Paradoxical effects of essential fatty acid supplementation on lipid profiles and sweat electrolytes in cystic fibrosis

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Supplements of evening primrose oil *(Oenothera biennis)*, which contains at least 72% linoleic (18:2*n*-6) and 7% γ-linolenic (18:3 *n*-6) acids (expressed as % fatty acid methyl esters) were given to sixteen cystic fibrosis patients for a period of 12 months. Clinical observations showed no significant changes in patients' weights or respiratory function throughout. Linoleic acid levels in plasma and erythrocyte membranes increased significantly during the first 6 months but this increase was not sustained at its initial level. After supplementation was discontinued reversion to baseline (low) levels occurred within 4 months. Levels of plasma prostaglandins (PG) and urinary PG metabolites varied among individuals over a wide range, and urinary $PGF_{2\alpha}$ metabolites fell during the supplementation. There was a significant fall in sweat sodium concentrations after 6 weeks of supplementation, but sweat chloride was unchanged. It is not known whether the effect of essential fatty acids on sweat Na^+ reflects changes in cell membrane conformation or if there is a direct effect on Na^+ pump activity.

Cystic fibrosis: Essential fatty acids: Sweat electrolytes

Deficiency of the essential fatty acid (EFA) linoleic acid, 18:2*n*-6, has been widely reported in cystic fibrosis (CF), particularly but not exclusively when there is impairment of pancreatic function (Hubbard *et al.* 1977; Lloyd-Still *et al.* 1981). Evidence that there is an increased turnover of certain fatty acids in CF has been provided by Rogiers *et al.* (1984). The metabolites of linoleic acid (Fig. 1) are prostaglandin (PG) precursors, and urinary metabolites of PGE_2 are increased in patients with CF and in heterozygotes (Burns & Dodge, 1982), although EFA deficiency is usually associated with reduction of PGE synthesis and a corresponding decrease in the excretion of its metabolites (Friedman *et al.* 1980). Thus, there appears to be increased metabolism of, and perhaps increased requirement for, EFA in CF. Further evidence for an increased turnover of the linoleic acid derivative arachidonic acid (20:4*n*-6) has been provided by Carlstedt-Duke *et al.* (1986) who showed that the normal inhibition of arachidonic acid release from lymphocytes by dexamethasone is not seen in CF. They postulated that many of the clinical and biochemical features of CF can be explained on this basis. The recent identification of the major CF mutation and its corresponding protein implicates an abnormal binding site for ATP as the primary defect (Riordan *et al.* 1989). Intracellular energy cycles are likely to be disturbed, and secondary effects on fatty acid metabolism might be expected.

For these and other reasons, various authors have attempted clinical trials of EFA supplements in CF. One of the earliest reports was of a single patient studied by Elliott & Robinson (1975) to whom Intralipid®, an intravenous emulsion of soya-bean oil, was given by regular infusion. Clinical benefit was reported, and perhaps most interestingly it was

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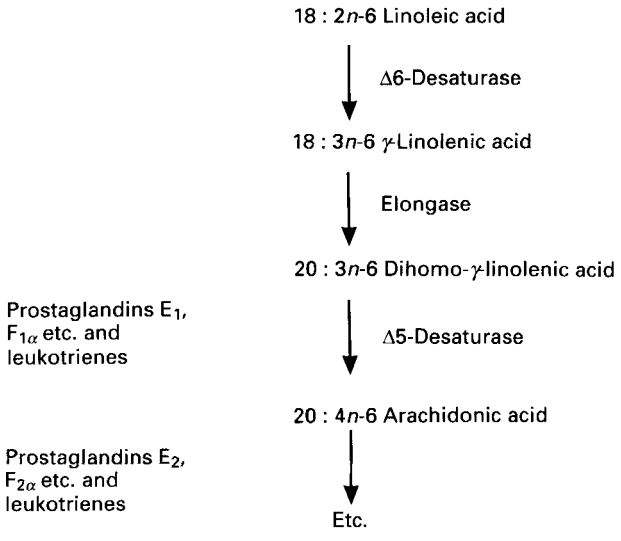

Fig. 1. The metabolic pathway of linoleic acid.

claimed that sweat electrolyte concentrations were reduced. Elevated sweat electrolytes are a characteristic feature of CF. Although another trial of Intralipid produced no fall in sweat chlorides (Kussavsky *et al.* 1983) others also found that in some patients sweat sodium does fall after EFA supplementation (Rosenlund *et al.* 1977). Oral supplements of maize oil (Rosenlund *et al.* 1977), safflower oil (Mischler *et al.* 1986; Lloyd-Still *et al.* 1979), and linoleic acid monoacylglycerol (Christophe *et al.* 1985) have also been used and again the results conflict. Some have claimed clinical benefit without any change in the EFA status (Chase *et al.* 1979) while others have been able to raise the blood levels of EFA with corresponding increases in the urinary excretion of PGE_2 metabolites (Mischler *et al.* 1986).

In the present study we observed the effects of oral supplementation with oil of evening primrose (*Oenothera biennis*), which is rich in linoleic (18:2*n*-6) and γ-linolenic acids (18:3*n*-6), on the clinical state, sweat electrolytes and fatty acid profiles of a group of children with CF.

SUBJECTS AND METHODS

Sixteen patients with CF were enrolled in the study, nine female and seven male. Informed consent was obtained from patients, parents, or both. Ages ranged from 3·4 to 23·0 (mean 9·2) years. Each subject received oil of evening primrose by mouth as a dietary supplement for a period of 12 months in a daily dose of 1 g/kg body-weight to a maximum of 25 g. The oil contains at least 72% linoleic acid and 7% γ-linolenic acid expressed as a percentage of total fatty acid methyl esters (Table 1). During the first 3 months the oil was given in standard commercial capsules (Efamol®) which also contain vitamin E, but because of patient difficulty with taking large numbers of capsules the preparation was changed to liquid oil (prepared in individual dark brown 10 ml bottles) supplied by the same manufacturer. The oil did not contain added vitamin E, which was therefore given as an additional supplement (200 mg daily) from 6 months on, and continued after oil supplements ceased.

The patients attended a special research clinic where baseline anthropometric and biochemical measurements were made (*a*) before supplementation began and the same

Table 1. *Composition of evening primrose* (Oenothera biennis) *oil (mol/mol total fatty acid)*

Fatty acid		mol %
Palmitic acid	16:0	6
Stearic acid	18:0	2
Oleic acid	18:1*n*-9	11
Linoleic acid	18:2*n*-6	72
γ-Linolenic acid	18:3*n*-6	7
Others		2

variables were monitored after (*b*) 6 weeks, (*c*) 6 months, (*d*) 12 months and (*e*) 16 months, the last measurements being made 4 months after the supplement had been discontinued. Height, weight, triceps skinfold thickness and simple lung function tests were measured using standard equipment. Sweating of the forearm was stimulated by the standard pilocarpine iontophoresis method of Gibson & Cooke (1959), sweat was collected into weighed Na^+-free gauze pads, and the Na^+ and Cl^- contents were measured by flame photometry and titration respectively. Plasma cholesterol, triacylglycerols, zinc, vitamin E and 24 h urinary creatinine were measured in the routine clinical laboratories of the University Hospital of Wales. Blood samples (5 ml) for fatty acid analysis were collected in heparinized tubes and centrifuged at 2000 rev./min for 10 min at 4° within 10 min of venepuncture. The erythrocytes were thrice washed with saline (9 g sodium chloride/l) and were again centrifuged between washings. Erythrocyte and plasma lipids were extracted using the Folch *et al.* (1957) extraction method, with chloroform–methanol (2:1, v/v) with 0·01% butylated hydroxytoluene (Lloyd-Still *et al.* 1981). Plasma lipids (1 ml) were separated into individual classes (free fatty acids, cholesteryl esters, triacylglycerols, phospholipids) by thin layer chromatography (TLC) using silica gel plates heated for 1 h at 110° and light petroleum (b.p. 40–60°), diethyl ether, and glacial acetic acid (90:10:1, by vol.). Plates were run twice for 30 min in the solvent system, dried, and spots were visualized using rhodamine and examined under u.v. light. The erythrocyte phospholipids were separated using TLC and chloroform–methanol–water–ammonium hydroxide (60:35:2:2 by vol.) Plates were run for 50 min, using standards of phosphatidyl choline, phosphatidyl serine, phosphatidyl inositol, phosphatidyl ethanolamine and sphingomyelin. Extracted lipids were methylated using methanol and sulphuric acid. The resulting fatty acid methyl esters were extracted with light petroleum (b.p. 40–60°) then analysed using a Pye 204 gas–liquid chromatograph with SCOT FFAP capillary column, inlet splitter and flame ionization detector with helium as a carrier gas. Blood samples for PG analysis were centrifuged within 10 min of venepuncture before the plasma was extracted and submitted to radioimmunoassay as previously described by Dodge *et al.* (1981). Collections (24 h) of urine for PG metabolites (PGM) were extracted with chloroform, separated on silicic acid columns using toluene–acetate–methanol systems (Haning *et al.* 1977) and assayed using antisera provided by Miles-Yeda Laboratories (Israel). Coefficients of variation for the PG assays were 4·2, 6·8, 8·1 and 12·1% for PGE_2, $PGF_{2\alpha}$, PGE_2M and $PGF_{2\alpha}M$ respectively. Controls for the plasma prostaglandins and urine metabolites (*n* 15) were healthy young adults, and plasma values were in the same range as those previously found in healthy children (Dodge *et al.* 1981). Control values for total plasma and erythrocyte fatty acids were obtained from healthy children (*n* 30).

Table 2. *Anthropometric measurements: height, weight and triceps skinfold (percentage of normal values appropriate for age and sex) for subjects with cystic fibrosis receiving an essential fatty acid supplement as evening primrose* (Oenothera biennis) *oil for 12 months*

(Mean values, range and standard deviations)

Stage of treatment...		Baseline	6 weeks	6 months	12 months	16 months
Height	Mean	94·46	94·51	94·63	94·69	94·67
	Range	86·8–103·6	87·71–104·44	87·7–104·23	89·08–105·15	89·22–103·60
	SD	4·4	4·66	4·54	4·45	4·44
Body-wt	Mean	78·6	79·68	78·94	77·93	76·99
	Range	53·3–101·6	58·61–102·89	59·20–103·00	55·61–104·04	56·07–100·90
	SD	12·1	12·15	11·66	13·08	12·61
Skinfold	Mean	74·0	71·70	70·42	75·47	64·94
	Range	40·0–102·2	47·5–90·24	43·9–108·16	52·3–101·23	42·85–109·52
	SD	17·4	13·44	15·33	14·42	18·94
	n	16	15	16	16	15

Table 3. *Lung-function tests (percentage of standards-for-height) for subjects with cystic fibrosis receiving an essential fatty acid supplement as evening primrose* (Oenothera biennis) *oil for 12 months*

(Mean values and standard deviations)

Stage of treatment...		Baseline	6 weeks	6 months	12 months	16 months
FEV_1	Mean	60·1	67·4	59·3	61·6	60·2
	Range	26·7–118·5	25·0–113·6	16·2–116·7	27·3–112·6	14·0–105·8
	SD	23·7	16·2	26·7	21·2	25·0
FVC	Mean	72·8	79·9	70·9	75·0	72·2
	Range	46·4–127·4	41·3–133·7	37·9–122·0	42·9–133·4	20·5–119·5
	SD	23·8	22·7	29·2	24·0	26·8
PFR	Mean	78·8	87·6	74·6	72·6	73·2
	Range	50·0–120·8	48·4–122·9	36·1–124·1	35·1	46·0–126·0
	SD	24·4	26·1	26·4	26·4	24·7
	n	16	14	16	12	15

FEV_1, forced expiratory volume at 1 s (litres); FVC, forced vital capacity (litres); PFR, peak expiratory flow-rate (litres/min)

Statistical analysis

Results were submitted to an analysis of variance for repeated measures using the P2V program in the BMDP package (Dixon *et al.* 1983). If a significant *F* ratio test result was obtained for the comparison of the means at different times, Dunnett's test was used to compare each mean with the mean at baseline (Winer, 1962). Some variables were transformed by taking logarithms before analysis of variance.

RESULTS

No significant changes in height, weight, triceps skinfold thickness or lung function test results (expressed as percentages of mean normal values appropriate for age and sex of the subjects) occurred during the study (Tables 2 and 3).

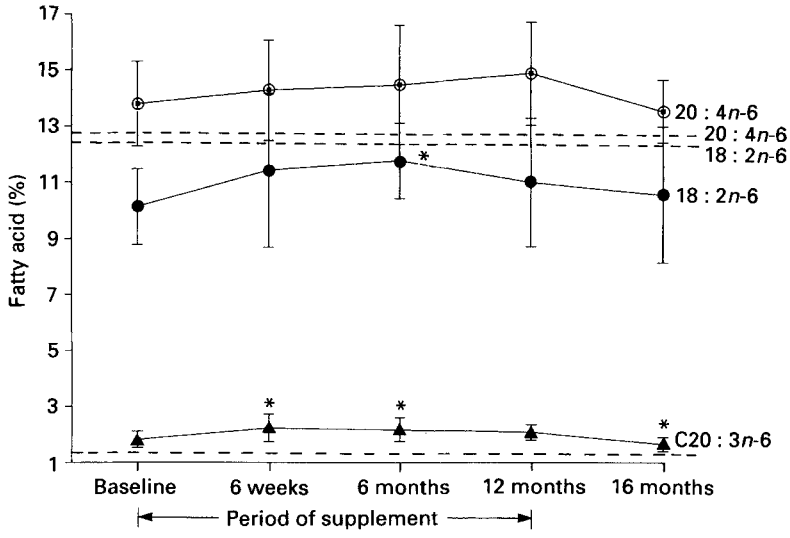

Fig. 2. Total erythrocyte fatty acids of patients with cystic fibrosis receiving an essential fatty acid (EFA) supplement as evening primrose (*Oenothera biennis*) oil. (---), Mean values in healthy children. Points are means, and standard deviations are represented by vertical bars. For details of supplement, see Table 1 and p. 260. * $P < 0{\cdot}05$. Comparisons are between baseline and 6 weeks/6 months/12 months, and between 12 and 16 months.

Changes in fatty acid composition of erythrocytes are shown in Fig. 2. It will be seen that linoleic (18:2*n*-6) acid levels increased significantly during the first 6 months, although never reaching normal mean values, but that the increase was not sustained. After supplementation was stopped, reversion to baseline (low) levels occurred within 4 months. A similar change occurred in the case of the EFA dihomo-γ-linolenic acid (20:3*n*-6), but in this case the rise was maintained until supplementation ceased at 12 months. Baseline values for 20:3*n*-6 were already increased above those of normal child controls and giving 18:2*n*-6 and 18:3*n*-6, which are both present in evening primrose oil, had the expected effect of further elevation. This was also seen in the case of the important structural fatty acid 20:4*n*-6 (arachidonic), which started from a high level and increased until 12 months despite the fall in the level of its major precursor, 18:2*n*-6, but reflecting the sustained rise in its immediate precursor, 20:3*n*-6. Statistically significant ($P < 0{\cdot}05$) increases in 18:2*n*-6 and 20:3*n*-6 in erythrocyte phospholipids at 6 and 12 months were noted in the phosphatidyl choline component only, but there was no significant increase in 20:4*n*-6. No significant changes in EFA were seen in the phosphatidyl ethanolamine fraction. Detailed fatty acid analyses of these phospholipid fractions are available on file; other phospholipids were present in very small amounts.

Total plasma fatty acids showed changes similar to those in the erythrocyte, also reverting quickly to baseline levels when supplementation was stopped (Fig. 3). Similar changes in the relative proportions of fatty acids during supplementation were also seen in the individual components of plasma lipids, i.e. cholesteryl esters, triacylglycerols, phospholipids and free fatty acids, with varying degrees of statistical significance. Details of these results have been omitted from the present report because they added nothing of importance to the observed changes in total plasma fatty acids, except that a continuing elevated level of EFA in the triacylglycerol fraction at a time when total erythrocyte and plasma 18:2*n*-6 was falling (6–12 months) is an indirect confirmation of continuing patient compliance in the supplement trial (Table 4).

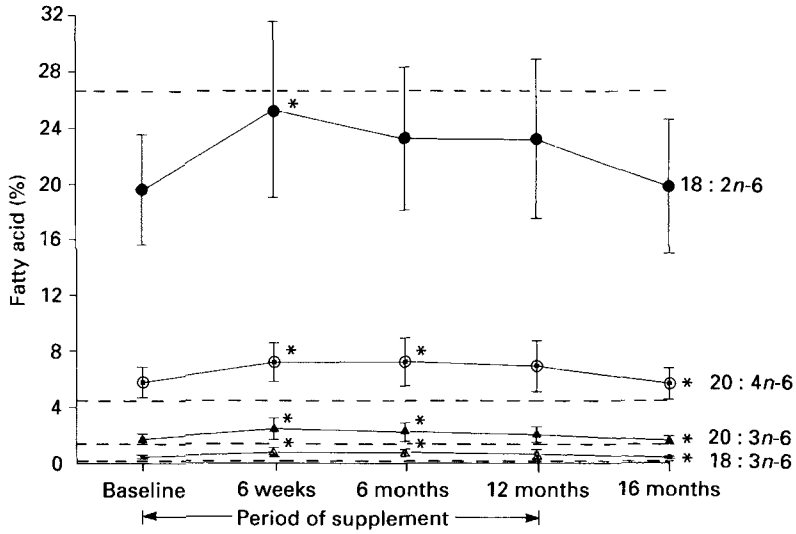

Fig. 3. Total plasma fatty acids of patients with cystic fibrosis receiving an essential fatty acid (EFA) supplement as evening primrose (*Oenothera biennis*) oil. (---), Mean values in healthy children. Points are means, and standard deviations are represented by vertical bars. For details of supplement, see Table 1 and p. 260. * $P < 0.05$. Comparisons are between baseline and 6 weeks/6 months/12 months, and between 12 and 16 months.

Table 4. *Plasma triacylglycerols: selected fatty acids* (%)

(Mean values and standard deviations)

Stage of treatment...	Baseline		6 weeks		6 months		12 months		16 months	
Fatty acid	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
6:1*n*-3	7·24	1·47	6·13	1·47	5·97	1·32	6·02	1·33	5·94	1·10
18:2*n*-6	9·50	5·53	13·90	6·45	13·49	3·27	15·54*	4·8	11·68*	4·95
18:3*n*-6	0·30	0·11	0·49	0·29	0·60	0·24	0·59	0·41	0·39	0·27
20:4*n*-6	0·96	0·41	1·23	0·62	1·72*	0·74	1·85*	0·83	1·11*	0·41

* Mean values were significantly different from baseline levels (or between 12 and 16 months) ($P < 0.05$).

PG levels in plasma and urine PGM varied among individuals over a wide range. Plasma PGE_2 and $PGF_{2\alpha}$ levels both fell during supplementation (without reaching statistical significance) and reverted to baseline levels later (Fig. 4 and Table 5). A consistent fall in urine PGF_2M was seen, but a less consistent upward trend was seen in the case of PGE_2M. The excretion of PGM related to urinary creatinine excretion is shown diagrammatically in Fig. 5. However, some of the samples for PGE_2M analysis were stored under varying conditions at the 12-month stage so that the apparent secondary fall in PGE_2M may not be real.

Urine 24 h creatinine excretion rose significantly ($P < 0.05$) during the first 6 months and then fell, returning to baseline values when supplementation stopped. Plasma creatinine fell during the first 6 months although this did not quite reach statistical significance. Thus, plasma creatinine was falling at a time when its urinary excretion was rising. Plasma cholesterol and triacylglycerol levels both fell during the first 6 months of EFA

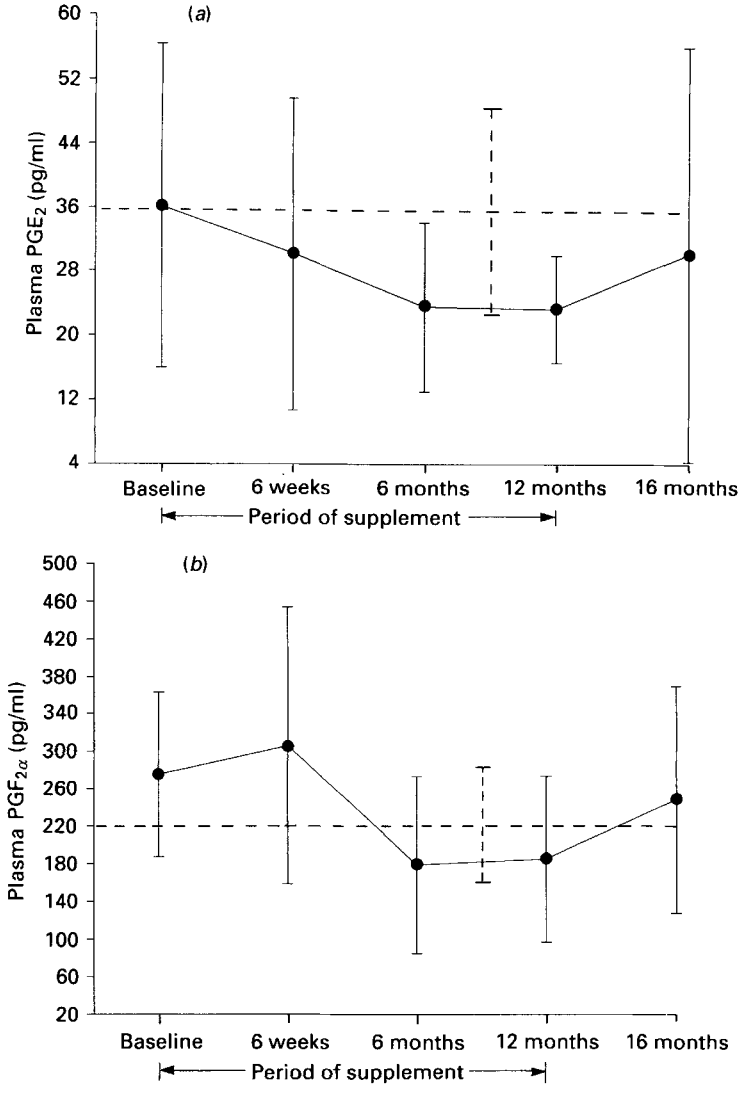

Fig. 4. (*a*) Plasma prostaglandin E_2 (PGE_2) and (*b*) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) of patients with cystic fibrosis receiving an essential fatty acid (EFA) supplement as evening primrose (*Oenothera biennis*) oil. Points are means, and standard deviations are represented by vertical bars. (---), Mean values in healthy children. For details of supplement, see Table 1 and p. 260.

supplementation. In the case of cholesterol, mean levels were almost back to baseline at 12 months. The fall in triacylglycerol reached statistical significance at 6 months and levels remained low up to 16 months. No significant changes occurred in plasma zinc levels, but serum vitamin E concentrations increased from a low baseline value and remained elevated at 16 months. The relatively low values observed at 6 months reflect the change-over from capsules containing vitamin E to the oil itself, before specific vitamin E supplements were given (Table 5).

Sweat electrolyte measurement showed no change in the Cl^- concentration (Fig. 6), but a significant fall in Na^+ after 6 weeks supplementation, following an initial rise (Fig. 7).

Table 5. *Biochemical changes during essential fatty acid supplementation*

(Mean values and standard deviations)

Stage of treatment...	Baseline		6 weeks		6 months		12 months		16 months	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Plasma zinc (μmol/l)	11·74	1·44	11·87	1·44	11·77	1·24	12·42	1·83	11·46	1·75
Plasma triacylglycerols (mmol/l)	1·59	0·53	1·45	0·66	1·19*	0·49	1·26	0·85	1·21	0·51
Plasma cholesterol (mmol/l)	3·04	0·48	2·99	0·45	2·73	0·40	2·99	0·47	2·95	0·60
Plasma creatinine (mmol/l)	57·50	7·58	48·33	8·76	45·00	18·17	59·83	9·92	52·50	10·37
Serum vitamin E (μmol/l)	5·10	4·06	9·30*	4·62	5·61	3·16	9·37*	4·50	10·58*	6·47
Urine creatinine (μmol/24 h)	3849	2736	4164	3053	5706**	4314	4192	2589	4038	3384

Mean values were significantly different from baseline levels: * $P < 0{\cdot}05$ and ** $P < 0{\cdot}05$ for log-transformed data.

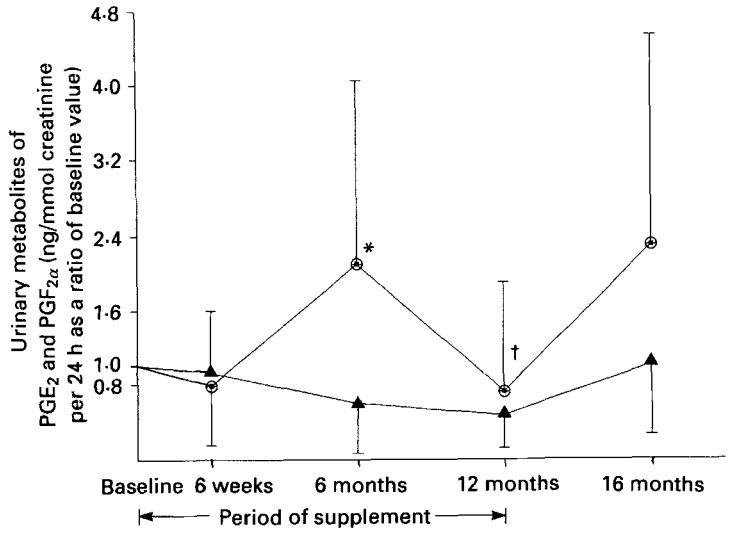

Fig. 5. Urinary metabolites of prostaglandins E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$) (ng/mmol creatinine per 24 h as a ratio of baseline value) in patients with cystic fibrosis receiving an essential fatty acid (EFA) supplement as evening primrose (*Oenothera biennis*) oil. Points are means, and standard deviations are represented by vertical bars. For details of supplement see Table 1 and p. 260. * $P < 0{\cdot}05$ on log-transformed data. Comparison with baseline. † Indicates a value that may be inaccurate (see text).

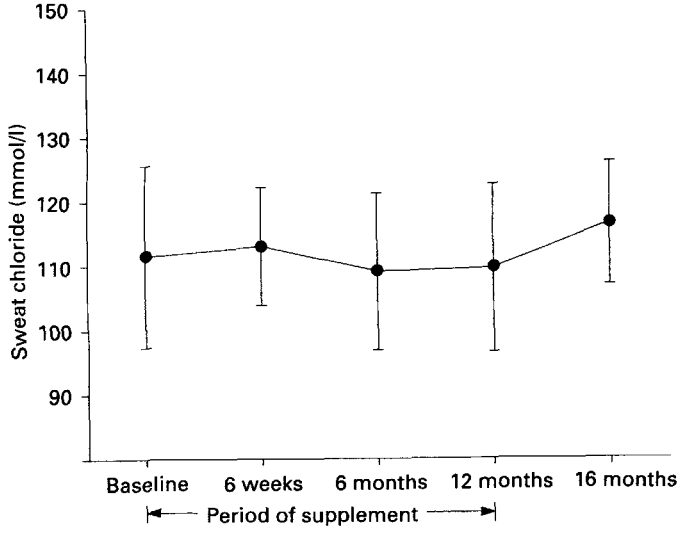

Fig. 6. Iontophoresis sweat chloride levels of patients with cystic fibrosis receiving an essential fatty acid (EFA) supplement as evening primrose (*Oenothera biennis*) oil. Points are means, and standard deviations are represented by vertical bars. For details of supplement see Table 1 and p. 260.

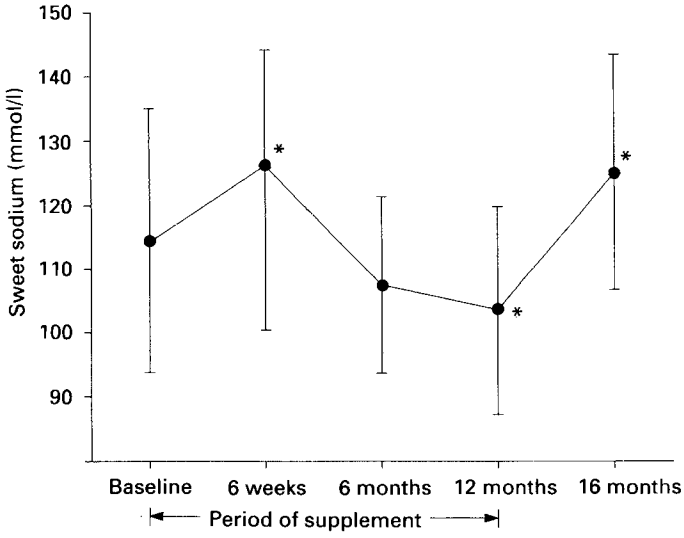

Fig. 7. Iontophoresis sweat sodium levels of patients with cystic fibrosis receiving an essential fatty acid (EFA) supplement as evening primrose (*Oenothera biennis*) oil. Points are means, and standard deviations are represented by vertical bars. For details of supplement, see Table 1 and p. 260. * $P < 0{\cdot}05$. Comparisons are between baseline and 6 weeks/6 months/12 months, and between 12 and 16 months.

DISCUSSION

Among the many biochemical changes observed in these patients during dietary supplementation with evening primrose oil, three are of particular interest and may shed some light on the underlying metabolic abnormality in CF. These are the failure to maintain levels of linoleic acid initially achieved despite continuation of the EFA supplement, the elevated levels of linoleic acid metabolites seen throughout the study, and the effects of EFA on sweat electrolyte concentrations.

It is clear from Figs 2 and 3 that the plasma linoleic acid level responds well to oral EFA supplementation at 6 weeks, and that at 6 weeks and 6 months this increased uptake of linoleic acid has been reflected in an increased incorporation into erythrocyte membranes. However, by 12 months the linoleic acid levels of both plasma and erythrocytes are reverting back towards baseline values, although the oral supplements were continuing. One possible explanation for failure to sustain the improvement in linoleic acid status (which only achieved normal mean values at any stage in a minority of subjects) is that the supplementary linoleic acid may have been used as a general source of energy, in which case increasing the total energy intake might have avoided the secondary fall that occurred after initial improvement (Parsons *et al.* 1988). However, if inadequate total energy intake is the explanation, the substantial early rise in linoleic acid would not have been expected. It is nevertheless possible that the increase in available linoleic acid was directed to one of its important functions as a regulator of intracellular metabolism, particularly in ion channel control (McKenna *et al.* 1985). The distribution of absorbed linoleic acid is to body cells for storage as triacylglycerol, oxidation to energy, metabolism to its derivatives (Fig. 1) or incorporation in membrane phospholipids, whence it can be mobilized again, particularly from phosphatidyl inositol. It is of interest that inositol, when complexed with phosphate, plays a key role in the maintenance of intracellular ionic homeostasis (McPherson & Dormer, 1987). We were unable, for practical reasons, to measure phosphatidyl inositol

fatty acids in our patients because although phosphatidyl inositol has high metabolic activity and turnover it forms only a small fraction of total membrane phospholipids, but others have shown a unique relationship between phosphatidyl inositol and dietary fatty acids. In guinea-pig lung, phosphatidyl inositol concentrations of arachidonic acid decrease when there is dietary linoleic acid deficiency, and also decrease with linoleic acid excess (Senne & Mathias, 1987). This suggests that linoleic acid may be the preferred fatty acid in phosphatidyl inositol, so that when available it replaces arachidonic acid, but if linoleic acid is not available then arachidonic acid will fall as it is used up. By contrast, in other classes of phospholipid, arachidonic acid tends to be conserved even when there is marked linoleic acid deficiency (Lefkowith *et al.* 1986). Previous studies in our laboratory on platelets (Burns & Dodge, 1982) and elsewhere on erythrocytes (R. T. Holman, personal communication) have found low linoleic acid levels not only in CF homozygotes but also in their parents, who have no impairment of linoleic acid absorption. This is consistent with an intrinsic abnormality of EFA metabolism in CF, resulting in enhanced turnover of linoleic acid (Rogiers *et al.* 1984), perhaps through the phosphatidyl inositol fraction, which is also present in heterozygotes.

There is no evidence of a defect in Δ5- or Δ6-desaturase in CF from this or other studies. In fact, plasma arachidonic acid levels as well as those of intermediate linoleic acid derivatives were normal or slightly elevated at the start of the EFA supplementation, and were increased and well maintained throughout. In the case of erythrocyte arachidonic acid and 20:3*n*-6 there was no secondary fall such as was seen in the case of linoleic acid, and the increases continued until EFA supplementation was stopped at 12 months, when the levels quickly reverted to pretreatment values (Fig. 2). Elevation of linoleic acid metabolites such as arachidonic acid has been seen before in CF (Lloyd-Still *et al.* 1981; Parsons *et al.* 1988) and indicates that it is the linoleic acid itself that is being actively utilized.

It is possible that the increased proportions of these linoleic acid derivatives in cell membranes might alter the permeability characteristics sufficiently to account for the consistent and significant decrease in stimulated sweat Na^+ concentrations that occurred during the study, but this would not in itself explain the initial elevation in sweat Na^+ during the first 6 weeks (Fig. 7), although the transient increase in membrane linoleic acid levels might do so. Current opinion favours control of the transport of Cl^- rather than Na^+ as the site of the primary abnormality in CF, but sweat Cl^- concentrations were unaffected. Changes in membrane conformation might not only affect ion channels, but also the exposure or masking of receptors, and the activity of ion-exchange pumps. Some previous reports have claimed that EFA supplements reduce sweat Na^+ (Elliott & Robinson, 1975; Rosenlund *et al.* 1977) while others have found no effect on Cl^- concentrations (Lloyd-Still *et al.* 1979; Kussavsky *et al.* 1983). The dissociated effects on the anion and cation seen in the present study are consistent with both conclusions, although in the one previous report where both electrolytes were measured no effect was observed (Mischler *et al.* 1986). The initial rise and subsequent fall of sweat Na^+ suggested it is directly related to the changes in fatty acid status and not to an indirect effect of the marked change in cholesterol and triacylglycerol levels, which, in plasma at least, fell for the first 6 months before beginning to revert to baseline values. However, the cholesterol content of erythrocyte membranes (which we did not measure) is known to have a marked effect on both active and passive Na^+ efflux (Ng & Hockaday, 1986) and similar effects might also occur in the sweat duct epithelium.

There is evidence that plasma non-esterified fatty acids may regulate human leucocyte Na^+ pump activity, with unsaturated fatty acids, including linoleic acid, having an inhibitory effect on ouabain-sensitive Na^+ efflux (Kroes & Ostwald, 1971). It is conceivable that a similar inhibitory effect of increased circulating plasma levels of non-esterified

linoleic acid might operate in the coil of the sweat glands, so that their primary secretory product would have a lowered Na^+ content which would still be reflected in the sweat Na^+ after partial reabsorption in the duct.

Changes in 24 h urine PGM levels (Fig. 4) are in keeping with previous observations (Mischler *et al.* 1986) and show a shift in the balance between PGE_2 and $PGF_{2\alpha}$ in the direction of PGE_2. This reflects the increased availability of linoleic acid, deficiency of which tends to increase $PGF_{2\alpha}$ synthesis (Chase & Dupont, 1978). Although this shift might be expected to decrease any tendency to broncho-constriction produced by $PGF_{2\alpha}$, no significant change in lung function was observed. Urinary PGE_2M levels are significantly higher in CF than in child controls generally (Burns & Dodge, 1982). It has been reported that in guinea-pig tracheal epithelium, desensitization of β-adrenergic receptors by repeated stimulation with isoproterenol is accompanied by increased PGE_2 synthesis (Omini *et al.* 1983). Increased PGE_2M excretion, and increased PGE_2 synthesis, could, therefore, reflect further downgrading of β-adrenoceptor sensitivity and thus possibly account for the reduction in sweat Na^+. However, the uncertainty about the PGE_2M measurements at 12 months means that these observations should be interpreted cautiously. Circulating plasma PGE_2 and $PGF_{2\alpha}$ values decreased proportionately during the trial. No measurements were made of the PG derivatives of 20:3*n*-6, PGE_1 and $PGF_{1\alpha}$, although the presence of 18:3*n*-6 in the supplement might be expected to increase their synthesis.

The increase in urinary creatinine occurred while plasma creatinine levels decreased (Table 5). Parents reported increased physical activity by the subjects while they were receiving EFA supplements, but we were unable to show any objective differences in their clinical state as measured by height, weight, skinfold thickness or lung function. No specific measurements of muscle bulk were made. The urinary excretion of creatinine is generally regarded as an indirect measurement of muscle mass, but it is possible that the increase in these subjects might indicate increased breakdown of muscle, reflecting the change in PG balance. $PGF_{2\alpha}$ stimulates ribosomal protein synthesis from amino acids and therefore tends to be anabolic, whereas PGE_2, whose output was probably increased relative to $PGF_{2\alpha}$, increases lysosomal protein degradation to amino acids and therefore has catabolic properties (Reeds *et al.* 1987). Although the subjects maintained their pre-existing height and weight centiles, they may therefore have had an increased protein turnover during EFA supplementation.

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