UNIVERSITÉ DE LAUSANNE - FACULTÉ DE BIOLOGIE ET DE MÉDECINE
Service de Médecine Intensive Adulte
Chef de service : Professeur René Chioléro

Effects of fish oil on the neuro-endocrine responses to an endotoxin challenge in healthy volunteers

THESE

présentée à la Faculté de biologie et de médecine de l'Université de Lausanne pour l'obtention du grade de

DOCTEUR EN MEDECINE

par

Burkhard MICHAELI

Médecin diplômé de la Bundesrepublik Deutschland
et reconnu par la Confédération Suisse
Originaire de Saarbrücken (D)

Lausanne
2007

Résumé

Introduction et hypothèse : Certains acides gras polyinsaturés de type n-3 PUFA, qui sont contenus dans l'huile de poisson, exercent des effets non-énergétiques (fluidité des membranes cellulaires, métabolisme énergétique et prostanoïdes, régulation génique de la réponse inflammatoire). Les mécanismes de la modulation de cette dernière sont encore mal connus.
L'administration d'endotoxine (LPS) induit chez les volontaires sains une affection inflammatoire aiguë, comparable à un état grippal, associé à des modifications métaboliques et inflammatoires transitoires, similaires au sepsis. Ce modèle est utilisé de longue date pour l'investigation clinique expérimentale.
Cette étude examine les effets d'une supplémentation orale d'huile de poisson sur la réponse inflammatoire (systémique et endocrinienne) de sujets sains soumis à une injection d'endotoxine. L'hypothèse était que la supplémentation d'huile de poisson réduirait les réponses physiologiques à l'endotoxine.

Méthodes : Quinze volontaires masculins (âge 26.0±3.1 ans) ont participé à une étude randomisée, contrôlée. Les sujets sont désignés au hasard à recevoir ou non une supplémentation orale : 7.2 g d'huile de poisson par jour correspondant à un apport de 1.1 g/jour d'acides gras 20:5 (n-3, acide écosapentaénoïque) et 0.7 g/jour de 22:6 (n-3, acide docosahexaénoïque).
Chaque sujet est investigué deux fois dans des conditions identiques : une fois il reçoit une injection de 2 ng par kg poids corporel de LPS intraveineuse, l'autre fois une injection de placebo. Les variables suivantes sont relevées avant l'intervention et durant les 360 min qui suivent l'injection : signes vitaux, dépense énergétique (EE) et utilisation nette des substrats (calorimétrie indirecte, cinétique du glucose (isotopes stables), taux plasmatique des triglycérides et FFA, du glucose, ainsi que des cytokines et hormones de stress (ACTH, cortisol, Adré, Nor-Adré).

Analyses et statistiques : moyennes, déviations standards, analyse de variance (one way, test de Scheffé), différences significatives entre les groupes pour une valeur de $p < 0.05$.

Résultats : L'injection de LPS provoque une augmentation de la température, de la fréquence cardiaque, de la dépense d'énergie et de l'oxydation nette des lipides. On observe une élévation des taux plasmatiques de TNF-α et IL-6, de la glycémie, ainsi qu'une élévation transitoire des concentrations plasmatiques des hormones de stress ACTH, cortisol, adrénaline et noradrénaline. L'huile de poisson atténue significativement la fièvre, la réponse neuro-endocrinienne (ACTH et cortisol) et sympathique (baisse de la noradrénaline plasmatique). Par contre, les taux des cytokines ne sont pas influencés par la supplémentation d'huile de poisson.

Conclusion : La supplémentation d'huile de poisson atténue la réponse physiologique à l'endotoxine chez le sujet sain, en particulier la fièvre et la réponse endocrinienne, sans influencer la production des cytokines. Ces résultats soutiennent l'hypothèse que les effets bénéfiques de l'huile de poisson sont principalement caractérisés au niveau du système nerveux central, par des mécanismes non-inflammatoires qui restent encore à élucider.

Clinical Nutrition (2007) 26, 70–77

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevierhealth.com/journals/clnu

ORIGINAL ARTICLE

Effects of fish oil on the neuro-endocrine responses to an endotoxin challenge in healthy volunteers ☆

Burkhard Michaeli[a], Mette M. Berger[a,*], Jean-Pierre Revelly[a], Luc Tappy[b], René Chioléro[a]

[a]*Department of Intensive Care Medicine, CHUV, Rue du Bugnon 46, CH-1011 Lausanne, Switzerland*
[b]*Department of Human Physiology, University of Lausanne, Rue du Bugnon 7, CH-1005 Lausanne, Switzerland*

Received 13 February 2006; accepted 13 June 2006

KEYWORDS
Endotoxin challenge;
Lipopolysacharide;
Fish oil supplement;
Cytokines;
Inflammatory response;
Neuro-endocrine response

Summary
Background & aims: Fish oil (FO) has been shown to modulate the acute and chronic inflammatory responses. Endotoxin (LPS) has been shown to mimic several aspects of sepsis. The study aimed at testing the effects of oral FO supplements in healthy subjects submitted to intravenous LPS on systemic and endocrine response.
Subjects and methods: Fifteen healthy men (aged 26.0±3.1 years, BMI 23.8±1.9 kg/m^2), were enrolled. Subjects were randomised to 3–4 weeks of oral FO supplementation (7.2 g/day, providing 1.1 g/day of 20:5(n-3) and 0.7 g/day of 22:6 (n-3) fatty acids) or no supplementation and then submitted to endotoxin challenge: 2 ng/kg of LPS. All subjects were studied twice (placebo and LPS). Measurements: vital signs, energy expenditure (EE), glucose and lipid metabolism ($^2H_2$-glucose), plasma cytokines and stress hormones for 6 h after LPS or placebo.
Results: LPS caused cytokine release, fever, increases in heart rate, resting EE and substrate oxidation, plasma glucagon and glucose concentrations; the neuro-endocrine response was characterised by increased plasma stress hormones. FO significantly blunted fever, ACTH and cortisol plasma levels (no effect on cytokine release). FO blunted the peak norepinephrine after LPS.
Conclusion: FO supplements blunted the endocrine stress response and the increase in body temperature, but had no impact on cytokine production after LPS. These findings conflict with the postulated anti-inflammatory effects of FO on arachidonic acid

☆Financial disclosure: None of the authors has any commercial associations or conflicts of interest to disclose in connection with the present paper. The study was partially supported by the Swiss national found of research Grant no.3200BO-1020064.

*Corresponding author. Tel.: +41 21 314 20 02; fax :+41 21 31 43 045.
E-mail address: Mette.Berger@chuv.ch (M. M. Berger).

doi:10.1016/j.clnu.2006.06.001

metabolism and cytokine release. These results suggest that FO may exert beneficial effects in sepsis though non-inflammatory which require further investigations

Introduction

Fatty acids constitute an important source of endogenous and exogenous energy, providing usually 30–45% of daily energy supply. Among them, two families are particularly important for human health, the n-6 and the n-3 polyunsaturated fatty acids (n-6 PUFAs and n-3 PUFAs). The n-6 PUFA linoleic acid is found in substantial amounts in vegetable seeds and oils and is the precursor of arachidonic acid. Some n-3 PUFA are also found in vegetable seeds and oil, but the main active of these compounds, docosahexaenoic acid (DHA, 20:5 (n-3)) and ecospentaenoic acid (EPA, 22:6 (n-3)) are essentially found in marine products and fish oils (FO).

N-3 and n-6 PUFAs are actively incorporated into cell membrane phospholipids and modify the membrane physical and chemical characteristics.[1,2] They exert numerous biological effects, influencing metabolic pathways and numerous cell functions. They have also been shown to modulate the acute and chronic inflammatory processes and to exert anti-inflammatory properties both in vitro in vivo—while n-6 PUFAs upregulate the inflammatory processes. The mechanisms of the anti-inflammatory effect of n-3 PUFAs are complex, including changes in membrane fluidity, synthesis of lipid mediators, cytokine production, inflammatory gene expression and remain yet not fully understood.[3,4] Recent in vivo and in vitro studies suggest that n-3 PUFAs may decrease the inflammatory response to both *acute and chronic* inflammatory diseases.[5–12]

Intravenous endotoxin (LPS) induces a reproducible and transient inflammatory condition in animals and healthy humans, which mimics several aspects of sepsis.[13] This healthy subject model was used in the present study to test the hypothesis that oral FO supplementation attenuates the physiologic response to intravenous endotoxin in healthy subjects, particularly the inflammatory response.

Subjects and methods

Sixteen healthy males were enrolled. All had a normal body weight, with no metabolic or endocrine disease, or medication. They all underwent thorough clinical evaluation of their cardiopulmonary status and had an ECG recording during an inclusion visit. Mean age was 26.0 years ($\pm$3.1), mean body weight 75.3$\pm$7.3 kg, and mean BMI 23.8$\pm$1.9 kg/m^2.

Experimental protocol

The study was conducted with Institutional Ethical committee approval and individual written informed consent. The experiments were performed under the supervision of critical care physicians.

Sixteen subjects were randomised to an intravenous LPS-challenge alone ($n = 8$) or to an intravenous LPS-challenge preceded by 3–4 weeks oral FO supplementation ($n = 8$) (7.2 g/day of OMEGA-3 Gisand® containing 17% EPA, 11% DHA). Each subject was studied twice: placebo and LPS-challenge were separated by 1 week at least and in a random order. One subject of the FO group interrupted the research protocol. The subjects came to the metabolic laboratory at 7 AM after an overnight fast. Upon arrival, they were requested to void and urine was discarded. Thereafter, they remained supine quietly in a bed for the next 10 h. An antecubital vein was cannulated for labelled glucose and lactate infusion. A dorsal hand vein of the opposite arm was cannulated to sample partially arterialised blood samples (infrared heating of the hand).[14]

After blood sampling for determination of basal glucose isotope enrichment and basal lactate, a primed continuous infusion of 6,6 $^2H_2$ glucose was infused (Cambridge Isotope Laboratory, Cambridge, MA; prime 3 mg/kg/min, continuous 30 μg/kg/min) from T-180 (180 min before intravenous LPS) throughout the study. After 2.5 h of tracer equilibration, blood samples were collected at a 30-min interval until the end of test. In addition, ^{13}C-labelled lactate at 0.03% excess (10 μmol/kg/min) was infused from T-90 until T270. Systemic lactate kinetics (lactate clearance and endogenous production) were assessed by means of a modified Connor's method.[15] Breath samples were collected every 30 min into 10 ml hexatainers (Europa Scientific Crew, UK) in quadruplate to determine $^{13}CO_2$ excretion (as reflect of lactate use as metabolic substrate). Glucose turnover (6,6 $^2H_2$-glucose) and lactate conversion into glucose (^{13}C-glucose) were measured at the same time points.

Endotoxin challenge: endotoxin 2 ng/kg, (*Escherichia coli*, USP Rockville, MD) or placebo (NaCl 0.9%) was administered as intravenous bolus at time T0. Heart rate, blood pressure, and respiratory rates were continuously monitored throughout the experimental protocol. In addition, the studies were performed in a metabolic laboratory located within the intensive care unit and under the supervision of a trained intensive care physicians.

The data concerning the metabolic, inflammatory and neuro-endocrine response in a LPS-challenge are reported in the present paper, while those of lactate and glucose metabolism will be reported separately.

Measurements

Blood was sampled at regular intervals to determine glucose, triglycerides, FFA, hormone levels (insulin, glucagon, cortisol, ACTH, norepinephrine, epinephrine), and

inflammatory markers (TNF-α, IL-6, C-reactive protein). Blood samples were collected into heparinised tubes and centrifuged to separate plasma. Plasma was stored at –20 and –80 °C until analysis. Urine was sampled from spontaneous voiding throughout the study to assess the urinary nitrogen excretion (see "analytical procedures").

The following vital functions were monitored: heart rate, rectal temperature (Hellige, Servomed), non-invasive arterial blood pressure, cardiac output by bioimpedance (NCCOM3 cardiodynamic monitor, BoMed, Irvine CA), respiratory rate, oxygen saturation (Ohmeda, Biox 3740 Pulse oximeter).

Resting energy expenditure (EE) and substrate metabolism

Indirect calorimetry was performed throughout the study. Oxygen consumption (V_{O_2}) and CO_2 production (V_{CO_2}) measured by indirect calorimetry, using a homemade calorimeter,[16] were used to determine the EE.[17]

Rates of glucose appearance and disappearance were calculated from plasma 6,6 $^2H_2$ glucose enrichments. Whole body glucose turnover was calculated with Steele's equation for non-steady-state conditions[18]:

$$R_a = \frac{F - p \cdot V_d(C_2 + C_1)/2 \cdot (IE_2 - IE_1)/\Delta t}{(IE_2 + IE_1)/2},$$

$$R_d = R_a - p \cdot V_d \cdot (C_2 - C_1)/\Delta t,$$

where F is the infusion rate of labelled glucose; C the glucose concentration; IE the isotopic enrichment; a pool fraction (p) of 0.75 and a distribution volume for glucose (V_d) of 0.2 l per kg body weight were used for these calculations.

Non-oxidative glucose utilisation was calculated as the difference of $R_{d(glucose)}$ and the net oxidation of carbohydrates calculated from indirect calorimetry.

Analytical procedures

Substrate analysis

Plasma glucose concentrations were measured enzymatically using a Yellow Spring Instrument YSI 2300 STAT PLUS (Yellow Spring, OH, USA); Plasma free fatty acid concentrations were measured with a colorimetric method, using a kit from Wako (Freiburg, Germany). Triglycerides levels were analysed enzymatically using the PAP 150 test (BioMérieux, France). The urine nitrogen excretion rate was determined with the micro-Kjehldahl method.

Concentrations of adrenocorticotropine (ACTH) were determined with a chemiluminescence assay kit (Nichols Institute Diagnostics, San Juan Capistrano, CA). Concentrations of insulin (kit from Adaltis, Casalecchio di Reno, Italy), cortisol (kit from Diagnostic Products Corporation, Los Angeles, CA) and glucagon (kit from Linco Research, St. Charles, MO) were determined by radioimmunoassay.[19] Plasma concentrations of bioactive IL-6 and TNF-α were determined using bioassays as previously described (7TD1 IL-6-dependent mouse-mouse hybridoma cells[20]; WEHI 164 clone 13 cell line).[21] CRP was analysed by turbidimetry on a Hitachi 917 (Roche) using reactants produced by DaKo.

Plasma catecholamines were determined by HPLC with electrochemical detection.[22]

Plasma glucose isotopic enrichments were determined on deproteinised samples partially purified over sequential cation–anion exchange resins (AG 1-X8 and AG 50W-X8; Bio-Rad, Richmond, CA). For glucose measurements, pentaacetyl glucose was analysed with gas chromatography–mass spectrometry (Hewlett Packard, Palo Alto, CA).

Statistical analysis

The data are expressed as mean ± SD. Comparisons between groups were carried out with one-way analysis of variance, and post-hoc comparisons with Scheffé tests. Variables measured repeatedly were compared by two-way analysis of variance for the effect of group and time, and their interaction. Post-hoc comparisons were made by Scheffe tests for the effect of group, Dunett's test for the effect of time. $P<0.05$ was considered statistically significant. Statistical analysis was performed using JMP Statistical software version 3.2.2 (SAS Institute, Cary, NC).

Results

Physiologic effects of endotoxin

Intravenous LPS induced flu-like symptoms after 75 min in all subjects; they were associated with significant systemic, endocrine and metabolic changes (Table 1). All subjects complained about headache, muscle ache and nausea; vomiting was observed in two subjects, without any other serious side effect.

There was a marked increase in heart rate (+38 min^{-1}) and cardiac output (+34%; Fig. 1A), and in body temperature (maximum +1.7 °C; Fig. 1B), while mean blood pressure and peripheral oxygen saturation did not change significantly.

Pro-inflammatory cytokines (TNF-α and IL-6) were not detectable at baseline, and increased sharply after LPS (Fig. 2). The endocrine response was substantial: plasma ACTH, cortisol levels, norepinephrine (NE +100%) and epinephrine (EPI +250%) increased strongly after LPS (Fig. 3). Plasma CRP was not detectable at baseline and increased 8 h after LPS-injection, ranging between 6 and 21 mg/l.

Sustained increases in EE (max. +40%) were observed after LPS. Plasma glucose showed a biphasic pattern, with a transient decrease at T120 (−8%) and moderate increase thereafter (T180+10%). Insulin levels were unchanged, while glucagon levels peaked at T240 (+71%). Net glucose oxidation decreased continuously (−45% by the end of the protocol). Both plasma free fatty acids and net fat oxidation were markedly increased after LPS (+94%) (Table 1).

Effect of FO

FO did not influence the basal physiological variables, nor the inflammatory response to LPS (TNF-α, IL-6, and CRP)

Table 1 Effects of physiologic changes due to LPS in fish oil treated (3–4 weeks oral FO supplementation) and control group (no FO).

Time (min); LPS injection at T0	T0	T90	T120	T180	T240	T360
Energy expenditure EE (kcal/min)						
Control	1.25±0.13	1.53±0.29[a]	1.56±0.36[a]	1.75±0.40[a]	1.64±0.31[a]	1.53±0.25[a]
Fish oil	1.34±0.17	1.69±0.26[a]	1.68±0.20[a]	1.83±0.34[a]	1.79±0.27[a]	1.62±0.24[a]
Respiratory quotient						
Control	0.82±0.06	0.80±0.06	0.76±0.06[a,b]	0.77±0.04[a]	0.75±0.05[a]	0.77±0.03[a]
Fish oil	0.80±0.04	0.81±0.03	0.80±0.06[b]	0.77±0.04[a]	0.76±0.04[a]	0.77±0.03[a]
Glycemia (mg/dl)						
Control	94±6	88±4[a]	87±4[a]	103±9[a]	102±8[a]	107±11[a]
Fish oil	96±10	86±9[a]	88±9[a]	103±9[a]	103±6[a]	106±8[a]
Free fatty acids (mEq/l)						
Control	0.38±0.20	0.36±0.23	0.58±0.37[a]	0.69±0.30[a]	0.84±0.31[a]	1.07±0.14[a,b]
Fish oil	0.33±0.21	0.43±0.27	0.51±0.23[a]	0.67±0.22[a]	0.78±0.19[a]	0.85±0.15[a,b]
Net glucose oxidation (mg/min)						
Control	105±63	114±75	60±88[a,b]	90±56	51±77[a]	73±37
Fish oil	96±50	148±48[a]	122±91[b]	84±54	59±60	76±40
Net fat oxidation (mg/min)						
Control	69±30	94±37[a]	119±42[a]	132±38[a]	134±39[a]	113±24[a]
Fish oil	77±25	93±32	101±39[a]	132±52[a]	137±41[a]	113±27[a]
Insulin (mU/l)						
Control	9.0±2.5	—	7.1±1.6	—	10.2±3.5	8.4±2.9
Fish oil	10.8±3.9	—	6.9±1.1[a]	—	9.6±3.5	8.6±1.6
Glucagon (ng/l)						
Control	63±13	—	91±23[a]	—	108±20[a]	99±19[a]
Fish oil	69±13	—	101±34[a]	—	103±47[a]	85±18[a]

Data as mean±SD
[a]Difference significant from baseline (T0).
[b]Difference significant between groups.

(Fig. 2). By contrast FO attenuated other important aspects of the physiological response to LPS. Body temperature was lower in the treated subjects from T240 ($P = 0.012$; Fig. 1B), while the cardiovascular response was not modified. The sympathoadrenal and adrenocortical responses to LPS were blunted by FO. While the basal levels of plasma ACTH and cortisol were similar, peak ACTH was decreased by 50% by FO ($P = 0.028$), and peak cortisol level was 30–40% lower in the treated group ($P = 0.002$, Fig. 3A). Peak NE level after LPS was 45% lower in the FO group ($P = 0.01$). Basal levels of plasma EPI did not differ. The EPI response to LPS was blunted in subjects treated with FO (trend: $P = 0.08$) (Fig. 3B).

FO supplementation did not influence the metabolic response to LPS: plasma glucose and FFA levels, glucose turnover and substrate net oxidation (glucose and FFA) were similar in both the groups (Table 1).

Discussion

To our knowledge, our study is the first human study testing the modulating effects of FO in healthy subjects submitted to an endotoxin challenge. FO decreased fever and blunted the neuro-endocrine response but had neither influence on the metabolic, nor on the inflammatory responses.

Effect of endotoxin

The general effects of experimental endotoxemia in man were first described in 1967, being characterised by a transient systemic inflammatory reaction.[23] Afterwards, the metabolic and endocrine effects of endotoxin were described[24,25] and the intravenous injection of endotoxin became a widely used experimental model.[26] In the present study 2 ng/kg was injected, as doses 2–4 ng/kg of LPS have been given to healthy humans on several occasions by other investigators, without any major adverse effect.[13,27]

Endotoxin is the common name for lipopolysaccharides, which are present in the outer cell wall of Gram-negative bacteria. Intravenous LPS elicits a transient self-limited flue-like state, associated with a short-lived release of proinflammatory and anti-inflammatory cytokines and other mediators. A full neuro-endocrine response is observed, involving the hypothalamo–pituitary–adrenal

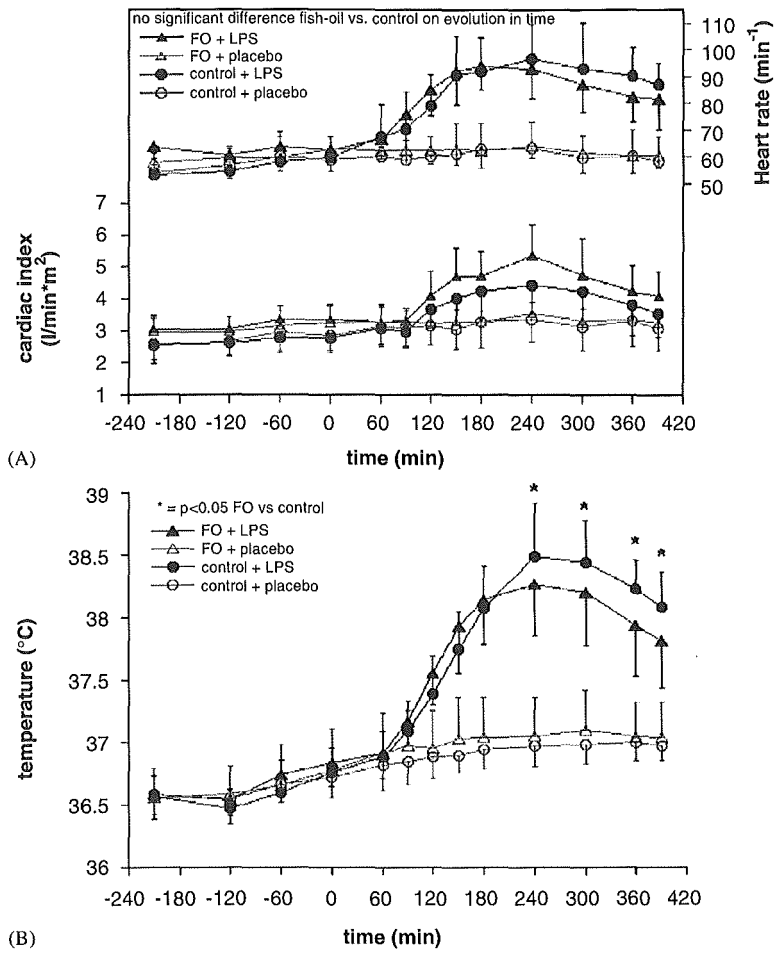


Figure 1 Systemic responses to endotoxin: (A) heart rate and cardiac output, (B) temperature.

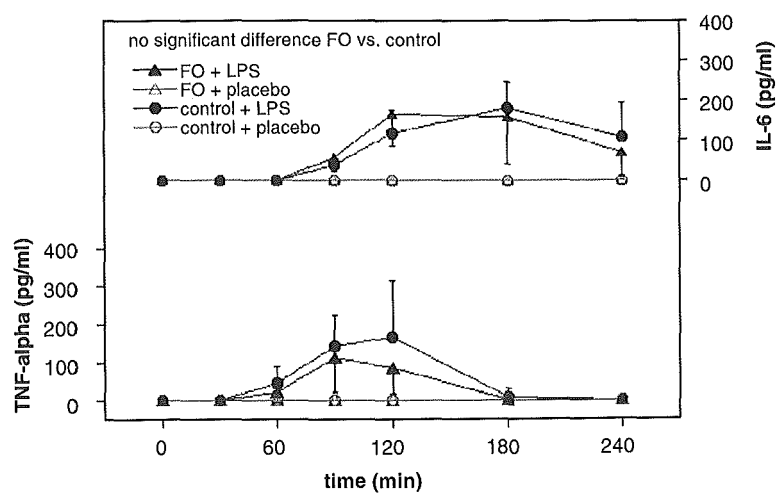
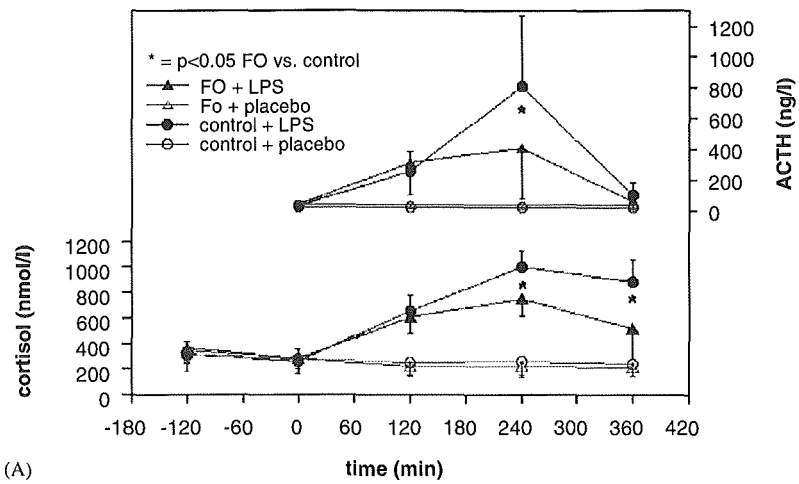


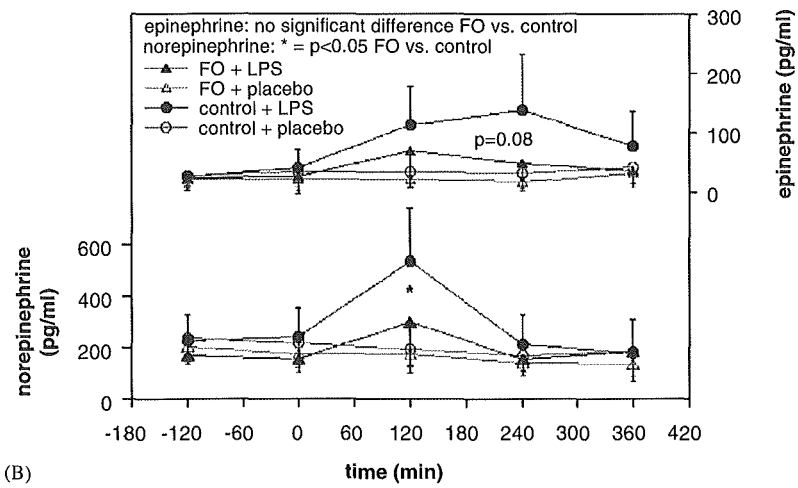
Figure 2 Effects of FO on plasma TNF-α and IL-6 after a LPS challenge.

axis (HPA) and the sympathoadrenal systems. This response is associated with cardiovascular, coagulation and metabolic changes, as well as alterations of endothelial functions.[27]

In the present study, an intravenous endotoxin challenge was applied after 3–4 weeks oral FO supplementation in healthy subjects. As expected, a full inflammatory response was observed after LPS.



(A)



(B)

Figure 3 Effects of FO on the endocrine responses to LPS.

Body temperature and neuro-endocrine response

Fever is a non-specific response mediated by complex peripheral and central mechanisms, involving prostaglandin E2, a metabolite of arachidonic acid, produced in response to cytokines and other mediators of inflammation.[28] The ability of short n-3 FO supplementation to inhibit the pyrogenic effects of intraperitoneally IL-1 was first demonstrated in rats.[29] After prolonged supplementation, fever was also reduced after intracerebrovascular IL-1, suggesting the existence of two distinct mechanisms by which FO modulates the thermoregulation. This antipyretic effect of FO was also documented in healthy humans subjected to the intramuscular injection of a typhoid vaccine.[30] Our present results fully corroborate these observations: the full blow pyrogenic response observed in the controls, was blunted in the FO subjects.

The HPA and the systemic sympathetic system are the two main peripheral limbs of the stress system. The classical stress reaction is elicited by pro-inflammatory cytokines, by the release of hypothalamic hormones (corticotropin, vasopressin) or by the direct activation of the central systemic sympathetic system.[31] Many stimuli can elicit stress response including tissue injury, infection, surgery, mental and psychological factors, and hypoglycemia. Our results suggest the central component of the stress response was modulated by FO supplementation. In support of this hypothesis, it has been documented that dietary FO significantly affects the fatty acid profile in the CNS cells (Y. Carpentier: personal communication). Animal and human studies have reported the potential of FO to reduce the stress response. In rats submitted to intracerebroventricular administration of corticotropin or fear-conditioned behaviour, DHA significantly reduced this response.[32] In medical students submitted to psychological tests during the exam period, DHA reduced the hostility reactions and decreased norepinephrine plasma levels, while hostility increased in the control group[33]; cortisol levels were unchanged. In another study assessing 9 weeks DHA of supplementation in students under exam-stress,[34] a 30% reduction of norepinephrine plasma levels was observed. In healthy subjects submitted to mental stress, 3–4 weeks FO supplementation blunted the neuro-endocrine, metabolic and cardiovascular responses to stress.[35] These data combined with ours show

that FO blunts the neuro-endocrine response to various types of stress. The central sympathetic effect seems to be more important than the peripheral, as suggested by the larger suppression of plasma norepinephrine compared to epinephrine. Based on these results, we hypothesize that oral FO modulated the stress response to LPS in our subjects, by a central nervous mechanism, which might open new potential therapeutic perspectives.

Inflammatory response

Oral intake of FO in humans is reported to be beneficial in chronic and acute inflammatory conditions, but the mechanism is not well elucidated. In many in vitro and in vivo animal experiments, FO was associated with a reduction of pro-inflammatory cytokines release.[13] The classical explanation of this effect is linked to the incorporation of n-3 PUFAs into cell membranes of immune competent cells, resulting in a lower production of proinflammatory interleukins.[36] FO has been shown to reduce mononuclear IL-1 production,[5] depress the immune response in bacteremic pigs,[6] and to blunt cytokine production in burned rats.[7] In critically ill humans with severe sepsis, intravenous FO reduced ex vivo cytokine release by monocytes in response to LPS.[37]

Surprisingly, although we observed a marked increase in IL-6 and TNF-α in our subjects, cytokine release was not significantly influenced by FO supplementation suggesting that FO did not act primarily by down regulating cytokines release. Similar observation was reported in healthy humans after an intramuscular injection of typhoid vaccine: prior supplementation with FO failed to blunt the response of TNF-α (although, in contrast to our present observation, it significantly reduced IL1 and IL6 concentrations).[30] It remains nonetheless possible that FO specifically down-regulates other pro-inflammatory cytokines which were not monitored in this study. The increase in CRP was modest and not influenced by FO administration. CRP was determined 8 h after LPS injection, when CRP production was only submaximal; in addition, CRP was not measured by means of a high-sensitivity method, which may have limited detection of early changes. Further study is required to determine if FO donwnregulates the acute phase proteins response after LPS.

Limitations in the study design may also provide some explanation for our results. Dose and duration of oral FO supplementation is controversial. We used a 3–4 week FO supplementation protocol providing about 2 g DHA and EPA per day. According to the published data this should result in significant membrane incorporation of n-3 PUFAs. Although we did not measure the membrane incorporation of n-3 PUFAs, it seems unlikely that this duration was insufficient to achieve the expected changes, as demonstrated by the effects on fever and endocrine response. In monks submitted to an endotoxin challenge after a prolonged FO supplementation (26–52 weeks supplementation) similar cytokine results were reported.[38] Altogether, these results suggest that FO does not exert a major effect on the inflammatory response to LPS, a very short-lived inflammation.

Metabolic response

The effects of FO on glucose metabolism have been extensively studied although conclusions of these studies remain disputed. Numerous observations show that FO does not alter basal glucose production or basal glycaemia in healthy individuals. It has also been observed that FO may enhance gluconeogenesis, and that such effect may be *detrimental* in insulin-resistant obese or diabetic patients.[39] Our results clearly show that FO does not affect energy metabolism and substrate utilisation in healthy subjects submitted to LPS. Based on the simultaneous decrease on plasma catecholamines, hypothalamo–pituitary axis, and body temperature, it is tempting to speculate that FO exerted its main effect at the level of the central nervous system, whereas disturbed glucose homeostasis related to the peripheral effects of endotoxin were not affected by FO. Further studies are required to better define the mechanisms of actions of FO and the clinical perspective in term of patient's care.

Conclusion

Three to four weeks of oral FO supplementation blunts important aspects of the response to intravenous LPS in healthy subjects: fever, central sympathetic and hypothalamo–pituitary–adrenal response are blunted. By contrast the inflammatory cytokine response was not affected.

Acknowledgements

The author expresses special thanks to Mrs. M-C. Cayeux (RN) for efforts in realizing this research and to Dr. M. Schuler-Barazzoni for helpful discussions.

References

1. Calder PC, Deckelbaum RJ. Dietary lipids: more than just a source of calories. *Curr Opin Clin Nutr Metab Care* 1999;2: 105–7.
2. Calder PC. Lipids and the critically ill patient. *Nestle Nutr Workshop Ser Clin Perform Programme* 2003:75–91 discussion 91–8.
3. Grimble RF, Tappia PS. Modulation of pro-inflammatory cytokine biology by unsaturated fatty acids. *Z Ernahrungswiss* 1998; **37**(Suppl 1):57–65.
4. Grimm H, Mayer K, Mayser P, Eigenbrodt E. Regulatory potential of n-3 fatty acids in immunological and inflammatory processes. *Br J Nutr* 2002;**87**(Suppl 1):S59–67.
5. Endres S, Ghorbani R, Kelley VE, et al. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N Engl J Med* 1989;**320**:265–71.
6. Murray MJ, Svingen BA, Holman RT, Yaksh TL. Effects of a fish oil diet on pigs' cardiopulmonary response to bacteremia. *J Parenter Enteral Nutr* 1991;**15**:152–8.
7. Hayashi N, Tashiro T, Yamamori H, et al. Effects of intravenous omega-3 and omega-6 fat emulsion on cytokine production and delayed type hypersensitivity in burned rats receiving total parenteral nutrition. *J Parenter Enteral Nutr* 1998;**22**:363–7.
8. Sadeghi S, Wallace FA, Calder PC. Dietary lipids modify the cytokine response to bacterial lipopolysaccharide in mice. *Immunology* 1999;**96**:404–10.

9. McCann ME, Moore JN, Carrick JB, Barton MH. Effect of intravenous infusion of omega-3 and omega-6 lipid emulsions on equine monocyte fatty acid composition and inflammatory mediator production in vitro. *Shock* 2000;**14**:222–8.
10. Vollmar B, Bauer C, Menger MD. n-3 Polyunsaturated fatty acid-enriched diet does not protect from liver injury but attenuates mortality rate in a rat model of systemic endotoxemia. *Crit Care Med* 2002;**30**:1091–8.
11. Liu YL, Li DF, Gong LM, Yi GF, Gaines AM, Carroll JA. Effects of fish oil supplementation on the performance and the immunological, adrenal, and somatotropic responses of weaned pigs after an *Escherichia coli* lipopolysaccharide challenge. *J Anim Sci* 2003;**81**:2758–65.
12. Grecu I, Mirea L, Grintescu I. Parenteral fish oil supplementation in patients with abdominal sepsis. *Clin Nutr* 2003;**22**:S23.
13. O'Brien Jr JM, Abraham E. Human models of endotoxemia and recombinant human activated protein C. *Crit Care Med* 2004;**32**:S202–8.
14. Abumrad NN, Rabin D, Diamond MP, Lacy WW. Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. *Metabolism* 1981;**30**:936–40.
15. Connor H, Woods HF, Ledingham JG, Murray JD. A model of L(+)-lactate metabolism in normal man. *Ann Nutr Metab* 1982;**26**: 254–63.
16. Tappy L, Schneiter P. Measurement of substrate oxidation in man. *Diabetes Metab* 1997;**23**:435–42.
17. Livesey G, Elia M. Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. *Am J Clin Nutr* 1988;**47**:608–28.
18. Steele R. Influences of glucose loading and of injected insulin on hepatic glucose output. *Ann NY Acad Sci* 1959;**82**:420–30.
19. van der Meer JW, Endres S, Lonnemann G, et al. Concentrations of immunoreactive human tumor necrosis factor alpha produced by human mononuclear cells in vitro. *J Leukoc Biol* 1988;**43**:216–23.
20. Van Snick J, Cayphas S, Vink A, et al. Purification and NH_2-terminal amino acid sequence of a T-cell-derived lymphokine with growth factor activity for B-cell hybridomas. *Proc Natl Acad Sci U S A* 1986;**83**:9679–83.
21. Espevik T, Nissen-Meyer J. A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. *J Immunol Methods* 1986;**95**:99–105.
22. Farnebo LO, Hallman H, Hamberger B, Jonsson G. Catecholamines and hemorrhagic shock in awake and anesthetized rats. *Circ Shock* 1979;**6**:109–18.
23. Ollodart RM, Hawthorne I, Attar S. Studies in experimental endotoxemia in man. *Am J Surg* 1967;**113**:599–607.
24. Santos AA, Wilmore DW. The systemic inflammatory response: perspective of human endotoxemia. *Shock* 1996;**6**(Suppl 1): S50–6.
25. Lin E, Lowry SF. The human response to endotoxin. *Sepsis* 1998:255–62.
26. Schultz MJ, van der Poll T. Animal and human models for sepsis. *Ann Med* 2002;**34**:573–81.
27. Van der Poll T, Romijn JA, Endert E, Borm JJ, Buller HR, Sauerwein HP. Tumor necrosis factor mimics the metabolic response to acute infection in healthy humans. *Am J Physiol* 1991;**261**:E457–65.
28. Ivanov AI, Romanovsky AA. Prostaglandin E2 as a mediator of fever: synthesis and catabolism. *Front Biosci* 2004;**9**: 1977–93.
29. Cooper AL, Rothwell NJ. Inhibition of the thermogenic and pyrogenic responses to interleukin-1 beta in the rat by dietary N-3 fatty acid supplementation. *Prostaglandins Leukot Essent Fatty Acids* 1993;**49**:615–26.
30. Cooper AL, Gibbons L, Horan MA, Little RA, Rothwell NJ. Effect of dietary fish oil supplementation on fever and cytokine production in human volunteers. *Clin Nutr* 1993;**12**:321–8.
31. Chrousos GP. The hypothalamic–pituitary–adrenal axis and immune-mediated inflammation. *N Engl J Med* 1995;**332**:1351–62.
32. Takeuchi T, Iwanaga M, Harada E. Possible regulatory mechanism of DHA-induced anti-stress reaction in rats. *Brain Res* 2003;**964**:136–43.
33. Hamazaki T, Itomura M, Sawazaki S, Nagao Y. Anti-stress effects of DHA. *Biofactors* 2000;**13**:41–5.
34. Sawazaki S, Hamazaki T, Yazawa K, Kobayashi M. The effect of docosahexaenoic acid on plasma catecholamine concentrations and glucose tolerance during long-lasting psychological stress: a double-blind placebo-controlled study. *J Nutr Sci Vitaminol (Tokyo)* 1999;**45**:655–65.
35. Delarue J, Matzinger O, Binnert C, Schneiter P, Chiolero R, Tappy L. Fish oil prevents the adrenal activation elicited by mental stress in healthy men. *Diabetes Metab* 2003;**29**: 289–95.
36. Calder PC. Dietary modification of inflammation with lipids. *Proc Nutr Soc* 2002;**61**:345–58.
37. Mayer K, Gokorsch S, Fegbeutel C, et al. Parenteral nutrition with fish oil modulates cytokine response in patients with sepsis. *Am J Respir Crit Care Med* 2003;**167**:1321–8.
38. Blok WL, Deslypere JP, Demacker PN, et al. Pro- and anti-inflammatory cytokines in healthy volunteers fed various doses of fish oil for 1 year. *Eur J Clin Invest* 1997;**27**:1003–8.
39. Delarue J, LeFoll C, Corporeau C, Lucas D. N-3 long chain polyunsaturated fatty acids: a nutritional tool to prevent insulin resistance associated to type 2 diabetes and obesity? *Reprod Nutr Dev* 2004;44:289–99.