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Evening primrose oil reduces urinary calcium excretion in both normal and hypercalciuric rats

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Abstract Hypercalciuria is an important risk factor in the aetiology of idiopathic urolithiasis and many treatment modalities in clinical practice are directed towards reducing urinary calcium excreation. There are no natural animal models of hypercalciuria, such as the spontaneous hypertensive rat; however, the streptozotocin-diabetic rat is accepted as a good model for studies of disordered renal function associated with diabetes mellitus. Hypercalciuria is a prominent feature of the streptozotocin-diabetic rat and the model was, therefore, used to study the influence of evening primrose oil on urinary clacium excretion. Twenty rats divided into two groups of ten rats each were maintained on either normal rat chow (group 1) or primrose oil enriched diet (group 2) for 10 weeks. At 4 weeks both groups of rats were made diabetic with streptozotocin. Urine calcium measurements were serially performed before commencement of the diet, during the pre-streptozotocin (pre-diabetic) phase and during the post streptozotocin (diabetic) phase. The urine calcium excretion was significantly less in the primrose oil fed animals during both the pre-diabetic phase and the diabetic phase compared with the rats on the normal rat chow. These results indicate that evening primrose oil, a rich source of γ-linolenic acid, helps to reduce urine calcium excretion in normal animals as well as in the hypercalciuric streptozotocin-diabetic rat. Dietary modifications with long-chain ω-6 and ω-3 fatty acids might be a useful adjunct in the treatment of idiopathic hypercalciuric urolithiasis.

Key words Evening primrose oil · Urinary calcium excretion · Streptozotocin-induced diabetes · γ-linolenic acid

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Essential fatty acids, such as arachidonic (AA) and eicosapentaenoic acid (EPA), bound to membrane phospholipid from an essential component of all cell membranes and are the precursors of the eicosanoid metabolites that determine a variety of cell functions including fluidity, permeability, ion channels and the behaviour of membrane-associated receptors and enzymes. Diabetes mellitus is characterised by changes in membrane permeability related to a deficiency in essential fatty acids (EFAs). It is known that both hyperglycaemia and glycosuria are associated with an increased urinary calcium excretion [8, 24, 26]. Previous studies have shown that renal prostaglandins (PGE_2) influence urinary calcium excretion, that idiopathic hypercalciuric stone formers have an increased urinary excretion of prostaglandin E_2 and that non-steroidal anti-inflammatory drugs (NSAIDs) reduce both urine calcium excretion and raised urinary PGE_2 levels in idiopathic hypercalciuric stone-formers [2, 3, 9, 15]. Experimental studies suggest that diabetic glomerulopathy is associated with striking changes in glomerular haemodynamics, which are compensated for by an increased prostaglandin production, and that an increased endogeneous synthesis of prostaglandin may act to maintain renal function in long-term streptozotocin-diabetic rats [18]. It has been shown that the hypercalciuria associated with streptozotocin-induced diabetes, in the absence of glycosuria, was inhibited by the prostaglandin synthetase inhibitor indomethacin, suggesting that hypercalciuria in the streptozotocin-diabetic rat is prostaglandin mediated [4].

Buck et al. [5] have reported the benefits of ω-3 fatty acids (EPA) derived from fish oil in the prevention of experimental nephrocalcinosis and the treatment of idiopathic hypercalciuria. Experimental and clinical studies have shown that the effects of the ω-3 fatty acids (EPA) can be enhanced by ω-6 fatty acids derived from γ-linolenic acid (GLA). Evening primrose oil is a rich source of GLA and has been shown to prevent and reverse the neuropathy and proteinuria of clinical diabetes mellitus. The aim of this experiment was to study the effect of evening primrose oil on urinary calcium excertion in

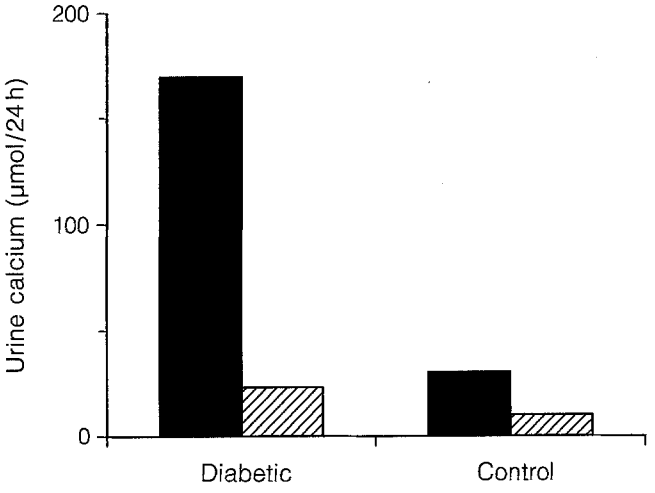

Fig. 1 Twenty-four-hour urine calcium excretion (μmol/24 h) in two groups of ten rats fed either normal chow (■) or a diet supplemented with 8.6% evening primrose oil (▨), before and after being rendered diabetic and hypercalciuric with streptozotocin

Table 1 Mean urine calcium excretion (μmol/24 h)

	Pre-diabetic phase	Diabetic phase
Rats fed a normal diet	21.24 ± 2.16	167.5 ± 60.9
Rats fed evening primrose oil 9.6%	10.6 ± 1.6	29.6 ± 2.02

normal rats and on the hypercalciuria associated with streptozotocin treatment.

Materials and methods

Twenty male Lewis rats (Bantin and Kingman, Hull, UK) weighing 100–125 g were divided into two groups of ten animals each and housed under standard conditions. The animals were placed in metabolic cages for 48 h for collection of urine. One group was then fed standard laboratory chow (Special Diet Services Ltd., Essex, UK) for 4 weeks while the other group was fed a fat-deficient diet supplemented with 8.6% evening primrose oil for 4 weeks (Scotia Pharmaceuticals, UK). Towards the end of this period urine was collected for a second 24-h period. The urines were centrifuged at 3000 rpm (0–4°C) for 15 min prior to freezing at −70°C whilst awaiting analysis. At the end of the 4-week period both groups of rats were anaesthetised with ether and rendered diabetic with streptozotocin (Zanosar, Upjohn, 80 mg/kg body wt. in citrate buffer, pH 4.5, final concentration 100 mg/ml). The two groups of rats were maintained on their respective diets for a further 6 weeks (post-streptozotocin diabetic phase). Following the streptozotocin injection diabetes was induced in all 20 rats. Urine was collected over 24-h periods during both the pre-diabetic and diabetic periods. Food and water was allowed ad libitum.

Human Ultratard (Novo), 4 units/day, was administered to the animals to maintain glycosuria at ++/+++, measured using Labstix (Ames). Urinary calcium was measured by an *o*-cresolphthalein method and creatinine by a kinetic Jaffé reaction (Boehringer Mannheim, Lewis, UK) on a Hitachi 704 discrete autoanalyser.

Statistical analysis was performed by the Wilcoxon Signed Rank test for paired data and by the Kruskall Wallace test for unpaired group data. Urinary calcium excretion and calcium : creatinine ratios were compared by linear regression. Urinary calcium and creatinine excretion was expressed in μmol/24 h and the calcium creatinine ratio derived by calculation.

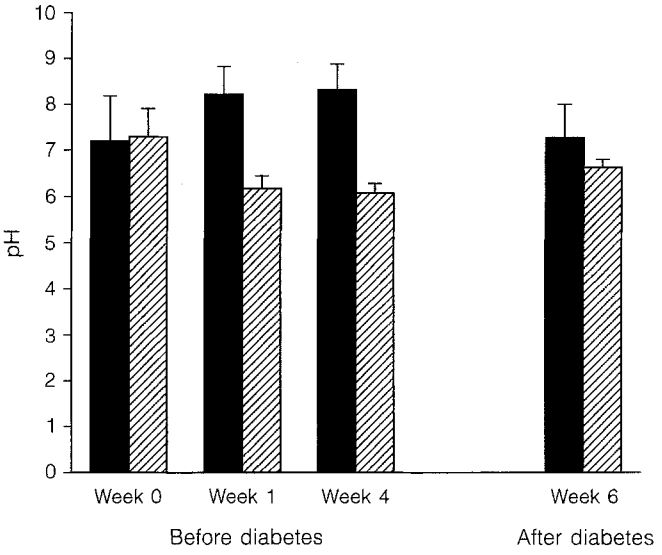

Fig. 2 Urine pH before and after diabetes showing a significant lowering in the animals on the evening primrose oil diet (▨) throughout the 10-week period of the study as compared with the animals on the standard diet (■). Lowering of the urine pH would increase the solubility of calcium salts in the urine

Results

Urinary calcium excretion and calcium creatinine ratios were strongly correlated ($r = 0.97$, $P < 0.001$) and, therefore, all statistical analyses were performed on only one representative 24-h period of urinary calcium excretion.

The mean urine calcium excretion during the pre-diabetic period in the rats fed a normal diet was 21.24 ± SE 2.16 μmol/24 h. In the rats fed the evening primrose oil diet the mean urine calcium excretion in this period was significantly lower at 10.6 ± SE 1.6 μmol/24 h ($P < 0.001$). After induction of diabetes with streptozotocin the urine calcium excretion in the rats fed a normal diet increased significantly to 167 ± SE 60.91 μmol/24 h. The urine calcium excretion during the diabetic phase in the rats fed evening primrose oil rose 29.9 ± SE 2.02 μmol/24 h, but this was significantly less than the calcium excretion in the rats fed a normal diet ($P < 0.001$) (Fig. 1, Table 1).

Polyuria and glycosuria appeared within 2 days of streptozotocin administration. Twenty-four-hour urine volumes were greatly increased in the diabetic phase as compared with the pre-diabetic, control period (109.2 ml/24 h vs. 5.75 ml/24 h; $P < 0.001$). However, there was no significant difference between urine volumes in the two groups; thus, evening primrose oil appeared to have no specific effect on urine output. There was a significant fall in urinary pH both pre- and post-streptozotocin in the evening primrose fed animals as compared with the animals on a normal diet (8.4 vs 6.1 before 7.0 vs 6.5 after streptozotocin; $P < 0.01$) (Fig. 2).

Discussion

The most common metabolic syndrome associated with idiopathic urolithiasis is hypercalciuria, which is observed in 40–60% of patients on a normal calcium intake. Thus, most dietary and therapeutic measures have been directed towards reducing urine calcium excretion in stone formers. There are now well-established studies to indicate that primary intestinal calcium over-absorption is the cause in most cases. However, in prospective studies the degree of hypercalciuria did not predict either the risk of recurrence or the efficacy of treatments which decreased urinary calcium [1, 10, 20, 21, 25]. There are no known treatment regimens to date that will completely abolish the risk of calcium stone recurrence.

Unfortunately, there are no suitable animal models of spontaneous hypercalciuria to represent clinical hypercalciuric states in humans and/or to faciliate the study of therapeutic modalities. The streptozotocin-diabetic rat is accepted to be a good experimental model for renal function in early human insulin-dependent diabetes [14, 19]. In an earlier study we demonstrated that streptozotocin-induced diabetes in the rat was associated with a marked degree of hypercalciuria, even in the absence of glycosuria. In this acute experiment over a 6-h period, we also confirmed that the calciuretic response was predominantly a renal tubular effect as there were no significant changes in inulin clearance or plasma calcium concentration and the response was independent of gut calcium absorption [4].

Streptozotocin-induced diabetes is associated with an increased prostaglandin synthesis compensating for the changes in glomerular haemodynamics associated with diabetes [18]. Our previous studies suggest that the hypercalciuria in this animal model of diabetes could be prostaglandin mediated. However, in both experimental animals and in humans it has been shown that there is impaired conversion of linoleic acid (LA) to GLA [7, 12, 22, 23]. In a double-blind, placebo-controlled study dietary substitution with γ-linolenic acid was associated with a significant clinical and neurophysiological improvement in distal diabetic neuropathy [17].

In both experimental animals and humans EPA and GLA are readily and rapidly incorporated into cell membrane phospholipids at the expense of arachidonic acid when fish oil and evening primrose oil enriched diets are administered. A high affinity of EPA for cyclooxygenase results in competitive inhibition of this enzyme equal to that of indomethacin [11, 16]. There is now substantial evidence that the prostanoids of the monoenoic series (i.e. PGE_1, etc.) derived from GLA have properties similar to the prostanoids derived from EPA and that a combination of GLA and EPA potentiates the desirable effects of both these compounds [13].

This study has shown that rats fed a diet supplemented with evening primrose oil had a significantly lower urine calcium excreation than rats fed a normal diet. Evening primrose oil also significantly reduced the hypercalciuria associated with streptozotocin-induced diabetes. It is suggested that the incorporation of GLA into the renal tubular cell membrane alters the renal handling of calcium in a favourable direction in both the normal and the diabetic state. However, it is not possible to rule out an effect on gut calcium absorption, though from our clinical studies this seems unlikely [6].

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