MINIREVIEW

Diet and Kidney Disease: The Role of Dietary Fatty Acids (43217)

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An important characteristic of chronic kidney disease is the tendency of the glomerular filtration rate (GFR) to decrease inexorably once a certain threshold of nephron destruction has been passed, even after resolution of the initiating renal injury (1). The progressive fall in GFR is marked histologically by increasing glomerulosclerosis and interstitial fibrosis, in which differentiated segments of the nephron are progressively obliterated by extracellular matrix (2). Renal diseases of immune, vascular, and metabolic etiologies culminate in nephrosclerosis, suggesting that a heterogeneous array of toxic stimuli can induce pathologic responses that converge upon a common avenue of fibrotic effacement (1). Transition from acute injury to renal fibrosis appears to reflect a summation of several different factors whose interaction reinforces the progression of chronic kidney disease to sclerosis.

It has recently become evident that lipid metabolism is one such factor. Dietary and pharmacologic manipulation of lipids can markedly accelerate or inhibit the development of glomerulosclerosis and interstitial fibrosis in a variety of disease models of glomerular and interstitital injury (3). Before describing the effects of dietary fatty acid manipulation on the progression of renal injury, it would be useful to review what is currently known about the pathophysiology of glomerulosclerosis in order to understand the potential paths by which fatty acids could modulate this process. Much of the emphasis will be on glomerulosclerosis, currently a better understood process than interstitial fibrosis. However, it should be emphasized that effacement of the renal vasculature anywhere along the nephron can produce interstitial fibrosis and contribute to loss of the glomerulus whose vascular tree subserves that nephron.

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The Process of Glomerulosclerosis

The essential process of glomerulosclerosis is the obliteration of the glomerular capillary bed by excess extracellular matrix (2, 4). The matrix in sclerosed regions generally contains type IV collagen, laminin, and fibronectin. These proteins are normal components of the glomerular matrix (2). Glomerulosclerosis does not appear to involve the synthesis of novel proteins but rather the inappropriate synthesis of normal components of the glomerulus. Certain types of sclerosis, including idiopathic focal segmental sclerosis and crescentic glomerulonephritis, can also be characterized by the presence of type III collagen and interstitial collagen. Glomerulosclerosis can occur as the end point of primary inflammatory diseases of the glomerulus, such as membranoproliferative glomerulonephritis or as a secondary result of systemic vasculitis, particularly systemic lupus erythematosus. Metabolic disorders inducing proteinuria, such as diabetes and amyloidosis, vascular pathology, including hypertension and intravascular thrombosis, and alterations in lipid storage, such as Fabry's disease, can all evolve into diffuse glomerulosclerosis. In amyloidosis and multiple myeloma, of course, there is the deposition of altered proteins within the glomerulus and the interstitium in addition to the excess synthesis of normal extracellular matrix.

Within the glomerulus, the initial lesions of sclerosis generally appear within the mesangium (4). The glomerular mesangium is centrally situated within the glomerulus, surrounded by the capillary tuft. It is composed principally of mesangial cells, which are vascular smooth muscle cells whose state of activation regulates the surface area of the glomerulus available for filtration (5). The mesangium is also occupied by resident, mar-

row-derived macrophages (6). There is considerable mesangial matrix, consisting of a mixture of mucopolysaccharides and glycoproteins. The endothelium overlying the mesangial region is fenestrated, permitting a passage of serum proteins and other molecules into the mesangial region. This parallel mesangial circulation filters through the mesangial region, and empties into the interstitium near the renin-producing juxtaglomerular area. From there, material enters the renal lymphatics (7). The intramesangial flow of serum-derived factors and the products of infiltrating cells or activated endogenous mesangial cells can thus disproportionately affect a region of the glomerulus responsible for maintaining the integrity of the capillary lumens, as well as for regulating glomerular filtration.

Experimental Models of Renal Disease

Experimental models of kidney disease have proven very useful in elucidating some of the factors contributing to the progression of kidney damage. Both systemic hypertension (1, 8) and increases in glomerular capillary pressure and glomerular plasma flow rate (1, 8) have been implicated in the progression of kidney disease in a partial nephrectomy model of glomerular sclerosis. In this model, removal of one kidney and two thirds of the remaining kidney induces a syndrome of glomerular overwork initially characterized by proteinuria followed by expansion of the mesangium, and eventually leading to glomerulosclerosis (1, 8). Lowering glomerular capillary pressure via the use of angiotensin-converting enzyme inhibitors or dietary protein restriction has been shown to be markedly effective in inhibiting the progression of renal histology to fibrosis (8). On the other hand, several experimental disease models induce progression of kidney failure in the absence of glomerular hyperfiltration or hypertension. These include a model of experimental nephrosis induced by the aminonucleoside of puromycin (9) and the genetically diabetic, obese Zucker rat (10). Both models are associated with proteinuria, hyperlipidemia, and eventual sclerosis. In both models, dietary-induced exaggeration of the preexisting tendency toward hypercholesterolemia and hypertriglyceridemia has been shown to accelerate the sclerosing process in the kidney. Pharmacologic intervention with respect to cholesterol synthesis, such as the use of lovastatin, has been shown to be remarkably protective of renal function (11, 12). Other factors that have been implicated in progression in kidney disease include the participation of peptide growth factors, particularly epidermal growth factor, associated with mesangial cell proliferation and enhanced synthesis of extracellular matrix, and insulin-like growth factor-1 (13). Two leukocyte factors, platelet-derived growth factor and interleukin 1, are cofactors in stimulating mesangial cell proflieration and matrix synthesis (13).

The Role of Leukocytes

Recently leukocytes have been implicated as active participants in the progression of kidney disease, in both immune and nonimmune models of injury. Many human glomerular and interstitial diseases are characterized by parenchymal infiltration by mononuclear leukocytes (14–17). The glomerulus is particularly susceptible to infiltration by macrophages. The interstitium is susceptible to infiltration by both macrophages and lymphocytes. In addition to the active glomerulonephritides and/or interstitial nephritis secondary to numerous etiologies, renal disease not thought of as immunologic in origin, such as diabetic nephropathy and focal glomerulosclerosis, are associated with significant glomerular and interstitial leukocyte infiltration. The glomerular syndromes characterized by monocyte infiltration are associated with hypertension, fluid retention, proteinuria, decreased glomerular filtration rate, and diminished renal blood flow, whereas inflammatory states of the renal interstitium are characterized by disorders in tubular epithelial function, including impaired acidification and concentration of the urine, alterations in sodium and potassium transport, and interstitial fibrosis (17). There is an emerging correlation between the intensity or chronicity of the glomerular and interstitial infiltrate and the lack of responsiveness to therapy and the tendency to progress toward renal obsolescence and nephrosclerosis (15, 18). Interest in the mediating role of macrophages in the chronicity of kidney disease is particularly strong. With respect to factors that have already been implicated in the progression of renal injury, macrophages can oxidize and take up low density lipoproteins and secrete lipoprotein lipase, secrete growth factors for mesangial cells and fibroblasts including interleukin 1 and platelet-derived growth factor, and secrete proteinases including neutral proteinases and collagenases that can affect matrix degradation (19). Macrophages can stimulate the synthesis of collagen and fibronectin via the release of factors such as interleukin 1, induce a tissue factor on endothelium that can activate coagulation, and activate platelets via the secretion of platelet-activating factor. Macrophages are extremely active producers of thromboxane A_2, but are also capable of inducing cyclooxygenase in mesenchymal cells via the release of interleukin 1 (20). Macrophages isolated from nephritic glomeruli release large amounts of both thromboxane A_2 and interleukin 1 (21–23).

Dietary Modification of Lipids

The role of dietary modification on the progression of chronic renal disease has generated renewed interest in recent years (24). Most of the studies have examined the effects of dietary protein and/or phosphorus restriction on the progression of renal disease in humans or animals with experimentally induced renal injury. In

addition, the role of reduced caloric intake on the progression of renal disease has been examined. In the last decade, a number of studies have addressed the potential role of dietary lipid composition in the progression of renal disease in animals with experimentally induced renal failure.

Several reports suggest a role for dietary lipids in the progression of renal disease (3). Data in experimental animals indicate that an increase in dietary cholesterol favors the development of focal glomerulosclerosis in rats (25), guinea pigs (26, 27), and New Zealand White rabbits (28). Foam cells resembling those seen in the early stages of atherosclerosis are seen frequently in the glomeruli of animals with dietary-induced hypercholesterolemia (28, 29). Foam cells very likely originate from circulating monocytes and develop their characteristic appearance by ingesting lipids. In guinea pigs placed on a high cholesterol diet, spontaneous monocyte infiltration into the glomerular mesangial region was observed, correlating with subsequent expansion of the mesangium and the development of proteinuria and hematuria (27). It has also been shown that an increase in dietary cholesterol in rats with endogenous hyperlipidemia secondary to aminonucleoside of puromycin-induced nephrotic syndrome increases glomerulosclerosis in such animals (30). In addition, several studies have demonstrated a beneficial effect of lowering serum lipid levels on the progression of renal disease in animals with endogenous hyperlipidemia (31) or animals with experimentally induced kidney damage (11, 32). Most of these studies suggest that abnormalities in lipid metabolism that occur either spontaneously or as a consequence of renal disease may be important in the pathogenesis of focal glomerulosclerosis (33). An analogy between the pathogenesis of focal glomerulosclerosis and atherosclerosis has been suggested (33, 34).

Fatty Acids

In the last decade, evidence has accumulated to indicate that the amount and composition of fatty acids in the diet as well as diets deficient in fatty acids have important effects on the progression of renal disease in experimental animals. This review summarizes some of the major studies in this area and proposes potential mechanisms by which changes in the intake of fatty acids and fatty acid deficiency may exert beneficial effects on the progression of renal disease.

Fatty acids are products of the hydrolysis of fats. They contain an even number of carbon atoms and are classified on the basis of the length of the hydrocarbon chain and the number and position of double bonds within the chain. Carbon atoms are numbered from the carboxyl carbon (carbon 1) to the methyl end carbon known as the ω-carbon. The position of the double bonds in naturally occurring fatty acids is related to the CH_3 or ω-end of the fatty acid. Thus, a series of fatty acids of increasing chain length or increasing desaturation based on oleic acid are ω-9 acids in which the first double bond is nine carbons from the ω-end, a series based on linoleic acid are ω-6 acids, and a series based on linolenic acid are ω-3 acids. In animals, additional double bonds are introduced between the existing double bonds (ω-9, ω-6, or ω-3, respectively) and carbon 1 (carboxyl carbon). Saturated fatty acids are those without double bonds. Monounsaturated fatty acids are those with a single double bond. Polyunsaturated fatty acids are those with two double bonds or more.

Linoleic acid is an essential dietary factor for mammals because they lack enzymes for desaturation of fatty acids distal to the ω-9 carbon (35, 36). Humans and other mammals have enzymes for desaturation and elongation of other dietary fatty acids and for the synthesis of 16:0 and 18:0 and their desaturation to 16:1, ω-9, and 18:1, ω-9. Competitive effects exist between fatty acids such that the 18:3 ω-3 family suppresses the metabolism of fatty acids of the 18:2 ω-6 family. The 18:2, ω-6 family inhibits the metabolism of the 18:3 ω-3 fatty acids although less strongly. Both 18:2, ω-6 and 18:3 ω-3 fatty acids inhibit the metabolism of 18:1 ω-9 fatty acids (37).

Polyunsaturated fatty acids of the ω-6 class are found as linoleic acid primarily in the oil of plant seeds (sunflower oil, corn oil, etc.). They are incorporated into cell membranes as the metabolic derivative, arachidonic acid (20:4), and can be released from phospholipids in the membrane by the action of phospholipase A_2. Since linoleic acid (18:2) is oxidized more rapidly than saturated or monounsaturated 18:C fatty acids, it is an efficient source of energy even when animals are fatty acid deficient. Linoleic acid is an integral part of cell membrane lipoprotein complexes and the structure of membrane bilayers (38).

Fish oils contain predominantly very long chain ω-3 fatty acids (Fig. 1). When marine fish, fish oils, or purified preparations of fish oil fatty acids are consumed, they are stored in tissue replacing part of the ω-6 very long chain fatty acids, arachidonic and docodecosopentanoic. Eicosapentanoic acid is converted to thromboxane A_3, which when concentrated in tissues inhibits the desaturation of linoleic acid (Fig. 1).

The studies designed to examine the effects of fatty acids on the progression of renal disease have involved either increases or decreases in the amount of polyunsaturated fatty acids provided in the diet. Experimental animal models utilized have included both nonimmune and immune mediated models of glomerular injury.

The Role of Eicosanoids in the Progression of Renal Disease

The cyclooxygenase and lipoxygenase pathways mediate some of the most significant effects of fatty

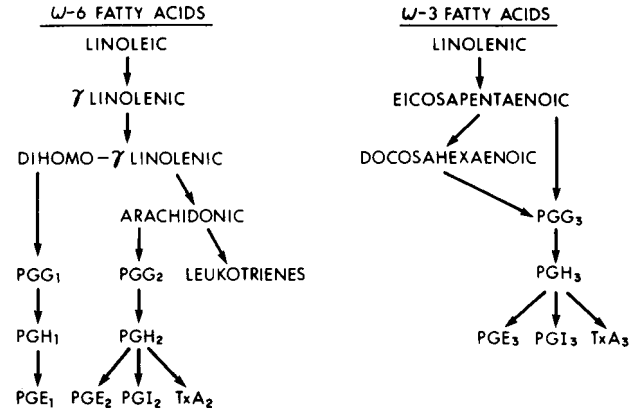

Figure 1. Synthesis of PGE_2, PGE_3, prostacyclin (PGI_2 or PGI_3), and thromboxane (TxA_2 or TxA_3) from ω-6 or ω-3 fatty acids. PGE_1 and PGH are endoperoxides. The synthesis of prostaglandins of the 1 series (PGE_1) is also shown. Eicosanoids of the 2 series have different biologic properties than eicosanoids of the 3 series (see text for details).

acid manipulation on renal structure and function (Fig. 1). Eicosanoid metabolites exert numerous stimulatory and inhibitory effects in renal processes. Increased production of thromboxane B_2, the stable metabolite of thromboxane A_2 (Fig. 1), has been reported in several experimental models of glomerular injury including subtotal renal ablation (39), adriamycin-induced nephrotic syndrome (40), nephrotoxic serum nephritis (41, 42), the normotensive strain of Milan rats (43), murine lupus nephritis (44), and also in models of interstitial renal disease such as urinary tract obstruction (45). The increased production of thromboxane A_2 in these models may be due to greater synthesis of this eicosanoid by intrinsic glomerular cells (45) and/or to its production by invading cells such as macrophages (46) and/or platelets. Reduction of thromboxane synthesis using selective inhibitors improved renal function, decreased proteinuria, and ameliorated the glomerulosclerosis of rats with subtotal renal ablation (39). Administration of these inhibitors also reduced significantly the proteinuria of rats with adriamycin-induced nephrotic syndrome (40) and partially prevented the initial fall in GFR seen in rats given nephrotoxic serum (41). Inhibition of thromboxane synthesis also ameliorated the progressive renal disease in the Dahl salt-sensitive rat, another model of glomerular injury (47). Thromboxane A_2 stimulates platelet aggregation and is also a powerful vasoconstrictor. Both of these mechanisms may play a role in the progression of renal disease.

Endogenous biosynthesis of vasodilatory prostaglandins (PG; PGE, prostacyclin-PGI_2) in the kidney modulates such diverse functions as regional blood flow (48), salt and water transport (49), renin secretion (50, 51), and neurotransmitter release (52). In states of decreased renal perfusion or chronic renal disease, prostaglandins are increased and serve to maintain renal blood flow and GFR. Several drugs block the production of eicosanoids at various points in their synthesis. Aspirin and nonsteroidal anti-inflammatory drugs inhibit the cyclooxygenase pathway. Imidazole derivatives block the synthesis of thromboxane. Enrichment of the diet with linoleic acid will preferentially augment the production of prostaglandins of the two series, and would be expected to support renal blood flow and GFR.

The effects of prostaglandins on renal disease have been examined in several models of glomerular injury. Zurier *et al.* (53) found increased survival in (NZB/NZN)F_1 hybrid mice with lupus nephritis injected once or twice daily with 200 μg of PGE_1 from 6 weeks to 1 year. Antinuclear antibody production was not affected. Increased survival was also reported in the same animals when injected with 15 methyl-PGE_1 (200 μg/kg/day), even when the therapy was initiated at 7 months of age, when the mice had established lupus nephritis. Animals with immune complex glomerulonephritis injected with PGE_1 had less renal pathologic changes and decreased proteinuria but higher levels of circulating antibodies than control animals (54). No beneficial effects were seen in mice receiving $PGF_{2\alpha}$. In a model of serum sickness induced by apoferritin, PGE_1 administration reduced immune complex deposition (55). Injections of arachidonic acid ameliorated glomerular damage in mice given apoferritin (56). The levels of anti-apoferritin antibodies were decreased and deposition of complexes occurred in the mesangium but not in capillary loops. Thus, the experiments summarized above suggest a beneficial effect of exogenous administration of prostaglandins or its precursor, arachidonic acid, in several models of renal disease. The mechanisms of action may involve multiple steps including decreased production of antibodies, diminished deposition of immune complexes, or decreased injurious response of the glomerulus to immune complexes.

Eicosanoid metabolites also affect the biology of glomerular cells. Prostaglandins modulate the hypertrophy and proliferation of mesangial cells (57). Ω-3 polyunsaturated fatty acids inhibit endothelial production of platelet-derived growth factor (58). They also inhibit the secretion of interleukin 1 and tumor necrosis factor by macrophages, which in turn may contribute to the development and progression of nephrosclerosis (59). Analogues of thromboxane A_2 increase collagen production by mesangial cells. Thus, thromboxane may directly augment the expansion of the mesangial matrix in the glomerulus entering its sclerosing phase (60).

Effects of Changing Linoleic Acid in the Diet on the Progression of Renal Disease

Three groups have reported (61–63) that rats with subtotal renal ablation fed a diet with a high content of

linoleic acid (safflower oil) develop less glomerulosclerosis, a lesser increase in serum creatinine, and blood urea nitrogen, and have less proteinuria and a greater GFR than similar rats fed a diet low in linoleic acid. Two of these reports (62, 63) described rats with a remnant kidney that were given a high linoleic acid and had lower systemic blood pressure values than rats fed a low linoleic acid diet. The third group of investigators did not find such an effect (61). In addition, there was an increase in the linoleic acid content in the kidney of rats fed a high linoleic acid diet as compared with those given a low linoleic acid diet (63). The mechanisms responsible for this beneficial effect of linoleic acid on renal disease in this experimental model may be multifactorial. The decrease in systemic blood pressure may ameliorate the degree of glomerulosclerosis in this model. Dietary linoleic acid supplementation increases glomerular production of PGE_2, a vasodilator, in rats with reduced renal mass (64). Changes in the lipid composition of membranes may affect the rheology of blood and consequently modify blood flow to the kidney. Fatty acids may decrease the production of interleukin-1 and tumor necrosis factor and other inflammatory products of macrophages (59). Platelets and coagulation may play a role in the progression of the renal disease in subtotally nephrectomized rats (65). It is of interest that studies in rats fed different concentrations of dietary linoleic acid from 0 to 6% have shown that increased amounts of linoleic acid were associated with decreased susceptibility of platelets to thrombin-induced aggregation and prolongation of the clotting time of platelet-rich plasma (66). Immune glomerulonephritis is associated with enhanced glomerular production of thromboxane A_2 (41), which would have a proaggregatory effect on platelets. Inhibition of thromboxane synthase reverses platelet hyperaggregability, decreases proteinuria, and inhibits crescent formation in this model (67).

Effects of Fish Oil on the Progression on Renal Disease

Conflicting results have been reported in the model of subtotal renal ablation with the use of fish oils. Scharschmidt *et al.* (68) found that rats fed menhaden oil for 5 weeks after subtotal nephrectomy had a greater decline in renal function and developed greater glomerulosclerosis than partially nephrectomized rats pair-fed beef tallow. The authors suggested that this detrimental effect of fish oil was due to decreased production of the vasodilatory prostaglandins E_2 and prostacyclin, which tend to maintain vasodilation and glomerular function in situations of altered renal hemodynamics. On the other hand, Barcelli *et al.* (69) reported that salmon oil ameliorated glomerulosclerosis in the absence of a decrease in urine PGE_2 excretion in rats with subtotal renal ablation studied 2 weeks after the decrease in renal mass. It is possible that the increased mortality and the anemia noted by Scharschmidt *et al.* (68) were related to toxicity of oxidized fish oil due to the absence of a specific antioxidant that was used by Barcelli and his associates (69). It is also possible that in the studies of Scharschmidt *et al.* (68) and Logan *et al.* (70) the amount of linoleic acid and arachidonic acid intake was too restricted. Consequently, the marked decrease in PGE_2 synthesis may be related to fatty acid deficiency in such rats (71).

Fatty acids also affect immune-mediated glomerular injury. A high linoleic acid diet has been reported to decrease proteinuria and inhibit crescent formation in a rat model of crescentic, antiglomerular basement membrane glomerulonephritis (72). Dietary supplementation of ω-3 polyunsaturated fatty acids reduces glomerular injury and mortality in the $(NZB/NZW)F_1$ mice (73, 74). These beneficial effects on the renal injury of immune-mediated disease in response to fish oil supplementation are dose dependent. No significant effects were seen when less than 5% fish oil was administered. Fish oil was beneficial even when administered after the onset of lupus nephritis (75). In MRL-1 mice, supplementation with ω-3 polyunsaturated fatty acid-enriched diets resulted in a decrease in albumin excretion and in the number of Ia-positive glomerular cells (76). Kher *et al.* (77) have shown that both ω-6 and ω-3 polyunsaturated fatty acids reduced significantly glomerular injury in an immune complex (apoferritin) model of glomerulonephritis. Thus, a number of studies reveal a beneficial effect of fish oils in the immune-mediated glomerular disease.

In summary, most of the evidence suggests that supplementation of the diets with polyunsaturated fatty acids can ameliorate progressive glomerular injury in both immune and nonimmune models of renal disease. The mechanisms of the beneficial effects of polyunsaturated fatty acids on progressive renal injury have not been elucidated, but these fatty acids are known to affect a number of important biologic events. Thus, the mechanisms involved may be multiple. In some of the studies, it is difficult to eliminate a potential beneficial effect on the progression of renal disease of lowering blood pressure as a consequence of dietary supplementation with polyunsaturated fatty acids, either linoleic acid or fish oil (78). Polyunsaturated fatty acids may affect the progression of renal disease by effects on cell metabolism, including membrane function and fluidity, eicosanoid production, and mitochondrial oxidation (79–86). The unsaturated fatty acids play an important role in the metabolism of prostaglandins. Administration of ω-3 polyunsaturated fatty acids may alter the ratio of prostaglandins, thereby favoring an antiaggregatory state as reflected in reduced platelet aggregation and prolonged bleeding times (87–92). In addition, a reduction in chemoattractant leukotrienes

has been demonstrated to occur as a result of ω-3 fatty acid supplementation (93). Initial data indicate that prostaglandins may modulate the proliferation of glomerular mesangial cells, suggesting that polyunsaturated fatty acids may indirectly modulate cell growth (57). Furthermore, ω-3 polyunsaturated fatty acids can inhibit endothelial synthesis of growth factors that resemble platelet-derived growth factor (58). Diets supplemented with ω-3 fatty acids have a marked effect on the production of macrophage secretory products such as tumor necrosis factor and interleukin 1. These latter two compounds are important effectors of the inflammatory response (59).

Polyunsaturated fatty acids can also regulate serum cholesterol. Polyunsaturated fatty acids of 18:C chain length lower serum cholesterol to a greater extent than would be expected from replacement of saturated fatty acids (94). Diets rich in ω-3 polyunsaturated fatty acids lower plasma triglycerides and total cholesterol in nephrotic rats (95). The effects upon lipoprotein cholesterol are not fully understood but possibly include changing morphology of the lipoproteins, an influence of the low density lipoprotein receptor activity, and an alteration in the synthesis and excretion of bile acids. In addition to preventing heart disease by lowering cholesterol, increasing the ratio of polyunsaturated fatty acids to saturated fatty acids changes the ratio of these two classes of fatty acids in membranes. It has also been shown that polyunsaturated fatty acids can modify the physical characteristics of cell membranes. For example, the membranes of erythrocytes are more deformable as the content of polyunsaturated fatty acids, particularly ω-3 fatty acids, increases (96). These effects, of course, have important implications in terms of blood flow and blood viscosity, factors that may play a role in the progression of kidney disease (97). Furthermore, changes in the composition of membranes in the kidney in terms of the amount of polyunsaturated fatty acids may per se have an important salutary effect, as shown recently in studies conducted in the Zucker obese rat (98) as well as in cholesterol-fed rats (99). Thus, changes in polyunsaturated fatty acids in the diet may influence an array of cell functions as well as the production of various mediators of cell growth and inflammation.

Effects of Restriction of Fatty Acids

We have been examining the dependence of macrophage recruitment to the kidney upon a novel lipid pathway affected by dietary restriction of essential fatty acids. The essential fatty acids (EFA), linoleate and its metabolite, arachidonate, were initially defined in 1929 (36). EFA-deficient rats were observed to develop dry, scaly skin, reduced growth, and infertility. The EFA-deficient state was subsequently defined biochemically when it was discovered that in the absence of linoleate, oleate (18:1) underwent desaturation and elongation to form a three-double bond analogue of arachidonate, eicosatrienoic acid, also known as Mead acid after its discoverer (36). In normal rats, Mead acid is absent or barely detectable. The ratio of Mead acid to arachidonate increases with time of consumption of an EFA-deficient diet. A ratio exceeding 0.4 comprises the biochemical definition of EFA deficiency (100). A dietary content of 1% linoleate is adequate to maintain the ratio <0.4 and prevent the symptomatology of EFA deficiency. Our interest in examining the effects of deficiency of the essential fatty acids arose from the findings of Hurd *et al.* (101) that (NZB/NZW)F_1 mice with systemic lupus erythematosus did not die of renal failure when fed an EFA-deficient diet. Such mice normally die of glomerulonephritis and interstitial nephritis as a result of renal involvement by their systemic vasculitis. EFA-deficient mice did in fact have circulating immune complexes, as well as deposits of immune complexes and complement in their glomeruli. Nonetheless, they did not die of inflammatory kidney disease. This is not due to immune deficiency. Cellular immunity is intact in EFA-deficient animals. They express normal allograft rejection, delayed-type hypersensitivity reactions in the skin (102), and will develop experimental allergic encephalomyelitis when primed with myelin basic protein in adjuvant (103). Two observations suggested, however, that macrophage biology might be affected by fatty acid deficiency. Essential fatty acid-deficient animals are markedly protected against the development of carrageenan-induced granulomas in their footpads (104). Carrageenan is a derivative of seaweed that induces a foreign body reaction that essentially consists entirely of macrophages. Second, EFA-deficient animals are markedly inhibited with respect to the formation of granulation tissue and long-term wound healing (105). The process of wound healing is known to be heavily dependent on the presence of platelets and macrophages as sources of growth factors regulating endothelial and fibroblast proliferation and secretion of extracellular matrix (19).

In collaboration with James Lefkowith, we have examined the role of EFA deficiency in monocyte/macrophage biology within the glomerulus. We have found that EFA deficiency induces a striking reduction in the number of macrophages that are normally resident in the uninflamed state of the kidney in the glomerular mesangium and in the interstitium (106). When animals are selectively repleted with (ω-6) fatty acid supplementation, a spontaneous repopulation of the kidney with macrophages occurs, indicating the presence of a lipid pathway regulating the basal seeding of the kidney with macrophages. The depletion of these resident macrophages, which are highly stimulatory in a mixed lymphocyte culture reaction, resulted in a marked decrease in the immunogenicity of EFA-deficient kidneys when transplanted across a major histo-

compatibility barrier (107). Allografts from EFA-deficient donors normalized their lipid composition within the allogeneic recipient, on a normal diet. The kidneys were repopulated with host macrophages within 5 days. The rapid repopulation of the kidney with resident macrophages closely paralleled the restoration of the arachidonate and linoleic acid content of renal phospholipids.

An analogous mechanism appears to underlie the inflammation-induced influx of macrophages in the kidney in a model of glomerulonephritis induced by the injection of antibody directed against the glomerular basement membrane. This disease model is characterized by the influx of polymorphonuclear leukocytes 1–3 hr after the deposition of antibody within the renal glomerulus followed by an infiltration of macrophages into the magnesium 12–24 hr later. This model of nephritis is characterized by azotemia, polyuria, sodium retention, and proteinuria. Concomitant with the influx of neutrophils is a marked enhancement of glomerular leukotriene B_4 and thromboxane production. The effects of EFA deficiency on the leukocyte invasion of the glomerulus in this model of inflammation are striking. EFA deficiency completely prevents the influx of macrophages into the glomerulus during the course of this disease. In contrast, neutrophil influx is unaffected. The marked increases in glomerular eicosanoid production normally observed in this model are substantially attenuated by the deficiency state. EFA deficiency completely prevents the polyuria, azotemia, sodium retention, and proteinuria observed in this model (108).

EFA-deficient macrophages are not impaired in their ability to move chemotactically toward C5A or platelet-activating factor. No circulating inhibitors of chemotaxis are found in EFA-deficient serum. Instead, the defect appears to lie at the level of tissue signaling of monocyte influx. When we place isolated nephritic glomeruli in short-term tissue culture, we have observed the release of a potent chemoattractant specific for monocytes. This material is heat stable and is not dependent on complement activation. Neutral lipid extraction of nephritic glomeruli from control diet animals has yielded the chemoattractant activity in the organic phase. *In vivo* studies employing inhibitors of cyclooxygenase and lipoxygenase and administration of antagonists to the platelet-activating factor receptor do not inhibit the glomerular influx of macrophages. EFA-deficient nephritic glomeruli do not release significant amounts of this novel monocyte chemoattractant (109).

Analogous observations have been carried out by Diamond *et al.* (110) in a model of glomerulosclerosis induced by the aminonucleoside of puromycin in which, as noted above, the presence of hyperlipidemia and proteinuria is associated with progressive scarring in the renal glomerulus and in the interstitium. Diamond *et al.* (110) found that animals deficient in essential fatty acids, deficient only during the period of acutely induced nephrosis, were protected against the development of glomerular sclerosis 4 months later even though the animals had been switched to a normal diet 1 month after the administration of puromycin aminonucleoside. The protection against glomerulosclerosis did not correlate with degree of proteinuria, hyperlipidemia, or hypertension. Rather, it directly correlated with the depletion of glomerular macrophages observed in the EFA-deficient animals. This model of proteinuria in the kidney is further associated with a marked interstitial infiltrate consisting predominantly of macrophages and lymphocytes. We have found that essential fatty acid deficiency is equally effective in blocking this interstitial infiltrate in the acutely proteinuric state and that the marked decreases in renal blood flow observed in the acute phases of the disease in this model directly correlate with the presence of interstitial macrophages. Inhibition of the macrophage infiltrate results in a 3-fold increase in renal blood flow (111).

The regulatory effects of essential fatty acid deficiency on monocyte traffic through tissues are not confined to the kidney. Wound healing in both the skin and the gut is impaired in this model (105). EFA deficiency has also been shown to inhibit the development of autoimmune insulitis in mice receiving low-dose streptozotocin and in the diabetes-prone BB/Wor rat (112, 113). Both are models of diabetes in which insulitis is preceded by islet infiltration by monocytes. We have recently observed that oxidatively stressed pancreatic islets release a neutral lipid chemoattractant that is very potent and specific for monocytes whose characteristics markedly resemble those of the lipid factor released by inflamed glomeruli (Muir A, Rovin B, Lacy P, Schreiner G, submitted for publication).

These findings suggest that a lipid pathway metabolically dependent upon essential fatty acids may be a generalized mechanism for the induction of monocyte infiltration into tissues under conditions of both immune and nonimmune stimuli. Essential fatty acid deficiency appears to prevent either the synthesis or the release of this potent monocyte chemoattractant in a number of pathologic states characterized by the accumulation of macrophages and the subsequent appearance of sclerosis. Its characterization is currently in progress.

Summary

Several general principles with respect to the role of the fatty acids in the progression of kidney disease have begun to emerge from the mass of observational detail. Interventions that increase renal exposure to prostaglandins of the E series appear to be beneficial. They include administration of prostaglandin analogues and dietary supplementation with their fatty acid precursor, linoleate. The beneficial effects may be at-

tributed to preservation of renal blood flow and glomerular filtration, reduction in blood pressure, direct effects on the lipid composition and function of cell membranes, and immune suppression. Interventions that inhibit thromboxane and leukotriene production, such as ω-3 fatty acid supplementation of the diet or administration of enzyme or receptor inhibitors, are also protective. Prevention of vasoconstriction, inhibition of platelet activation, and regulation of cell proliferation and matrix production have all been implicated in the mediation of the observed retardation of sclerosis. Fish oil may have synergistic, suppressive effects on various parameters of immune activation. Essential fatty acid deficiency, of course, inhibits both prostaglandin E and thromboxane production, canceling out the protective and injurious components of arachidonate oxidation. Yet, studies on its beneficial effects have revealed another aspect of eicosanoid metabolism, independent of cyclooxygenase and lipoxygenase activity, that appears to regulate monocyte migration into injured tissue. Dietary interruption of this pathway has proven protective to renal structure and function.

Alterations in lipid metabolism may represent a common, mediating pathway of glomerular and interstitial susceptibility to progressive sclerosis in the kidney. The process appears to be amenable to manipulation by pharmacologic or dietary modulation of fatty acid metabolism. Eicosanoid metabolites and tissue-leukocyte signaling are two mechanisms by which lipid alterations can affect renal function. There are doubtless many others awaiting elucidation. Delineation of all the mechanisms whereby fatty acid metabolism can contribute to progressive kidney injury may provide a useful model for the examination of progressive sclerosis affecting other tissues subsequent to immune, vascular, or metabolic injury.

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