The effect of n-6 fatty acid supplementation on pro-inflammatory cytokine

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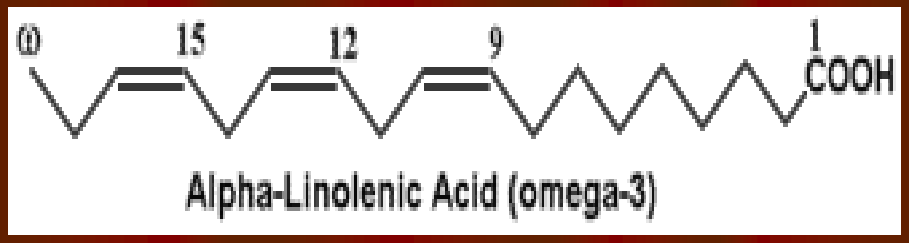
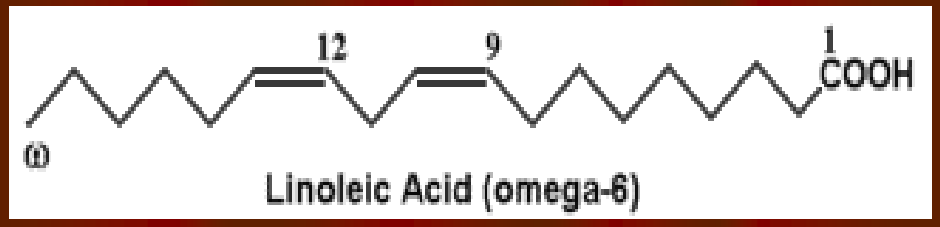
Introduction

There are several families of polyunsaturated fatty acids including the n-3, n-6 and n-9. Essential fatty acids cannot be synthesized by humans, so must be obtained through diet.

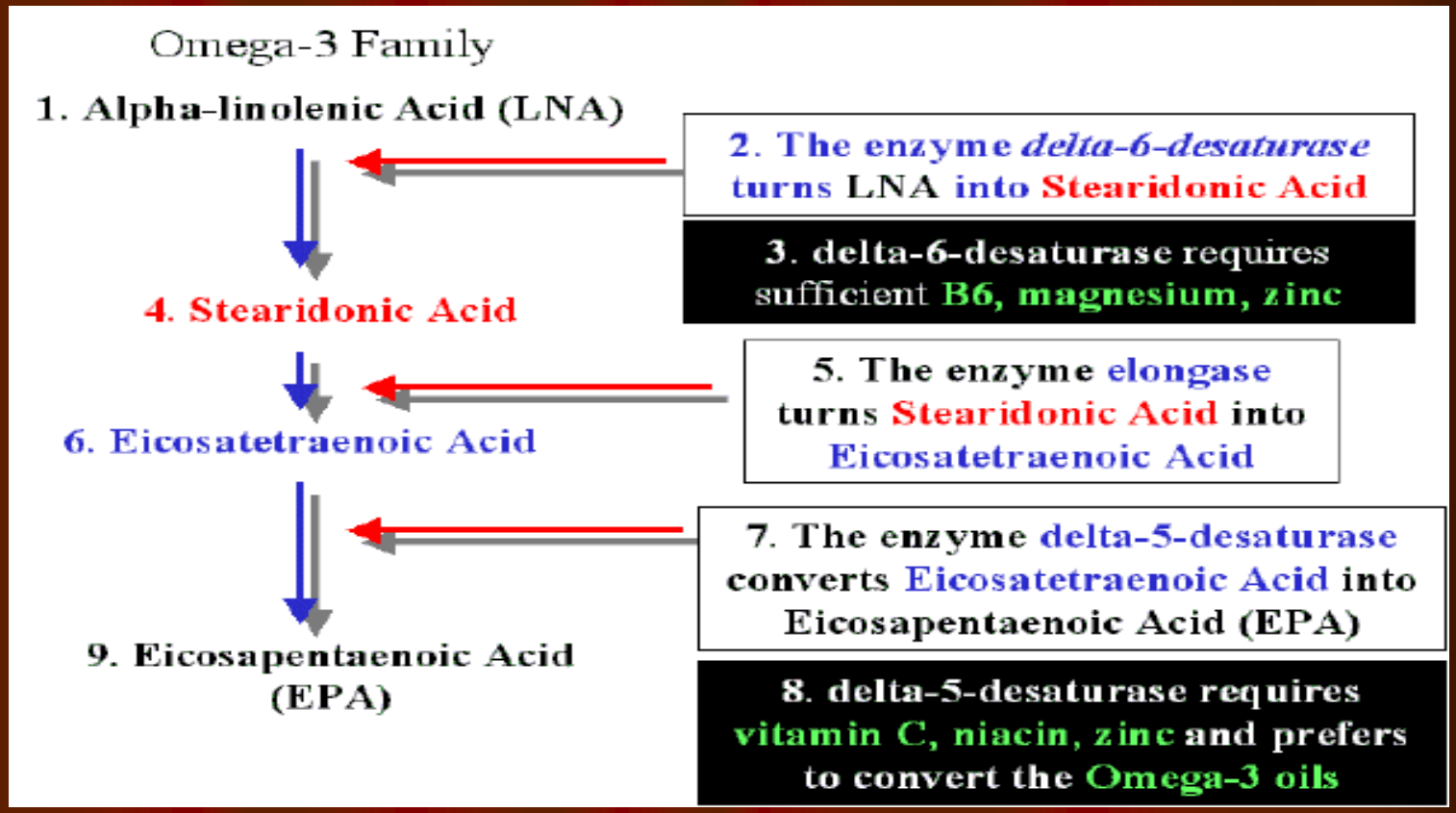


Alpha-Linolenic Acid (omega-3)

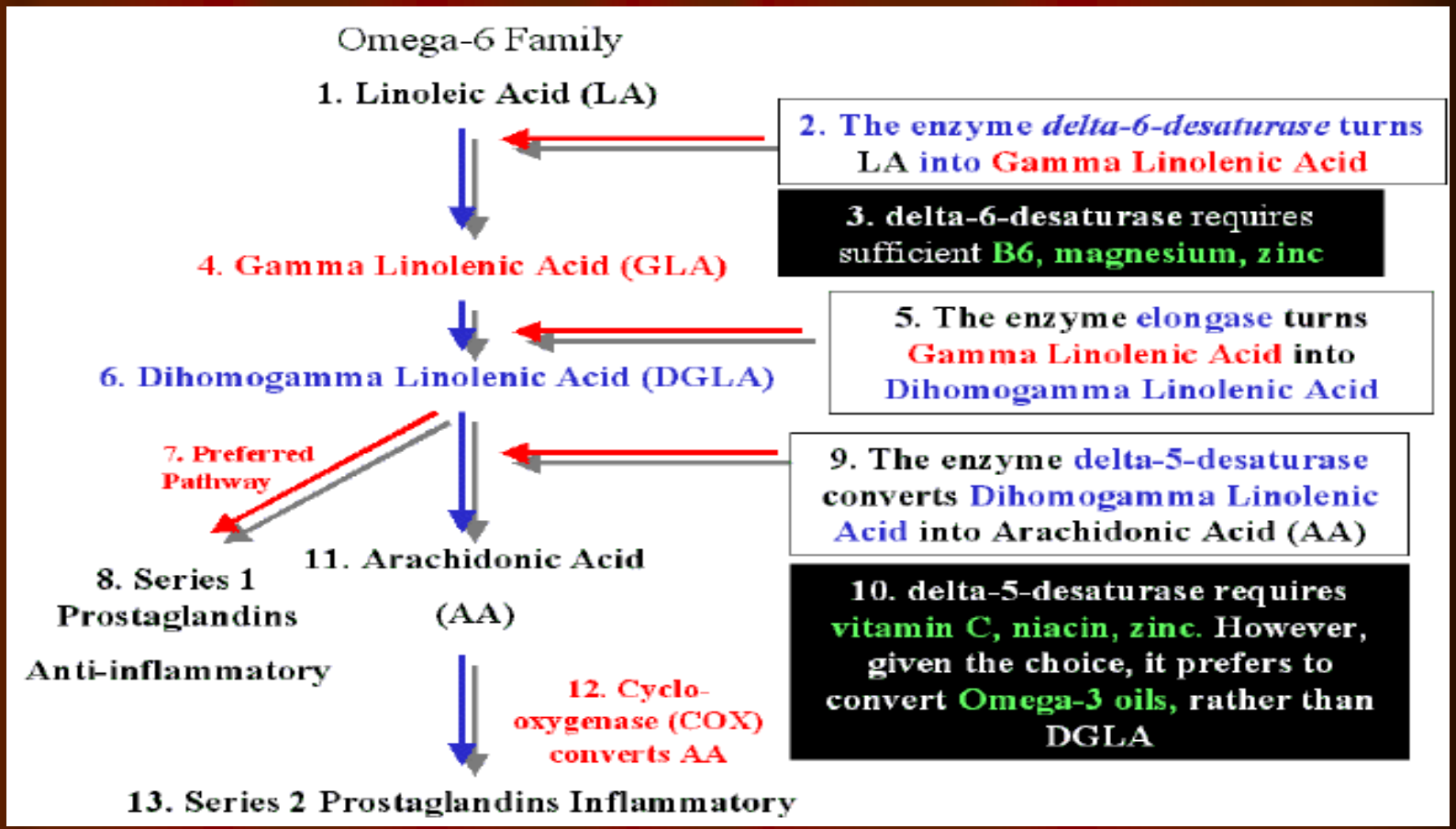


Linoleic Acid (omega-6)

Metabolism of n-3 fatty acids

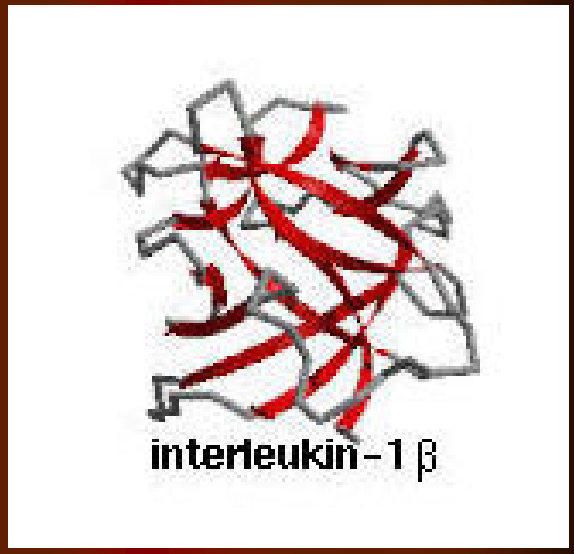


Metabolism of n-6 fatty acids



Interleukin 1-β

- **The *interleukin-1* family of cytokines are 17-20k polypeptides having essential physiological roles i.e. host defence, wound healing, programmed cell death ,inflammation, (Dinarello et al, 1996).**
- **IL-1β activates cyclooxygenase-2 (Cox-2) expression with a release of prostaglandin E2 (PGE2) (Bachman *et al*,1998).**



Structure of IL-1 β

Anti-inflammatory effects of PUFAs.

- Epidemiological evidence suggests that n-3 PUFA can influence inflammation and immune function.
- Endres et al (1989,1993), Meydani et al (1991,1993) and others have found that supplementation of the human diet with n-3 PUFA decreases the production of TNF-α and IL-1β by human PBMCs.
- However, the effects of n-6 PUFAs upon cytokine production have not been studied.

Aim / objective:

- To investigate the effects of n-6 supplementation in healthy volunteers in relation to PBMC pro-inflammatory cytokine IL-1β.

Subjects

- Eight healthy male (mean age 34 years; range 22-52) and female volunteers (mean age 34; range 22-45) were enrolled from laboratory personnel. The study was approved by the Ethics Committee of the Lambeth, Southwark and Lewisham Health Commission, St Thomas' Hospital London, UK.
- The subjects (4 males and 4 females) ingested 15 ml of refined borage seed oil (Quest Vitamins, Birmingham, UK) daily for 12 weeks, while maintaining their normal dietary habits.
- The borage oil contained (w/w): 37 % linoleic acid (18:2n-6), 22 % γ-linolenic acid (18:3n-6), 17.8% oleic acid (18: 1n-9) and 9.7 % palmitic acid (16:0). The oil was stabilised with dl α-tocopherol (50 mg /kg) and ascorbyl palmitate (200mg/kg).

Method

Separation of blood to obtain peripheral blood mononuclear cells.

- **Peripheral venous blood (50 ml) was obtained byvenepuncure into centrifuged tubes with heparin (50 IU). The remainder was diluted 1:1 with Hanks Balanced Salt Solution (HBSS,Gibco) before being layered onto lymphoprep (Nycomed) density gradient.**
- **A further centrifugation at 2000rpm for 25 minutes, separated the mononuclear cells and neutrophils. Peripheral blood mononuclear cells (PBMC) at the interface were removed by using plastic pipette.**
- **Giemsa staining routinely revealed the proportions of lympocytes (77-83%)and monocytes (11-18%) in PBMC preparations.**

Method

Production and Detection of IL-1 β by PBMC

- ***Ex vivo*** **PBMC were stimulated by bacterial lipopolysaccharide (LPS) at concentrations of 0, 50, 100 ng/ml, 1µg/ml and 5 µg/ml.**

- **The concentration of IL-1β in PBMC supernatants was detecting using commercially available paired antibodies in an ELISA format(DuoSeTs).**

Statistical Analysis

- An **Independent t-test** was used to determine any significant difference in IL-1β levels at different concentrations of LPS.
- A **Pearson's Correlation Coefficient Test** was to measure the strength of a linear correlation between LPS concentrations and the mean values of IL-1β.
- A **Mann Whitney Test for Independent Samples** to determine whether significant differences in IL-1β production between baseline and week 12.
- A ***P* value < 0.05** was taken to indicate statistical significance.

RESULTS

1a.Effects of LPS on PBMC IL-1β production

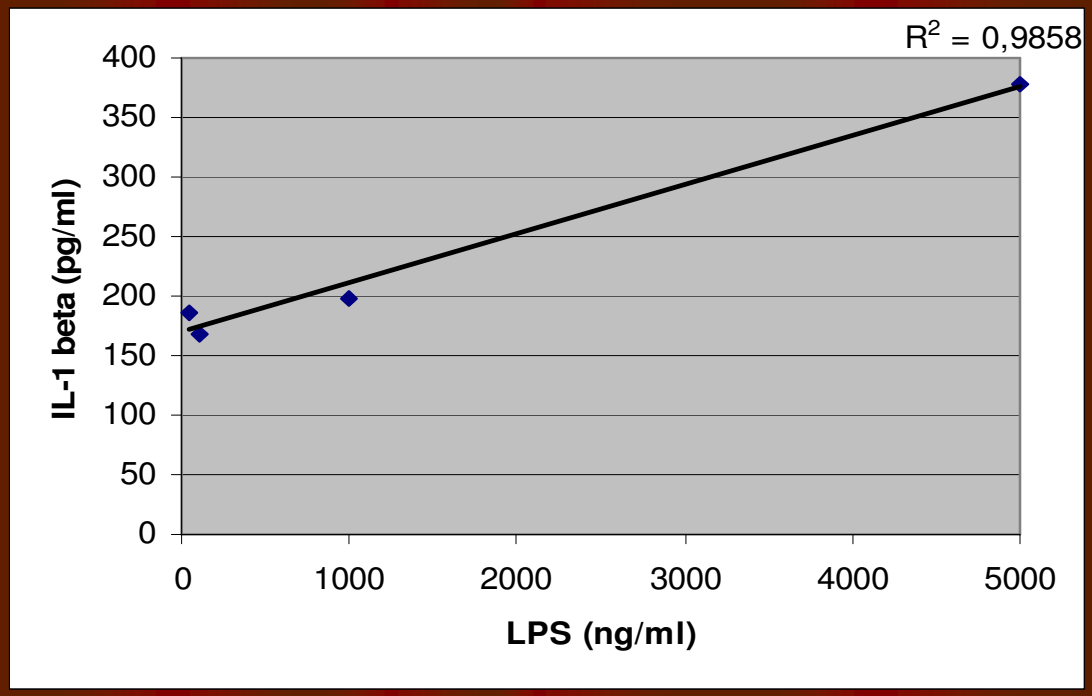


Fig 1. Effects of LPS on individual values of *IL-1β* release from human peripheral blood mononuclear cells (PBMC).

Results

1b.Effects of LPS on PBMC IL-1β production

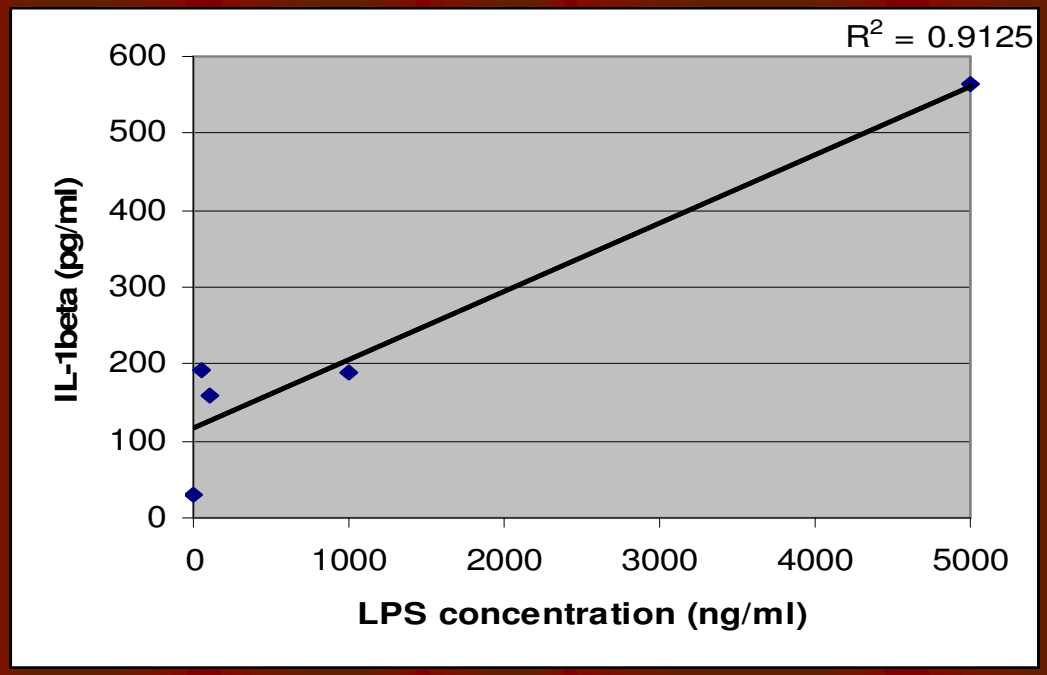


Fig 2. Dose-dependent effects of LPS on PBMC IL-1β production. IL-1β values are presented as means.

Results

1c.Effects of LPS on PBMC IL-1β production

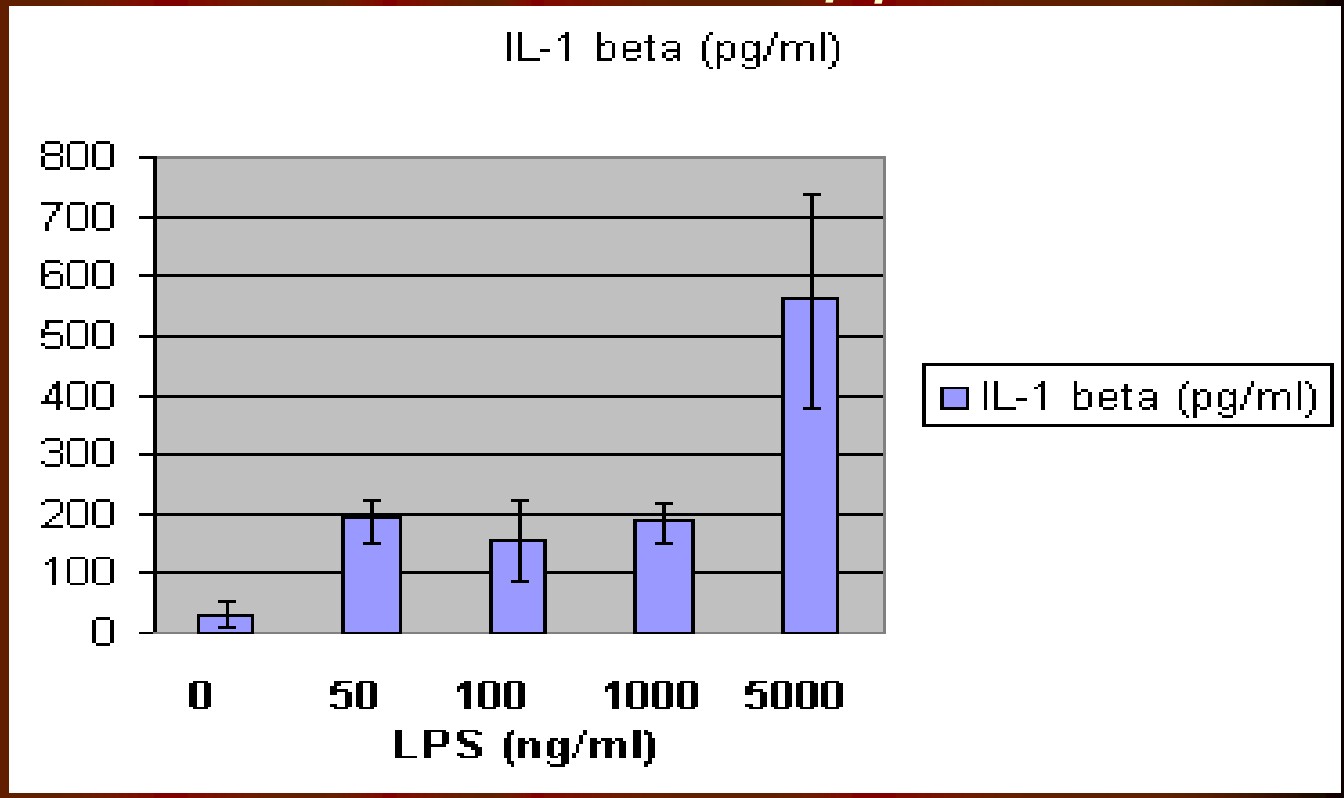


Fig 3. The cytokine production at higher LPS levels was significantly different from that at lower LPS levels ($P < 0.05$). Values are means and standard deviations represented by vertical bars for the five concentrations of LPS.

Results

2. *Effects of n-6 PUFA in the production of IL-1β*

- Fig 4. shows the effects of GLA-rich oil supplementation on the mean IL-1β production, at week 0 and 12.
- The concentration of LPS used to stimulate the PBMCs was 5 µg/ml.
- Mean values obtained at week 0 and 12 were not significantly different (P> 0.05).
- Abbreviations used: w0, week 0; w12, week 12.

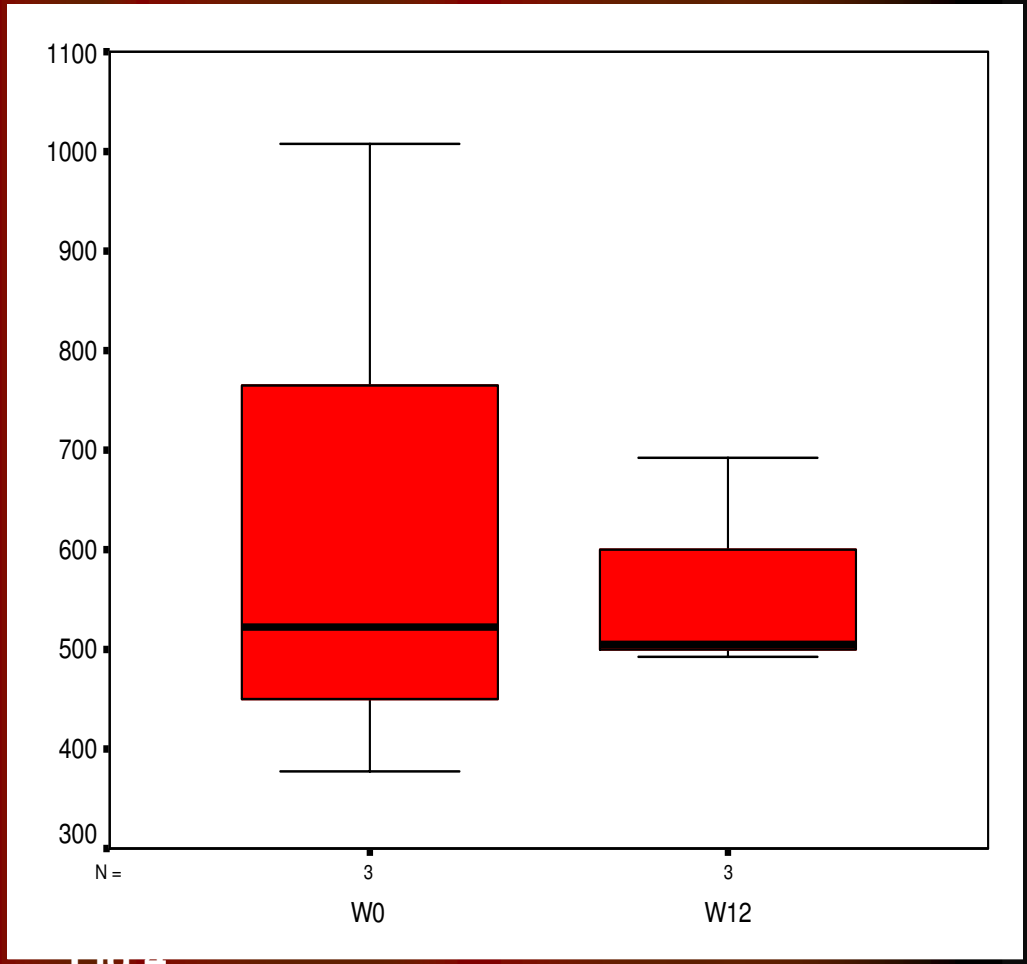


Fig 4

Results

3a. Individual variation in IL-1β production in control group

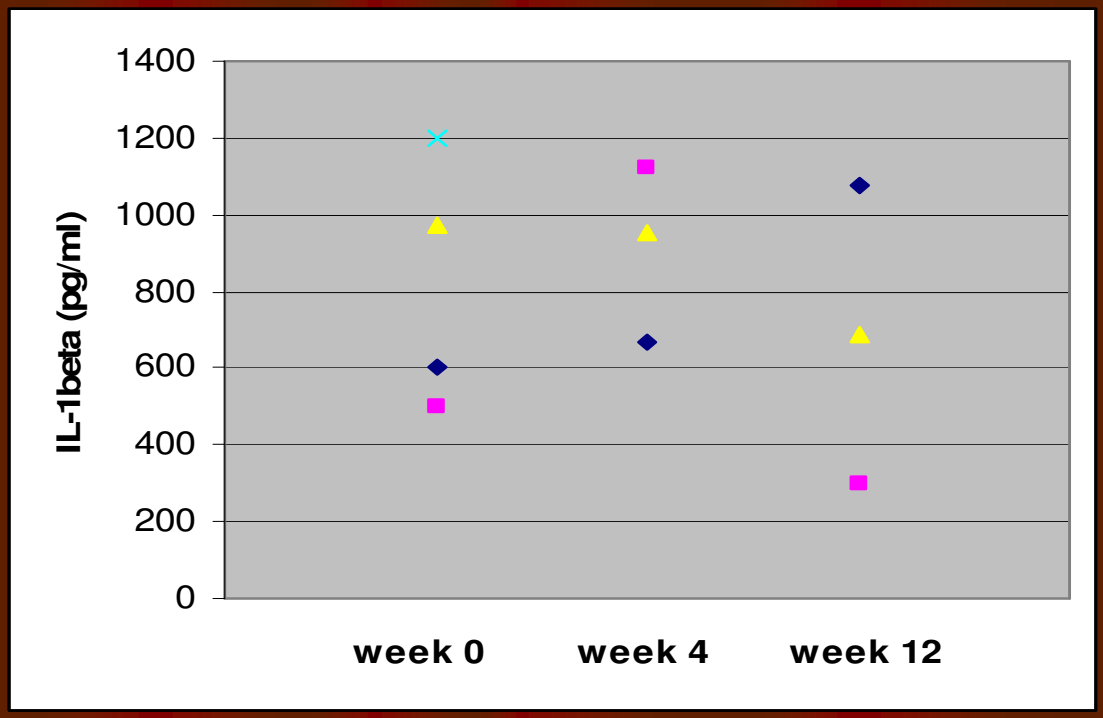


Fig 5. Individual values from normal – control (unsupplemented) group at week 0,4 and 12. The cytokine IL-1β was detected after a stimulation of peripheral blood mononuclear cells with 5 µg/ml LPS.

Results

3b. Individual variation in IL-1β production in BO supplemented group

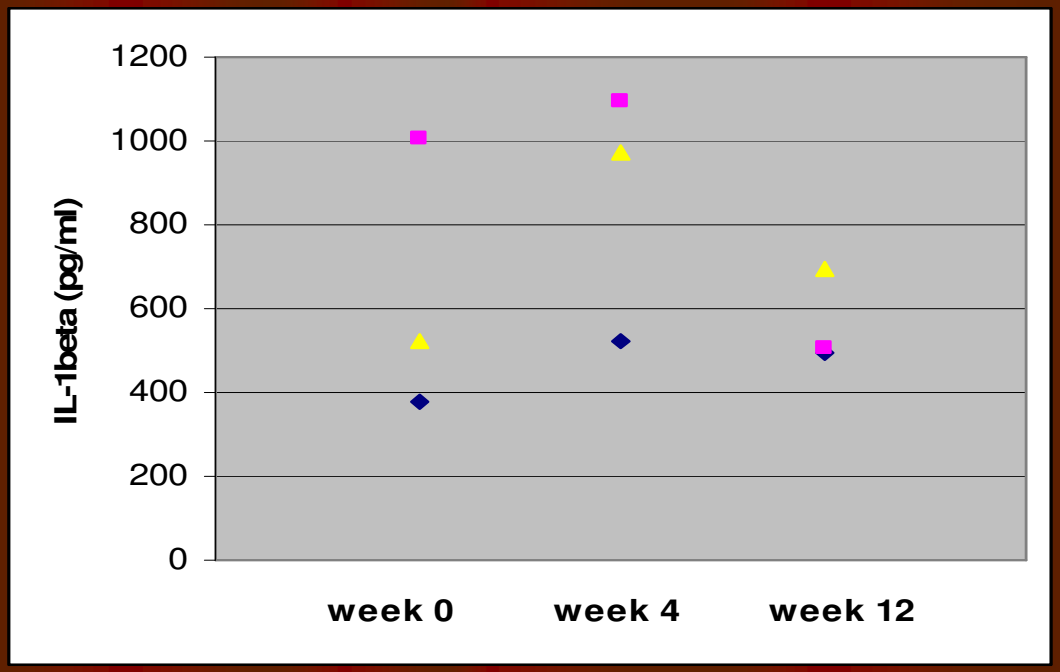


Fig 6. Variations in individual values from borage oil group at week 0,4 and 12. The cytokine IL-1β was detected after a stimulation of PBMCs with 5 µg/ml LPS.

Discussion

- In this study involving dietary supplementation with borage seed oil, an increase in the intake of n-6 fatty acids (LA and GLA in the form of borage oil) did not show any significant difference in the PBMC IL-1 β production after 12 weeks of supplementation.
- However, it might be a decrease in IL-1 β production in some individuals from both groups. This can be explained ,since some individuals may respond to the n-6 PUFA supplementation, while some others may not.
- In previous studies, Thies *et al* (2001) also reported no significant alteration in the production of TNF-a, IL-1β and IL-6 after a daily ingestion of 2g ALA, and approximately 700 mg GLA, ARA or DHA or 1g EPA and DHA.
- However, the current experimental observations are not consistent with those of other studies in which GLA significantly alters the IL-1 β production (De Luca *et al* ,1999) .

Discussion

The pro-inflammatory effects of GLA are independent of IL-1 beta secretion.

Other suggested mechanisms:

- ✓Suppression in LTB_4 and PG_2 release (Pullman-Mooar *et al* ,1990 & Tate *et al,* 1989) .
- ✓Increase the production interferon –γ (IFN-γ) (Harbige & Fisher,unpublished results).
- ✓Elevation of the Transforming Growth Factor- beta 1(TGF-β1) from PHA-stimulated PBMCs.
- ✓Suppressed Th2 production of IL-4 and IL-10 (Harbige *et al*, 2003, Fisher, and Harbige ,1997).
- ✓GLA can modify the signal transduction pathway of T cells (Vassilopoulos *et al*, 1990).

Discussion

- In this study, ingestion of borage seed oil was confirmed by membrane fatty acid analysis. Biochemical analysis by GLC within Dr.Harbige's laboratory at the University of Greenwich, showed significantly increased levels of DHGLA and AA in the PBMC membrane phospholipids.

- Although many studies have confirmed prothromnotic, and pro-inflammatory properties of high n-6 PUFA intakes, a moderate consumption may have a protective role mainly due to the anti-inflammatory action of GLA, DGLA and AA.

- Further research is required to identify the effects of n-6 PUFA on other immunoregulatory molecules such as TNF- α, TGF-β1 and PGE2.

Conclusion

- This study found no difference in the *ex vivo* PBMC production of IL-1β between healthy volunteers taking 15 g borage oil for 12 weeks and those who were not taking supplementation.

- Regardless of the action of n-6 PUFA, an optimal balance between n-3 and n-6 PUFAs should be recommended to maintain healthy physiological responses.

References

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- Thies F, Miles EA, Nebe-von-Caron G, Powell JR, Hurst TL, Newsholme EA, Calder PC.(2001) Influence of dietary supplementation with long-chain n-3 or n-6 polyunsaturated fatty acids on blood inflammatory cell populations and functions and on plasma soluble adhesion molecules in healthy adults.*Lipids.* 36(11):1183-93 .