Bone turnover and nutritional status in Crohn's disease: relationship to circulating mononuclear cell function and response to fish oil and antioxidants

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Crohn's disease is associated with osteoporosis, malnutrition and altered function of peripheral blood mononuclear cells (PBMC). The responses of circulating immune cells and extra-intestinal manifestations to increased inflammatory activity and to modulation by dietary supplementation are uncertain. The relationships between disease status, bone turnover and body mass and composition, PBMC function and fatty acid availability have been investigated in patients with Crohn's disease. The availability of *n*-3 and *n*-6 PUFA is altered in adult patients and interferon (IFN)-γ production by PBMC is lower. Increased inflammatory activity is associated with increased bone resorption in adult patients and decreased body mass in paediatric patients. In healthy male subjects there is a proportionate relationship between supplementary intake of EPA and DHA (0·3–2·0 g as fish oil/d) in combination with antioxidants (vitamins A, C and E and Se) and incorporation into plasma phospholipids and PBMC, and a non-linear relationship with PBMC synthesis of TNF-α, IL-6 and prostaglandin E$_2$ (decrease) and IFN-γ (increase). In adults with Crohn's disease high-dose fish oil (2·7 g EPA + DHA/d) in combination with antioxidants (vitamins A, C and E and Se) increases the EPA and DHA content of PBMC and decreases the production of IFN-γ by PBMC, but is not associated with effects on bone turnover or nutritional status.

Crohn's disease: Mononuclear cell: Fish oil and antioxidants: Bone turnover

Crohn's disease is a relapsing inflammatory condition of the gastrointestinal tract associated with inappropriately activated T-cells within the mucosal and mucosally-associated lymphoid tissue, and characterised by increased local production of the cytokine interferon (IFN)-γ. Crohn's disease is associated with extra-intestinal manifestations and raised laboratory markers of inflammation, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). CRP is produced by hepatocytes in response to pro-inflammatory mediators released from activated peripheral blood mononuclear cells (PBMC; Wigmore *et al*. 1998, 2002). PBMC, typically comprising 10–15 % monocytes and 85–90 % T and B lymphocytes (Calder *et al*. 2002), circulate within the peripheral blood system and migrate between foci of chronic inflammation, the systemic circulation and extra-vascular lymphoid and non-lymphoid tissue. Raised CRP and ESR, which may

occur in Crohn's disease without clinically-active intestinal disease, are predictive for disease relapse and may indicate the presence of subclinical inflammation within the gastrointestinal mucosa (Brignola *et al*. 1986*a,b*; Schreiber *et al*. 1999).

Osteoporosis is a recognised extra-intestinal manifestation of Crohn's disease. Osteoporosis represents a state of reduced bone density resulting from an imbalance in bone turnover between bone formation by osteoblasts and bone resorption by osteoclasts and is associated with an increased risk of fracture, including low-impact fractures (Jordan & Cooper, 2002). The development of osteoporosis in Crohn's disease is associated with corticosteroid use and low BMI (Andreassen *et al*. 1997; Habtezion *et al*. 2002). An independent effect of Crohn's associated inflammation, within the gastrointestinal mucosa, on bone turnover has not been confirmed in man but is supported

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by the results of animal studies (Lin *et al.* 1996). A potential mechanism to explain such a relationship may involve circulating mononuclear cells (PBMC) with an altered profile of synthesised soluble inflammatory mediators, e.g. TNF-α, IFN-γ and prostaglandin (PG) E$_2$.

Crohn's disease is associated with malnutrition (Capristo *et al.* 1998), dietary restrictions (Rigaud *et al.* 1994), decreased body fat mass (Capristo *et al.* 1998; Geerling *et al.* 1998) and nutritional deficiencies (Harries & Heatley, 1983; Geerling *et al.* 1998). The relationship between changes in nutritional status and increased disease activity has not been adequately quantified. Chronic inflammation within the gastrointestinal tract may indirectly inhibit digestion and absorption of nutrients through damage to the intestinal mucosa, but whether there is a direct effect of active inflammation in Crohn's disease or the circulation of activated or inappropriately functioning immune cells on nutritional status is uncertain. Crohn's disease is associated with altered dietary intake (Rigaud *et al.* 1994) and utilisation of nutrients (Mingrone *et al.* 1999); furthermore, in other conditions release of inflammatory mediators, including TNF-α, may influence both nutritional status (Beisel, 1995) and the utilisation of endogenous fat and protein (Warren *et al.* 1987; Starnes *et al.* 1988). These responses may result in quantitative or qualitative changes in the availability of essential nutrients.

EPA and DHA are long-chain *n*-3 PUFA derived directly from the diet or metabolised from the essential fatty acid α-linolenic acid (Fig. 1). EPA is a substrate for synthesis of eicosanoids (PG, leukotrienes and thromboxanes) and competes with the *n*-6 PUFA arachidonic acid (AA), itself derived from the essential fatty acid linolenic acid. In Crohn's disease increased inflammatory activity may be associated with an increase in production of eicosanoids (Zifroni *et al.* 1983; Lauritsen *et al.* 1988) and therefore substrate demand for PUFA. In the presence of reduced dietary intake Crohn's disease may therefore be associated with altered availability of EPA and AA and other *n*-3 and *n*-6 PUFA. This altered availability has been demonstrated in studies of plasma fatty acid composition in patients with Crohn's disease compared with healthy subjects in both the clinically-active and quiescent disease state (Esteve-Comas *et al.* 1992, 1993; Geerling *et al.* 1999*b*). The consequences of this altered availability for immune cell function are uncertain. Studies in healthy subjects suggest that dietary supplementation with high
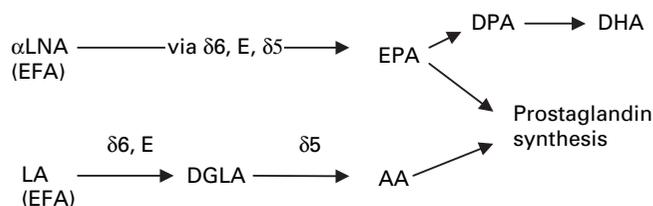
Fig. 1. Schematic representation of synthetic pathways of *n*-3 and *n*-6 long-chain PUFA from dietary essential fatty acids (EFA). The *n*-3 PUFA are: α-linolenic acid (αLNA); EPA; docosapentaenoic acid (DPA); DHA. The *n*-6 PUFA are: linoleic acid (LA); dihomo-γ-linolenic acid (DGLA); arachidonic acid (AA). δ5, δ5-desaturase; δ6, δ6-desaturase; E, elongase.

doses of EPA- and DHA-rich fish oil inhibit production of TNF-α, IL-1 and -2, consistent with an anti-inflammatory effect (Calder, 2001, 2003). Furthermore, a single clinical study in patients with Crohn's disease has demonstrated that high doses of an EPA- and DHA-rich fish oil reduce rates of disease relapse and values for laboratory markers of inflammatory disease activity consistent with an inhibitory effect on both the intestinal and systemic inflammatory responses (Belluzzi *et al.* 1996). It is uncertain whether these responses reflect a primarily pharmacological effect or repletion of an unrecognised deficiency in *n*-3 PUFA.

Long-chain PUFA are susceptible to free radical-mediated degradation, with the release of further free radical moieties, a process known as lipid peroxidation, that may also alter antioxidant availability (Foulon *et al.* 1999). This process may be stimulated by dietary supplementation with fish oils and exposure to high intakes of EPA and DHA (Eritsland, 2000). Release of free radicals may increase intestinal mucosal damage and inflammation in Crohn's disease (Simmonds & Rampton, 1993; Grisham, 1994) and reduce bone mass through increased bone resorption and decreased bone formation (Parhami, 2003). Dietary supplementation with antioxidants decreases oxidative stress (Aghdassi *et al.* 2003) and inhibits production of inflammatory mediators by PBMC (Reimund *et al.* 1998) in Crohn's disease. Reduced availability of vitamin C and other antioxidant micronutrients, which may be noted in Crohn's disease (Kuroki *et al.* 1993; Ringstad *et al.* 1993; Buffinton & Doe, 1995), are associated with bone loss in post-menopausal women (Kaptoge *et al.* 2003; Maggio *et al.* 2003), but whether antioxidant supplementation has an effect on bone turnover is uncertain.

Disease activity, bone turnover and nutritional status in Crohn's disease

The lack of reports of a relationship between increased Crohn's disease activity and altered bone turnover may reflect a number of factors relating to the design of published studies. First, patients with characteristics that may independently affect bone metabolism, i.e. post-menopausal women and corticosteroid use, have been included. Second, clinical disease scores that may not accurately reflect biochemical or metabolic changes during the inflammatory response have been used to assess disease activity. By comparison, CRP and ESR are objective markers of the acute-phase or 'systemic' inflammatory response (Baumann & Gauldie, 1994; Barland & Lipstein, 1996; Moshage, 1997). Third, direct measurements of bone density, which are influenced by pre-morbid bone mass and reflect the sum of temporal fluctuations in bone turnover, have been used to assess the effect of interventions or aetiological factors on the risk of osteoporosis in Crohn's disease. By comparison, biochemical markers of bone metabolism are dynamic tests that are predictive for osteoporotic fracture (Clowes & Eastell, 2000; Garnero, 2000; Stepan, 2000) and may be used to assess bone turnover during inflammatory episodes or therapeutic interventions.

As there have been no reports of a relationship between bone turnover and disease severity, as determined by clinical symptom score, in Crohn's disease (Robinson *et al.* 1998; Schoon *et al.* 2001), this relationship has therefore been assessed using markers of the inflammatory response, CRP and ESR (Trebble *et al.* 2004*c*). Bone turnover markers, urinary deoxypyridinoline (DPD) and serum osteocalcin concentrations, were compared in males and premenopausal females with 'active' Crohn's disease (CRP ≥ 10 mg/l and/or ESR ≥ 20 mm/h; *n* 22) and controls with 'quiescent' Crohn's disease (CRP < 10 mg/l and ESR < 20 mm/h; *n* 21). DPD, a peptide derived from non-reducible pyridinium cross-links within mature collagen that is excreted in the urine following bone degradation and is therefore a marker of bone resorption (Clowes & Eastell, 2000), was corrected for urinary creatinine (Cre) concentrations (DPD/Cre). Osteocalcin, which is a non-collagenous protein synthesised by osteoblasts and incorporated within the bone matrix, is a marker of bone formation (Clowes & Eastell, 2000). The study also applied strict exclusion criteria for the cohorts, including the use of oral or intravenous corticosteroid therapy within the previous 4 weeks, or pharmacological interventions for the prevention or treatment of osteoporosis, e.g. hormone-replacement therapy or bisphosphonates, recent nutritional support or use of Ca or vitamin D dietary supplements.

The results of the study (Trebble *et al.* 2004*c*) show that DPD/Cre is higher in active Crohn's disease compared with quiescent Crohn's disease ($P = 0.02$), consistent with higher rates of bone resorption. Values for DPD/Cre were found to be above the reference range in thirteen of twenty-one (62%) patients with quiescent Crohn's disease and seventeen of twenty-two (77%) patients with active Crohn's disease, but these groups were not significantly different ($P = 0.27$). There is no difference in serum osteocalcin between active and quiescent Crohn's disease ($P = 0.24$). A comparison of the DPD/Cre:osteocalcin ratio in patients with active and quiescent Crohn's disease shows that values are significantly higher in active Crohn's disease ($P = 0.01$). These findings are not explained by differences in urinary Cre levels for which the DPD was adjusted.

Although the strict use of comprehensive inclusion and exclusion criteria for the study resulted in small cohorts, the findings are consistent with a relationship between laboratory markers of the inflammatory response and increased rates of bone resorption without a compensating increase in rates of bone formation.

In an investigation of nutritional status within the study cohorts values for BMI suggest normal levels of nutrition (BMI 19–25 kg/m^2) in nineteen patients (44%), overweight (BMI 25–30 kg/m^2) in nineteen patients (44%) and obesity (BMI > 30 kg/m^2) in five patients (12%), but no malnourished patients (BMI < 19 kg/m^2). When compared with age- and sex-matched healthy controls the active and quiescent Crohn's disease groups show no differences in anthropometric, body composition or BMI measurements, or in BMI alone ($P = 0.47$).

The relationship between nutritional status and the systemic inflammatory response has also been investigated in paediatric Crohn's disease (Trebble *et al.* 2003*b*). In this population disease activity may be associated with delayed pubertal growth spurt and reductions in growth velocity and, if not managed appropriately, final adult height (Kanof *et al.* 1988; Griffiths *et al.* 1993; Savage *et al.* 1999; Bremner & Beattie, 2002). Furthermore, children exhibit increased nutrient demands as a result of growth, and therefore the effects of inadequate dietary intake may be more clinically apparent. When thirty children with Crohn's disease were stratified first by paediatric Crohn's disease activity index (a hybrid clinical and laboratory score; Hyams *et al.* 1991) as exhibiting active (*n* 15) or the quiescent (*n* 15) disease, and then by laboratory markers of inflammation alone as having the active (CRP ≥10 mg/l and/or ESR ≥20 mm/h; *n* 14) or quiescent (CRP <10 mg/l and ESR <20 mm/h; *n* 16) disease, mean values for CRP ($P < 0.01$), paediatric Crohn's disease activity index ($P < 0.01$) and ESR ($P < 0.05$) were found to be significantly higher in the active disease groups compared with the quiescent disease groups, by either type of stratification (Trebble *et al.* 2003*b*).

Patients with active disease (by paediatric Crohn's disease activity index or by laboratory markers of inflammation) have significantly lower mid-upper arm circumference and triceps skinfold thickness compared with patients with quiescent disease. Additionally, patients with active disease identified by laboratory markers of inflammation exhibit a lower BMI centile, body-weight centile and percentage weight/weight-for-height centile compared with the quiescent disease group. Comparison of BMI centiles in patients with active and quiescent Crohn's disease (by CRP and ESR; Fig. 2) demonstrates contrasting distributions for each group. There are no significant differences between active and quiescent
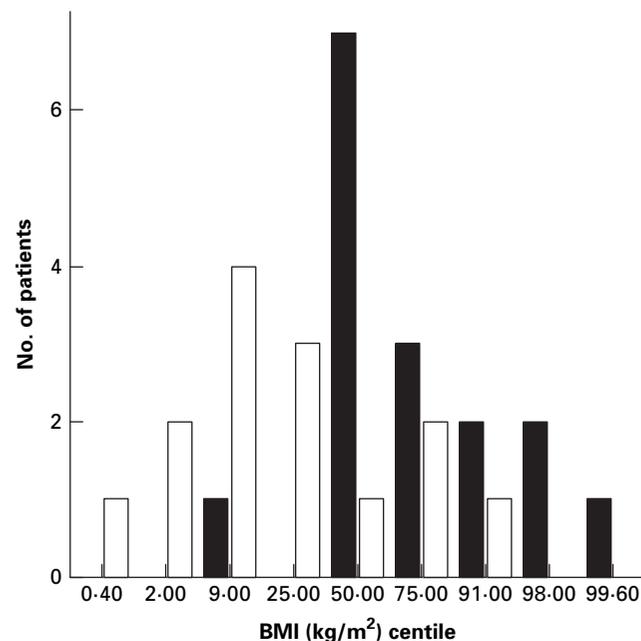

Fig. 2. Comparison of BMI centile in paediatric patients with Crohn's disease stratified by laboratory markers (C-reactive peptide (CRP) and erythrocyte sedimentation rate (ESR)) into active (■; *n* 14) and quiescent (□; *n* 16) disease.

disease groups in dietary energy or fat intake (using a food frequency questionnaire), but there are trends towards a lower intake of fish products in the active disease group compared with the quiescent disease group when stratified by paediatric Crohn's disease activity index ($P = 0.098$) and by laboratory markers of inflammation ($P = 0.059$). Mean CRP, ESR and paediatric Crohn's disease activity index are all significantly higher amongst patients with a BMI < 50th centile compared with those with a BMI >50th centile ($P = 0.004$, $P = 0.008$ and $P = 0.012$ respectively). These findings therefore support a relationship between nutritional status and laboratory markers of a systemic inflammatory response in paediatric Crohn's disease.

Peripheral blood mononuclear cell fatty acid composition and function in Crohn's disease

Local exposure to soluble inflammatory mediators, including cytokines and eicosanoids, may influence both inflammatory and physiological processes, including bone remodelling and protein and fat turnover, which may affect bone density and body mass respectively (Warren et al. 1987; MacDonald & Gowen, 1992; Baumann & Gauldie, 1994; Beisel, 1995). PBMC synthesise inflammatory mediators, relating to cell type, that may be altered in Crohn's disease (Pallone et al. 1987; Mazlam & Hodgson, 1992; Nakamura et al. 1992; Bouma et al. 1995; Bernstein et al. 1997).

EPA and AA compete in the PG and leukotriene synthetic pathways resulting in eicosanoid products of different C-skeleton lengths and saturations and with contrasting effects on the inflammatory response (Calder, 2003). Release of eicosanoids such as PGE_2 may influence cytokine synthesis by PBMC, including IFN-γ by T-cells. In healthy subjects dietary fish oil supplementation increases EPA availability and decreases AA availability in PBMC (Calder, 2003) and is associated with lower production of PGE_2 and TNF-α (Endres et al. 1989; Meydani et al. 1991). Thus, different availabilities of EPA and AA may influence PBMC production of inflammatory mediators and may explain the noted differences between Crohn's patients and healthy controls. There is evidence that differences in plasma concentrations of EPA and AA occur in Crohn's disease in the basal state compared with healthy subjects (Esteve-Comas et al. 1992, 1993; Kuroki et al. 1997; Geerling et al. 1999a,b). However, the relative availability of these fatty acids in the PBMC is not known.

The availability of n-3 and n-6 PUFA within PBMC and their production of inflammatory mediators (TNF-α, PGE_2 and IFN-γ) has been measured in fifty-two patients with Crohn's disease and in age- and sex-matched healthy controls (Trebble et al. 2004b). Dietary fat intake, quantitatively and qualitatively, was found to be similar for patients with active and quiescent Crohn's disease, and when compared with the controls. The proportion of EPA in PBMC is significantly higher in the cohort with Crohn's disease compared with controls, and AA is significantly lower ($P < 0.001$ in both cases). There are no significant differences in the PUFA composition of PBMC between active and quiescent Crohn's disease (defined by clinical or inflammatory markers). Although the DHA in plasma phosphatidylcholine is significantly lower ($P = 0.005$) for the cohort with Crohn's disease compared with controls, there are no other significant differences.

Using ELISA to measure the production of inflammatory mediators by PBMC incubated with and without the monocyte–macrophage stimulant lipopolysaccharide or the T-cell stimulant concanavalin A for 24 h, the cohort with Crohn's disease show a significantly lower production of IFN-γ by concanavalin A-stimulated PBMC ($P = 0.003$) compared with controls, but there are no other differences (Trebble et al. 2004b). Quiescent Crohn's disease (by inflammatory markers) is associated with significantly lower production of PGE_2 by unstimulated PBMC ($P = 0.008$) compared with controls, but there are no differences in cytokine production between active and quiescent Crohn's disease.

These findings suggest that Crohn's disease is associated with a greater availability, and not a deficiency, of n-3 PUFA in PBMC, but lower concentrations of AA, and lower rates of production of PGE_2 and IFN-γ, compared with healthy controls. There are, however, no significant differences in the production of inflammatory mediators between patients with the active and quiescent disease (defined by CRP and ESR). Nevertheless, the assessment of IFN-γ production by PBMC in the patients with active and quiescent disease (and fulfilling the strict study inclusion and exclusion criteria) who demonstrated differences in DPD/Cre (bone resorption marker), indicates that lower production of IFN-γ ($P = 0.02$) is associated with higher values for CRP and/or ESR and, therefore, higher values for DPD/Cre (reflecting bone resorption).

Lower production of IFN-γ by PBMC from patients with Crohn's disease compared with healthy controls has been described by other authors (Miura & Hiwatashi, 1985; Mutchnick et al. 1988; Sasaki et al. 1992). These findings contrast with the increase in IFN-γ production by mononuclear cells from the lamina propria reported in the literature (Sasaki et al. 1992; MacDonald et al. 2000; Colpaert et al. 2002) and would suggest that PBMC and mononuclear cells from the lamina propria exhibit contrasting responses to the inflammatory response in Crohn's disease. The results of Trebble et al. (2004b) also suggest that relatively high rates of bone loss are associated with lower rates of production of IFN-γ by PBMC, within the context of a systemic inflammatory response, identified by higher levels of laboratory markers (CRP and ESR).

Production of inflammatory mediators by peripheral blood mononuclear cells and bone turnover in Crohn's disease

A mechanism by which the systemic inflammatory response (increased CRP and ESR) may be associated with altered bone turnover remains unidentified, but the findings of Trebble et al. (2004b) are consistent with a role for PBMC that is characterised by altered production of inflammatory mediators, in particular the T-helper 1 cell cytokine IFN-γ. This possible mechanism is supported by evidence in the literature to suggest that altered production
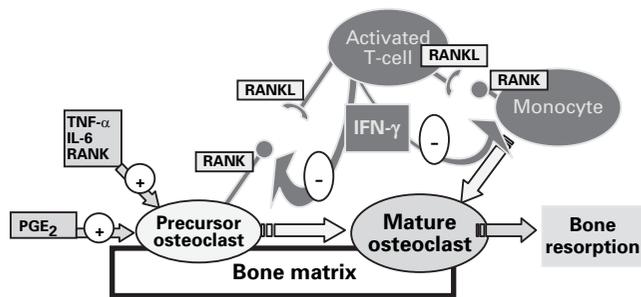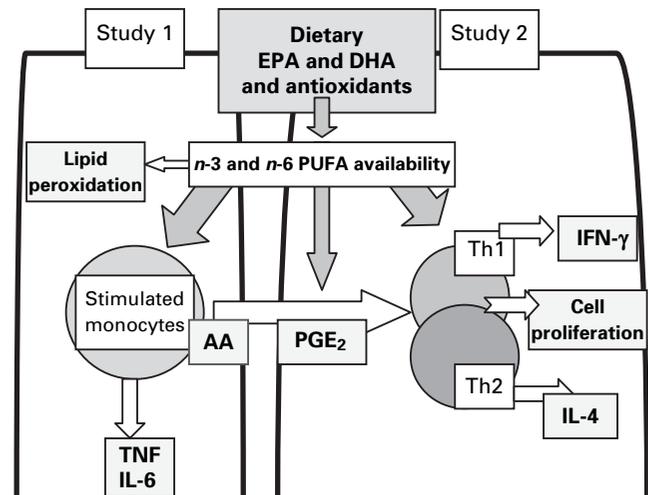
Fig. 3. Proposed relationship between T-cell function and osteoclast differentiation. PGE$_2$, prostaglandin E$_2$; IFN-γ, interferon-γ; +, promotes; –, inhibits.

Fig. 4. Proposed relationship between n-3 and n-6 PUFA availability and peripheral blood mononuclear cell function, and overview of fish oil and antioxidant dietary supplementation intervention studies in healthy subjects (Trebble et al. 2003b,c). AA, Arachidonic acid; PGE$_2$, prostaglandin E$_2$; IFN-γ, interferon-γ; Th, T-helper cell.

of cytokines by PBMC is associated with osteoporosis secondary to other inflammatory diseases (MacDonald & Gowen, 1992) and post-menopausal status (Pietschmann et al. 2001). There is also evidence of an association between osteoclasts, responsible for bone resorption, and macrophage–monocytes involved in the inflammatory response. Both cell types are derived from common progenitor cell lines (Miyamoto et al. 2001) and both respond to the release of TNF-α and other pro-inflammatory cytokines (Manolagas & Jilka, 1995; Roux & Orcel, 2000). Activated T-cells can directly promote osteoclast differentiation and function through expression of the membrane protein RANKL (Takayanagi et al. 2002; Fig. 3), which interacts with RANK on osteoclast precursor cells (Horwood et al. 1999; Kotake et al. 2001). IFN-γ inhibits the osteoclastic response to RANK activation and is released by activated T-cells during the inflammatory response, possibly as a counter-regulatory mechanism to prevent uncontrolled bone resorption (Arron & Choi, 2000). The higher rates of bone resorption noted in patients with Crohn's disease with a systemic inflammatory response, and noted by Trebble et al. (2004c), may therefore be explained by lower rates of production of IFN-γ by activated circulating T-cells and the subsequent loss of an inhibiting influence on osteoclast differentiation and function.

Fish oil and antioxidant supplementation and peripheral blood mononuclear cell function in healthy subjects

Dietary fish oil supplementation in healthy subjects has demonstrated the potential to alter production of inflammatory mediators including TNF-α, the T-cell cytokine IL-2 and the eicosanoid PGE$_2$ (Calder, 2001, 2003; Calder et al. 2002). However, the results of intervention studies are inconsistent and uncertainties remain relating to the dose–response relationship between EPA and DHA and the modulation of PBMC function, the effect of antioxidant co-supplementation and the relative response of cytokine production reflecting monocyte–macrophage and T-cell function (Fig. 4). These factors have been investigated in sixteen healthy male subjects allocated randomly, in a double-blind manner, to receive a daily antioxidant preparation containing 200 μg Se, 3 mg Mn, 30 mg D-α-tocopheryl succinate, 450 μg β-carotene and retinol (retinol

equivalent) and 90 mg ascorbic acid, or a placebo (maltose and lactose), over a 12-week period (Trebble et al. 2003b,c). Both groups received a concurrent 12-week course of increasing intakes of fish oil equivalent to a total EPA + DHA (+ DPA) intake of 0·3, 1 and 2 g/d for 4 weeks. The results suggest that production of TNF-α and IL-6 by PBMC is decreased compared with baseline with each intake of fish oil (all $P < 0·05$), but tends towards a 'U-shaped' dose response (Trebble et al. 2003a). However, fish oil supplementation decreases PGE$_2$ production (all $P < 0·05$) and increases IFN-γ production (at 2 g/d, $P < 0·05$), and lymphocyte proliferation, from baseline values (Trebble et al. 2003c). Co-supplementation with antioxidants does not consistently affect cytokine production or lymphocyte proliferation.

Fish oil and antioxidant supplementation and peripheral blood mononuclear cell function in Crohn's disease

The studies of Trebble et al. (2003b,c) suggest an association between dietary supplementation with the long-chain n-3 PUFA EPA and DHA as fish oil and PBMC function in healthy subjects, both with and without antioxidants. There is evidence of an anti-inflammatory effect of fish oil on disease activity assessed symptomatically (by clinical disease score) in Crohn's disease (Belluzzi et al. 1996). However, the response of PBMC function to dietary supplementation with fish oil or antioxidants in Crohn's disease is not known and has therefore been investigated in a randomised double-blind placebo-controlled trial in adult patients with Crohn's disease (Trebble et al. 2004a). The fish oil intervention was equivalent to an additional intake of 2·7 g EPA and DHA/d. Patients received fish oil or a placebo in addition to estimated habitual dietary intakes of n-3 and n-6 PUFA

consistent with estimated mean values for the UK population of 1·8 and 10·2 g/d respectively (British Nutrition Foundation, 1999). In the UK population estimated habitual EPA and DHA intake accounts for <0·25 g/d, and *in vivo* synthesis from the essential fatty acid α-linolenic acid, the major component of *n*-3 PUFA intake, is inefficient (Sanderson *et al*. 2002). The level of supplementary intake of EPA and DHA used was derived from a previous fish oil study demonstrating anti-inflammatory effects in Crohn's disease (Belluzzi *et al*. 1996), and represented an increase of more than ten times the estimated habitual intake in the general UK population. Fish oil was administered with a nutritional antioxidant co-supplement that contained quantities of Se and vitamins A and C equivalent to ≥50 % of the reference nutrient intakes (Department of Health, 1991), and of vitamin E equivalent to four times the estimated average requirement in the UK population. In the cohort with Crohn's disease the antioxidant supplement was found to result in a higher plasma concentration of Se and a trend towards higher plasma concentrations of vitamin E. In healthy subjects antioxidant co-supplementation has a negligible effect on either EPA incorporation in plasma phospholipid or cytokine release by PBMC (Trebble *et al*. 2003*b*,*c*).

The inclusion criteria for this intervention study included CRP >6·9 mg/l and/or ESR >18 mm/h within the previous 4 weeks, and the exclusion criteria included: severe disease (Crohn's disease activity index (Best *et al*. 1976) >450); oral or intravenous corticosteroid medication within the previous 4 weeks; introduction of other immunosuppressant medication within the previous 8 weeks (Trebble *et al*. 2004*a*). Patients consumed daily nine capsules of fish oil or placebo (olive) oil in addition to their habitual diet. Fish oil plus antioxidants was found to be associated with significantly greater incorporation of EPA ($P < 0·001$), docosapentaenoic acid ($P = 0·001$) and DHA ($P < 0·001$) within PBMC compared with placebo, and a significantly lower incorporation of AA ($P = 0·006$), with other fatty acids, including oleic acid, showing no significant differences. Fish oil plus antioxidants was shown to be associated with significantly lower production of IFN-γ by concanavalin A-stimulated PBMC ($P = 0·012$) and of PGE$_2$ by lipopolysaccharide-stimulated PBMC ($P = 0·047$) compared with the placebo group. The production of TNF-α by lipopolysaccharide-stimulated PBMC was not found to be different between groups.

Fish oil and antioxidants and bone turnover and nutritional status in Crohn's disease

Cohorts with Crohn's disease show no significant differences between supplementation with fish oil plus antioxidants (*n* 31) and a placebo (*n* 30) in the response of absolute values for Crohn's disease activity index, CRP or ESR (TM Trebble, unpublished results). There are no significant differences between groups in the response of BMI, anthropometry or body composition nor any significant differences between groups in the response of absolute values for DPD/Cre ($P = 0·290$), osteocalcin ($P = 0·562$) or DPD/Cre:osteocalcin ($P = 0·400$). The

failure to demonstrate an effect on Crohn's disease activity contrasts with the response to fish oil alone noted by Belluzzi *et al*. (1996). The latter study, however, was designed to assess continued disease remission in patients with a high risk of disease relapse, while the former study aimed to demonstrate an absolute or quantitative change in bone turnover that may reflect a relative improvement in gastrointestinal Crohn's disease.

Conclusion

The theoretical basis for the studies of Trebble *et al*. (2004*a*,*b*,*c*) relates to the possible role of the circulating and inappropriately-functioning mononuclear cells as the vector for the dissemination of the extra-intestinal manifestations of Crohn's disease. As fish oil is an intervention with recognised modulatory effects on PBMC function, relationships between fatty acid availability and function of PBMC, clinical disease activity, nutritional status, bone turnover and the systemic inflammatory response state were investigated.

The results are consistent with a role for the PBMC in pathological bone modelling in Crohn's disease and the modification of its synthetic function by fish oil in combination with antioxidants. However, fish oil and antioxidants are not associated with a reduction in clinical disease scores (Crohn's disease activity index) or laboratory markers of inflammation (CRP or ESR), or an effect on bone turnover or nutritional status. There are a number of possible explanations for the absence of a clinical response in the presence of altered PBMC function. First, altered PBMC function may be only one of a number of factors that may modulate bone remodelling in inflammation. Second, effective therapeutic interventions for metabolic bone disease may act by modulating bone via an effect on gastrointestinal inflammation and the systemic inflammatory response independent of normal or altered PBMC function. Third, altered bone turnover during the inflammatory response may reflect the function of mononuclear cell populations within the bone and not PBMC. Finally, the effect of vitamin A, within the antioxidant co-supplement, is uncertain but may have detrimentally increased bone loss, negating any positive effects of the fish oil and antioxidant intervention.

The results of these studies have a number of clinical implications. Fish oils and antioxidants may have important pharmacological effects on the function of immune cells from the peripheral blood. There is a theoretical basis for an associated therapeutic effect on extra-intestinal manifestations of Crohn's disease, but there are no demonstrated effects. The immunological effects of high-dose fish oils and antioxidants do not appear to lead to any of the common side-effects seen with other anti-inflammatory interventions, e.g. the effects on bone turnover noted following corticosteroid use. This finding would support the use of high-dose fish oils and antioxidants as potent, but 'comparatively' safe, pharmacological agents. The effect of lower doses of fish oil on bone turnover, or other extra-intestinal manifestations, in Crohn's disease is uncertain. Further work in this area is indicated.

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