		0.7-1.7	
ApoB(g/l)	1.0(0.04)		0.6-1.4	
Lp(a)(g/l)	0.12(0.07)		<0.3	
Blood glucose (mmol/l)	4.6(0.07)			

Results are expressed as mean ± SEM

Fig 3.1 Endothelial Function Study

Blood Pressure Cuff

Pneumatic cuff

Ultrasound Scanner
(7.0 mHz probe, Acuson 128 XP/10)

Wall tracking System

- Resting flow
- Brachial arterial diameter

Cuff up to 300 mmHg for 4.5 mins

- Post hyperemia flow
- Brachial arterial diameter

79
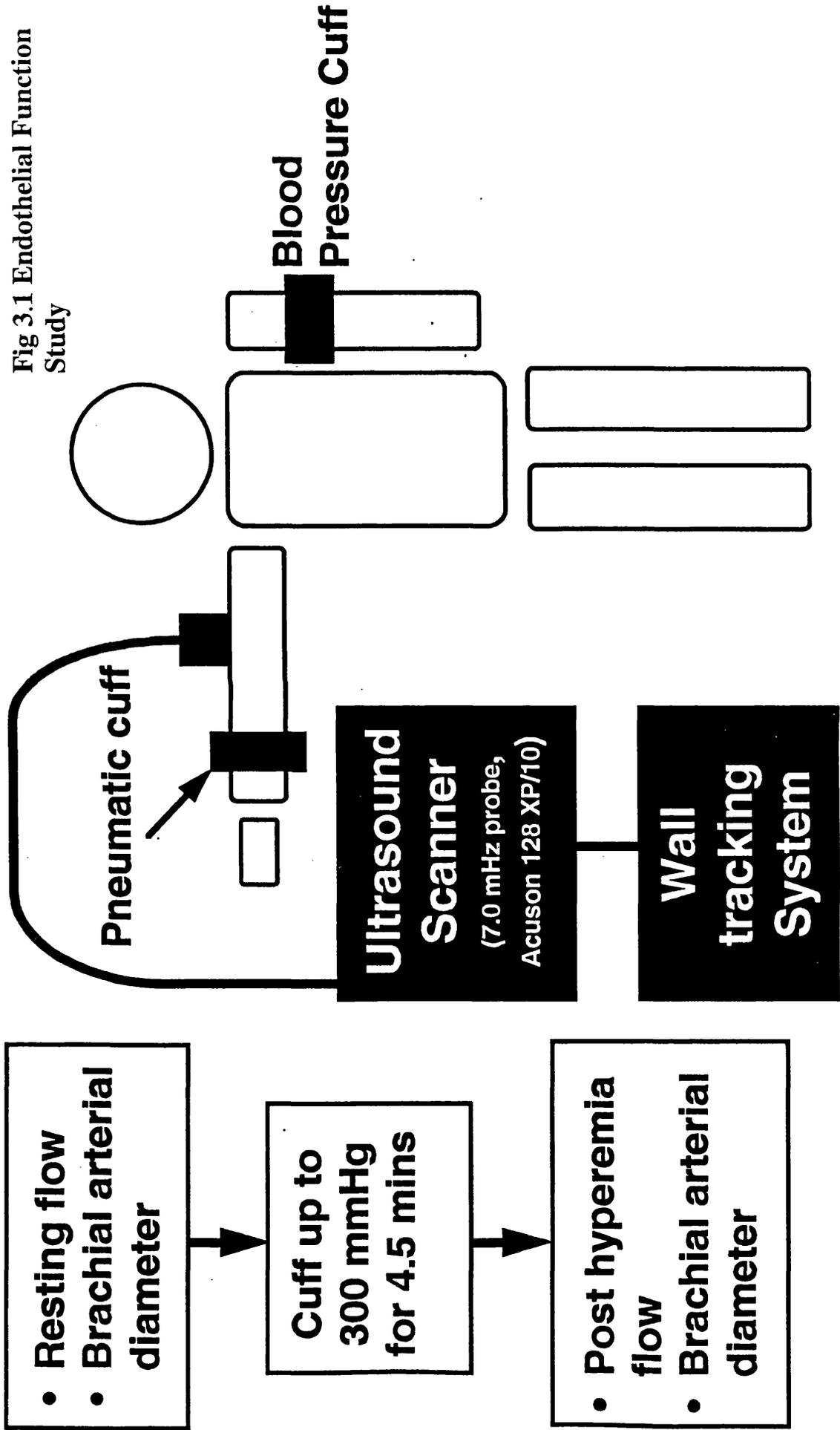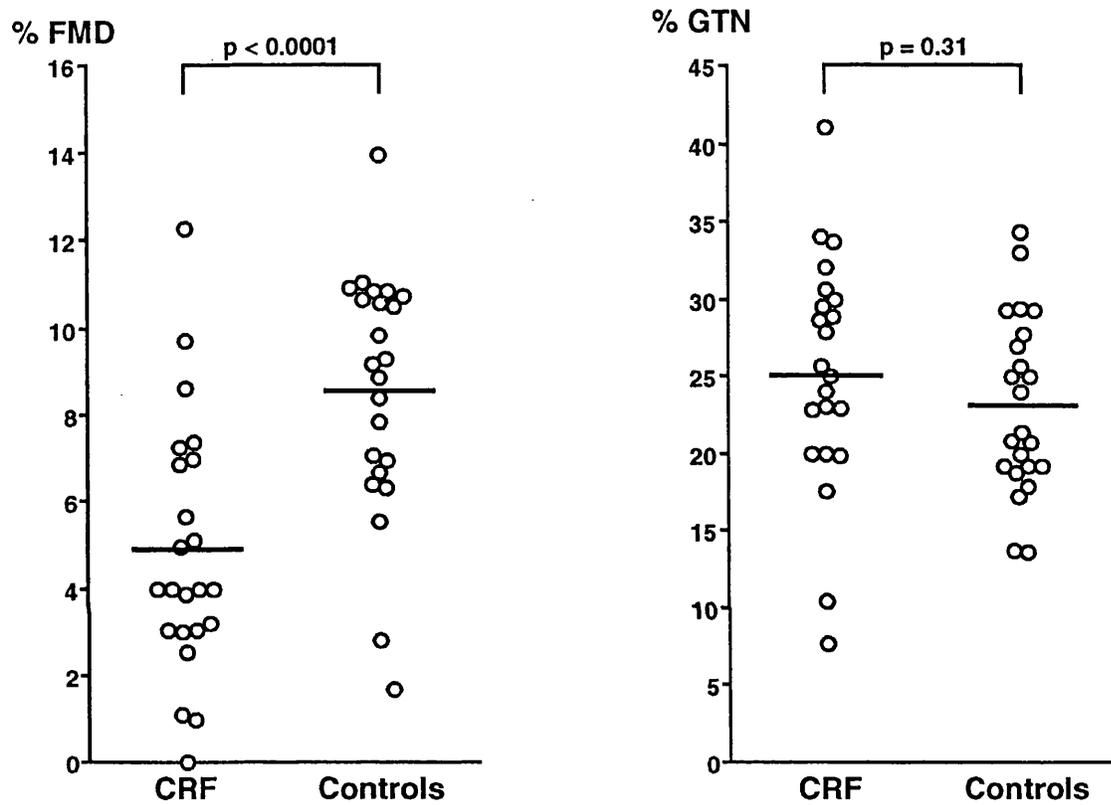
Fig. 3.2. Flow mediated (FMD) and glyceryl trinitrate (GTN) induced dilatation in the controls and children with CRF. Horizontal lines are group means. FMD was significantly impaired in CRF whereas GTN response was normal.
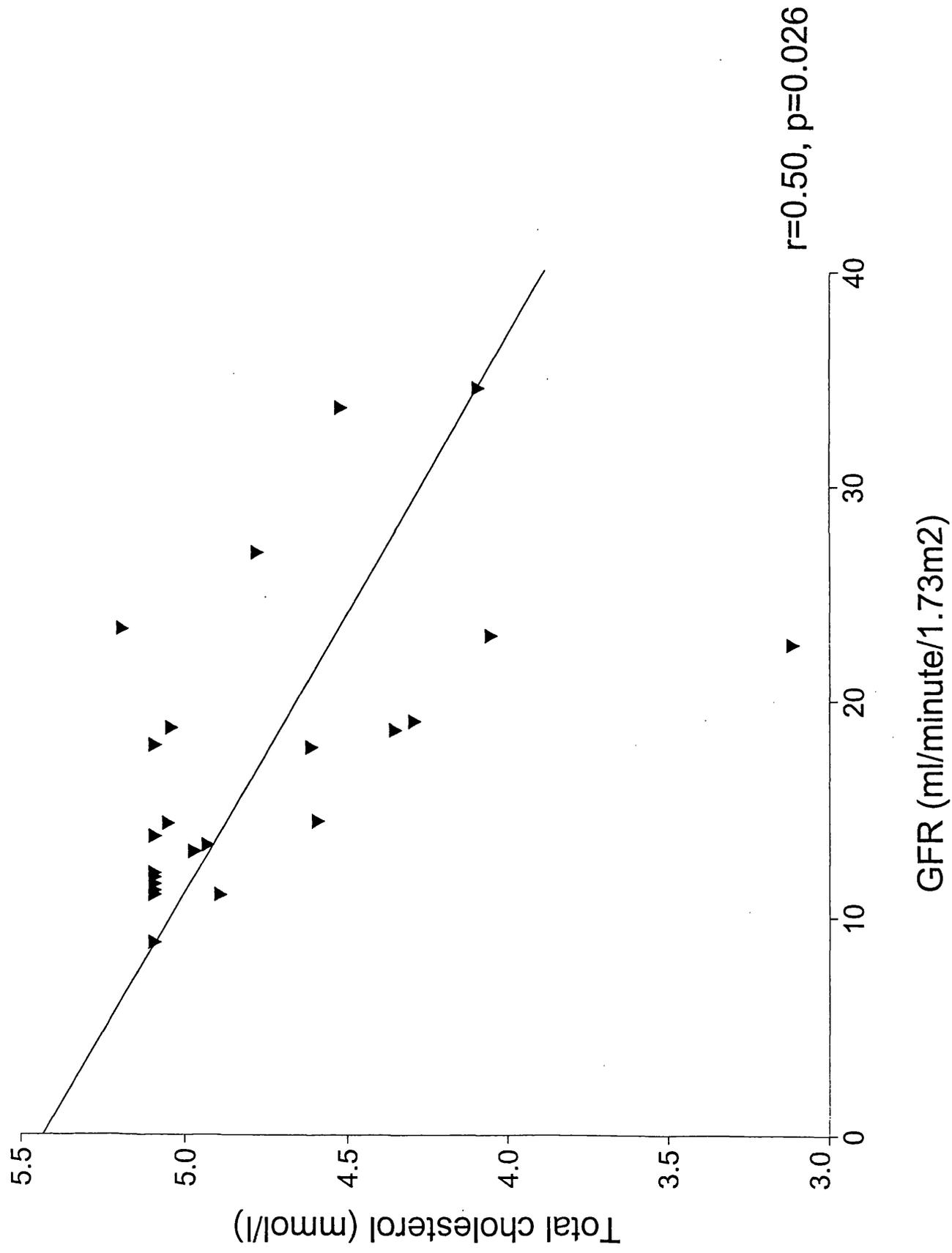
Fig.3.3. Effect of GFR on total cholesterol in children with CRF

r=0.50, p=0.026

81
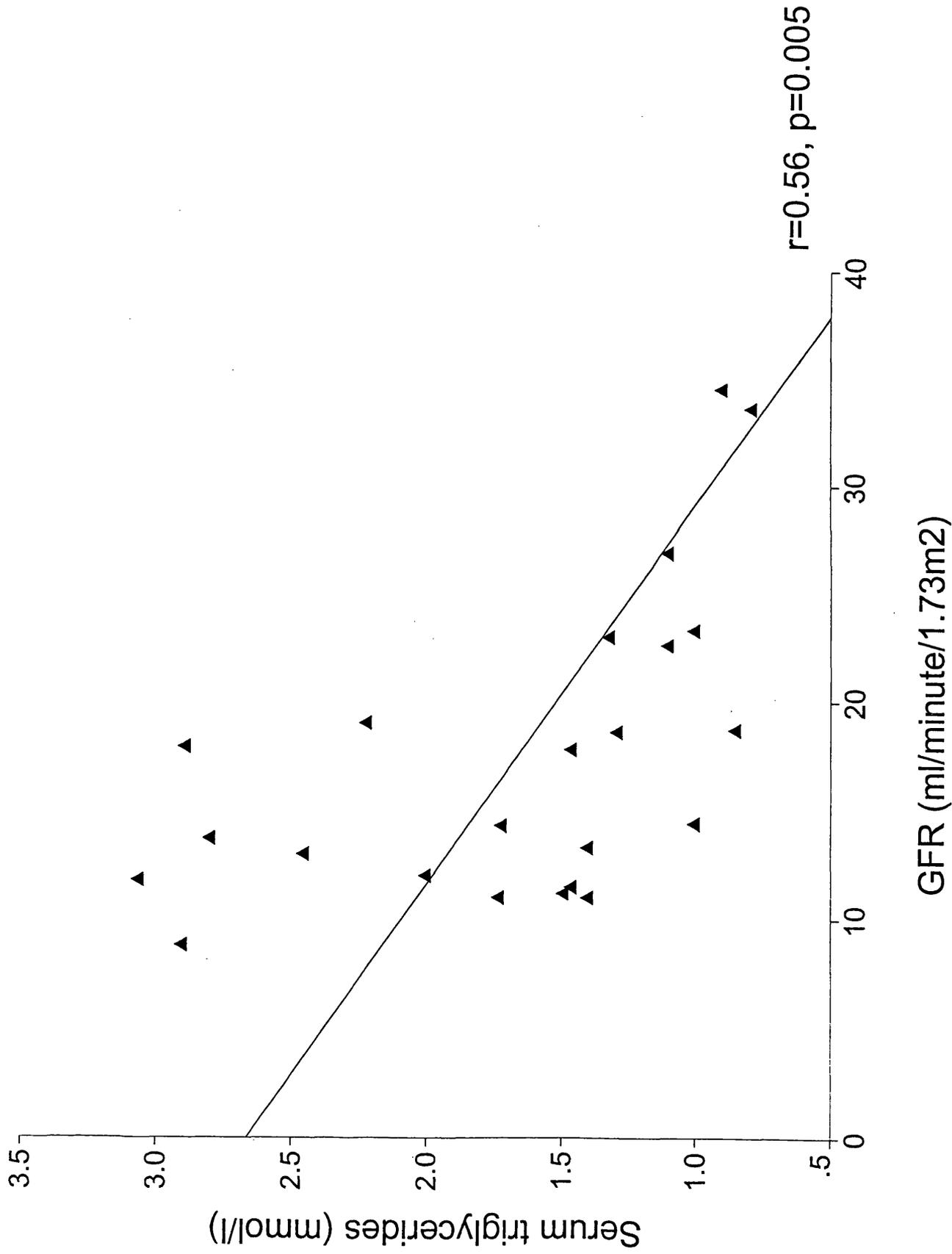
Fig.3.4. Effect of GFR on serum TGs against GFR

r=0.56, p=0.005

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CHAPTER (4)

ABNORMALITIES OF NITRIC OXIDE

METABOLISM IN CHILDREN WITH CRF

Nitric Oxide Metabolites in Children with CRF

4.1 Introduction

Nitric oxide (NO) is a major regulator of arterial smooth muscle tone and blood flow. It has an important biological role as endothelium-derived relaxing factor, a key agent in the maintenance of normal vascular tone (108). It protects against atherogenesis by the relaxation of vascular smooth muscle and inhibition of the interaction of platelets and white blood cells with the endothelium (65). NO is synthesized from the amino acid L-arginine by a family of calcium (Ca^{2+}) and calmodulin-dependent and independent enzymes, NO synthases (NOS)(109). Synthesis of NO requires L-arginine and NADPH and results in the production of citrulline as well as NO(110). Three different NOS isoenzymes are involved; constitutive (endothelial [eNOS] and neuronal [nNOS]) and inducible [iNOS](macrophage, smooth muscle, liver cells, etc)(111). Vascular endothelium releases NO continuously by the constitutive enzyme endothelial nitric oxide synthase (112)(Figure 4.1). NO is rapidly metabolized, hence each millimeter of the vessel wall is regulated by its adjoining endothelium. NO diffuses across from the generator cell to the target cell and stimulates its receptor guanylate cyclase, leading to a rise in intracellular concentration of cyclic GMP (cGMP)(113), thus functioning as physiological messenger. NO reacts readily in the presence of thiol to yield biologically active and more stable S-nitrosothiols(115,116). S-

nitrosothiols also possess NO-like effects, causing vasodilatation and platelet inhibition, and may serve as an intermediary in the cellular metabolism of NO(117). Low molecular weight thiol may be the carrier molecule responsible for the paracrine effect of NO (118). Direct measurement of NO is difficult due to its very short half-life (approximately microsecond) (119), therefore, indirect methods of measurement have been used. These are either physiological (the endothelial dependent relaxation which is mainly mediated by NO)(106), or biochemical, by the measurement of the metabolic products of NO: nitrate and nitrite (120), citrulline (121), and cGMP(122). Endogenous NOS inhibitors such as asymmetric dimethyl arginine (ADMA) and symmetric dimethyl arginine (SDMA) can be synthesized and metabolized by human endothelial cells (123) and are mainly excreted via the kidney (124). Abnormalities of ADMA elimination have been reported in adults with CRF (66,68). In addition to their interference with the anti-platelet properties of NO (125,126), NO inhibitors promote endothelial activation (70,71), with increased expression of adhesion molecules that may form the beginnings of atherosclerosis.

In this study we have measured NO metabolites and inhibitors in children with CRF as biochemical indicators of endothelial function in the same group who had the vascular physiological measurements (endothelium dependent relaxation) in the previous chapter.

4.2 Methods

Plasma nitrite and nitrate (NO oxidation products), high and low molecular weight nitrosothiols (intermediate metabolites of NO), eNOS inhibitors [asymmetrical dimethylarginine (ADMA) and symmetrical dimethylarginine (SDMA)] in the CRF patients were compared to values from 6 control children, aged 6 - 16 (median 11 years), who were having blood taken for family genetic screening of non-cardiac abnormalities. All blood samples were taken after an overnight fast (although tap water was allowed), were immediately centrifuged, and the plasma was stored at -70°C. Each subject and/or their parents gave informed consent to the study, which was approved by the Local Committee on Ethical Practice.

4.2.1 Nitric Oxide Biochemistry

Nitrite and nitrate were measured using high performance capillary electrophoresis (127). Nitrosothiols were measured after separating the plasma into two molecular weight fractions by ultracentrifugation (5,000 Mwt filter at 5000g). Mercury salts were then added to displace the NO from the thiol to generate nitrite, which was assayed by capillary electrophoresis (127).

ADMA and SDMA were measured by high performance liquid chromatography using electrochemical detection after precolumn

derivatization with o-phthalaldehyde (OPA)/b-mercaptoethanol (128).

4.3 Results

Plasma nitrate, ADMA and SDMA levels were significantly elevated in the

CRF children (Appendix 4.1-2) (Table 4.1). There was a significant inverse

correlation in the CRF group between GFR and total dimethylarginines (r= -

0.48, p= 0.03) and SDMA (r= -0.46, p= 0.04), although the correlation with

ADMA was not significant (r=0.41, p=0.07) (Figure 4.2). Nitrite levels however

did not differ from controls. Low and high molecular weight nitrosothiol levels

were significantly lower in the CRF group, and there was a positive

correlation between GFR and low molecular weight nitrosothiol (r=0.52, p=

0.017) (Figure 4.3).

Although it would be expected that high levels of eNOS inhibitors might

decrease NO production, there was no correlation between ADMA and SDMA

and any NO metabolites.

4.4 Discussion

In this study we found a high level of eNOS inhibitors (ADMA and SDMA) in children with CRF. This is similar to findings in adults(66,68,129), Vallance et al found accumulation of these inhibitors in proportion to serum creatinine, and low plasma arginine in 9 uraemic patients (68). Macallister et al found a 3-fold increase in plasma ADMA concentration and an 8-fold increase in SDMA concentration in uraemic patients compared to controls (129). Arese et al demonstrated that most (79%) end-stage uraemic plasmas and many plasmas from patients with moderate renal failure (pre-dialysis) inhibited eNOS activity of murine endothelial cells transformed by mT oncogene of polyomavirus (tEnd.1) and in iNOS in J774 murine macrophages induced by cytokine(66). Nitrate, which is the stable end product of NO, is excreted in the urine (130). The reduced clearance could explain the high nitrate levels in the CRF children even if they do have low NO production. These separate mechanisms would explain also the lack of correlation between dimethylarginines and nitrate. Nitrite concentrations in plasma are low (about 5%)(131) compared with those of nitrate and eventually all NO metabolites are excreted as nitrate in urine (130). Interestingly, nitrosothiols were not affected by renal clearance, and were lowest in the patients with the lowest GFR measurements. S-nitrosothiols, such as S-nitrosocysteine and S-nitrosoglutathione, are formed by S-nitrosylation of free thiol groups by NO (116). These nitrosothiols have been shown to have biological properties

similar to those of NO (117), which may be released from them (115). While the biological significance of S-nitrosothiols remains unclear, they may represent a measure of NO bioavailability, and low levels in CRF may be one mechanism whereby NO activity is impaired. Biochemical measurements are difficult to interpret in CRF due to the effects of abnormal renal clearance. This may explain the lack of correlation between measures such as nitrite and nitrate and endothelium dependent dilatation (FMD).

NO not only acts as a physiological regulator of vascular tone (64), but it is also an important anti-atherogenic molecule by inhibiting platelet activation, monocyte and endothelial cell interaction, and smooth muscle cell proliferation (65).

Endothelial dysfunction in CRF may involve abnormalities of both NO production and breakdown. Decreased synthesis may be due to the presence of elevated levels of L-arginine analogues such as ADMA and SDMA in CRF in proportion to its severity. ADMA and SDMA competitively antagonise eNOS, accumulate in CRF, and correlate with its progression (66,68). They have been shown experimentally to increase vascular tone (68) and promote early atherogenic changes (71). Other molecules that accumulate in uraemia, such as the cytokine IL8, also inhibit eNOS (66). Additionally, in CRF circulating levels of L-arginine, the substrate for NO production, are reduced (68). Increased inactivation of NO may also be important due to increased oxidative stress and free radical production in CRF (130,131).While total LDL in our patients did not differ from the controls

(as we had excluded children with hypercholesterolaemia), levels of antibodies to ox-LDL were elevated in our patients with CRF. Ox-LDL is a critical factor in promoting atherogenesis (132) because it interferes with NO metabolism (133,134), promotes monocyte chemotaxis and transformation, and has a direct effect on endothelial cell survival (135). Thus in CRF, as in other high risk factor groups such as insulin dependent diabetes mellitus, LDL levels even within the normal range may have an impact on endothelial function (97) A similar situation thus may apply in children with CRF.

Table 4.1. Nitric oxide biochemistry results

	CRF	Controls	p
nitrate(μmol/l)	100.9 (9.4)	32.1 (4.3)	<0.00001
nitrite((μmol/l)	1.2 (0.1)	1.4 (0.2)	0.38
ADMA((μmol/l)	3.8 (0.4)	0.7 (0.1)	0.001
SDMA((μmol/l)	1.8 (0.2)	0.3 (0.1)	0.001
low mol.wt. nitrosothiol((μmol/l)	0.9 (0.1)	1.6 (0.2)	0.02
high mol.wt. nitrosothiol((μmol/l)	2.5 (0.1)	3.4 (0.3)	0.0001

Results are expressed as mean (SEM)
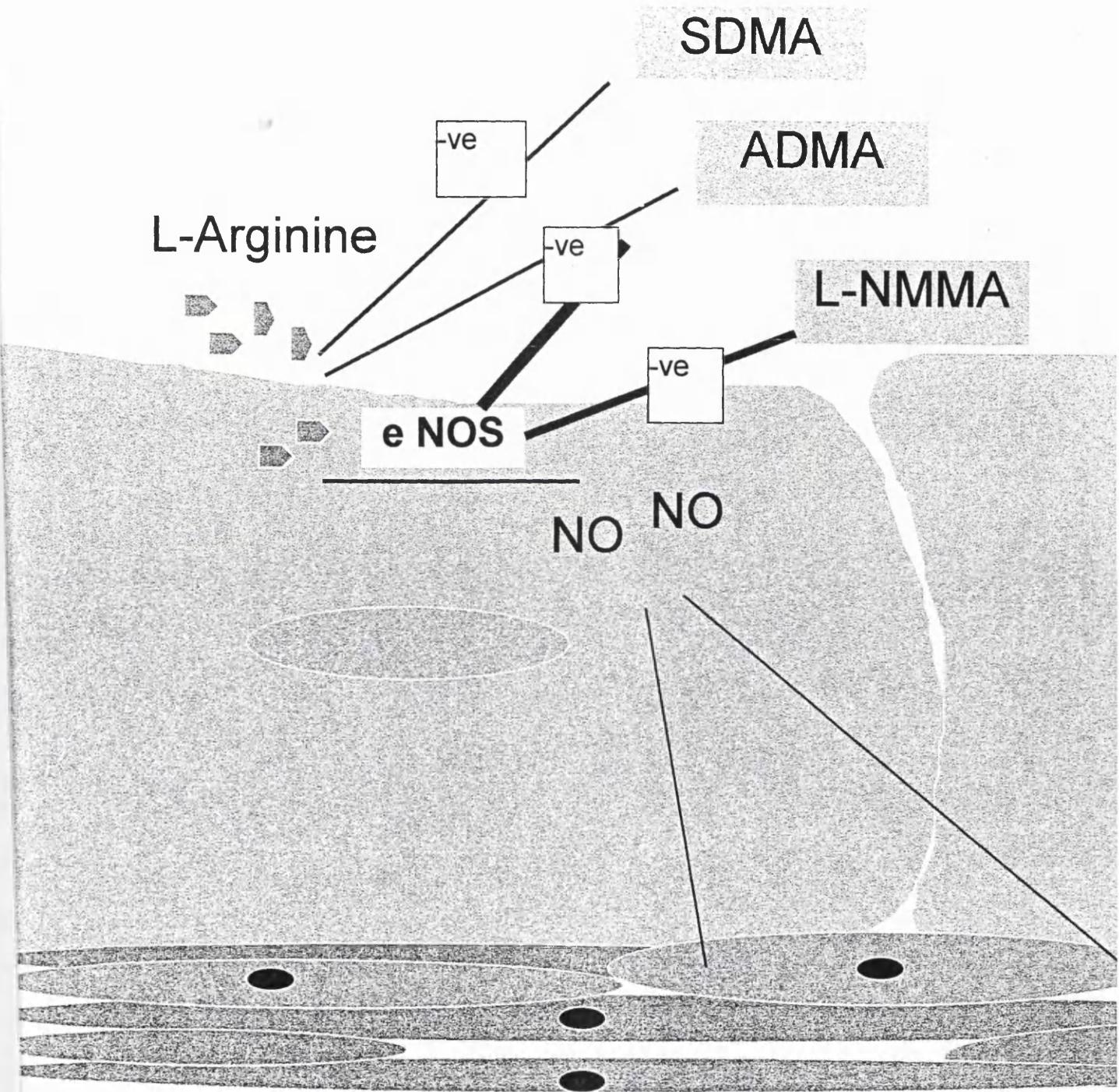
Fig. 4.1. Nitic Oxide is formed from L-arginine by NO synthase. ADMA, SDMA and LNMMA inhibit NO synthase
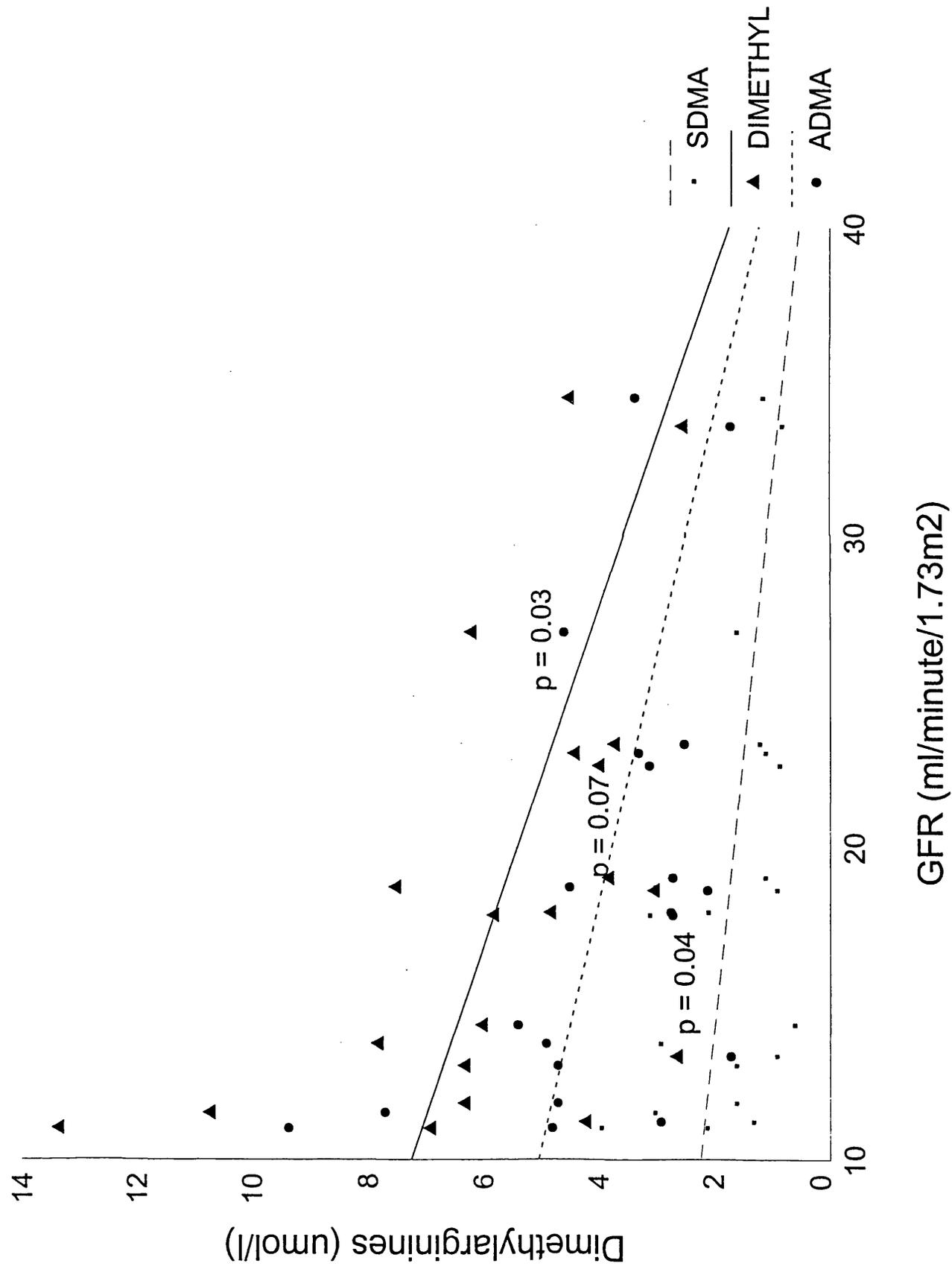
Fig.4.2. Relation between GFR and dimethylarginine, ADMA and SDMA.

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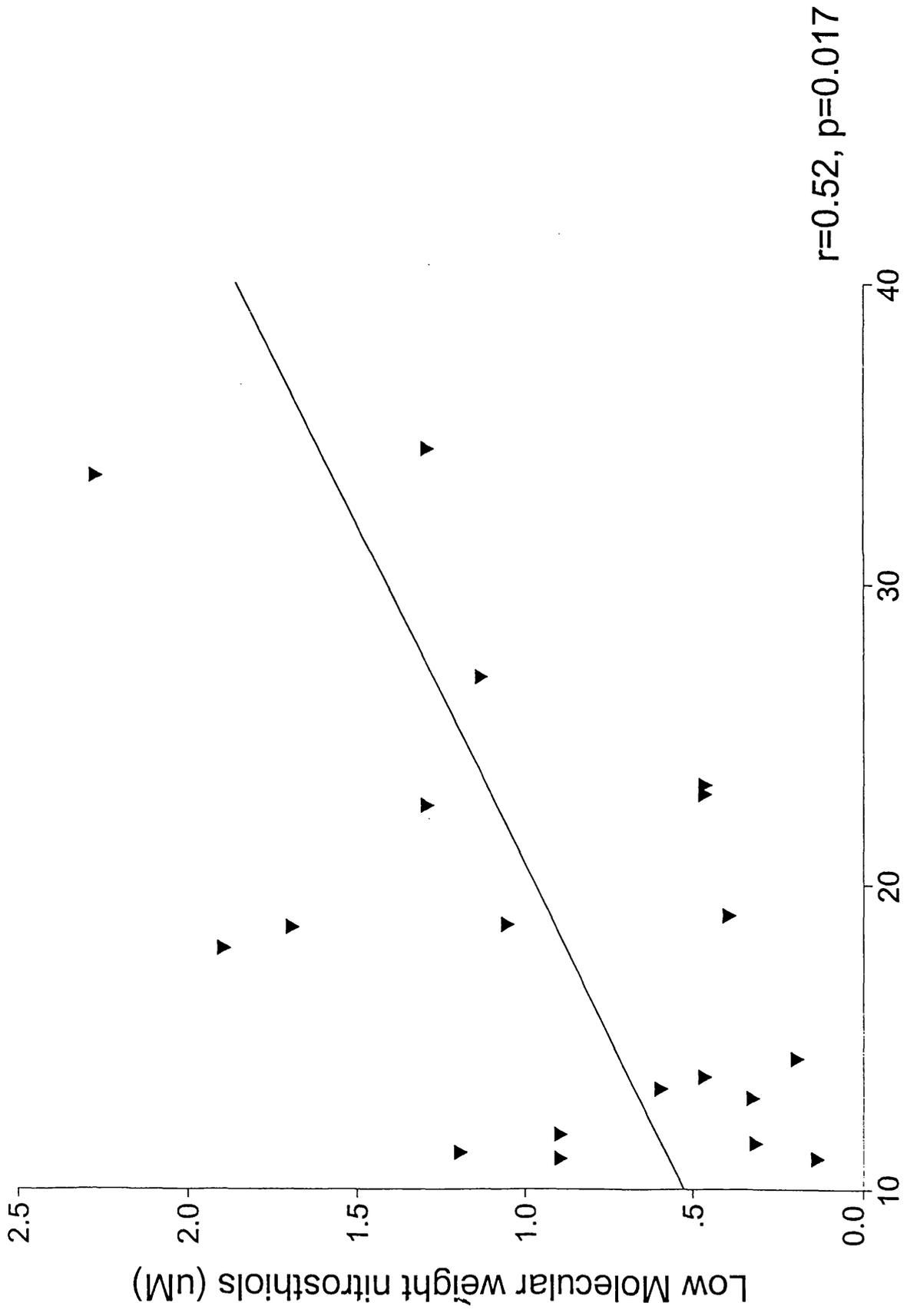
Fig. 4.3. Cerrelation between LMW nitrosothiols and GFR

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CHAPTER (5)
LIPID PROFILES IN CHILDREN WITH CRF

Lipid Profiles in Children with CRF

5.1 Introduction

Hyperlipidaemia is one of the factors believed to be responsible for the

high incidence of atherosclerosis in chronic renal failure (41).

Abnormalities of lipids and lipoproteins that have been reported in CRF

are: high low density lipoprotein (LDL), apoprotein B (apo B),

triglycerides (TGs) and lipoprotein (a) (Lp (a)); reduced high density

lipoprotein (HDL), apo A1 and apo A2; and high total cholesterol

(TC)(60), all of which are believed to predispose to atherosclerosis (see

chapter 1:C-B). Studies in children with CRF have demonstrated similar

findings, but with a higher incidence of hypercholesterolaemia (43).

As well as the metabolic effects of CRF, the blood lipid profiles of

patients may be influenced by their diet. High intakes of saturated fatty

acids (FAs) increase serum LDL and TGs. Polyunsaturated FAs reduce

LDL, but at the same time also reduce HDL (134), which is protective

against coronary heart disease (135). Monounsaturated FAs lower both

LDL and TGs, and are associated with higher levels of HDL. High

intakes of refined carbohydrate (CHO) increase TGs and reduce HDL

(134).

It is recommended to institute early enteral feeding in children with CRF with a declining growth velocity (90). Such feeds are based on whole protein (and occasionally protein hydrolysate) complete feeds, and are supplemented with glucose polymers and / or peanut oil emulsions as additional energy sources. The children eat little or no complex CHO or non-starch polysaccharides (fibre), so most of their intake of CHO is refined. It is a concern, therefore, that, while providing adequate nutrition for growth, tube-feeding regimens might have an adverse effect on the blood lipid profiles of the children.

Our purpose was to study the lipid profiles of children attending our CRF clinic who were eating a high energy, low phosphate, but otherwise unrestricted diet; and to compare the results with those of a group of children who were receiving at least 50% of their energy as an enteral feed.

5.2 Methods

Older children were fasted overnight, but younger children were fasted for at least 4 hours (although they were allowed water). This length of time has been shown to be adequate in infants and younger children

(136) . Blood samples were collected for measurement of serum TC,

TG, LDL, HDL, apo A1, apo A2, apo B, Lp(a) and blood glucose .

Plasma creatinine and plasma albumin concentrations were measured

at the same time.

Ten of the children had received enteral feeds by pump overnight for a

median (range) of 19.0 (6-32) months. The feeds were prepared from

whey-based infant formulae or cows milk protein-based adult enteral

feeds. One child received a soya-based feed because of parental

suspicion of cows milk protein intolerance. One received a whey

hydrolysate to enhance stomach emptying. All feeds included a

comprehensive range of vitamins and minerals. The aim was to offer

adequate nutrition for growth while maintaining blood chemistry within

acceptable parameters by the provision of at least the estimated

average requirement for energy for chronological age using additional

glucose polymers and peanut oil emulsions, and reference nutrient

intake for protein for height age (dietary reference values, table 5.1) .

Appendix 5.1 shows the tube feed composition for protein, CHO and

fatty acids (FA) for each tube fed child. Table 5.1 shows mean (SEM) of

tube feed composition, and the corresponding UK recommended dietary

intakes for a healthy population. Also shown are the observed dietary

intakes of British adults (comparable figures are not available for

children). The dietary intake of the non -tube fed children was not

assessed formally: they were recommended to eat the family diet but with an emphasis on high energy, low phosphate foods.

Lipid subfractions were compared between the four patient groups (Table 5.2) using analysis of variance. Analysis of covariance (ANCOVA) was used to adjust for age and creatinine when a significant difference was found between groups.

To enable comparison of the results of the 10 tube fed children (6 boys), they were matched for age by selecting the 10 youngest non-tube fed children (8 boys). Five of the tube fed and 2 of the non-tube fed children were on peritoneal dialysis. There was no significant difference in the creatinine or GFR between the 2 groups (Table 5.3). The lipid fractions were compared between the two groups using Student's t-test assuming equal variance.

A significant p value was defined as <0.05 Each subject and/or their parents gave informed consent to the study, which was approved by the Local Committee on Ethical Practice.

5.2.1 Lipid Analysis

TC was measured using the cholesterol C system high performance

cholesterol oxidase 4-aminophenazone (CHOD-PAP) method and TGs

by glyceryl phosphate oxidase 4-aminophenazone (GPO-PAP) high

performance enzymatic colorimetric test (both Boehringer Mannheim

Diagnostica GmbH)(102). HDL was measured following precipitation of

Apo B containing lipoproteins a phosphotungstic acid method and the

CHOD-PAP cholesterol method. LDL was calculated using the

Friedewald formula (103). Apo A1 and Apo B were measured using

immunoturbidimetry (Immuno ltd, Sevenoaks, Kent) (104), and Lp(a) y

enzyme-linked immunosorbent assay (ELISA) (Immuno ltd)(105). All

assays were validated by the national external quality assessment

scheme.

5.2.2 Glomerular Filtration Rate (GFR)

GFR was estimated from the plasma clearance of chromium-51 EDTA

(137) or by the Schwartz formula (138).

5.3 Results

Children who were managed medically and on PD without enteral feeds

(groups 1 and 3) were older than the enterally fed children. Medically

managed children were taller, but there was no difference in body mass index between the four groups (Table 5.2).

The results of the lipid subfractions are shown in table 5.2. TGs were elevated in all groups. Figure 5.1 illustrates the relationship between serum TGs and method of feeding, plasma creatinine, and treatment modality. There was an overall positive correlation between TGs and creatinine (r=0.63, p< 0.0001). However, there was no difference in TGs between the patient groups when the results were corrected for age and creatinine (ANCOVA p= 0.07). There was also a negative correlation between TGs and GFR in children in group 1 and 2 (p=0.0001), even when age was taken into consideration (Figure 5.2)

Children managed by PD (group 3) were the only group with levels of the atherogenic lipids TC, LDL and Apo B that were above the normal range, although TC levels were at the upper limit of normal in the other groups. However, only apo B was significantly higher in children on PD when adjusted for age and creatinine (ANCOVA p=0.01). No other lipid subfractions were abnormal in any other group. There was no correlation between GFR and any lipid subfraction other than TGs.

There were no differences in the mean plasma levels of TC, TG, LDL, HDL, apo A1, apo A2 or Lp(a) between tube fed and non-tube fed

children (Table 5.3). Apo B (g/l) was significantly lower in tube fed children (0.9 ± 0.1 (SEM) vs. 1.4 ±0.2 mmol/l, p=0.049).

5.4 Discussion

In this study we have confirmed previous reports that hypertriglyceridaemia correlates inversely with GFR in CRF, and that TC is at the upper limit of the normal range (43,60). Our patients did not, however, have abnormalities of apo A1, apo A2 , apo B and Lp(a), which have been found to be abnormal in some, although not all, previous studies (43,60) . Angiographic studies have shown that low apo A and high apo B (or their ratio) may be better indicators of future coronary heart disease than HDL levels (139,140). HDL, high levels of which protect against vascular disease, has been reported to be reduced in previous studies (43,60), but was also normal in our patients.

We were concerned that the enteral feeds we give to our patients might have an adverse effect on their blood lipids and lipoproteins. The value of tube feeding in the promotion of catch-up growth is well established (78,91). However, such feeds contain added glucose polymers and fat emulsions which may be atherogenic. Ingestion of a bolus of refined carbohydrate causes an increase in TGs and reduces HDL, and a high

intake of saturated fat raises TGs and LDL (134). Furthermore, an imbalance of mono- and polyunsaturated fat can also promote atherogenesis by reducing HDL (134).

However, we achieved a balanced energy intake with our enteral formula composition, which did not differ significantly from published recommendations for dietary intake for a normal population. Indeed, the total fat intake, and particularly the saturated fat intake, was less in the tube fed children than in a normal adult population eating an unrestricted diet (Table 5.1). Despite a CHO intake comprised mainly of refined sugars rather than a mixture of sugars, starch and fibre, there was no adverse effect on their serum TGs and HDL.

Although the children were under regular dietary review, we were not able to fully analyse the intakes of those who were not tube fed because there are only a few foods that have been analysed for their fatty acid composition. As these children were eating a relatively free diet rather than receiving a precisely prescribed enteral feed, it is possible that their diet was less balanced than that of the tube fed children.

It might be expected that the glucose load during PD would have an adverse effect on plasma lipids, resulting in hypertriglyceridaemia and decreased HDL (141). Although the patients on PD were the only group

to have levels of atherogenic lipids above the normal range, those on PD who were enterally fed did not. One possible explanation is that the enteral feed was beneficial to the plasma lipids, but the numbers are too small to draw any conclusions.

All the children had high TG levels. The importance of TGs in the process of atherogenesis is controversial, but recently it has been found that hypertriglyceridaemia is associated with a high proportion of small, dense LDL, which is now recognised to be particularly atherogenic (142).

In conclusion, this study would suggest that an enteral feeding regimen providing an appropriate energy intake with a balanced profile of fat and carbohydrate can be administered to children with CRF who are both conservatively managed and on PD, without detrimentally affecting their serum lipids.

Table 5.1. Enteral Feed Composition

Source	%Total Energy Intake		
	Enteral feeds	Dietary Reference Values*	Dietary and Nutritional Survey (OPCS)**
Feed	86.5 (5.0)		
Protein	8.1 (1.2)	15	15
Carbohydrate	58.3 (3.8)	50	42
Total fat	32 (3.7)	35	38
Saturated FAs	10.1 (1.6)	11(+2% trans)	16
Monounsaturated FAs	13.3 (2.4)	13	12
Polyunsaturated FAs	6.6 (0.8)	6.5 ***	6

values expressed as mean (SEM)

* Dietary Reference Values for Food Energy and Nutrients for the United Kingdom (143).

** The Dietary and Nutritional Survey of British Adults (144).

*** maximum recommended 10% of total energy intake.

FA: Fatty acid. trans: transpolyunsaturated FA.

Table 5.2. Serum Lipid Subfractions

Groups	(1) Medically Managed	(2) Enterally fed	(3) Peritoneal dialysis (PD)	(4) Enterally fed and on PD	Normal Values	P Value (ANOVA*)
Number	32	5	5	5		
Age (years)	11.1(0.8)	2.3(0.6)	12.3(2.0)	3.9(0.8)		<0.0001
Creatinine (μmol/l)	344 (25)	415(98)	777(130)	516(40)		<0.0001
Height SDS	-1.1(0.2)	-2.7(1.3)	-2.5(0.4)	-2.1(0.2)		0.025
Body Mass Index	18.9(0.5)	17.1(1.4)	20.3(0.9)	20.5(2.6)		0.33
TG(mmol/L)	1.8(0.2)	2.4(0.5)	3.9(0.4)	2.7(0.8)	<1.7	0.002**
TC(mmol/L)	5.1(0.3)	4.2 (0.6)	6.1(0.6)	5.1(0.4)	<5.2	0.25
LDL(mmol/L)	3(0.3)	2.4(0.2)	3.6(0.7)	2.8(0.3)	<3.3	0.55
HDL(mmol/L)	1.3(0.1)	1.3(0.5)	1.1(0.1)	1.0(0.1)	1.0-2.0	0.59
ApoA1(g/L)	1.7(0.1)	1.8(0.5)	1.5(0.1)	1.5(0.2)	0.7-1.7	0.75
ApoA2(g/L)	0.6(0.02)	0.7(0.04)	0.8(0.07)	0.7(0.04)	0.3-0.8	0.0002**
ApoB (g/L)	1.1(0.07)	0.9(0.17)	1.5(0.18)	0.9(0.20)	0.6-1.4	0.03
Lp(a)(g/L)	0.18(0.03)	0.14(0.06)	0.3(0.04)	0.23(0.03)	<0.3	0.47
Glucose (mmol/l)	4.4(0.07)	4.7(0.29)		4.55(0.47)		

Values expressed as mean (SEM)

* ANOVA: Analysis of variance
**Insignificant when adjusted for age and creatinine by ANCOVA(analysis of covariance)

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Table 5.3. Plasma Lipid Subfractions

(Number)	All (47)	Enterally fed (10)	Matched non-enterally fed (10)	Normal values	p
Age (years)	9.3 (0.8)	3.2 (1)	5.5 (0.5)		0.06
GFR (ml/min/1.73m^2)	14.9 (1.2)	8.9 (2.1)	12.5 (1.7)		0.22
Creatinine (μmol/l)	425 (30)	468 (51)	423 (78)		0.56
Albumin (g/L)	40.8 (0.6)	41.6 (1.6)	37.8 (1.4)	35-50	0.10
TG (mmol/L)	2.1 (0.17)	2.7 (0.3)	3.1 (0.5)	<1.7	0.42
TC (mmol/L)	5.2 (0.2)	4.7 (0.4)	5.9 (0.8)	<5.2	0.19
LDL (mmol/L)	3.0 (0.2)	2.6 (0.8)	3.1 (0.2)	<3.3	0.63
HDL (mmol/L)	1.3 (0.1)	1.1 (0.2)	1.4 (0.3)	1.0-2.0	0.47
Apo A1 (g/L)	1.7 (0.1)	1.8 (0.2)	1.7 (0.1)	0.7-1.7	0.62
Apo A2 (g/L)	0.61 (0.02)	0.67 (0.1)	0.69 (0.03)	0.3-0.8	0.86
Apo B (g/L)	1.1 (0.1)	0.9 (0.1)	1.4 (0.2)	0.6-1.4	0.049
Lp(a)(g/L)	0.19 (0.03)	0.18 (0.04)	0.2 (0.04)	<0.3	0.60

Results expressed as mean (SEM)
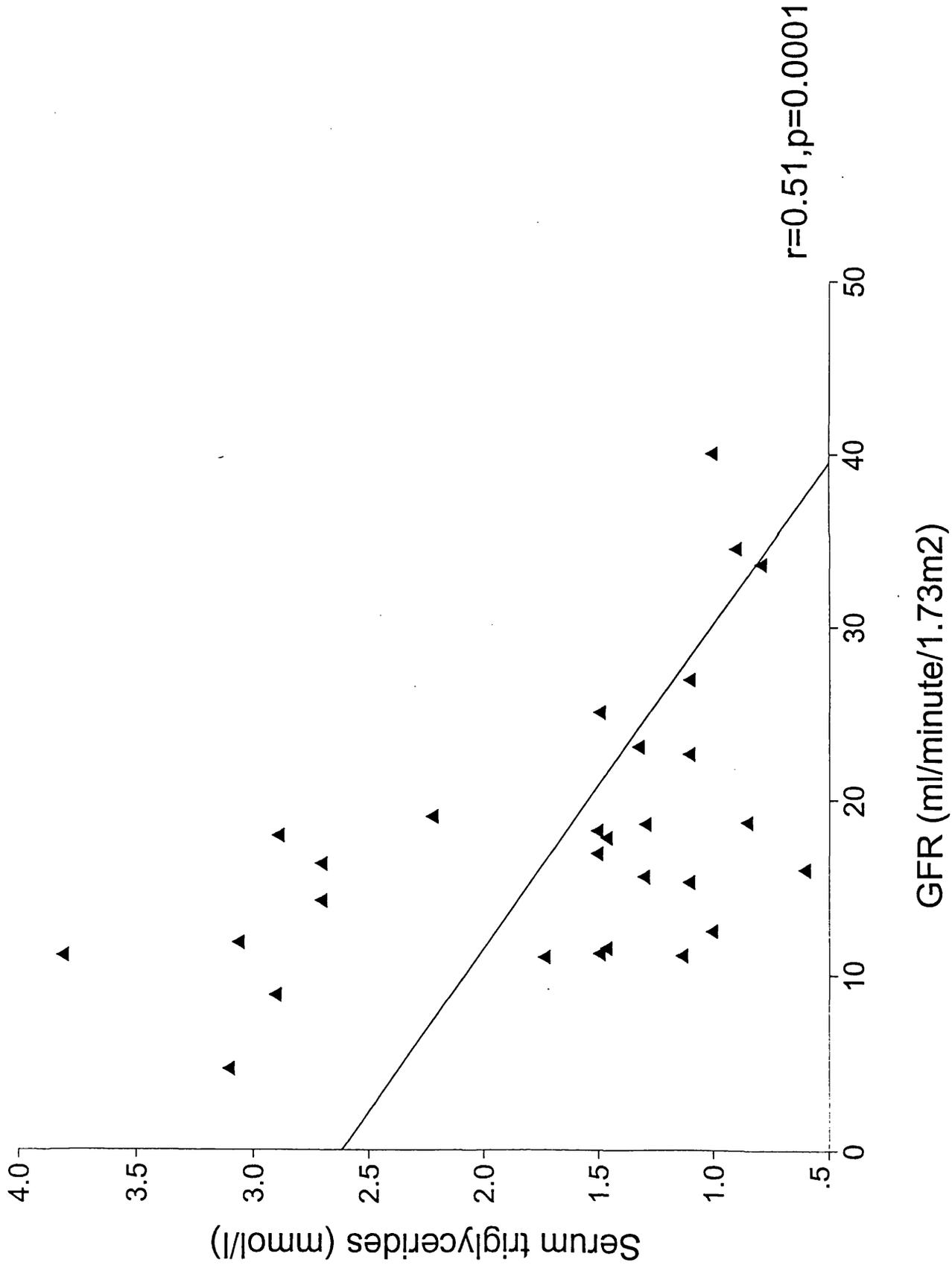
Fig.5.2. Effect of GFR on serum TGs in conservatively managed children

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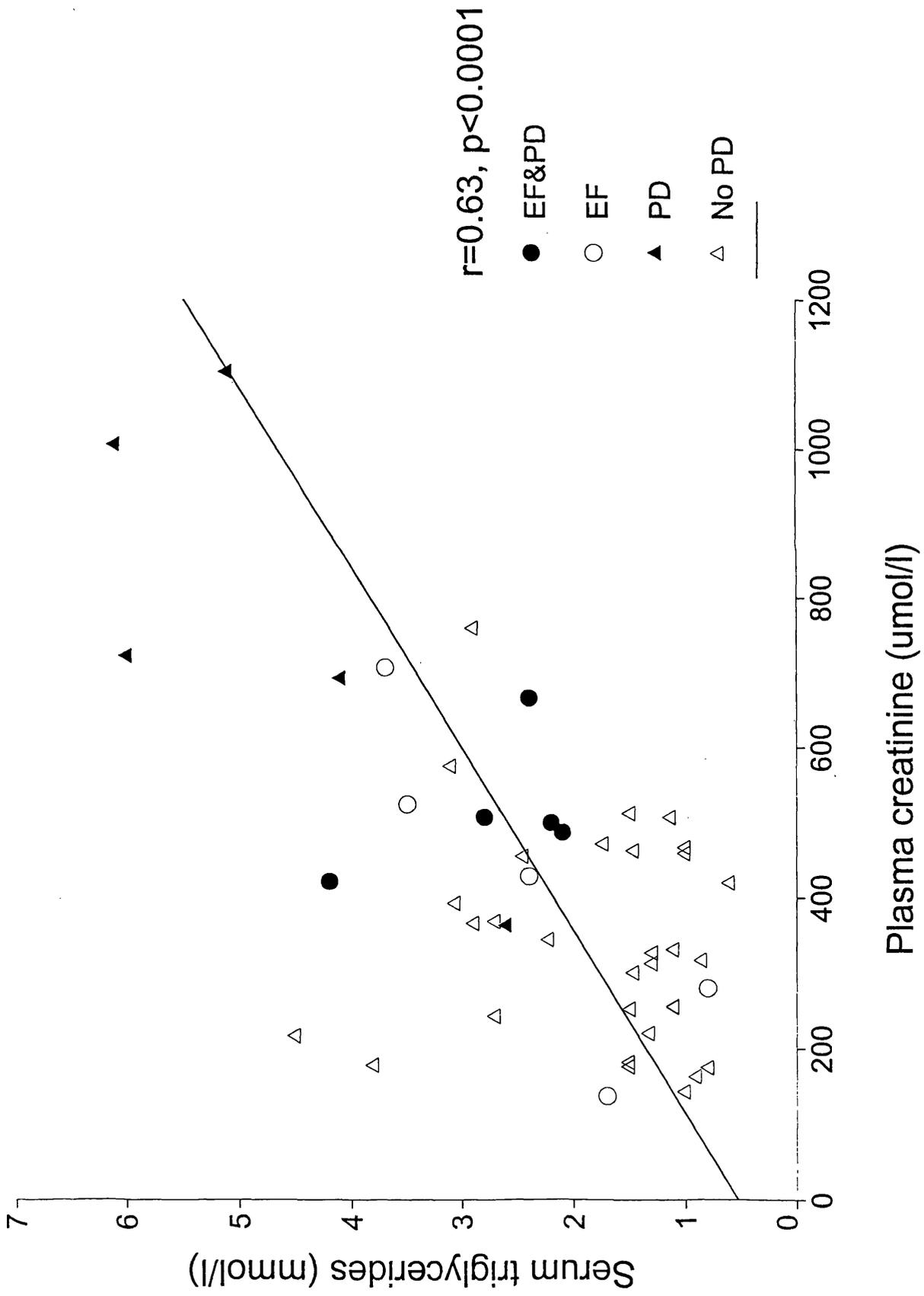
Fig.5.1. Effect of method of feeding, p.creatinine and PD on TGs

EF, Enteral feeding; PD, peritoneal dialysis

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CHAPTER (6)

CONCLUSION AND FINAL DISCUSSION

CONCLUSION AND FINAL DISCUSSION

6.1 Principal Observations

The data presented in this thesis show that there is already evidence of impaired endothelial function in the conduit arteries of children with CRF in the first decade of life. It is likely that this represents an early manifestation of the atherosclerotic complications which are the leading cause of death in CRF patients (145), even in the phase of conservative management (146).

The accelerated atherosclerotic disease process in uraemia has been attribured to several factors (145): dyslipidaemia , drug therapy, increased oxidative stress, and the metabolic consequences of CRF. The latter has been suggested as a specific pathological entity (uraemic arterial disease) which is characterized by medial degeneration and calcification rather than by accumulation of cholesterol (147). In the endothelial function study, we have avoided other confounding factors by selecting children with no other recognized risk factors for cardiovascular disease, i.e. normotensive, normal plasma total cholesterol (TC) concentration, and were not on vasoactive drugs.

We measured endothelial function using physiological and biochemical measures. The endothelium makes a significant contribution to the regulation of vascular tone through the release of potent vasodilator agents such as nitric oxide (NO) and prostacyclin (PGI_2) as well as vasoconstrictor compounds such as endothelin (148). Therefore one of the early consequences of arterial pathology, such as atherosclerosis, hypertension or ischaemia, is a reduction in endothelium-dependent vasodilatation, both at a basal level and in response to endogenous and exogenous stimuli. The hypothesis that vascular disease results from attenuated NO production was used to examine endothelial function by testing the response to flow mediated dilatation which is endothelial dependent, in comparison to nitroglycerine induced dilatation which is endothelium independent. In addition to the ultra-sonographic physiological measures, we measured NO metabolites as NO has very short half life and is itself very difficult to measure (118).

Loss of endothelium-dependent dilatation in the systemic arteries occurs in the pre-clinical phase of vascular disease and is associated with interaction of the same risk factors known to predispose to atherosclerosis and its complications in later life (149). The endothelium lies in a strategic anatomical position between the circulating blood and vascular smooth muscle and hence is a major

local mediator of cardiovascular function. In addition, endothelial cells are exposed to mechanical forces and cardiovascular risk factors in the circulation. Thus it is not surprising that their function becomes impaired at an early stage in the disease process.

Endothelial function and the effects of nitric oxide and endothelin in particular can be evaluated in the coronary circulation by quantitative coronary angiography and Doppler flow wire, and in the peripheral circulation with plethysmography and new ultrasound/Doppler devices. The least invasive technique of these is the recently developed ultrasound technique, which allows non-invasive evaluation of endothelial function in large systemic arteries such as the brachial artery. The technique is accurate, reproducible and able to differentiate between subjects with and without vascular dysfunction (150) . Reduced flow-mediated dilation has been found to be significantly related to other risk factors for atherosclerosis such as; hypercholesterolemia, cigarette smoking, higher blood pressure, male gender, older age, family history of premature vascular disease (150) and hyperhomocysteinemia (151) . Furthermore, the impairment of flow mediated dilatation in the brachial artery, a marker of systemic endothelial function, is closely related to the angiographic extent of coronary artery disease (152). Endothelial dysfunction was found in adults with CRF on haemodialysis (153), but we found that

even at an early stage of CRF (i.e before dialysis treatment required) in children with low risk factors for atherosclerotic disease, there is already evidence of endothelial dysfunction. The observation of abnormal vascular function at such an early stage in the decline of renal function permits serial studies of interventions such as risk factor modification or therapeutic intervention aimed at preventing or retarding large vessel atherosclerosis, which is such an important contributor to clinical morbidity and mortality in these patients.

Although NO and NO metabolites are useful indicators of endothelial function, they are not very helpful in CRF, because of the effect of impaired renal function in their excretion. The impaired renal clearance is most likely to be the cause of high nitrate in our patients, while they were expected to have low NO production and therefore low nitrate levels. However S-nitrosothiols were found to be low. They are believed to serve as active intermediates in the inhibitory action of nitric oxide, and related nitrogen oxides, on platelet aggregation (154) and possess EDRF-like effects of vasodilation (155). S-nitrosothiols stimulate the formation of both cyclic AMP and cyclic GMP (113), and possibly act as intermediates in the activation of guanylate cyclase by glyceryl trinitrate, $NaNO_2$ and possibly nitroprusside (156,157). S-nitrosothiols are formed of thiols such as glutathione, cysteine, N-acetylcysteine and albumin upon reaction

with nitric oxide (NO) in the presence of oxygen (158). Low

nitrosothiol levels indicate low levels of EDRF and NO activity.

The finding of high endogenous NOS inhibitors is similar to the

finding in adults with CRF (66,68). They contribute with low NO

precursor (L-arginine) in CRF to low NO production (68). However

NOS activity in CRF results from a balance between inhibitors and

activators. Arese et al found that plasma from patients with

moderate or end-stage CRF tested on tEnd.1 (murine endothelial

cells transformed by mT oncogene of polyomavirus) could activate,

inhibit or have no effecton NOS activity. The accumulation of the

inhibitory molecules is in proportion to the progression of renal

failure, with a reduction in the inhibitory activity after haemodialysis

and the accumulation of molecules which inhibit NOS in the

ultrafiltrate. In addition to ADMA, aminoguanidine, methylguandine

and cytokines (IL8) accumulate in CRF and inhibit NOS. NO may be

inactivated in the presence of the increased stress and free radical

production in CRF. Noris et al studied subgroup of patients with CRF

characterized with a history of bleeding symptoms and found their

plasma potently induced NO synthesis of cultured endothelial cells

(159).

In this thesis, I did not measure other endothelial dependent vaso-active compounds, such as endothelin-1 and thromboxane-A2, because neither has been shown to have a major role in clinical vascular disease (160). Our findings of markedly reduced FMD in young subjects from as early as the first and second decades of life indicate that CRF may be contributing to endothelial abnormalities in addition to the influences of other vascular risk factors. The failure to detect a relationship between FMD and eNOS inhibitors could be explained by the fact that abnormal endothelial function in CRF may involve abnormal NO production and breakdown (as explained in chapter 4). Reduced production results from the accumulation of eNOS inhibitors and reduced levels of circulating L-arginine in patients with CRF (68). on the other hand, increased oxidative stress and free radicals accelerate NO inactivation in CRF (130). The relatively small number of patients studied could contribute to the lack of significance as well.

In the lipid study, our findings of hypertriglyceridaemia which correlates with the progression of CRF, and TC of upper limit of the normal range, are similar to previous reports (43,60) . However we did not find reduced HDL or abnormalities of apo A1, apo A2 , apo B and Lp(a), which have been found to be abnormal in some, although not all, previous studies (43,60) . Interestingly, when we chose

children with normal TC and normal LDL for the endothelial study, we found them to have elevated antibodies to oxidized LDL. Oxidised LDL is taken up more readily by monocyte-derived macrophages than LDL (161). Antibodies to oxidised LDL are found in atherosclerotic lesions and their levels represent a indicator to the progression of atherosclerotic process (101,162). Increased risk of ischaemic heart disease is associated with a preponderance of small dense LDL particles, which are more susceptible to oxidation (161). Ox-LDL is a critical factor in promoting atherogenesis (131) because it interferes with NO metabolism (132,133), promotes monocyte chemotaxis and transformation, and has a direct effect on endothelial cell survival (134). Furthermore, oxidised LDL may contribute to the pathogenesis of atherogenesis by stimulating migration of smooth muscle cells from media to the intima via abolishing the physiological inhibitory effect of normal endothelium (163). They also stimulate both outgrowths of smooth muscle cells from vascular tissue and their proliferation (164,165). LDL modification by oxidation or glycosylation interferes with NO metabolism by cytotoxic reactions with endothelial cells (164,165). Thus in CRF, as in other high risk factor groups such as insulin dependent diabetes mellitus, LDL levels even within the normal range may have an impact on endothelial function (97)·

The last part of this thesis was to examine the effect of enteral feeding on blood lipids and lipoproteins. The difficulties associated with feeding the infants and young children with CRF are well recognized. Refusal of feeds, reduced spontaneous oral intake and vomiting result in failure to provide an adequate nutritional intake and therefore growth loss in this important early phase of growth (167). It has been clearly shown in previous studies that inadequate energy intake occurs in children with CRF, if no intervention and advice is offered (168,169). Enteral feeding has been an integral part of the care of infants and children with CRF at Great Ormond Street Hospital for Children since 1989. Such intervention can prevent or revert weight loss and growth retardation, and lead to significant catch up growth in those less than 2 years of age (90). Our concern that such intensive feeding might aggravate hyperlipidaemia in these children proved to be invalid. The balanced enteral feed (EF) which was used in these young children did not worsen their hyperlipidaemia but was even associated with lower levels of apo B than those that were not tube fed: it is recognised that high levels of apo B are a risk factor for coronary vascular disease (170). Children on peritoneal dialysis (PD) and on EF had less atherogenic lipid profiles than those on PD who were not tube fed. This could be explaind by the fact that those children were eating a relatively free diet rather than receiving a precisely prescribed enteral feed and

possibly that their diet was less balanced than that of the tube fed

children.

6.2 Therapeutic Possibilities

6.2.1 L-Arginine

In this thesis we have demonstrated that large vessel endothelial

function is abnormal in children with CRF in the absence of classical

risk factors for vascular disease, such as hypertension, diabetes and

hypercholesterolaemia. This direct adverse effect of CRF on

endothelium in our young patients may involve abnormalities of both

NO production and NO breakdown. Deceased synthesis of NO may

be due to decreased L-arginine levels in CRF (68). L-arginine is the

substrate for eNOS (109);increases bioavailability of NO due to its

superoxide scavenging properties (171) ; and inhibits the effects of

ox-LDL on NO synthase activity (172). Therefore there are several

reasons for the use of dietary supplementation with L-arginine in the

prevention of atherosclerosis in CRF; L-arginine might improve the

bioavailability of NO in CRF by correction of substrate deficiency;

overcoming competitive antagonism; and inhibiting the oxidation of

LDL. Correction of endothelial dysfunction was demonstrated in

hypercholesteraemic patients following short term infusion of

intracoronary (173) or intravenous (174,175) L-arginine. Furthermore, dietary L-arginine supplementation has already been shown to improve endothelial function in the brachial artery of young people with hypercholesterolaemia (176) and experimentally to inhibit atherogenesis in animal models (177,178). Long-term L-arginine supplementation improves small-vessel coronary endothelial function in humans with significant coronary artery disease on coronary angiography and intravascular ultrasound. It was shown that endothelium-dependent coronary blood flow reserve to acetylcholine improves in the subjects who takes L-arginine, compared with the placebo group. This was associated with a decrease in plasma endothelin concentrations and an improvement in patients' symptoms scores (179). The next step, therefore will be to examine the effect of dietary supplementation with L-arginine on endothelial function in the conduit arteries of young subjects with CRF using the non-invasive ultrasound method. The US technique assesses the integrity of the L-arginine/NO pathway in conduit arteries by contrasting vascular response to endothelial dependent (flow mediated) and endothelial independent (GTN which supplies the endothelium directly with NO) dilatation. We have demonstrated that this technique is reproducible in children with CRF and it was shown in earlier studies to be reproducible and reliable.

6.2.2 Anti-oxidant

Increased oxidative stress in CRF occurs as a result of excessive free radical production and low anti-oxidant levels (130). Our finding of high antibodies against oxidised LDL, which is similar to a previous study in dialysis patients, provides indirect evidence for stimulation of lipid peroxidation processes (180). Another indirect proof is the observation that lipoprotein from haemodialysis patients is oxidised more easily (56). Oxidative stress emerges as an important causative factor for the development of endothelial dysfunction and atherogenesis (181,182). Antioxidants such as probucol and vitamin E reduce the formation of free radicals and the oxidative modification of LDL that lead to the impairment of EDRF responses and may prevent the same dysfunction in hypercholesterolaemia and atherosclerosis (183,184). Antioxidants prevent the inhibition of endothelium dependent relaxation by low density lipoprotein and could be used in conditions with risk factors for CVD. Other antioxidants such as vitamin C might have a role in prevention of atherogenesis as well (185). Treatment trials in CRF have not been undertaken and further intervention studies with antioxidants are required to prove whether this concept holds true.

reducing fat and/or carbohydrate intake, can hardly be recommended for them (190).

6.2.3.2 Lipid Lowering Drugs

The use of lipid lowering drugs in the dyslipidaemia of CRF is still a controversial issue (191). Experience of this therapy in uraemic adults has mainly been gained with fibric acids, including clofibrate, gemfibrozil and bezafibrate. These drugs normalise lipoprotein profiles in adult patients with CRF and on HD or PD by stimulating LPL and HTGL activity (192-195). However, even with dose reduction, severe side effects, and particularly myopathy (195) ,have been observed in patients with CRF. Experience with other lipid lowering agents in uraemic patients is limited. Probucol lowers TC and LDL-cholesterol and can also prevent lipid oxidation, which all play an important role in atherogenesis, but thus far no studies with probucol have been reported in uraemic patients. Hydroxymethyl-glutaryl coenzyme A reductase inhibitors (e.g. lovastatin) lower hepatic cholesterol synthesis and thereby enhance expression of LDL receptors, resulting in increased clearance of apo B-containing lipoproteins. They have been used successfully in the treatment of hypercholesterolaemia in adult renal transplant recipients (196,197).

6.2.3.3 Fish Oil

Fish oil contains large amounts of omega-3 polyunsaturated fatty acids. It has been shown to reduce serum TGs levels in young patients receiving renal replacement therapy (RRT) and to improve their atherogenic serum lipoprotein profile (198). However it has a detrimental effect on renal function and histology in rats with subtotal nephrectomy[1] [199] and experience of its use is as yet too limited to recommend its use in children.

6.2.3.4 Exercise and Life Style

Endurance exercise training is an effective therapeutic modality in adult HD patients (200). Aerobic exercise (201) and resistive training (202)have beneficial effects on lipoprotein profiles in healthy children and adolescents, but long term experience in young uraemic patients has not been reported.

6.2.4 Antihypertensive Therapy

Antihypertensive therapy normalizes endothelium dependent relaxation to acetylcholine, ADP and thrombin in hypertensive rats (203). Lowering of blood pressure by itself will reduce arterial wall tension, reduce pulse wave velocity and delay wave reflections. In CRF, good control of blood pressure leads to a reduction in the incidence of myocardial ischaemia (37) and it is a potentially effective way to slow the rate of decline in renal function (204).

6.2.5 Anigiotensin Converting Enzyme Inhibitors

Angiotensin converting enzyme inhibitors augment the endothelium dependent relaxation to bradykinin in most blood vessels, including the human coronary artery but not the mammary artery (205). This indicates that inhibition of angiotensin converting enzyme may play an important role in the circulation by augmenting the release of NO and endothelium derived relaxing (factor in the blood vessel wall in certain but not all blood vessels (206).

6.2.6 Ca^{2+} Antagonists

Ca^{2+} antagonists facilitate the effects of endothelium derived

vasodilators by reducing the contractile responses of vascular

smooth muscle (206). They may at least partially reverse the

increase in tension induced by endothelin-1(207). Whether the

inhibitory effects of Ca^{2+} antagonists on the vasoconstrictive or

proliferative actions of endothelin-1 exert protective vascular effects

in patients in vivo remains to be demonstrated. However, it was

demonstrated in cholesterol fed rabbits that Ca^{2+} antagonists partially

suppress the development of atherosclerosis and augment

endothelium dependent relaxation to acetylcholine (208)

6.2.7 β- Blockers

Certain β-blockers augment the release of endothelium derived

relaxing factor evoked by alpha2-adrenergic agonists (209). However

the effect of chronic β-blockade on endothelial function has not yet

been studied. In addition, some β-blockers may elevate blood lipid

levels (206).

6.2.8 Folic Acid

Folic acid can ameliorate endothelial dysfunction in young patients with peripheral arterial occlusive disease and hyperhomocysteinaemia, as it normalizes homocysteine metabolism (210). It has also been shown that its active form (5-methyltetrahydrofolate) restores endothelial function in familial hypercholesterolemia, through reduced catabolism of NO and an increase in its bioavailability (211). However other studies in haemodialysis patients (212) and peritoneal dialysis patients (213), detected no change in endothelial function after long-term folic acid therapy.

6.3 Conclusion

In this thesis the physiology and biochemistry of endothelial function in young children with CRF has been explored. These studies have demonstrated, for the first time, that the atherosclerotic process is already established in young children with CRF who are not hypertensive or hypercholesterolaemic. The underlying causes of the endothelial dysfunction need further exploration.

It is reassuring that enteral feeding, which is so important in the

maintenance of normal growth in children with CRF, is not causing worsening of the dyslipidaemia of CRF.

It is hoped that this work will lead to interventional studies that may prevent or retard the atherosclerotic process at a time when this is still possible.

APPENDICES

Appendix 2.1. Clinical and laboratory features of children recruited for endothelial function

and nitric oxide study

Patient (sex)	Age(ys)	Sys.BP. SDS	Dia.BP SDS	G.F.R.(ml/minute /1.73m²)	T chol (mmol/l)	LDL chol (mmol/l)	Fasting blood glucose (mmol/l)	Plasma Albumin (g/l)
P1 (M)	9.5	-1.29	-1.09	13.3	4.94	3	4.2	43
P2 (M)	10.5	-0.41	0.23	12	5.1	3.2	5	43
P3 (F)	8.7	-1.31	-1.48	13.7	5.1	2.9	4.2	45
P4 (F)	9.2	-0.08	0.35	14.4	4.6	2.8	5	43
P5 (M)	9.5	-1.08	-1.14	11	4.9	3.1	4.4	44
P6 (M)	10	-0.29	0.72	14.3	5.06	2.9	4.7	43
P7 (M)	12	-1.39	-0.21	23.3	5.2	3.2	4.7	41
P8 (M)	12.8	-0.23	-0.69	23	4.06	2.3	5	42
P9 (M)	12.6	-0.21	-2.2	11.5	5.1	3.2	4.7	37
P10 (F)	11.3	-0.45	0.37	18.6	4.36	2.7		46

P11 (F)	11.4	0.51	0.36	11	5.1	3.2		44
P12 (M)	14.3	-0.45	0.37	17.9	5.1	2.9	4.5	39
P13 (M)	17.1	0.45	0.94	8.8	5.1	3.1	4.2	44
P14 (M)	7.8	0.89	0.5	11.8	5.1	3.1	5	45
P15 (M)	13.2	0.05	0.56	13	4.98	2.7	4.3	45
P16 (M)	13.9	-1	-0.34	26.9	4.79	3.2	4.7	
P17 (M)	13	0.1	0.57	33.6	4.53	2.8	4.4	41
P18 (M)	9.9	0.59	0.72	34.5	4.1	3.2	4.1	46
P19 (M)	16	0.23	0.24	19	4.3	2.4	4.3	41
P20 (F)	14.4	-0.08	0.71	18.7	5.05	3.1	4.7	39
P21 (M)	10.4	-0.81	-0.21	17.8	4.62	3	4.7	46
P22 (M)	14.5	-0.29	-0.42	11.2	5.1	3.2		44
P23 (M)	14.8	-2.08	-1.35	22.6	3.12	1.7	4.7	39

Appendix 2.3.C: Clinical and laboratory features of children recruited for the lipid study

(Group 3): Peritoneally dialyzed (PD)

Patient (Sex)	AGE (ys)	Diagnosis	Plasma Creatinine (µmol/l)	P.Albumin (g/l)	Ht SDS	Body mass Index (centile)
P45 (M)	15.5	VUR	1104	40	-4.1	17.7 (9-25%)
P46 (M)	8.3	Dys	722	44	-2.4	19.3 (91-98%)
P47 (M)	7.5	FSGS	692		-2	19.8 (98%)
P48 (F)	13	VUR	363	41	-1.7	22.7 (91%)
P49 (M)	16.5	VUR	1006	36	-2.2	22.1(75%)

Appendix 2.3.D: Clinical and laboratory features of children recruited for the lipid study

(Group 4): Enterally fed and on peritoneal dialysis (PD)

Patient (Sex)	AGE (ys)	Diagnosis	Plasma Creatinine (μmol/l)	P.Albumin (g/l)	Ht SDS	Body mass Index (centile)
P50 (M)	4.9	Wilms tumor	507	33	-2.1	19.1 (98-99.6%)
P51 (F)	4.4	CNS	487	36	-2.6	15.9 (50-75%)
P52 (F)	6	HUS	666	38	-1.3	19.6 (98%)
P53 (M)	2.1	CNS	421	34	-2	17.5 (75-91%)
P54 (F)	2.5	Dysplasia	500	36	-2.3	30.5 (>99.6%)

Appendix 3.1. Vascular study results in children with CRF

Patients	FMD (%)	GTN (%)	Peak reactive hyperaemia (RH %)	RH at 15 seconds (%)
P1	7.03	20	436	184.2
P2	3.10	20	237	157.4
P3	4.13	30	236	114.4
P4	4.24	24	250	385.2
P5	4.09	23	239	255.7
P6	4.05	25	371	244.5
P7	0.00	23	271	137.8
P8	7.25	28.7	304	283.6
P9	9.73	19.84	123	293.5
P10	3.2	30.67	173	554.5
P11	5.66	27.92	168	258.0
P12	5.11	22.82	129	410.2
P13	0.99	7.67	197	402.7
P14	12.3	29.51	241	287.5
P15	1.10	41.03	504	572.2
P16	3.04	25.68	205	92.3
P17	6.87	33.97	446	209.8
P18	4.94	32.1	431	174.2
P19	8.64	17.59	298	179.4
P20	7.38	28.86	510	128.9
P21	2.55	10.48	240	188.7
P22	3.03	22.9	314	337.9
P23	3.87	33.7	219	181.3

Appendix 3.2. Vascular study results in controls

Controls	FMD (%)	GTN (%)	Peak reactive hyperaemia (RH %)	RH at 15 seconds (%)
C1	2.80	13.68	204	177.2
C2	6.67	19.17	268	363.1
C3	9.28	18.72	162	312.3
C4	7.07	29.29	389	357.4
C5	10.48	25.71	322	180.5
C6	9.81	25.00	710	177.7
C7	1.67	25.00	337	188.6
C8	9.17	19.17	195	274.8
C9	10.75	20.86	600	160.0
C10	6.40	29.29	390	348.9
C11	6.31	17.86	337	255.9
C12	11.05	13.57	286	269.0
C13	10.64	21.41	375	79.9
C14	6.96	24	560	281.6
C15	10.93	27.75	330	313.7
C16	13.98	33.08	330	235.8
C17	10.86	34.39	448	292.9
C18	8.38	29.39	471	179.0
C19	8.87	17.25	187	224.1
C20	7.84	20.73	194	168.4
C21	10.56	19.2	352	261.5
C22	5.54	27	241	246.0
C23	10.86	20	448	288.1

Appendix 3.3. Serum Lipid Subfractions

Patient	HDL	Trig	Apo A1	Apo B	Lp (a)	AbOXLDL
(sex)	(mmol/l)	(mmol/l)	(g/l)	(g/l)	(g/l)	(Mu/ml)
P1 (M)	1.3	1.4	1.64	1	0.2	
P2 (M)	1.2	2	1.7	1.26	0.155	
P3 (F)	1	2.8	1.5	1.13	0.185	450
P4 (F)	1.35	1	1.6	0.96	0.355	
P5 (M)	1.2	1.4	1.6	1	0.13	297
P6 (M)	1.3	1.72	1.7	1	0.16	401
P7 (M)	1.5	1	1.9	1.1	0.05	
P8 (M)	1.12	1.32	1.56	0.86	0.31	639
P9 (M)	1.62	1.46	1.9	1.27	0.09	172
P10 (F)	1.11	1.29	1.46	0.89	>0.7	333
P11 (F)	1.22	1.73	1.71	1.48	<.05	
P12 (M)	0.91	2.89	1.44	1.19	0.07	337
P13 (M)	1.03	2.9	1.52	1.14	0.09	155
P14 (M)	0.92	3.06	1.51	1.2	<.05	
P15 (M)	1.15	2.45	1.5	1.05	0.28	149
P16 (M)	1.09	1.1	1.41	1.07	0.66	146
P17 (M)	1.37	0.79	1.53	0.9	0.05	111

P18 (M)	1.21	0.9	1.59	1.02	0.24	
P19 (M)	0.91	2.22	1.45	0.92	0.06	272
P20 (F)	1.52	0.85	1.77	0.94	0.05	322
P21 (M)	0.97	1.46	1.29	0.83	0.07	99
P22 (M)	1.16	1.49	1.63	0.87	0.11	103
P23 (M)	0.94	1.1	1.26	0.64	0.11	187

Appendix 4.1. Nitric oxide biochemistry results (patients)

Patient	Nitrate	Nitrites	Total Dimehyls	ADMA	SDMA	Nitoso-thiols(L.M.W)	Nitroso-thiols (H.M.W)(µmol/l)
(sex)	(µmol/l)	(µmol/l)	(µmol/l)	(µmol/l)	(µmol/l)	(µmol/l)	
P1 (M)	108.32	1.27	2.6	1.7	0.9	0.6	1.87
P2 (M)	100.1						
P3 (F)	180.6	1.4	7.8	4.9	2.9	0.47	2.4
P5 (M)	33.6	2.1	13.31	9.37	3.94	0.9	
P6 (M)	141.0	2.13	6	5.4	0.6	0.2	2.1
P7 (M)		1.4	3.7	2.5	1.2	0.47	3
P8 (M)	80.5	0.73	4.4	3.3	1.1	0.47	2.2
P9 (M)	161.0	1.53	10.7	7.7	3	0.32	2.33
P10 (F)	78.4	0.2	3	2.1	0.9	1.7	2.4
P11 (F)	74.1	0.93	6.9	4.8	2.1	0.14	2.4

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Appendix 2.2. Clinical and laboratory features of controls recruited for endothelial study

Control (Sex)	Age (ys)	VS. Diameter (mm)	T.chol (mmol/l)	LDL (mmol/L)
C1 (M)	10	2.85		
C2 (M)	9	3.00	4.50	2.6
C3 (F)	11	2.59		
C4 (M)	12	2.49		
C5 (M)	10	2.63	5.00	3.2
C6 (M)	16	2.70	4.6	3.22
C7 (M)	12	3.00		
C8 (F)	11	3	4.4	2.3
C9 (M)	15	3.12	3.26	1.56
C10 (M)	15	3.13	4.12	2.48
C11 (M)	16	3.33	4.4	3.18
C12 (M)	16	3.96	3.92	3.85
C13 (M)	15	3.53	6.9*	
C14 (M)	15	2.88	5.52*	4.16
C15 (M)	15	2.98	4.72	3.19
C16 (M)	15	2.04	4.7	3.32
C17 (F)	12	2.76		
C18 (M)	11	2.86		
C19 (F)	10	2.82		
C20 (F)	10	3.07		
C21 (M)	11	2.61		
C22 (F)	10	2.98		
C23 (F)	12	2.72		

* No known risk factors or a significant family history

Appendex 2.3.A: Clinical and laboratory features of children recruited for the lipid study
(Group 1): Conservatively managed CRF

Patient (Sex)	AGE(Ys)	Diagnosis	Plasma Creatinine (μmol/l)	G.F.R.(ml/ min/1.73m²)	P.Albumin (g/l)	Ht SDS	Body mass Index (centile)
P8 (M)	12.8	Dys	220	23	41	-1.7	18.4 (25-50%)
P9 (M)	12.6	CPN	462	11.5	42	-1.7	16.4 (25%)
P10 (F)	11.3	PUV	326	18.6	37	1.4	18.4 (50-75%)
P11 (F)	11.4	PUV	471	11	46	-2	24 (98%)
P12 (M)	14.3	FSGS	365	17.9	39	-1	18.1 (25-50%)
P13 (M)	17.1	Dys	759	8.8	44	-1	20.7 (50%)
P14 (M)	7.8	Dys	392	11.8	45	-1.5	17.5 (75-91%)
P15 (M)	13.2	Alports sy	455		45	-0.8	18.9 (50-75%)
P16 (M)	13.9	CPN	255	26.9		-0.3	18.7 (50%)
P17 (M)	13	VUR	175	33.6	41	-0.7	19.7 (50-75%)
P18 (M)	9.9	Dys	507	11.1	45	-2.2	14.5 (9%)

P19 (M)	16	Dys	344	19	41	-1.4	24.8 (91-98%)
P20 (F)	14.4	Dys	317	18.7	39	-2.1	20.8 (50-75%)
P21(M)	10.4	Dys	300	17.8	46	-0.8	15.5 (25%)
P22(M)	14.5	Dys	512	11.2	44	-2.2	16.1 (2-9%)
P23(M)	14.8	Dys	256	22.6	39	-3.4	20.4 (50-75%)
P24(M)	10.9	Prune Belly	331	15.3	45	-0.7	15.2 (9-25%)
P25 (M)	9.9	Dys	163	34.5	46	0.7	20 (91%)
P26 (F)	15.4	FSGS	368	16.3	41	-2	22.8 (75-91%)
P27(M)	10.5	Dys	312	15.6	40	-2	15.3 (9-25%)
P28 (M)	4.5	PUV	176	18.2	43	-1.2	16.9 (75-91%)
P29 (M)	1.6	Dys	178	11	42	-1	14.9 (2-9%)
P30 (F)	2.5	CPN	182	16.9	45	-2	15.7 (25-50%)
P31(M)	1	Dys	575	4.6	42	-2.6	17.5 (50%)
P32 (F)	3.9	Dys	243	14.2	40	-0.5	19.1 (98%)
P33 (M)	13.5	IPKD	467	12.5	46	-1.3	18.8 (50-75%)
P34 (F)	10.7	PUV	143	40	49	0.8	20.3 (75-91%)
P35 (M)	15.5	PUV	420	16		-0.3	24.4 (91-98%)
P37 (M)	8.5	CPN	217		31	-1.6	15.9 (50%)
P38 (F)	9.9	PUV	459		45	-0.3	24.4 (98-99.6%)
P39 (M)	15.5	FSGS	420	16		-0.3	24.4 (91-98%)

Appendex 2.3.B: Clinical and laboratory features of children recruited for the lipid study

(Group 2): Conservatively managed CRF and enterally fed

Patient (Sex)	AGE(ys)	Diagnosis	Plasma Creatinine(μmol/l)	G.F.R.(ml/minute/1.73m²)	P.Albumin (g/l)	Ht SDS	Body mass Index (centile)
P40 (F)	2.5	Dys	280	11.5	42	-1.3	13.1 (<0.4%)
P41 (M)	0.9	Dys	706	4.6	33	-0.2	20.8 (98%)
P42 (M)	3.2	Dys	524	6.2	46	-3.4	17 (75-91%)
P43 (M)	3.8	Dys	157	27.2	38	-7.6	14.9 (25%)
P44 (M)	1.2	PUV	428	6.2	39	-1.1	19.7 (91-98%)

P12 (M)	71.5	1	4.81	2.73	2.08	1.9	2.4
P13 (M)	79.6						2.3
P14 (M)	84.7	0.2	6.3	4.7	1.6	0.9	
P15 (M)	63.0	1.5	5.28	2.02	3.26	0.33	2.87
P16 (M)	112.0	0.93	6.2	4.6	1.6	1.14	2.33
P17 (M)	56.4	0.2	2.5	1.7	0.8	2.27	3.13
P18 (M)	100.5	1.8	4.48	3.35	1.13	1.3	2.6
P19 (M)	137.4	1	3.8	2.7	1.1	0.4	2.5
P20 (F)	98.4	1.27	7.5	4.5	3	1.06	2.53
P21 (M)	192.1		5.8	2.7	3.1		2.42
P22 (M)	65.3	0.87	4.2	2.9	1.3	1.2	2.6
P23 (M)		1.3	3.97	3.11	0.86	1.3	2.5

Appendix 5.2 (D). Serum Lipid Subtractions in Peritoneally dialyzed (PD) and enterally fed children

Patient (Sex)	T chol (mmol/l)	LDL chol (mmol/l)	HDL chol (mmol/l)	Trig (mmol/l)	Apo A1 (g/l)	Apo B (g/l)	Lp (a) (g/l)	Glucose (mmol/l)
P50 (M)	5.9	3.6	1	2.8	2.1	1.6	0.28	4.4
P51 (F)	4.5	2.2	1.3	2.1	1.6	0.9	0.15	4.2
P52 (F)	4.63	2.71	0.82	2.4	1.22	0.24		3.7
P53 (M)	6.1			4.2	1.5	0.9	0.3	5.9
P54 (F)	4.41	2.63	0.78	2.2	1.22	0.82	0.21	

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Practical Procedures:

Endothelial function study: The ultrasound study was done by Ann Donald with the assistance of Dr. Jameela Kari. Dr Kari, reviewed the video tapes, calculated reactive hyperaemias and analysed the data

Nitric oxide study: Laboratory analysis of NO metabolites were done by Professor K. R. Bruckdorfer team (Timothy Bunce and Belen Dorado)

Lipid study: Lipid studies were done by Dr David Vallance and measurement of antibodies against ox-LDL by Dr Zac Vargese.

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Physiology and biochemistry of endothelial function in children with chronic renal failure

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Physiology and biochemistry of endothelial function in children with chronic renal failure. Premature atherosclerosis is a major cause of morbidity and mortality in chronic renal failure (CRF). Endothelial dysfunction is a key early event in atherogenesis. The aim of this study was to assess the effect of CRF on endothelial function using physiological and biochemical measures. To focus on the effect of CRF itself, 23 children (matched with 23 controls for age and vessel diameter) were selected because they were normotensive, had normal total cholesterol (TC) levels, and were not on vasoactive drugs. Their mean (range) age was 12.0 (7.8 to 17.0) years; GFR 17.5 (8.8 to 34.5) ml/min/1.73 m². The physiology of endothelial function in the brachial artery was assessed using high resolution ultrasound by measuring its diameter at rest, during reactive hyperemia (endothelium dependent dilation) and after sublingual glyceryl trinitrate (GTN; endothelium independent dilation). Nitric oxide (NO) metabolites and endogenous NO synthetase (eNOS) inhibitors were measured as an assessment of endothelial metabolism. Brachial artery dilation to flow [FMD, mean (SEM)%] was reduced in CRF to 4.9 (0.6) and controls 8.6 (0.6), $P < 0.0001$. In contrast, the response to GTN was similar in both groups: CRF 25.1 (1.6), controls 23.3 (1.2), $P = 0.31$. There was no difference in TC, low density lipoprotein (LDL) or high density lipoprotein (HDL) between the patients and the controls. Triglycerides (TG) were higher in the patients but within the normal range. Antibodies against oxidized LDL (ox-LDL) were high in CRF. Endogenous NOS inhibitors were high in CRF, and intermediate NO metabolites were low. There was no correlation between FMD of the brachial artery and lipid subfractions, or with NO metabolites or eNOS inhibitors. Endothelium dependent dilation of the brachial artery is impaired in children with CRF who do not have co-existing risk factors for atherosclerosis. This may represent early evidence of atherogenic vascular disease.

Premature atherosclerosis is a major cause of morbidity in adults with chronic renal failure (CRF), and is responsible for a mortality rate ten times greater than in the normal population [1]. An increasing number of children with CRF are surviving to adulthood, both because of advances in dialysis and transplantation, and because treatment is being extended to younger patients. Their long-term morbidity and mortality are uncertain, but they

might be expected to have similar vascular complications to adults with CRF, at an even earlier age.

Although the clinical manifestations of atherosclerosis do not usually occur before adulthood, the process begins in childhood [2]. Endothelial dysfunction is a key early event that precedes the formation of atherosclerotic plaques, and results in reduced bioavailability of nitric oxide (NO), which may be an important anti-atherogenic agent [3]. In CRF, abnormal endothelial function and NO activity may result from both the metabolic consequences of CRF, such as reduced clearance of endogenous NO synthetase (eNOS) inhibitors [4, 5] and increased oxidative stress [6], as well as from the presence of other classical risk factors such as hyperlipidemia and hypertension [1].

We have developed a non-invasive technique using high resolution ultrasound to assess vascular reactivity in the conduit arteries of the systemic circulation, which can be used to study endothelial function from as early as the first decade of life [7]. We have previously shown that endothelial dysfunction may occur before clinical evidence of vascular disease in subjects with hypercholesterolemia [8], diabetes [9] and in cigarette smokers [10].

In the current study we have examined the influence of CRF on endothelial function in young subjects. In order to avoid confounding variables, we purposefully selected subjects with CRF who were not smokers, hypertensive, hypercholesterolemic or diabetic, and were not receiving vasoactive drug therapy.

Our findings suggest that CRF has a direct adverse effect on endothelial function in this young patient group. This may influence later morbidity and mortality from large vessel atherosclerotic disease independently of other risk factors.

METHODS

Patients

Twenty-three children (18 boys), aged 7.8 to 17.0 years (median and mean 12.0), with CRF on conservative management, none of whom were on maintenance dialysis [mean (range) glomerular filtration rate (GFR) 17.5 (8.8 to 34.5) ml/min/1.73 m²], were studied. Their diagnoses were renal dysplasia (19), reflux nephropathy (2), Alport's syndrome (1) and focal glomerulosclerosis (1). They were selected from an outpatient population because they were normotensive [mean (SEM) systolic blood pressure SD score (BPSDS) for age −0.04 (0.15), diastolic −0.11 (0.18)]; with

sma total cholesterol (TC) < 5.2 mmol/liter and low density oprotein (LDL) < 3.3 mmol/liter; were not diabetic or nerotic [mean (SEM) serum albumin 42.9 (0.6) g/liter, 24-hr otein excretion 0.85 (0.2) g/liter]; and were not taking vasoactive lipid-lowering medications. They were matched with 23 control bjects (friends or relatives of hospital staff) for age and brachial ery diameter. Twenty of the controls were gender matched with e CRF patients (87%). Ten boys and 3 girls in the CRF group, mpared to 9 boys and 4 girls in the controls were over the mal age for onset of puberty (over 11 years in boys and 10 ars in girls).

Plasma nitrite and nitrate (NO oxidation products), high and w molecular weight nitrosothiols (intermediate metabolites of O), eNOS inhibitors [asymmetrical dimethylarginine (ADMA) d symmetrical dimethylarginine (SDMA)] in the CRF patients ere compared to values from 6 control children, aged 6 to 16 nedian 11 years), who were having blood taken for family genetic reening of non-cardiac abnormalities. Plasma TC, triglycerides G), LDL and high density lipoprotein (HDL) were measured in e CRF group and in 12 of the controls who were willing to have ood tests. Other lipid subfractions such as Apo A1, ApoB, poprotein(a) [Lp(a)] and antibodies against oxidized LDL (ox-DL) were measured in the CRF group. All blood samples were ken after an overnight fast (although tap water was allowed), ere immediately centrifuged, and the plasma was stored at 70°C. Each subject and/or their parents gave informed consent the study, which was approved by the Local Committee on thical Practice.

ndothelial function study

Endothelial and smooth muscle function were studied non-nvasively by examining brachial artery responses to endothelial lependent and independent stimuli as we have previously re-orted [7]. Serial diameter changes were measured at rest, in esponse to reactive hyperemia (with increased flow producing ndothelial dependent vasodilatation), again at rest, and finally fter sublingual glyceryl trinitrate (GTN), which is an endothelial ndependent vasodilator. All subjects were scanned in a supine osition following a 10-minute rest period. A high resolution B-mode ultrasound image of the brachial artery was obtained in a ongitudinal section, 5 to 10 cm above the antecubital fossa, using a 7 MHz linear array transducer and Acuson 128XP/10 system (Acuson, Mountain View, CA, USA), connected to a wall-tracking system (Ingenious Systems, Netherlands) allowing accurate on line diameter measurements. The center of the artery was identified when the clearest picture of the anterior and posterior vessel wall layers was obtained. Depth and gain settings were set to optimize the lumen/arterial wall interface, and machine operating parameters were not changed throughout the study. The arm remained in the same position and a satisfactory transducer position was maintained using a stereotactic clamp. All the ultrasound scan data were recorded on superVHS video for later flow analysis and all scans were performed by the same operator. To measure the brachial artery diameter an M-line was placed perpendicular to the vessel walls on the B-mode image, the radio-frequency signals from the M-mode output were then relayed to the wall-tracking system. On completion of five seconds of data acquisition, the first radio-frequency signal was displayed and electronic markers placed at the vessel wall/lumen interface and a beat by beat computation of the end diastolic diameter and

mean over 5 to 10 cardiac cycles was obtained. Reproducibility and repeatability of this method have been previously reported [11, 12]. A resting scan was recorded and arterial flow velocity was measured using a pulsed Doppler signal at a 70° angle to the vessel with the range gate (1.5 mm) in the center of the artery. Volume blood flow was calculated by multiplying the velocity time integral of the Doppler flow signal (corrected for angle) by the heart rate and the vessel cross-sectional area (πr^2). A pneumatic tourniquet placed around the forearm was then inflated to 300 mm Hg for 4.5 minutes followed by rapid release, inducing increased flow. The post-hyperemic diameter was measured between 55 and 65 seconds after cuff deflation. Peak reactive hyperemia was calculated as the maximal flow change within 15 seconds of cuff deflation divided by the flow during the resting (baseline) scan reported as percentage increase in flow. A further reactive hyperemia was calculated in the same way 15 seconds after cuff release. Since the velocity is taken from the center of the artery, absolute values may be overestimated, but the relative values before and after cuff inflation are accurate. A further 10 minutes was allowed for vessel recovery, after which a second resting scan was recorded. A 400 µg sublingual dose of GTN was then administered and a final scan was recorded three minutes later. Flow mediated dilation (FMD) in the brachial artery following reactive hyperemia and endothelium independent dilation following GTN administration were expressed as percentage diameter change relative to the first base line scan.

To assess the reproducibility of the ultrasound technique in CRF, seven of the children were seen on three occasions, all within four months of the first study.

Lipid analysis

TC was measured using the cholesterol C system high performance cholesterol oxidase 4-aminophenazone (CHOD-PAP) method and TG by glyceryl phosphate oxidase 4-aminophenazone (GPO-PAP) high performance enzymatic colorimetric test (both Boehringer Mannheim Diagnostica GmbH, Mannheim, Germany) [13]. HDL was measured following precipitation of ApoB containing lipoproteins and LDL was calculated using the Friedewald formula [14]. ApoA1 and ApoB were measured using immunoturbidimetry (Immuno Ltd, Sevenoaks, Kent, UK) [15], and Lp(a) by enzyme-linked immunosorbent assay (ELISA) (Immuno Ltd) [16]. All assays were validated by the National External Quality Assessment Scheme. Antibodies against ox-LDL were measured by ELISA with a 450 nm filter, based on a set of standardized serum and controls obtained from a O-lab-ELISA kit (Biomedica Gruppe, Austria) [17].

Nitric oxide biochemistry

Nitrite and nitrate were measured using high performance capillary electrophoresis [18]. Nitrosothiols were measured after separating the plasma into two molecular weight fractions by ultracentrifugation (5,000 Mwt filter at 5000 g). Mercury salts were then added to displace the NO from the thiol to generate nitrite, which was assayed by capillary electrophoresis [18].

ADMA and SDMA were measured by high performance liquid chromatography using electrochemical detection after precolumn derivatization with o-phthalaldehyde (OPA)/b-mercaptoethanol [19].

A   *P<0.0001*

% FMD

16
14
12
10
8
6
4
2
0

CRF   Controls

B   *P=0.31*

% GTN

45
40
35
30
25
20
15
10
5
0
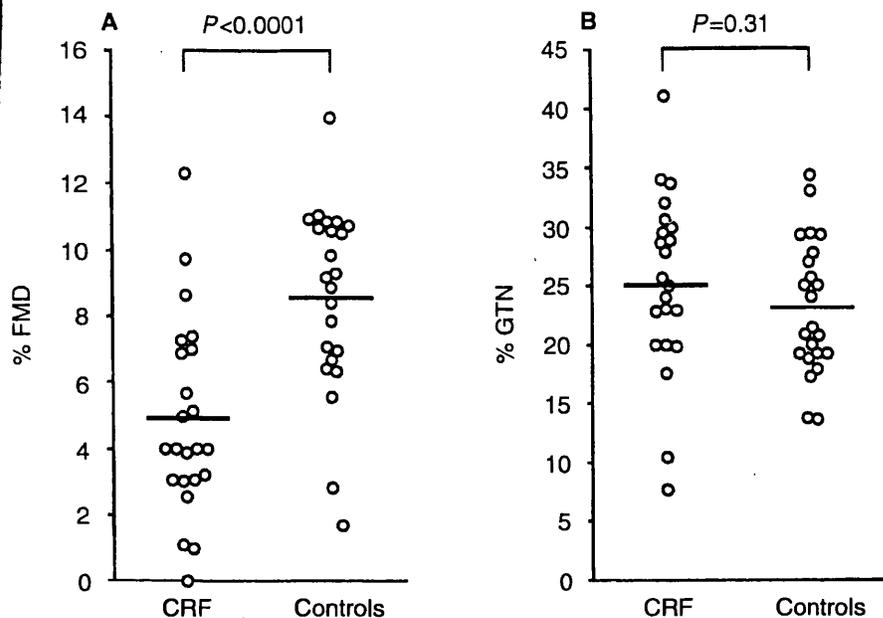
CRF   Controls

Fig. 1. Flow mediated (FMD, *A*) and glyceryl trinitrate (GTN, *B*)-induced dilation in the controls and children with CRF. Horizontal lines are group means. FMD was significantly impaired in CRF whereas GTN response was normal.

Table 1. Vascular study results in children with CRF and controls

	CRF	Controls	P
Peak reactive hyperemia %	284.4 (23.7)	357.6 (29.3)	0.06
Reactive hyperemia at 15 seconds %	262.3 (27.2)	241 (24.5)	0.61
Vessel size mm	2.9 (0.1)	2.9 (0.1)	0.72
FMD %	4.9 (0.6)	8.6 (0.6)	<0.0001
GTN %	25.1 (1.6)	23.3 (1.2)	0.31

Results are expressed as mean (SEM).

Table 2. Nitric oxide biochemistry results

	CRF	Controls	P
Nitrate μM	100.9 (9.4)	32.1 (4.3)	<0.00001
Nitrite μM	1.2 (0.1)	1.4 (0.2)	0.38
ADMA μM	3.8 (0.4)	0.7 (0.1)	0.001
SDMA μM	1.8 (0.2)	0.3 (0.1)	0.001
Low molecular wt nitrosothiol μM	0.9 (0.1)	1.6 (0.2)	0.02
High molecular wt nitrosothiol μM	2.5 (0.1)	3.4 (0.3)	0.0001

Results are expressed as mean (SEM).

Statistics

Descriptive statistics are expressed as mean ± SEM. The CRF and control groups were compared using two sample *t*-tests. Univariate regression analysis was used to assess the relationship between the two dependent variables, flow mediated and GTN induced dilation, and sex, age, vessel size, and TC in all the children, and with TG, HDL, LDL, ApoA1, ApoB, Lp(a), antibodies against ox-LDL, GFR and NO metabolites in the 23 children with CRF. Variability between results of the high resolution ultrasound technique (repeatability) for the seven patients who had been studied on more than one occasion was calculated as ratio of the within subject SD (the square root of the residual mean square taken from analysis of variance for repeated measures) to the overall mean. This gives the estimated coefficient of variation (%). Statistical significance was inferred at a *P* value of < 0.05.

RESULTS

Endothelial physiology

There was no difference in resting vessel size, peak reactive hyperemia, or reactive hyperemia at 15 seconds after cuff release between CRF and controls (Table 1). However, FMD(%) in CRF was significantly impaired in comparison to the controls [4.9 (0.6) vs. 8.6 (0.6), *P* < 0.0001]. In contrast, dilation to GTN did not differ from the controls [25.1 (1.6) vs. 23.3 (1.2), *P* = 0.31] (Fig. 1).

On univariate analysis, there was no correlation between endothelium dependent (FMD) or independent brachial artery dilation and measures of renal function, lipid subfractions and NO biochemistry.

Study of repeatability

For each child, there was a directionally similar response to increased flow and to GTN with reproducible failure to dilate to increased flow on the three study occasions. The mean (range) across visits of observed FMD was 5.1% (1.5 to 7.3). From analysis of variance for repeated measures, the estimated coefficient of variance between visits was 4.3%.

Nitric oxide biochemistry

Plasma nitrate, ADMA and SDMA levels were significantly elevated in the CRF children (Table 2). There was a significant inverse correlation between GFR and total dimethylarginines ($r = -0.48, P = 0.03$) and SDMA ($r = -0.46, P = 0.04$), although the correlation with ADMA was not significant ($r = 0.41, P = 0.07$). Nitrite levels however did not differ from controls. Low and high molecular weight nitrosothiol levels were significantly lower in the CRF group, and there was a positive correlation between GFR and low molecular weight nitrosothiol ($r = 0.52, P = 0.017$).

Although it would be expected that high levels of eNOS inhibitors might decrease NO production, there was no correlation between ADMA and SDMA and any NO metabolites.

Table 3. Lipid subfractions

	CRF	Controls	Normal values	P
ge *years*	12.1 (0.5)	12.6 (0.5)		0.53
otal cholesterol *mmol/liter*	5 (0.1)	4.8 (0.3)	<5.2	0.52
ow density lipoprotein *mmol/liter*	3.0 (0.1)	3.0 (0.2)	<3.3	0.97
igh density lipoprotein *mmol/liter*	1.2 (0.04)	1.2 (0.04)	1.0–2.0	0.92
riglycerides *mmol/liter*	1.5 (1.1)	0.76 (1.2)	<1.7	0.003
ntibodies against OX-LDL *μ/ml*	260 (37.5)		<250	
ApoA1 *g/liter*	1.6 (0.03)		0.7–1.7	
ApoB *g/liter*	1.0 (0.04)		0.6–1.4	
Lp(a) *g/liter*	0.12 (0.07)		<0.3	

Results are expressed as mean (SEM).

ipid studies

There was no significant difference between TC, HDL and LDL evels in the patients and the controls (Table 3). However, TG evels were higher in the CRF patients and antibodies against x-LDL were elevated. GFR correlated with TC ($r = 0.50$, $P = 026$) and log TG ($r = -0.64$, $P = 0.001$) levels even when age vas taken into consideration, but not with other lipid subfractions.

DISCUSSION

Our results show that impaired endothelial function is already resent in the conduit arteries of children with CRF by the first decade of life. It is likely that this represents an early manifestation of the atherosclerotic process, which causes important morbidity and mortality in CRF patients in later life [1].

A number of factors may contribute to endothelial dysfunction in CRF, including dyslipidemia [1], drug therapy, increased oxidative stress [6] and the metabolic consequences of CRF themselves. In this study, we set out to determine the influence of CRF on endothelial function as directly as possible by excluding patients with hypertension, high plasma cholesterol levels and those receiving vasoactive drugs. We were able to study endothelial function from a very early stage before acquired risk factors are likely to play a major role, because of the availability of a non-invasive technique to examine vascular physiology in conduit arteries of the systemic circulation. Vasodilation to increased flow (an endothelium dependent stimulus) is contrasted with response to GTN (which acts independently of the endothelium). This technique, both in earlier studies and in the CRF patients, has been shown to be accurate and reproducible [11, 12]. As FMD in the brachial artery can be attenuated by intra-arterial infusion of L-NMMA, it is likely that this method assesses the integrity of the L-arginine/NO pathway in conduit arteries [20]. Furthermore, a close correlation has been demonstrated between endothelial function in the brachial artery, assessed using our method, and endothelial function in the coronary arteries assessed invasively using acetylcholine [21].

NO not only acts as a physiological regulator of vascular tone [22], but it is also an important anti-atherogenic molecule that inhibits platelet activation, monocyte and endothelial cell interaction, and smooth muscle cell proliferation [3]. We chose to use a physiological measure of NO-dependent endothelial function because biochemical measurements are difficult to interpret in

CRF due to the effects of abnormal renal clearance. This may explain the lack of correlation between measures such as nitrite and nitrate and FMD. Interestingly, nitrosothiols were not affected by renal clearance, and were lowest in the patients with the lower GFR. S-nitrosothiols, such as S-nitrosocysteine and S-nitrosoglutathione, are formed either by S-nitrosation of free thiol groups in the presence of NO [23] or by the reaction of thiols with peroxynitrite, which is derived from the reaction of NO with superoxide anion [24]. The nitrosothiols have have been shown to have biological properties similar to those of NO [25], which may be released from them [26]. While the biological significance of S-nitrosothiols remains unclear, they may represent a measure of NO bioavailability, and low levels in CRF may be one mechanism whereby NO activity is impaired.

In this study, we did not measure other endothelial dependent vasoactive compounds, such as endothelin-1 and thromboxane-A2, because neither has been shown to have a major role in clinical vascular disease [27]. Our findings of markedly reduced FMD in young subjects from as early as the first and second decades of life indicate that CRF may be contributing to endothelial abnormalities in addition to the influences of other vascular risk factors.

Endothelial dysfunction in CRF may involve abnormalities of both NO production and breakdown. Decreased synthesis may be due to the presence of elevated levels of L-arginine analogues such as ADMA and SDMA in CRF in proportion to its severity, which competitively antagonize eNOS, accumulate in CRF and correlate with its progression [4, 5]. They have been shown experimentally to increase vascular tone [5] and promote early atherogenic changes [28]. Other molecules that accumulate in uremia, such as the cytokine IL-8, also inhibit eNOS [4]. Additionally, in CRF circulating levels of L-arginine, the substrate for NO production, are reduced [5]. Increased inactivation of NO may also be important with increased oxidative stress and free radical production in CRF [6]. While total LDL in our patients did not differ from the controls (as we had excluded children with hypercholesteremia), levels of antibodies to ox-LDL were elevated in our patients with CRF. Ox-LDL is a critical factor in promoting atherogenesis [17] because it interferes with NO metabolism [29], promotes monocyte chemotaxis and transformation, and has a direct effect on endothelial cell survival [30]. Thus, in CRF as in other high risk factor groups, such as insulin-dependent diabetes mellitus, LDL levels even within the normal range may have an impact on endothelial function [9]. A similar situation thus may apply in the children with CRF. In addition, the higher TG levels in the CRF patients may play a role in endothelial dysfunction, but their influence on atherogenesis remains controversial [1].

In conclusion, even young children with CRF, whose outlook for vascular disease would be expected to be relatively good because of the absence of hypertension and hypercholesteremia, have evidence of endothelial dysfunction that may be an early manifestation of atherogenesis. Detection at this early stage of abnormal vascular function permits serial studies of interventions such as risk factor modification, anti-oxidants [31], or L-arginine administration [8], aiming to prevent or retard large vessel atherosclerosis, which is such an important contributor to clinical morbidity and mortality in these patients.

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Pediatric Nephrology

Original article

Effect of enteral feeding on lipid subfractions in children with chronic renal failure

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Abstract. The anorexia of chronic renal failure (CRF) is frequently managed with enteral feeds using combinations of commercial preparations, glucose polymers and fat emulsions. Such feeds might predispose to atherogenic blood lipid profiles. Our aim, therefore, was to compare the blood lipid profiles of enterally fed and non-enterally fed children. Plasma lipid subfractions were measured in 37 children with CRF managed conservatively and 10 managed with peritoneal dialysis (PD); 10 of the children were tube fed, 5 of whom were on PD. Results were compared between these groups. Overall, triglycerides (TGs, mean ± SD) were high (2.3 ± 1.4 mmol/l) and total cholesterol (TC) was at the upper limit of normal (5.2 ± 1.5 mmol/l). Low-density lipoprotein (LDL), high-density lipoprotein (HDL), apoprotein A1 (apo A1), A2 (apo A2) and B (apo B), and lipoprotein (a) [Lp(a)] were within the normal range. There was an inverse correlation between TGs and glomerular filtration rate ($P = 0.0001$). There were no differences in the levels of TC, TG, LDL, HDL, apo A1, apo A2 or Lp(a) between tube-fed and non-tube-fed children. We conclude that enteral feeding does not enhance hyperlipidaemia.

Key words: Chronic renal failure – Lipids – Lipoproteins – Enteral feeding

Introduction

Hyperlipidaemia is one of the factors believed to be responsible for the high incidence of atherosclerosis in chronic renal failure (CRF) [1]. Abnormalities of lipids and lipoproteins reported in CRF include: increased triglycerides (TGs), total cholesterol (TC), low-density lipoprotein (LDL), apoprotein B (apo B) and lipoprotein(a) [Lp(a)], and reduced high-density lipoprotein (HDL), apo A1 and apo A2 [2], all of which are believed to predispose to atherosclerosis. Studies in children have demonstrated similar findings, but with a higher incidence of hypercholesterolaemia [3].

As well as the metabolic effects of CRF, the blood lipid profiles of patients may be influenced by their diet. High intakes of saturated fatty acids (FAs) increase serum LDL and TGs. Polyunsaturated FAs reduce LDL, but at the same time also reduce HDL [4], which is protective against coronary heart disease [5]. Monounsaturated FAs lower both LDL and TGs, and are associated with higher levels of HDL. High intakes of refined carbohydrate (CHO) increase TGs and reduce HDL [4].

It is our policy to institute early enteral feeding in children with CRF with a declining growth velocity. Such feeds are based on whole-protein (and occasionally protein hydrolysate) complete feeds and are supplemented with glucose polymers and/or peanut oil emulsions as additional energy sources. The children eat little or no complex CHO or non-starch polysaccharides (fibre), so most of their intake of CHO is refined. We were concerned, therefore, that while providing adequate nutrition for growth, tube feeding regimens might have an adverse effect on the blood lipid profiles of the children.

Our purpose was to study the lipid profiles of children attending our CRF clinic who were eating a high-energy, low-phosphate, but otherwise unrestricted diet and to compare the results with those of a group of children who were receiving at least 50% of their energy as an enteral feed.

Patients and methods

Patients. Forty-seven children (32 boys) aged 1–17 years [mean 9.3 ± 5.2 (SD)] with CRF [defined for the purposes of this study as a plasma creatinine concentration > 150 µmol/l (1.7 mg/dl)] were studied. Thirty-seven were managed medically, 5 of whom were enterally fed. The other 10 were receiving peritoneal dialysis (PD), 5 of

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Table 1. Serum lipid subfractions[a]

Groups Number	(1) Medically managed 32	(2) Enterally fed 5	(3) Peritoneal dialysis (PD) 5	(4) Enterally fed and on PD 5	Normal values	P value (ANOVA)
Age (years)	11.1 (0.8)	2.3 (0.6)	12.3 (2.0)	3.9 (0.8)		<0.0001
Creatinine (μmol/l)	344 (25)	415 (98)	777 (130)	516 (40)		<0.0001
Height SDS	-1.1 (0.2)	-2.7 (1.3)	-2.5 (0.4)	-2.1 (0.2)		0.025
Body mass index	18.9 (0.5)	17.1 (1.4)	20.3 (0.9)	20.5 (2.6)		0.33
TG (mmol/l)	1.8 (0.2)	2.4 (0.5)	3.9 (0.4)	2.7 (0.8)	<1.7	0.002*
TC (mmol/l)	5.1 (0.3)	4.2 (0.6)	6.1 (0.6)	5.1 (0.4)	<5.2	0.25
LDL (mmol/l)	3.0 (0.3)	2.4 (0.2)	3.6 (0.7)	2.8 (0.3)	<3.3	0.55
HDL (mmol/l)	1.3 (0.1)	1.3 (0.5)	1.1 (0.1)	1.0 (0.1)	1.0-2.0	0.59
apo A1 (g/l)	1.7 (0.1)	1.8 (0.5)	1.5 (0.1)	1.5 (0.2)	0.7-1.7	0.75
apo A2 (g/l)	0.6 (0.02)	0.7 (0.04)	0.8 (0.07)	0.7 (0.04)	0.3-0.8	0.0002*
Apo B (g/l)	1.1 (0.07)	0.9 (0.17)	1.5 (0.18)	0.9 (0.20)	0.6-1.4	0.03
Lp(a) (g/l)	0.18 (0.03)	0.14 (0.06)	0.3 (0.04)	0.23 (0.03)	<0.3	0.47

ANOVA, Analysis of variance; SDS, standard deviation score; TG, triglyceride; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; apo A1, apoprotein A1; Lp(a), lipoprotein (a)

* Insignificant when adjusted for age and creatinine by analysis of covariance
[a] Values expressed as mean (SEM)

Table 2. Enteral feed composition[a]

Source	Total energy intake (%)		
	Enteral feeds	Dietary reference values[b]	Dietary and nutritional survey (OPCS)[c]
Feed	86.5 (5.0)		
Protein	8.1 (1.2)	15	15
Carbohydrate	58.3 (3.8)	50	42
Total fat	32 (3.7)	35	38
Saturated FAs	10.1 (1.6)	11 (+2% trans)	16
Monounsaturated FAs	13.3 (2.4)	13	12
Polyunsaturated FAs	6.6 (0.8)	6.5[d]	6

FA, Fatty acid; trans, transpolyunsaturated FA
[a] Values expressed as mean (SEM)
[b] Dietary reference values for food energy and nutrients for the United Kingdom [17]
[c] The Dietary and Nutritional Survey of British Adults [18]
[d] Maximum recommended 10% of total energy intake

whom were enterally fed (Table 1). The mean (range) glomerular filtration rate (GFR) of the medically managed children was 15 (5-35) ml/min per 1.73 m². Children with nephrotic syndrome were excluded because of its effect on lipid metabolism [5].

The mean (range) length of time on enteral feeds was 13.2 (6-32) months. The feeds were prepared from whey-based infant formulae or cows' milk protein-based adult enteral feeds, and were delivered by pump overnight. One child received a soya-based feed because of parental suspicion of cows' milk protein intolerance. One received a whey hydrolysate to enhance stomach emptying. All feeds included a comprehensive range of vitamins and minerals. The aim was to offer adequate nutrition for growth, while maintaining blood chemistry within acceptable parameters by the provision of at least the estimated average requirement for energy for chronological age using additional glucose polymers and peanut oil emulsions, and reference nutrient intake for protein for height age (dietary reference values, Table 2).

Table 2 shows the tube feed composition for protein, CHO and FAs, and the corresponding United Kingdom recommended dietary intakes for a healthy population. Also shown are the observed dietary intakes of British adults (comparable figures are not available for children). The dietary intake of the non-tube-fed children was not assessed formally: they were recommended to eat the family diet but with an emphasis on high-energy, low-phosphate foods.

Older children were fasted overnight prior to blood sampling, but younger children were fasted for at least 4 h (although they were allowed water). This length of time has been used in other large studies [6]. Serum TC, TG, LDL, HDL, apo A1, apo A2, apo B and Lp(a) and plasma creatinine were measured.

Lipid subfractions were compared between the four patient groups (Table 2) using analysis of variance. Analysis of covariance (ANCOVA) was used to adjust for age and creatinine when a significant difference was found between groups. A significant P value was defined as <0.05. Each subject and/or their parents gave informed consent to the study, which was approved by the local committee on ethical practice.

Methods. TC was measured using the cholesterol C system high-performance cholesterol oxidase 4-aminophenazone method and TG by glyceryl phosphate oxidase 4-aminophenazone high-performance enzymatic colorimetric test (both Boehringer Mannheim Diagnostica) [7]. HDL was measured following precipitation of apo B-containing lipoproteins and LDL was calculated using the Friedewald formula [8]. apo A1 and apo B were measured using immunoturbidimetry (Immuno, Sevenoaks, Kent, UK) [9], and Lp(a) by enzyme-linked immunosorbent assay (Immuno) [10]. GFR was estimated from the clearance of ⁵¹chromium EDTA [11] or by the Schwartz formula [12].

Results

Children who were managed medically and on PD without enteral feeds (groups 1 and 2) were older than the enterally fed children. Medically managed children were taller, but there was no difference in body mass index among the four groups.

The results of the lipid subfractions are shown in Table 1. TGs were elevated in all groups. Figure 1 illustrates the relationship between serum TGs and method of feeding, plasma creatinine and treatment modality. There was an overall positive correlation between TGs and creatinine ($r = 0.63$, $P < 0.0001$). However, there was no difference among the patient groups when the results were corrected for age and creatinine (ANCOVA $P = 0.07$). There was also a negative correlation between TGs and GFR in children in
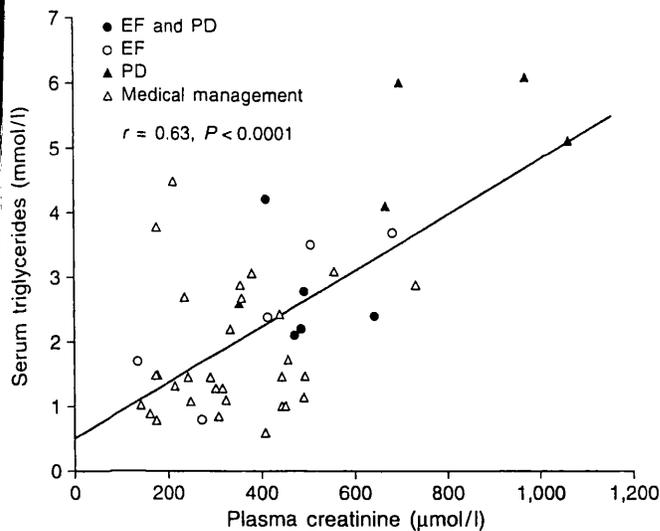
Fig. 1. Effect of method of feeding, plasma creatinine and treatment modality on serum triglycerides. *EF*, Enteral feeding; *PD*, peritoneal dialysis

group 1 ($P = 0.0001$), even when age was taken into consideration.

Children managed by PD (group 3) were the only group with levels of the atherogenic lipids TC, LDL and apo B that were above the normal range, although TC levels were at the upper limit of normal in the other groups. However, only apo B was significantly higher in children on PD when adjusted for age and creatinine (ANCOVA $P = 0.01$). No other lipid subfractions were abnormal in any other group. There was no correlation between GFR and any lipid subfraction other than TGs.

Discussion

In this study we have confirmed previous reports that hypertriglyceridaemia correlates inversely with GFR in CRF, and that TC is at the upper limit of the normal range [2, 3]. Our patients did not, however, have abnormalities of apo A1, apo A2, apo B and Lp(a), which have been found in some, although not all, previous studies [2, 3]. Angiographic studies have shown that low apo A and high apo B (or their ratio) may be better indicators of future coronary heart disease than HDL levels [5]. HDL, high levels of which protect against vascular disease, was reduced in previous studies [2, 3], but was also normal in our patients.

We were concerned that the enteral feeds we give to our patients might have an adverse effect on their blood lipids and lipoproteins. The value of tube feeding in the promotion of catch-up growth is well established [13, 14]. However, such feeds contain added glucose polymerase and fat emulsions which may be atherogenic. Ingestion of a bolus of refined CHO causes an increase in TGs and reduces HDL, and a high intake of saturated fat raises TGs and LDL [4]. Furthermore, an imbalance of mono- and polyunsaturated fat can also promote atherogenesis by reducing HDL [4].

However, we achieved a balanced energy intake with our enteral formula composition, which did not differ significantly from published recommendations for dietary intake for a normal population. Indeed, the total fat intake, and particularly the saturated fat intake, was less in the tube-fed children than in a normal adult population eating an unrestricted diet (Table 2). Despite a CHO intake comprised mainly of refined sugars rather than a mixture of sugars, starch and fibre, there was no adverse effect on serum TGs and HDL.

Although the children were under regular dietary review, we were not able to fully analyse the intakes of those who were not tube fed because there are only a few foods that have been analysed for their FA composition. As these children were eating a relatively free diet rather than receiving a precisely prescribed enteral feed, it is possible that their diet was less balanced than that of the tube-fed children.

It might be expected that the glucose load during PD would have an adverse effect on plasma lipids, resulting in hypertriglyceridaemia and decreased HDL [15]. Although the patients on PD were the only group to have levels of atherogenic lipids above the normal range, those on PD who were enterally fed did not. One possible explanation is that the enteral feed was beneficial to the plasma lipids, but the numbers are too small to draw any conclusions.

All the children had high TG levels. The importance of TGs in atherogenesis is controversial, but recently it has been found that hypertriglyceridaemia is associated with a high proportion of small, dense LDL, which is now recognised to be particularly atherogenic. Although overall the lipid fractions that we measured were at acceptable levels, we did not study those subfractions that are now recognised as important [16].

In conclusion, this small study would suggest that an enteral feeding regimen providing an appropriate energy intake with a balanced profile of fat and CHO can be administered to children with CRF who are both conservatively managed and on PD, without detrimentally affecting their serum lipids.

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Literature abstracts

Pharmacokinetics of tacrolimus (FK 506) in children and adolescents with renal transplants

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Background. Only few data exist on pharmacokinetics of tacrolimus in children.

Patients. In 1995 and 1996, 14 children (mean age 13 years, range 5–23 years) received tacrolimus after renal transplantation; 10 of these after biopsy-proven steroid-resistant rejection (2 with vascular rejection), two for cyclosporin A (CsA)-induced severe nephrotoxicity, one for untreatable gingival hyperplasia on CsA, and one child was treated primarily after transplantation because of severe liver involvement in nephronophthisis. Pharmacokinetic investigations were performed after establishing a stable maintenance dose with trough levels in the desired window of 5–12 ng/ml.

Results. Mean follow-up time was 6 months (range 3–25 months). Eleven patients are still on tacrolimus. Two were discontinued because of severe aggravation of chronic persistent hepatitis C (one of them also developed diabetes mellitus), and one patient was subsequently switched to conventional immunosuppression because of tacrolimus-associated nephrotoxicity. All tacrolimus levels were measured by a modified assay (MEIA, Tacrolimus, Abbott) with improved sensitivity.

At the time of switch, median serum creatinine was 234 ± 82 μmol/l and 6 months after switch 201 ± 99 μmol/l. All grafts are still functioning. Mean FK-506 dose was 0.16 mg/kg body weight/day (range 0.036–0.30 mg/kg). Mean trough level was 7.1 ± 2.6 ng/ml in the morning and 6.5 ± 2.0 ng/ml in the evening. Median time of maximum concentration (t_{max}) was 120 min after application, and the mean maximum concentration (C_{max}) was 15.2 ± 6.7 ng/ml. Mean area under the curve (AUC) was 104 ± 33 ng * h/ml, with a range from 65 to 169 ng * h/ml. No patient had unsatisfactorily low trough levels during the study. There was only a weak but significant ($P < 0.05$) correlation between dose per kg body weight and AUC and, as expected, an excellent correlation ($r = 0.73$, $P < 0.001$) between AUC and trough level.

Conclusion. Because of interindividual variation between patients, therapeutic drug monitoring of tacrolimus is mandatory. In this study, a daily dose of 0.15 mg/kg was sufficient in most patients. We recommend the performance of at least one pharmacokinetic study after establishing stable FK 506 trough levels to ascertain a safe profile.

Immunity of diphtheria and tetanus in a young population on a dialysis regimen or with a renal transplant

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In 54 transplant recipients diphtheria and tetanus immunity after primary vaccination was significantly lower than in 57 control subject and 35 patients on a dialysis regimen. After a booster, tetanus antibodies developed in the transplant recipients and dialysis patients but no diphtheria antibodies developed in two transplant recipients. No adverse reactions, including acute graft rejection episodes, occurred.