Discussion

This patient showed a combination of proximal muscle weakness and a great excess of triglyceride in type 1 muscle fibres. He did not develop muscle pain on exercise, his plasma lipid concentrations were normal, and he could produce ketones on fasting. This syndrome accords with previous descriptions of the clinical features of the lipid storage myopathy associated with carnitine deficiency.[8 12] Spontaneous remissions have been reported in this condition,[12] but in view of our patient's 12-year history of continuous profound muscle weakness with persistently raised activities of creatine kinase and lactate dehydrogenase it seems unlikely that treatment with propranolol coincided with a spontaneous remission in his case.

The mode of action of propranolol, however, is a mystery. Fasting plasma concentrations of non-esterified fatty acids, triglycerides, and cholesterol were not appreciably altered by the drug in this patient. Its effect, therefore, is unlikely to reside in altering the amount of circulating long-chain fatty acids available for muscle metabolism. Changes in numbers of mitochondria in rabbit ventricular myocardium after exposure to propranolol have been reported,[13] and possibly the action of the drug is directly on the muscle mitochondria and has nothing to do with beta-adrenergic blockade.

We are obliged to Professor A E H Emery, who first suggested the diagnosis of lipid myopathy in this case.

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Vitamin A toxicity and hypercalcaemia in chronic renal failure

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Abstract

Serum vitamin A concentrations were measured in 38 patients undergoing haemodialysis, 24 of whom were taking multivitamin preparations containing vitamin A. Vitamin A concentrations were significantly higher in patients undergoing haemodialysis than in 28 normal controls ($p < 0.001$). Patients taking vitamin A supplements had significantly higher vitamin A concentrations than those not taking them ($p < 0.05$), and hypercalcaemic patients had higher concentrations than normocalcaemic patients ($p < 0.005$). Withdrawal of vitamin A supplements in seven patients caused significant falls in serum vitamin A concentrations and plasma calcium concentrations ($p < 0.01$ at two and three months in both cases) and in plasma alkaline phosphatase concentrations ($p < 0.01$ at two months).

Vitamin A toxicity can contribute to hypercalcaemia in patients undergoing haemodialysis, probably by an osteolytic effect. Multivitamin preparations containing vitamin A should therefore be prescribed with caution in these patients.

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Introduction

Serum vitamin A concentrations are raised in chronic renal failure,[1-3] but little is known about the potential toxic effects of the excess vitamin A. In tissue culture vitamin A has been shown to have an osteolytic action,[4 5] and in rats toxic quantities of vitamin A produced an intensification of resorption processes.[6 7] Hypercalcaemia and skeletal lesions have also been described in chronic vitamin A overdose in man.[8-10] Hence vitamin A toxicity may contribute to the abnormal calcium and skeletal metabolism that are features of renal bone disease. To study this we examined the relation between serum vitamin A concentrations and various biochemical, radiological, and histological indicators of bone disease in patients with chronic renal failure who were receiving regular haemodialysis treatment. Water-soluble vitamins are prescribed routinely in these patients to replace losses during dialysis. Many of the patients studied, however, were taking multivitamin preparations containing vitamin A, often in large quantities, and the contribution of this to the development of vitamin A toxicity was also examined. Withdrawal of vitamin A supplements in a subgroup of these patients provided an additional and more direct means of studying the effects of hypervitaminosis A on calcium and skeletal metabolism in chronic renal failure.

Methods

Thirty-eight adults with chronic renal failure who were receiving regular haemodialysis treatment were studied. The mean age of the patients was 42·0 years ± SD 12·7 and the mean duration of dialysis was 55·4 months ± 43·5. None of the patients were receiving treatment with vitamin D analogues or metabolites, but 24 were taking a multivitamin preparation (Multivite pellets: Duncan, Flockhart and Co Ltd) containing 2500 IU vitamin A and 250 IU vitamin D_2 per tablet. These patients were taking from one to six tablets daily. Many of the patients were taking aluminium hydroxide preparations. None of the patients had undergone parathyroidectomy. Blood samples were taken and tested for vitamin A, calcium, phosphate, and alkaline phosphatase concentrations in all patients, for hydroxyproline concentrations in 30 patients, and for immunoreactive parathyroid hormone concentrations in 20 patients. All samples were taken after an overnight fast and at least 36 hours after dialysis. Specimens for vitamin A were taken into containers protected from light. Samples for vitamin A were also taken from 28 normal controls (mean age 29·5 years ± 8·4) to establish a normal range. A radiological skeletal survey incorporating radiographs of hands, feet, chest, pelvis, and lumbar spine was performed on each patient and analysed for the presence of subperiosteal erosions, osteosclerosis, and vascular calcification.

Iliac crest bone biopsy specimens were obtained in 10 patients and processed as described.[11] Estimates of total bone volume (% total tissue), osteoid volume (% total bone), active resorption surfaces (% calcified surface), and active formation surface—that is, percentage of osteoid surface covered by active osteoblasts—were obtained by a computerised histomorphometric method.[11]

In seven patients multivitamins were withdrawn and serial blood samples were taken for biochemical analysis at monthly intervals for three months under the same conditions as above. The clinical and biochemical details of these patients are shown in the table. Cases one to five only were restudied at one month, and all patients at two and three months.

Calcium, phosphate, and alkaline phosphatase concentrations were measured by standard autoanalyser methods, and hydroxyproline and immunoreactive parathyroid hormone concentrations by methods described elsewhere.[12,13] Vitamin A concentration was measured by the macromethod of Neeld and Pearson.[14] Statistical methods used were Student's t test for the difference between means of unpaired samples, linear regression analysis, and Wilcoxon's matched pairs rank sum test where appropriate.

Results

The mean serum vitamin A concentration in patients with chronic renal failure (4·8 ± 1·8 μmol/l (136·2 ± 50·5 μg/100 ml)) was significantly higher ($p < 0·001$) than that in controls (1·8 ± 0·4 μmol/l (51·9 ± 11·9 μg/100 ml)) (fig 1). There was a statistically significant correlation between serum vitamin A concentrations and plasma calcium concentrations ($r = 0·34$, $p < 0·05$), but vitamin A concentrations did not correlate with age, duration of dialysis, plasma phosphate, alkaline phosphatase, hydroxyproline, or immunoreactive parathyroid hormone concentrations. The mean vitamin A concentration in patients taking multivitamins (5·2 ± 1·9 μmol/l (150·0 ± 54·0 μg/100 ml)) was significantly higher ($p < 0·05$) than in those not taking the preparation (3·9 ± 1·3 μmol/l (112·6 ± 36·0 μg/100 ml)).

Eighteen patients were hypercalcaemic and these patients had a significantly higher ($p < 0·005$) mean serum vitamin A concentration (5·6 ± 2·1 μmol/l (160·5 ± 59·0 μg/100 ml)) than normocalcaemic patients (4·0 ± 1·3 μmol/l (114·4 ± 36·0 μg/100 ml)) (fig 2). There was no significant difference in immunoreactive parathyroid hormone concentration between hypercalcaemic and normocalcaemic patients. Vitamin A concentrations did not differ significantly in those patients with normal plasma concentrations of alkaline phosphatase, hydroxyproline, and immunoreactive parathyroid hormone and those patients with raised concentrations. Subperiosteal erosions were present in 10 patients, osteosclerosis in 12 patients, and vascular calcification in 16 patients. There were, however, no statistical differences in mean serum vitamin A concentrations between groups with and groups without these features. Periosteal calcification was not detected in any patient. There was no correlation between serum vitamin A concentrations and any of the histomorphometric indices studied.

The effects of withdrawing vitamin A supplements are seen in figure 3. Serum vitamin A concentrations fell dramatically in all patients and mean values were significantly different from baseline values at two and three months ($p < 0·01$) though still significantly higher than values found in controls ($p < 0·01$). There was a concomitant fall in plasma calcium concentrations, which was also significant ($p < 0·01$) at two and three months. Six of the seven patients were hypercalcaemic before withdrawal of vitamin A (table), but only two remained hypercalcaemic at three months. There was also a fall in plasma alkaline phosphatase concentration, which was significant at two months ($p < 0·01$). Plasma phosphate, hydroxyproline, and immunoreactive parathyroid hormone concentrations did not change significantly.

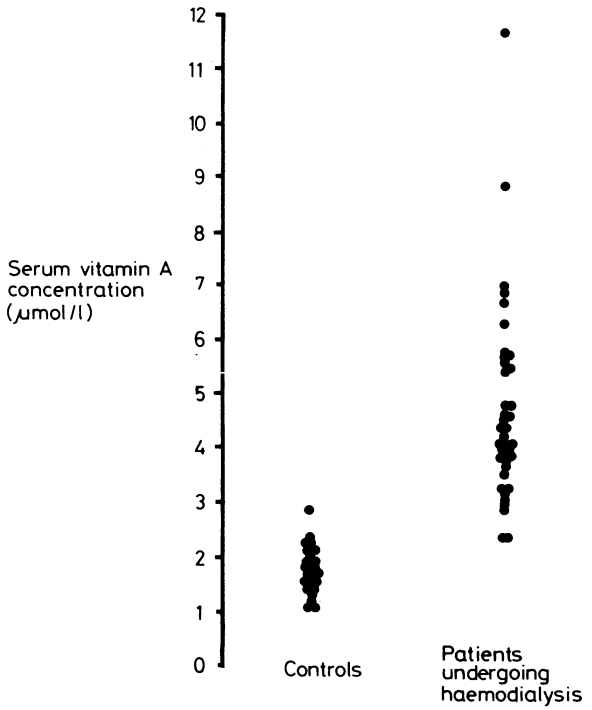

FIG 1—Vitamin A concentrations in 38 patients undergoing haemodialysis and 28 normal controls.

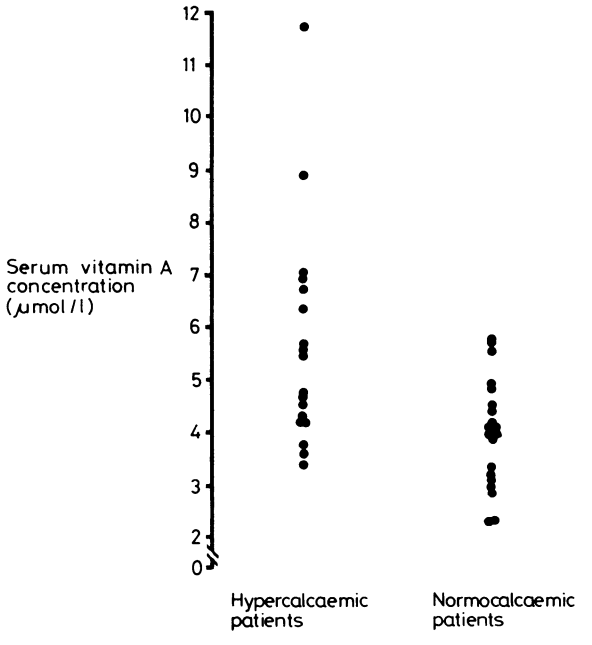

FIG 2—Vitamin A concentrations in 20 normocalcaemic and 18 hypercalcaemic patients undergoing haemodialysis.

Initial clinical and biochemical details of seven patients in whom vitamin A supplements were withdrawn

Case No	Sex	Age (yr)	Duration of dialysis (months)	Daily vitamin A supplement (IU)	Serum vitamin A (μmol/l)	Plasma calcium (mmol/l)	Plasma phosphate (mmol/l)	Plasma alkaline phosphatase (KA units/100 ml)	Plasma hydroxyproline (μmol/l)	Plasma i-PTH (ng/l)
1	F	33	24	7 500	7·0	2·75	2·45	23·4	54	1700
2	M	51	24	7 500	4·1	2·75	2·45	15·8	26	920
3	M	57	72	15 000	8·8	2·68	2·33	17·7	44	1350
4	M	29	47	7 500	4·3	2·75	1·55	18·0	53	1100
5	F	28	27	7 500	5·8	2·58	1·84	28·0	53	1200
6	F	59	20	7 500	4·5	2·90	3·04	13·2	31	1900
7	M	28	49	10 000	5·4	2·95	2·16	14·0	89	2500
Normal (mean ± 2 SD)					1·8 ± 0·8	2·43 ± 0·20	1·16 ± 0·26	6 ± 4	12 ± 9	541 ± 404

Conversion: SI to traditional units—Vitamin A: 1 μmol/l ≈ 28·6 μg/100 ml. Calcium: 1 mmol/l ≈ 4 mg/100 ml. Phosphate: 1 mmol/l ≈ 3 mg/100 ml. Hydroxyproline: 1 μmol/l ≈ 0·131 mg/l.

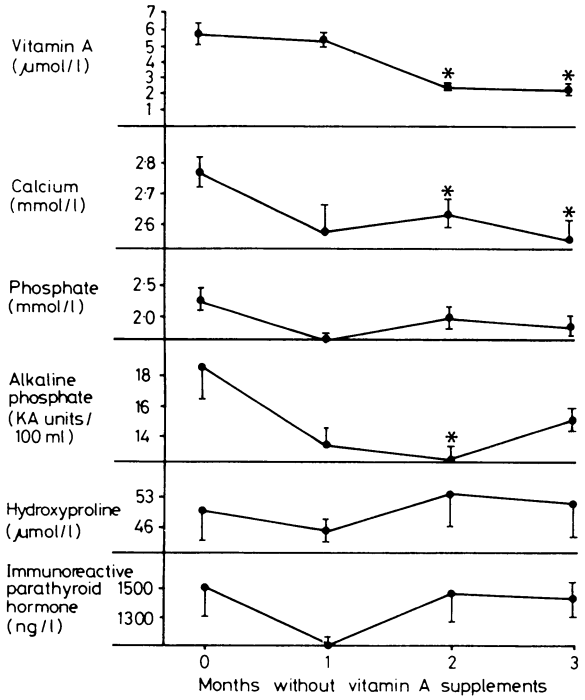

FIG 3—Serial biochemical data in seven patients undergoing haemodialysis after withdrawal of vitamin A supplements. Each point represents the mean of seven estimations except at one month, when only five patients were studied. Bars indicate standard error of mean. *$p < 0.01$.

Discussion

We found raised serum concentrations of vitamin A in patients with chronic renal failure who were undergoing haemodialysis, which agrees with previous findings.[1-3] Factors that may contribute to the high vitamin A concentrations in these patients are diminished metabolism of retinol to retinoic acid, which is a function of the kidney,[15] and increased concentrations of retinol-binding protein found in chronic renal failure.[2]

The significantly higher serum vitamin A concentrations in patients taking vitamin A supplements and the significant fall in serum vitamin A concentrations on withdrawal of vitamin A supplements indicate that these agents make an important contribution to vitamin A toxicity. The recommended daily requirement for vitamin A in adults is 5000 IU, these patients were taking up to 15 000 IU daily in addition to dietary vitamin A. Although this level of intake would be unlikely to produce problems in patients with normal renal function, care should be taken in the prescribing of such preparations to patients with impaired renal function.

We also found a positive correlation between serum vitamin A concentrations and plasma calcium concentrations in patients with chronic renal failure, in agreement with others.[16] The significantly higher concentrations of vitamin A in hypercalcaemic patients than in normocalcaemic patients and the significant reduction in plasma calcium concentration that occurred after withdrawal of vitamin A supplements indicate that vitamin A toxicity contributes to hypercalcaemia in patients undergoing haemodialysis. Hypercalcaemia is a well-documented effect of chronic vitamin A overdose in man and occurred in the presence of similar concentrations of vitamin A to those found in this study.[8-10] Since each Multivite tablet also contains a small amount of vitamin D_2 (250 IU) this might have contributed to the hypercalcaemia in these patients. This, however, is unlikely since such small quantities of vitamin D_2 are insufficient to overcome the "vitamin-D resistance" of chronic renal failure.[17,18]

The mechanism by which hypervitaminosis A causes hypercalcaemia is far from clear. In tissue culture experiments vitamin A has been shown to produce osteolysis[4,5] and to potentiate the osteolytic action of parathyroid extract.[5] In the rat hypervitaminosis A causes increased bone resorption[6,7] and, at least in the rapidly growing animal, may produce an inhibition of osteoblastic activity.[7,19] These osteolytic effects may occur as the result of the effect of vitamin A on lysosomal membranes.[20,21] In man skeletal lesions, radiological evidence of hyperostosis,[22] and histological evidence of osteolysis[23] have been described in hypervitaminosis A. Vitamin A has also been shown to stimulate parathyroid hormone secretion both in bovine parathyroid tissue and in man.[24]

We found no correlation between vitamin A concentrations and any of the radiological and histological variables of bone disease studied. The significant reduction in alkaline phosphatase activity which occurred on withdrawal of vitamin A suggests that the high concentrations of vitamin A were having an effect on bone, though we found no change in hydroxyproline concentrations. No change in immunoreactive parathyroid hormone concentration occurred after withdrawal of vitamin A, in spite of the significant reduction in plasma calcium concentration, which may have been expected to cause a rebound rise in plasma immunoreactive parathyroid hormone concentration. This may reflect a summation of effects of vitamin A on both parathyroid and bone. Alternatively, vitamin A may increase the sensitivity of bone cells to the osteolytic action of parathyroid hormone.

In conclusion, it appears that the high serum vitamin A concentration that occurs in patients with chronic renal failure has toxic effects. Hypercalcaemia probably occurs as a consequence of the effect of vitamin A on bone, though further work is necessary to establish the precise mechanisms.

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A protein in urine associated with muscle disease and muscle damage

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Abstract

Analysis of the protein composition of human urine by high-resolution two-dimensional electrophoresis showed that several features are associated with neuromuscular diseases, the best defined being the appearance in the urine of a small amount of a protein that migrates on the electropherogram as a characteristic spot (spot C). This spot consists of a protein of apparent molecular weight 26 000 and isoelectric point 5·3. The spot was usually present in the urine of patients suffering from diseases in which the musculature was directly affected but was rarely found in other patients and normal subjects.

The protein responsible for spot C appears to be an index of muscle damage caused by a number of conditions. Attempts are being made to isolate enough of the protein to permit its identification.

Introduction

The analysis of the protein composition of human urine by high-resolution two-dimensional electrophoresis provides a highly sensitive index of the functional state of an individual.[1 2] By applying this technique to patients with neuromuscular diseases we have observed several features in the urinary protein pattern that are associated with these diseases.[1] The best defined and most consistent feature is the appearance in the urine of a small amount of a protein that migrates on the electropherogram as a characteristic spot; we have designated this spot C.

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The protein and its prevalence

From its electrophoretic behaviour spot C consists of a protein of apparent molecular weight 26 000 and isoelectric point 5·3.

We studied the prevalence of spot C in electropherograms of urine from 107 normal subjects and 130 patients with various neuromuscular diseases. Spot C was most pronounced and was invariably observed on electropherograms of the urine of boys with Duchenne muscular dystrophy, who were estimated to excrete about 100 μg a day (table). It was also usually present in the urine of patients suffering from limb-girdle dystrophy, spinal muscular atrophies, and dystrophia myotonica. Spot C was not observed in the urine of patients partially immobilised by other disabilities such as spina bifida, cerebral palsy, and congenital deformity, in which the musculature is not directly affected. In preliminary studies we did not detect the protein responsible for spot C in the urine of patients with myasthenia gravis and multiple sclerosis.

Discussion

We believe that the presence of the protein responsible for spot C in urine is an index of muscle damage produced by a number of conditions and not the direct product of a modified gene associated with a particular neuromuscular disease. The protein is probably present in normal muscle, although the evidence for this is so far circumstantial for we have not been able to show its presence by electrophoresis of extracts of this tissue and have not detected it in the serum of normal people or patients with Duchenne muscular dystrophy. It was detected in the urine of normal men for a day or two after a bout of vigorous exercise. It was also observed in the urine of patients with a history of circu-