Carcinogenesis vol.35 no.7 pp.1613–1619, 2014
doi:10.1093/carcin/bgu068
Advance Access publication March 14, 2014

Olive oil and omega-3 polyunsaturated fatty acids suppress intestinal polyp growth by modulating the apoptotic process in $Apc^{Min/+}$ mice

Michele Barone[†], Maria Notarnicola[1,†], Maria G.Caruso[1], Maria P.Scavo[2], Maria T.Viggiani[2], Valeria Tutino[1], Lorenzo Polimeno[1,2], Barbara Pesetti[1], Alfredo Di Leo[2] and Antonio Francavilla[1,*]

Gastroenterology Unit, Department of Medical and Surgical Sciences, University of Foggia, Foggia 71122, Italy, [1]Laboratory of Biochemistry, National Institute for Digestive Diseases 'S. de Bellis', Castellana Grotte, Bari 70013, Italy and [2]Gastroenterology Unit, Department of Emergency and Organ Transplantation (D.E.T.O.), University of Bari, Bari 70124, Italy

*To whom correspondence should be addressed. Tel: +39 0804994175; Fax: +39 0804994313;
Email: direzionescientifica@irccsdebellis.it

The promotion and progression of carcinogenesis are susceptible to nutritional interventions aimed at counteracting cancer development. Lipid metabolism is essential in the onset and progression of tumors and for cancer cell survival. In this study, we tested the effects of diets enriched with natural compounds, such as olive oil and salmon oil, in mice that spontaneously develop intestinal polyps ($Apc^{Min/+}$ mice). For this purpose, we evaluated polyp number and volume, intestinal mucosa proliferation/apoptosis, estrogen receptors (ERs) expression, fatty acid synthase and 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase gene expression and enzymatic activity. Compared with the standard diet, the salmon oil-enriched diet, containing a high percentage of omega-3 polyunsaturated fatty acids, and, to a lesser extent, olive oil-enriched diet reduced polyp number and volume through a reduction of proliferation and a marked proapoptotic effect. These biological effects were mediated by an inhibition of fatty acid synthase and HMGCoA reductase gene expression and activity and an increase of ERβ/ERα ratio. Our findings suggest that a proper dietary lifestyle could contribute to primary cancer prevention.

Introduction

Alterations of lipid metabolism are essential in the onset and progression of tumors and for cancer cell survival (1). Fatty acid synthase (FAS) is overexpressed in many types of malignancies (2–4). Moreover, the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, the key regulator of cholesterol synthesis and the checkpoint regulating the availability of cholesterol for cell membrane synthesis, is upregulated severalfold in neoplastic cells (5–8).

The promotion and progression of carcinogenesis are susceptible to nutritional interventions aimed at counteracting cancer development. Olive oil has been demonstrated to reduce the incidence of aberrant crypt foci in azoxymethane-treated rats (9) and to downregulate the expression of Bcl-2, decreasing cancer cell survival (10). The main phenolic compounds present in olive oil, oleuropein and hydroxytyrosol, determine a reduction of proliferation and an increase of apoptosis in human colorectal cancer cell lines by downregulating FAS activity (11).

Abbreviations: ER, estrogen receptor; FAS, fatty acid synthase; HMGCoA, 3-hydroxy-3-methylglutaryl coenzyme A; MUFA, monounsaturated acid; OM-3, omega-3 PUFA-enriched diet; OO, olive oil-enriched diet; PCNA, proliferating cell nuclear antigen; PUFA, polyunsaturated fatty acid; ST, standard diet; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling.

[†]These authors contributed equally to this work.

As suggested by epidemiological studies, the dietary consumption of fish oil, rich in omega-3 polyunsaturated fatty acids (omega-3 PUFAs), is also associated with a reduced risk of colorectal cancer in humans (12,13). These findings are supported by *in vitro* studies showing a growth-inhibiting effect of omega-3 PUFAs in MDA-MB-231 and Michigan Cancer Foundation-7 breast cancer cells (14,15) and *in vivo* both in mice with an Apc defect ($Apc^{Min/+}$ mice) and in patients with familial adenomatous polyposis (16,17). Interestingly, the proapoptotic effects of omega-3 PUFAs in breast cancer cells involve estrogen receptors (ERs) (18), an important aspect upon consideration of the fact that ER beta (ERβ) exerts antitumor effects in colon carcinogenesis (19–23).

This study describes the molecular pathway(s) supporting the anticarcinogenetic activity of olive oil and omega-3 PUFAs using the $Apc^{Min/+}$ mouse. For this purpose, $Apc^{Min/+}$ mice were fed on either a standard diet or a standard diet enriched with olive oil or omega-3 PUFAs and, on intestinal tissue, we evaluated (i) the number and volume of polyps; (ii) cell proliferation [proliferating cell nuclear antigen (PCNA) positive cells]; (iii) cell apoptosis (STAT3 phosphorylation, Bax and Bcl-2 expression and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL); (iv) FAS and HMGCoA reductase enzymatic activity and gene expression; and (v) ERα and ERβ protein expression.

Materials and methods

Animals and experimental study design

Five-week-old C57BL/6J male mice with a heterozygote mutation for the *Apc* gene ($Apc^{Min/+}$) were obtained from Charles River Laboratories Italia (Calco, Lecco, Italy). Mice were maintained under temperature-, air- and light-controlled conditions and received food and water *ad libitum*; they did not receive any surgical or hormonal manipulation. All animals received care in compliance with the 'Guide for the Care and Use of Laboratory Animals'. The procedures relating to animal use were communicated to the Italian Ministry of Health and approved.

$Apc^{Min/+}$ male mice were randomly divided into 3 groups of 10 animals each and fed for 10 weeks as follows: control (ST) group, receiving a purified AIN-93M standard diet (12.5% protein, 12% soybean oil, 3% cellulose fiber); olive oil (OO) group, receiving a purified AIN-93M standard diet in which soybean oil was replaced with olive oil (12.5% protein, 12% olive oil, 3% cellulose fiber); omega-3 PUFA (OM-3) group, receiving a purified AIN-93M standard diet in which soybean oil was replaced with salmon oil, rich in omega-3 PUFAs (12.5% protein, 12% salmon oil, 3% cellulose fiber). Fatty acid composition depended on the source of oil used. Soybean oil contained 27.55% of monounsaturated acids (MUFAs), 13.25% of saturated fatty acids, 57.8% of PUFAs (5.8% omega-3 PUFAs and 52% omega-6 PUFAs); olive oil contained 72–75% MUFAs, 13–16% saturated fatty acids and 6–11.5% PUFAs (23); salmon oil fatty acid profile was 15% MUFAs, 8% saturated fatty acids and 77% omega-3 PUFAs (informative schedule given by the producer). All diets were isocaloric and supplied as pellets (Mucedola Srl, Settimo Milanese, Italy). Mice body weight and food intake were measured every 3 days.

After 10 weeks of dietary treatment, all animals were killed by cervical dislocation and the entire intestinal tract was immediately removed and washed with cold phosphate-buffered saline.

The small intestine and colon were cut along the mesenteric insertion, placed on a paper strip at 0–4°C and analyzed through a stereomicroscope at ×3 magnification by two independent observers. The volume of polyps was calculated considering them as hemispheres ($1/2 \times 3/4\ \pi\ r^3$).

All other evaluations were performed in distal tract of the small intestine because $Apc^{Min/+}$ mice develop the majority of tumors in the small intestine (19). Part of the distal small intestine underwent scraping of the mucosa, which in part was immediately put into RNA-later and stored at −20°C, and in part in liquid nitrogen in order to run real-time PCR and western blotting analyses, respectively. The remaining part of the distal small intestine was fixed in 10% neutral buffered formalin for 24 h and embedded in paraffin in a 'Swiss roll' fashion. Paraffin-embedded tissues were processed for immunohistochemical evaluations or confocal microscopy studies. Tissue sections cut

at 4 μm were used for hematoxylin and eosin stain and immunohistochemical preparations, whereas 7 μm-thick sections were used for confocal microscopy evaluations.

Cell proliferation (PCNA)

For PCNA determination, tissue sections were processed with polyclonal anti-PCNA antibody (Abcam, Ab 2496, Cambridge, UK). After antigen retrieval [Tris–ethylenediaminetetraacetic acid, pH 9, in microwave (850 W) for 10 min], cold water was added for 10 min. Then 10% goat serum and 1% bovine serum albumin in phosphate-buffered saline were used as a blocking buffer (2 h at room temperature). The primary antibody was diluted 1:100 and added to the slides. After washing, the reaction was detected by a secondary antibody (Alexa 555 anti-rabbit, Invitrogen, OR) diluted 1:100. After further washings, sections were counterstained with benzothiazolium-4-quinolinium monomer iodide (Invitrogen Molecular Probes, Eugene, OR) diluted 1:7000. All sections were observed at ×400 magnification by confocal microscopy (Leica TCS SP2 confocal laser scanning microscope). The percentage of PCNA positive cells over the total number of counted cells, i.e. the PCNA labeling index (PCNA-LI), was used to quantify epithelial cell proliferation.

Apoptosis

Apoptotic cells were detected in tissue sections by TUNEL assay according to the manufacturer's instructions (*In situ* Cell Death Detection Kit, Roche, Germany), as described previously (19). Briefly, deparaffinized slides were immersed in 0.1 M citrate buffer (pH 6.0) and microwave irradiated at 350 W for 10 min. After phosphate-buffered saline rinsing, slides were incubated with the TUNEL reaction mix at 37°C for 60 min and then counterstained with benzothiazolium-4-quinolinium monomer iodide (Invitrogen Molecular Probes, Eugene, OR). All sections were screened using the Leica TCS SP2 (Leica, Germany) confocal laser scanning microscope at ×400 magnification. A minimum of 10 randomly selected fields were examined. The percentage of TUNEL positive cells over the total number of counted cells, i.e. the TUNEL labeling index (TUNEL-LI), was used to quantify cell apoptosis.

Western blotting

STAT3 and p-STAT3 (Ser-727), Bax, Bcl-2, ERα, ERβ and β-actin protein expression levels were evaluated in tissue specimens by western blot analysis. Briefly, 50 μg aliquots of total protein were separated in 4–12% precast polyacrylamide gels (Invitrogen, Life Technologies) and transferred onto a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Milan, Italy) with Transblot Turbo (Bio-Rad Laboratories). The primary antibodies (anti-p-STAT3 Ser and STAT3, Cell Signaling, Beverly, MA; anti-Bax, Bcl-2, ERα, ERβ and β-actin, Santa Cruz Biotechnology, Santa Cruz, CA) were diluted 1:500 in blocking buffer. After overnight incubation, the membranes were further incubated with a horseradish peroxidase-conjugated secondary antibody (Bio-Rad Laboratories). The proteins were detected by chemiluminescence (enhanced chemiluminescence, Thermo Scientific, Rockford, IL) and the densitometric analysis of each protein-related signal was obtained using the Molecular Imager ChemidocTM (Bio-Rad Laboratories) and normalized against β-actin expression.

Gene expression analysis

Gene expression analysis of FAS and HMGCoA reductase in tissue was evaluated by real-time PCR. The reactions were performed using master mix with SYBR Green (iQ SYBR Green Supermix Bio-Rad, Milan, Italy) and sense and antisense primers for each target gene and the β-actin gene (FAS primer sense: 5′-GAT CCT GGA ACG AGA ACA CGA-3′ and antisense: 5′-GAG ACG TGT CAC TCC TGG ACT TG-3′; HMGCoA reductase primer sense: 5′-GCT TGA GCA TCC TGA CAT AC-3′ and antisense: 5′-GAA CCA TAG TTC CCA CGT CT-3′; β-actin primer sense: 5′-GCC TCT GGT CGT ACC ACT GGC-3′ and antisense: 5′-AGG GAG GAA GAG GAT GCG GCA-3′). Real-time PCR was carried out in a CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Philadelphia, PA) using the following protocol: 45 cycles at 95°C for 3 min, 95°C for 10 s, and 55°C for 30 s, followed by a melting curve step at 65–95°C with a heating rate of 0.5°C per cycle for 80 cycles. The PCR products were quantified by external calibration curves, one for each tested gene, obtained with serial dilutions of known copy number of molecules (10^2–10^7 molecules). All expression data were normalized by dividing the target amount by the amount of β-actin used as internal control for each sample (no. molecules of FAS or HMGCoA reductase messenger RNA (mRNA)/no. molecules of β-actin mRNA). The specificity of PCR products was confirmed by gel electrophoresis.

FAS activity assay

FAS activity was determined on frozen intestinal samples, as described previously (24), and expressed as picomoles of incorporated 2-^{14}C-malonyl-CoA/min/milligram of total proteins.

Microsomal HMGCoA reductase activity assay

HMGCoA reductase activity was measured in intestinal tract as [^{14}C]-mevalonolactone formed in resuspended microsomal pellets by radiochemical assay using DL-3-hydroxy-3-methyl-[3-14C]-glutaryl-coenzyme A (^{14}C-HMGCoA) as substrate, according to the Pallottini method (25). HMGCoA reductase activity was expressed as picomoles of [^{14}C]-mevalonate formed per minute per milligram of microsomal proteins (pmol/min/mg prot). In all experiments, enzyme assays were carried out in duplicate.

Statistical analysis

The significance of the differences among experimental groups was evaluated by one-way analysis of variance and Tukey's multiple comparison test. Differences were considered significant at a 5% probability level. Correlation analyses were performed using two-tailed Spearman's correlation (non-parametric test).

Results

After 10 weeks of dietary treatment, no statistically significant difference was found in body weight among the three groups of mice. None of the animals showed any gross changes at necropsy except for the intestinal tract.

Figure 1 reports the number (A) and volume (B) of polyps evaluated along the entire intestinal tract after 10 weeks of dietary treatment. Compared with mice treated with standard diet, the number of

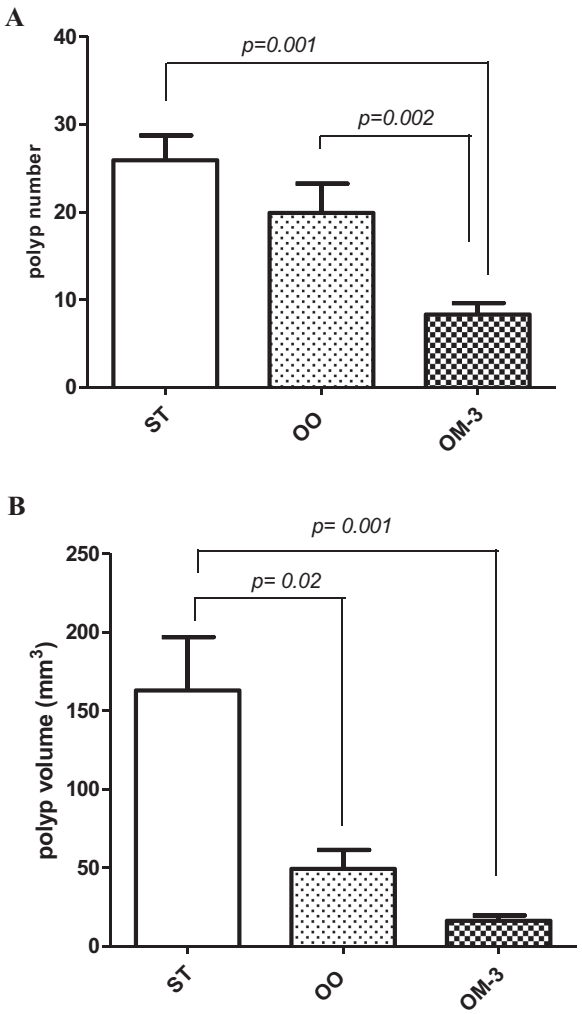

Fig. 1. Number of polyps (**A**) and total polyp volume (**B**) in the whole intestinal tract from *Apc*$^{Min/+}$ mice treated groups: ST, standard diet; OO, olive oil-enriched diet; OM-3, omega-3 PUFA-enriched diet. Data are expressed as the mean ± SE. $P < 0.05$ shows statistically significant differences (one-way analysis of variance and Tukey's multiple comparison test).

polyps decreased in both the OO- and OM-3-treated groups although a significant variation ($P = 0.001$, Tukey's multiple comparison test) was observed only in the OM-3-treated mice. A statistically significant difference in polyp number was also present between OO and OM-3 groups ($P = 0.002$, Tukey's multiple comparison test). Analysis of polyp volume revealed a dramatic and statistically significant reduction in both the OO and the OM-3-treated groups, compared with animals fed with standard diet ($P = 0.02$ and $P = 0.001$, respectively, Tukey's multiple comparison test).

Figure 2A shows intestinal cell proliferation, expressed as PCNA-LI, in the polyp tissue and 'healthy' surrounding intestinal mucosa of the small intestine in $Apc^{Min/+}$ mice. No variation regarding cell proliferation of 'healthy' intestinal mucosa was detected among the three different groups of mice and these PCNA-LI values were similar to those found in intestinal mucosa of wild-type mice (data not shown). As expected, a statistically significant increase of proliferative activity was observed in polyp colonic tissue with respect to 'healthy' adjacent mucosa in the group fed on standard diet ($P = 0.002$, Tukey's multiple comparison test). In the OO-treated and, more evidently, OM-3-treated groups, the proliferative activity in the polyps was not statistically different compared with the corresponding 'healthy' surrounding mucosa. Finally, a statistically significant reduction of cell proliferation was observed in polyp tissue of OM-3-treated mice compared with mice fed a standard diet ($P = 0.01$, Tukey's multiple comparison test).

Figure 2B reports the apoptotic activity in small intestine, expressed as TUNEL-LI, of the adenomatous tissue and 'healthy' surrounding mucosa from the three groups of mice. As observed for cell proliferation, dietary treatment did not influence apoptosis in 'healthy' surrounding tissue. On the other hand, a striking increase of apoptosis was observed in polyp tissue of OO- and OM-3-treated mice, compared with the corresponding 'healthy' surrounding mucosa ($P = 0.01$, Tukey's multiple comparison test). The apoptosis increased also between the polyp tissue of the ST group and polyp tissue of the

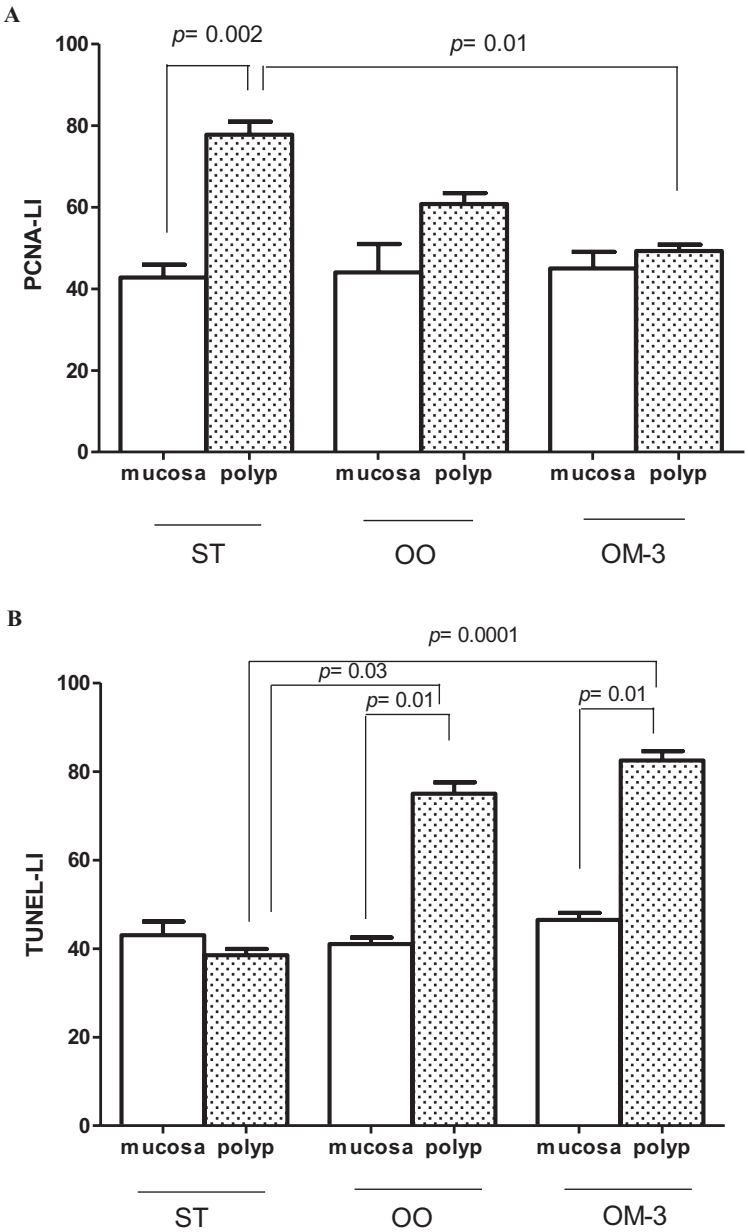

Fig. 2. Cell proliferation expressed as PCNA-LI (**A**) and cell apoptosis expressed as TUNEL-LI (**B**) in the polyp tissue and 'healthy' surrounding intestinal mucosa of $Apc^{Min/+}$ mice. ST, standard diet; OO, olive oil-enriched diet; OM-3, omega-3 PUFAs-enriched diet. Data are expressed as the mean ± SE. $P < 0.05$ shows statistically significant difference (one-way analysis of variance and Tukey's multiple comparison test).

OO- and OM-3-treated groups ($P = 0.03$ and $P = 0.0001$, respectively, Tukey's multiple comparison test).

The apoptotic protein expression (Bax, Bcl-2 and p-STAT3 Ser/STAT3) evaluated in the adenomatous tissue of all three groups of mice is reported in Figure 3. We observed a significant reduction of p-STAT3 Ser/STAT3 and of Bcl-2 expressions (Figure 3A and 3C). Figure 3B shows a slight reduction of Bax expression in OO- and OM-3- treated groups compared with the ST group. However, this reduction did not reach statistical significance. In addition, as shown in panel D, compared with ST group, Bax/Bcl-2 ratio markedly increased in the OO- and OM-3-treated groups as a consequence of a statistical significant reduction of Bcl-2 protein expression.

All dietary-supplemented groups presented a significant reduction of FAS and HMGCoA reductase activity, compared with mice fed a standard diet ($P < 0.05$, Tukey's multiple comparison test; Figure 4A). These results were confirmed by evaluating the levels of mRNA encoding for lipogenic enzymes (Figure 4B). In fact, the groups fed dietary supplements presented significantly lower FAS and HMGCoA reductase mRNA levels than the group fed a standard diet ($P < 0.05$, Tukey's multiple comparison test).

Western blot analysis of ERβ and ERα protein expression is shown in Figure 5. A striking increase of ERβ protein expression was evident in both dietary-treated groups (Figure 5A). However, in the OO-treated group, this increase was associated to an enhanced expression of ERα, whereas in the OM-3-treated group a reduction of ERα protein expression was observed (Figure 5B). In addition, ERβ/ERα ratio increased 3-fold and 2-fold in mice treated with omega-3 PUFAs and olive oil, respectively, compared with the ST group (Figure 5C).

Notably, a significant highly positive correlation (Figure 6) was detected between FAS activity and p-STAT3 Ser protein expression in the OM-3-treated group (Spearman's correlation $r = 0.77$, $P = 0.009$). No statistically significant correlation was found between other biological parameters studied both in OO and OM-3 groups.

Discussion

Different studies reported in the literature demonstrate that dietary manipulations are able to modulate physiological activities or to prevent neoplastic disease (26,27). Many micronutrients such as polyphenols or vitamins have been identified as possible chemopreventive agents in colon cancer (28).

We have previously demonstrated that the administration of natural substances, such as lignin and silymarin, counteract the carcinogenic process in $Apc^{Min/+}$ transgenic mice (19), confirming the importance of natural products for the prevention of neoplastic disease.

Conflicting data are reported on the effect of olive oil in modulating inducted or spontaneous intestinal tumorigenesis in animal model (29–31).

In this study, we demonstrated that both olive oil- and fish oil-enriched diets are able to reduce cancer development in the $Apc^{Min/+}$ mouse model. However, although the latter diet significantly reduced both the number and volume of polyps, olive oil was effective only on the reduction of polyp volume and to a lesser extent on polyp number. These differences were probably related to the fact that the beneficial effect of salmon oil is due to its high content of omega-3 PUFAs, as demonstrated by the use of purified omega-3 PUFA (16,17). On the other hand, olive oil contains a low percentage of omega-3 PUFAs (32). Moreover, some clinical and experimental evidences suggest that its beneficial effects are due to the high content of MUFAs (33) or to the presence of phenolic compounds such as oleuropein and hydroxytyrosol and alpha-tocopherol, which reduce the risk of cardiovascular events, inflammation and several varieties of cancer (33,34) and inhibit colon cancer cell growth *in vivo* and *in vitro* (9–11).

An interesting and innovative aspect of our study is represented by the fact that in the diets we used commercially available olive oil and salmon oil, with evident translational implications considering their common presence in human diet.

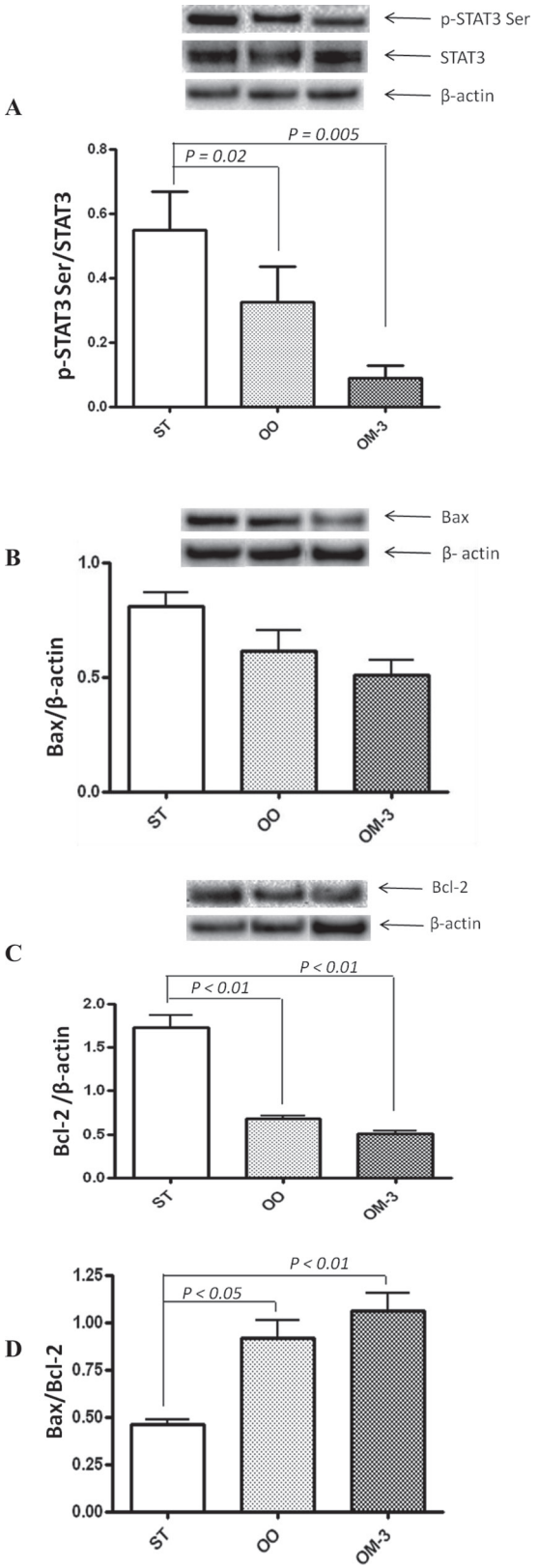

Fig. 3. Western blotting analysis of the expression of proteins involved in the apoptotic process in adenomatous tissue of $Apc^{Min/+}$ mice. (**A**) Levels of p-STAT3 Ser/STAT3 ratio; (**B**) levels of Bax expression normalized with β-actin; (**C**) levels of Bcl-2 expression normalized with β-actin; and (**D**) levels of Bax/Bcl-2 ratio. ST, standard diet; OO, olive oil-enriched diet; OM-3, omega-3 PUFAs-enriched diet. Data are expressed as the mean ± SE. $P < 0.05$ shows statistically significant difference (one-way analysis of variance and Tukey's multiple comparison test).

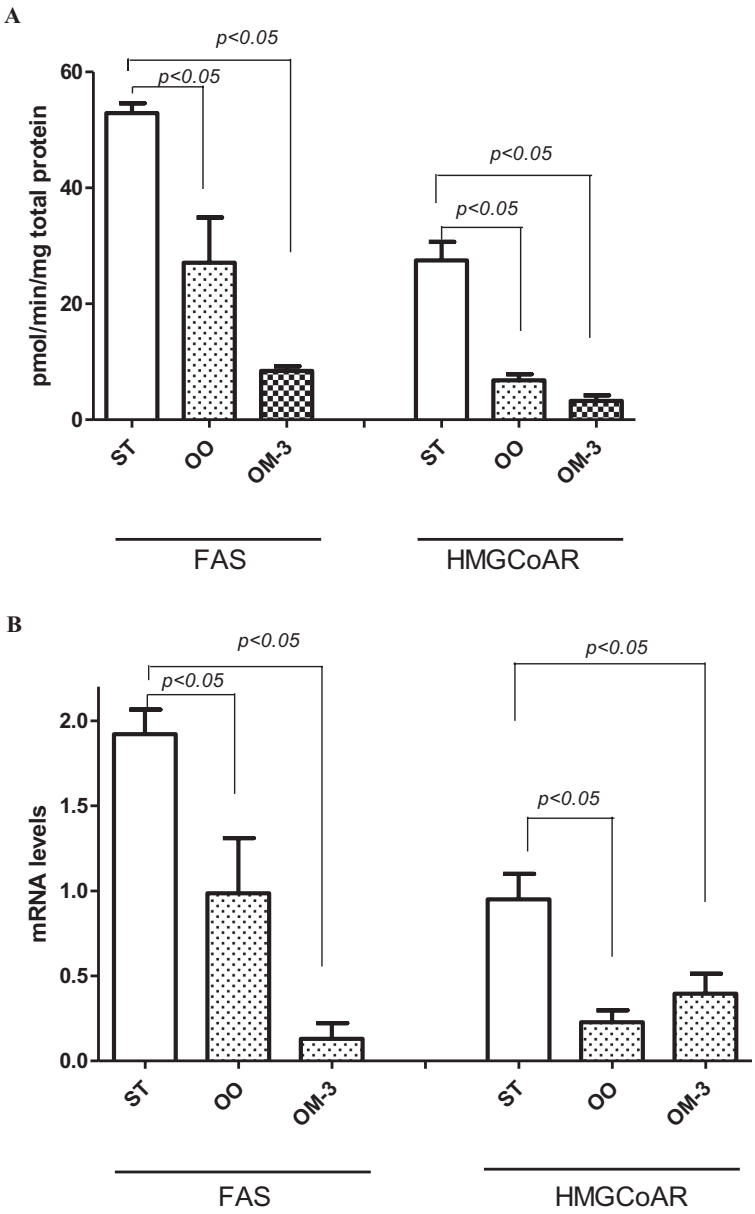

Fig. 4. FAS and HMGCoA reductase enzymatic activity (**A**) and mRNA levels (**B**) in polyp tissue from $Apc^{Min/+}$ mice treated groups. ST, standard diet; OO, olive oil-enriched diet; OM-3, omega-3 PUFAs-enriched diet. Data are expressed as the mean ± SE. $P < 0.05$ shows statistically significant difference (one-way analysis of variance and Tukey's multiple comparison test).

In addition, we explored new possible molecular mechanisms that could mediate the effects of olive oil and omega-3 PUFAs, which are alternative to those reported in the literature. It has been previously suggested that omega-3 PUFAs could act selectively modulating the subcellular localization of lipidated signaling proteins (35) or replacing the omega-6 PUFAs with a consequent reduction of inflammation (36), one of the major contributing factor to carcinogenesis (37,38). Other studies report that these effects were associated to a suppression of cyclooxygenase-2 expression and reduction of β-catenin nuclear translocation (16).

In this study, we describe three new mechanisms by which omega-3 PUFAs and olive oil could reduce cell proliferation and increase apoptosis in the intestinal polyps: (i) the decrease of p-STAT3 Ser, known to be responsible for the activation of metabolic pathways involved in regulation of cell proliferation; (ii) the reduction of FAS and HMGCoA reductase activity and gene expression, confirming our previous findings '*in vitro*' (11,39); (iii) the increase of ERβ/ERα ratio, a diagnostic and prognostic parameter for colon cancer progression (19,20,40,41).

It is known that p-STAT3 upregulates the expression of antiapoptotic genes (42–45). Our data clearly demonstrated that p-STAT3 Ser expression was significantly reduced in OM-3 and OO groups. This was associated to a significant reduction of Bcl-2 levels in mice fed on both olive oil and omega-3 PUFAs compared with mice receiving the standard diet.

Lipid metabolism alterations such as increased lipogenic enzyme activity are essential for cancer cell proliferation and survival. The effect of olive oil and omega-3 PUFAs diets on FAS strongly suggests an important role of this enzyme in reducing cell proliferation and increasing apoptosis in this intestinal cancer model. Moreover, p-STAT3 Ser positively correlated with FAS enzymatic activity, suggesting that the reduction of p-STAT3 Ser could to have a causative role in regulating cell proliferation and cell apoptosis also through its inhibitory effect on FAS activity.

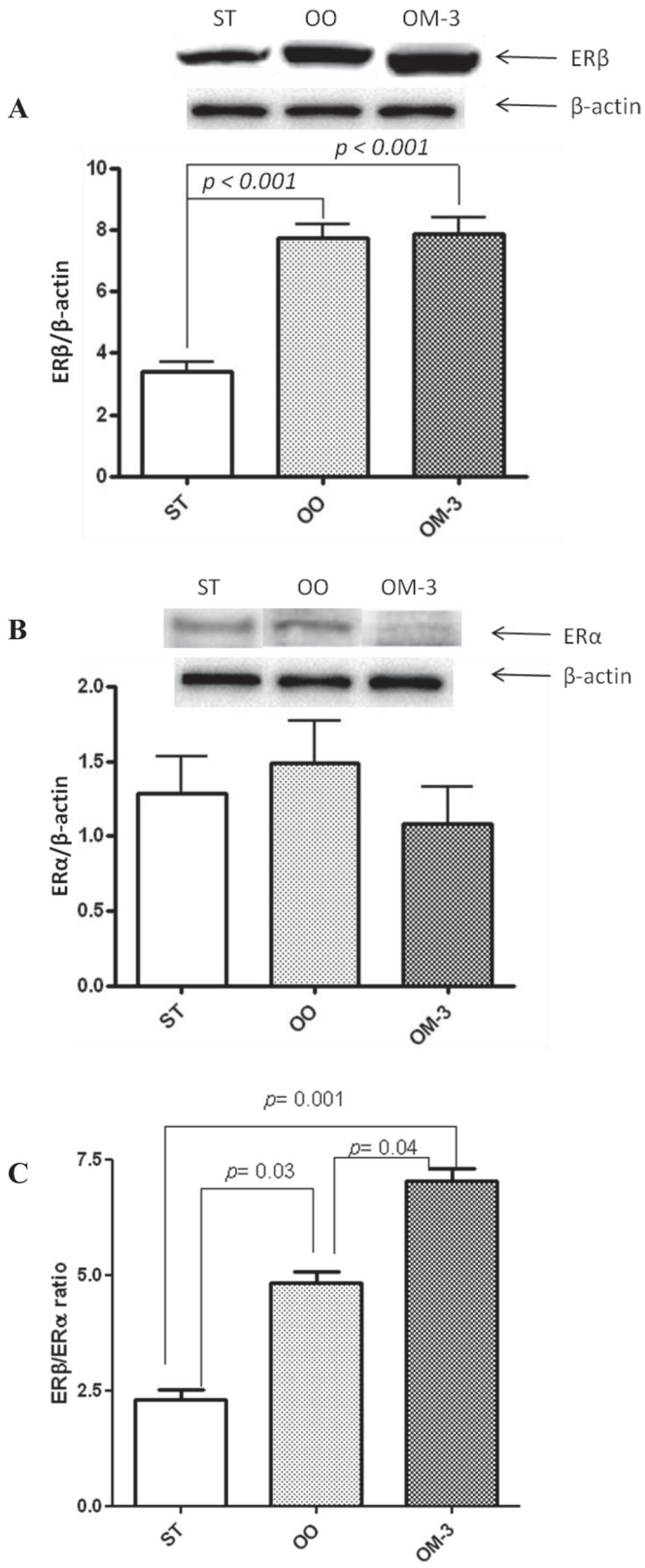

Fig. 5. Western blotting analysis of ER levels in polyp tissue from *Apc*$^{Min/+}$ mice treated groups. (**A**) Levels of ERβ expression normalized with β-actin; (**B**) levels of ERα expression normalized with β-actin; (**C**) levels of ERβ/ERα ratio. ST, standard diet; OO, olive oil-enriched diet; OM-3, omega-3 PUFAs-enriched diet. Data are expressed as the mean ± SE. $P < 0.05$ shows statistically significant difference (one-way analysis of variance and Tukey's multiple comparison test).

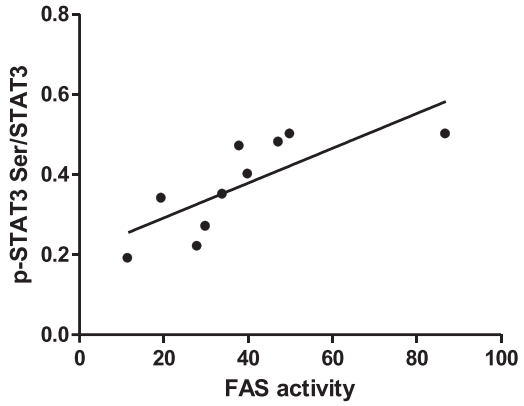

Fig. 6. Spearman correlation between FAS activity and p-STAT3 Ser expression in mice fed with omega-3 PUFAs-enriched diet (OM-3 group). Spearman $r = 0.77$, $P = 0.009$.

Finally, we have already demonstrated that hydroxytyrosol, one of the main phenolic compounds present in olive oil, induces a dose- and time-dependent antiproliferative effect in liver cancer cells by reducing cellular interleukin-6 levels (46). This suggests a possible involvement of interleukin-6 in the antitumorigenic effect of olive oil and omega-3 PUFAs, considering that p-STAT3 Ser activation is controlled by interleukin-6 (20,41).

The dietetic treatment with olive oil and omega-3 PUFAs deeply modified ERβ/ERα ratio in Apc$^{Min/+}$ mice, compared with standard diet. This mechanism has already been proven to be involved in polyp growth reduction in the same animal model (19). Interestingly, the higher increase of ERβ/ERα ratio in OM-3 group compared with OO group could explain the greater reduction of polyps number and volume detected in mice treated with omega-3 PUFAs.

The major limitation of our study is represented by the high percentage of olive oil and omega-3 PUFAs used in the diets. However, we had similar results on polyp formation (number and volume) using a 3% olive oil diet (data not shown). In addition, commercially available fish oils are supplied as ethyl esters, which are up to 5-fold less bioavailable compared with free fatty acids used in other studies (16,17,47,48).

In conclusion, our findings suggest that an appropriate dietary treatment has beneficial effects on health, being also important in primary colon cancer prevention. A better understanding of the effect of dietary manipulation on colonic polyp growth could benefit from a study of intestinal microbiota changes induced by the diet in these mice. Studies in progress in our laboratory are aimed at clarifying this issue.

Conflict of Interest Statement: None declared.

References

1. Chuang,H.Y. *et al.* (2011) Antitumor effect of orlistat, a fatty acid synthase inhibitor, is via activation of caspase-3 on human colorectal carcinoma-bearing animal. *Biomed. Pharm.*, **65**, 286–292.
2. Menendez,J.A. *et al.* (2000) Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nat. Rev. Cancer*, **7**, 763–777.
3. Kuhajda,F.P. (2000) Fatty acid synthase and cancer: new perspective on its role in tumor biology. *Nutrition*, **16**, 202–208.
4. Kuhajda,F.P. (2006) Fatty acid synthase and cancer: new application of an old pathway. *Cancer Res.*, **66**, 5977–5980.
5. Notarnicola,M. *et al.* (2004) Up-regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in left-sided human colon cancer. *Anticancer Res.*, **24**, 3837–3842.
6. Caruso,M.G. *et al.* (1999) Enhanced 3-hydroxy-3-methyl-glutaryl coenzyme A reductase activity in human colorectal cancer not expressing low density lipoprotein receptor. *Anticancer Res.*, **19**, 451–454.
7. Caruso,M.G. *et al.* (2002) 3-hydroxy-3-methylglutaryl Coenzyme A reductase activity and low density lipoprotein receptor expression in diffuse-type and intestinal-type human gastric cancer. *J. Gastroenterol.*, **37**, 504–508.

8. Thurnher,M. *et al.* (2013) Regulation of mevalonate metabolism in cancer and immune cells. *Biochim. Biophys. Acta*, **1831**, 1009–1015.
9. Waterman,E. *et al.* (2007) Active components and clinical applications of olive oil. *Altern. Med. Rev*., **12**, 331–342.
10. Corona,G. *et al.* (2007) Inhibition of p38/CREB phosphorylation and COX-2 expression by olive oil polyphenols underlies their anti-proliferative effects. *Biochem. Biophys. Res. Comm*., **362**, 606–611.
11. Notarnicola,M. *et al.* (2011) Effects of olive oil polyphenols on fatty acid synthase gene expression and activity in human colorectal cancer cells. *Genes Nutr*., **6**, 63–69.
12. Tavani,A. *et al.* (2007) n-3 polynsaturated fatty acid intake and cancer risk in Italy and Switzerland. *Int. J. Cancer*, **105**, 113–116.
13. Dupertuis,Y.M. *et al.* (2007) Colon cancer therapy: new perspectives of nutritional manipulations using polyunsaturated fatty acids. *Curr. Opin. Clin. Nutr. Metab. Care*, **10**, 427–432.
14. Altenburg,J.D. *et al.* (2009) Omega-3 polyunsaturated fatty acids down modulate CXCR4 expression and function in MDA-MB-231 breast cancer cells. *Mo. Cancer Res*., **7**, 1013–1020.
15. Kang,K.S. *et al.* (2010) Docosahexaenoic acid induces apoptosis in MCF-7 cells *in vitro* and *in vivo* via reactive oxygen species formation and caspase 8 activation. *PLoS One*, **5**, e10296.
16. Fini,L. *et al.* (2010) Highly purified eicosapentaenoic acid as free fatty acids strongly suppresses polyps in ApcMIN/+ mice. *Clin. Cancer Res*., **16**, 5703–5711. doi:10.1158/1078-0432.CCR-10–1990.
17. West,N.J. *et al.* (2010) Eicosapentaenoic acid reduces rectal polyp number and size in familial adenomatous polyposis. *Gut*, **59**, 918–925.
18. Cao,W. *et al.* (2012) n-3 poly-unsaturated fatty acids shift estrogen signaling to inhibit human breast cancer cell growth. *PLoS One*, **7**, e52838.
19. Barone,M. *et al.* (2010) Dietary-induced ERβ upregulation counteracts intestinal neoplasia development in intact male ApcMin/+ mice. *Carcinogenesis*, **31**, 269–274.
20. Di Leo,A. *et al.* (2008) ER-beta expression in large bowel adenomas: implications in colon carcinogenesis. *Dig. Liv. Dis*., **40**, 260–266.
21. Barone,M. *et al.* (2010) ERβ expression in normal, adenomatous and carcinomatous tissues of patients with familial adenomatous polyposis. *Scandinavian J. Gastroenterol*., **45**, 1320–1328.
22. Barone,M. *et al.* (2012) Phytoestrogens as nutritional modulators in colon cancer prevention. In Georgakilas,A. (ed.) *Cancer Prevention – From Mechanisms to Translational Benefits*. Intech, Rijeka, Croatia, pp. 321–330.
23. Edvardsson,K. *et al.* (2013) Estrogen receptor β expression induces changes in the microRNA pool in human colon cancer cells. *Carcinogenesis*, **34**, 1431–1441.
24. Notarnicola,M. *et al.* (2012) Low levels of lipogenic enzymes in peritumoral adipose tissue of colorectal cancer patients. *Lipids*, **47**, 59–63.
25. Pallottini,V. *et al.* (2005) 3-Hydroxy-3-methylglutaryl coenzyme A reductase deregulation and age-related hypercholesterolemia: a new role for ROS. *Mech. Aging Dev*., **126**, 845–851.
26. Agnoli,C. *et al.* (2013) Italian Mediterranean Index and risk of colorectal cancer in the italian section of the EPIC cohort. *Int. J. Cancer*, **132**, 1404–1411.
27. Estruch,R. *et al.* (2013) Primary prevention of cardiovascular disease with a Mediterranean diet. *N. Engl. J. Med*., **368**, 1279–1290.
28. Half,E. *et al.* (2009) Colon cancer: preventive agents and the present status of chemoprevention. *Exp. Opin. Pharmacother*., **10**, 211–219.
29. Bartolí,R. *et al.* (2000) Effect of olive oil on early and late events of colon carcinogenesis in rats: modulation of arachidonic acid metabolism and local prostaglandin E(2) synthesis. *Gut*, **46**, 191–199.
30. Femia,A.P. *et al.* (2008) No effects of olive oils with different phenolic content compared to corn oil on 1,2-dimethylhydrazine-induced colon carcinogenesis in rats. *Eur. J. Nutr*., **47**, 329–334.
31. Mai,V. *et al.* (2003) Calorie restriction and diet composition modulate spontaneous intestinal tumorigenesis in ApcMin mice through different mechanisms. *Cancer Res*., **63**, 1752–1755.
32. Lin,S. *et al.* (2013) Mammary inflammation around parturition appeared to be attenuated by consumption of fish oil rich in n-3 polyunsaturated fatty acids. *Lipids Health Dis*., **12**, 190.
33. Stark,A.H. *et al.* (2002) Olive oil as a functional food: epidemiology and nutritional approaches. *Nutrition Rev*., **60**, 170–176.
34. Rafehi,H. *et al.* (2012) Investigation into the biological properties of the olive polyphenol, hydroxytyrosol: mechanistic insights by genome-wide mRNA-Seq analysis. *Genes Nutr*., **7**, 343–355.
35. Chapkin,R.S. *et al.* (2008) Mechanisms by which docosahexaenoic acid and related fatty acids reduce colon cancer risk and inflammatory disorders of the intestine. Chem. *Phys. Lipids*, **153**, 14–23.
36. Dupertuis,Y.M. *et al.* (2007) Colon cancer therapy: new perspectives of nutritional manipulations using polyunsaturated fatty acids. *Curr. Opin. Clin. Nutr. Metab. Care*, **10**, 427–432.
37. Balkwill,F. *et al.* (2001) Inflammation and cancer: back to Wirchow? *Lancet*, **357**, 539–545.
38. Mantovani,A. *et al.* (2006) RalB signaling: a bridge between inflammation and cancer. *Cell*, **127**, 42–44.
39. Notarnicola,M. *et al*. (2011) Polyunsaturated fatty acids reduce fatty acid synthase and hydroxy-methyl-glutaryl CoA-reductase gene expression and promote apoptosis in HepG2 cell line. *Lipids Health Dis*., **10**, 10.
40. Bardin,A. *et al.* (2004) Loss of ERbeta expression is a common step in estrogen-dependent tumor progression. *Endocr. Relat. Cancer*, **11**, 537–551.
41. Jassam,N. *et al.* (2005) Loss of expression of oestrogen receptor beta in colon cancer and its association with Dukes' staging. *Oncol. Rep*., **14**, 17–21.
42. Goodman,W.A. *et al.* (2011) Stat3 phosphorylation mediates resistance of primary human T cells to regulatory T cell suppression. *J. Immunol*., **186**, 3336–3345.
43. Gao,S.P. *et al.* (2007) Mutations in the EGFR kinase domain mediate STAT3 activation via IL-6 production in human lung adenocarcinomas. *J. Clin. Invest*., **117**, 3846–3856.
44. Koukos,G. *et al.* (2013) MicroRNA-124 regulates STAT3 expression and is down-regulated in colon tissues of pediatric patients. *Gastroenterology*, **145**, 842–852.
45. Behera,R. *et al.* (2010) Activation of JAK2/STAT3 signaling by osteopontin promotes tumor growth in human breast cancer cells. *Carcinogenesis*, **31**, 192–200.
46. Tutino,V. *et al.* (2012) Antiproliferative, antioxidant and anti-inflammatory effects of hydroxytyrosol on human hepatoma HepG2 and Hep3b cell lines. *Anticancer Res*., **32**, 5371–5377.
47. Lawson,L.D. *et al.* (1988) Human absorption of fish oil fatty acids as triacylglycerols, free acids or ethyl esters. *Biochem. Biophys. Res. Commun*., **152**, 328–335.
48. Gudbrandsen,O.A. *et al.* (2009) The absorption, distribution and biological effects of a modified fatty acid in its freeform and as an ethyl ester in rats. *Chem. Biol. Interact*., **179**, 227–232.

Received November 6, 2013; revised February 11, 2014; accepted March 10, 2014