European Review for Medical and Pharmacological Sciences 2014; 18: 3311-3314

# Effects of polyunsaturated fatty acids on endometrial carcinoma Ishikawa cells

X.-Y. LU, T. ZENG

Department of Obstetrics and Gynecology, The Affiliated Hospital of Xuzhou Medical College, Xuzhou, Jiangsu, China

**Abstract. – OBJECTIVE: This study aimed to explore the effects and mechanisms of polyunsaturated fatty acids in the endometrial carcinoma Ishikawa cells.**

**MATERIALS AND METHODS: The Ishikawa cells were treated with n-6 PUFAs, n-3 PUFAs, or the mixture of n-6/n-3 PUFAs at a ratio of 1:1/10:1. Cell proliferation was quantified by MTT assay, while expressions of Akt, mTOR and VEGF mRNAs by PCR.**

**RESULTS: Treatment with n-6 PUFAs and 10:1 n-6/n-3PUFAs markedly promoted cell proliferation and up-regulated expressions of Akt, mTOR and VEGF mRNAs. Further, n-3 PUFAs and 1:1 n-6/n-3 PUFAs markedly suppressed cell proliferation and down-regulated expressions of Akt, mTOR and VEGF mRNAs.**

**CONCLUSIONS: Different ratios of n-6/n-3PUFAs exhibit differential effects on the endometrial carcinoma Ishikawa cells through the PI3k/Akt/mTOR signaling pathway and differentially regulate VEGF expression.**

*Key Words:*
Polyunsaturated fatty acids, Endometrial carcinoma Ishikawa cells, PI3k/Akt, mTOR.

## Introduction

The incidence of endometrial cancer is increasing and now presents with an annual morbidity of 189,000 cases and mortality of 45,000 cases[1]. The most important risk factor for endometrial cancer is exposure to unopposed estrogen[2]. Further, unhealthy diet also plays a role in the development of endometrial cancer[3]. Specifically, an unbalanced ratio of dietary n-6 polyunsaturated fatty acids (n-6 PUFAs) and n-3 polyunsaturated fatty acids (n-3 PUFAs), with a prevalence of the former, is thought to lead to many diseases, including endometrial cancer. It is further believed that n-3 PUFAs may play a role as potential anti-cachectic agents[4,5]. In the present study, we examined the effects and mechanisms of different ratios of polyunsaturated fatty acids in the endometrial carcinoma Ishikawa cells to gain a scientific evidence for the use of n-3 PUFAs in the treatment of endometrial cancer.

## Materials and Methods

### Cell Culture

The endometrial carcinoma Ishikawa cells were obtained from the Institute of Obstetrics and Gynecology of The Affiliated Hospital of Xuzhou Medical College. The cells were cultured at 37°C in a humidified incubator supplemented with 5% $CO_2$ in high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS).

### Drug Treatment

Linoeic acid (LA) and docosahexaenoic acid (DHA) were obtained from Sigma-Aldirich (St. Louis, MO, USA), diluted in ethanol, and stored at -20°C after flushing with nitrogen. The Ishikawa cells were treated with different PUFAs (n-6 PUFAs, n-3 PUFAs, or the mixture of n-6/n-3 PUFAs at a ratio of 1:1 or 10:1) diluted in culture medium. The n-6 PUFAs and n-3 PUFAs were used at a concentration of $2.0 \times 10^{-4}$ mol/L. Cells in control group were treated with ethanol vehicle only.

### Cell Viability

After cell treatments, 20 μl of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution were added to each well in the 96-well culture plate and incubated with cells for 4 hours at 37°C. Then, culture medium was removed, and 150 μl of dimethyl sulfoxide (DMSO) were added to dissolve the formed formazan. Cell solubilization proceeded for 30 min. After that, cell optical densities were documented at 490 nm.

*Corresponding Author:* Xiaoyuan Lu, MD; e-mail: xy_lu68@aliyun.com

**Table I.** Primer sequences.

| Primer | Sequence | Temperature | Length |
|---|---|---|---|
| β-actin | 5′-TCACCCACACTGTGCCCATCTACG -3′<br>5′-CAGCGGAACCGCTCATTGCCAATGG-3′ | 53°C | 295 bp |
| Akt | 5′- ACGGGCACATTAAGATCACAG-3′<br>5- GGCTGAGCTTCTTCTCGTACA-3′ | 53°C | 414 bp |
| mTOR | 5′-CTGGGACTCAAATGTGTGCAGTTC-3′<br>5′- GAACAATAGGGTGAATGATCCGGG-3′ | 53°C | 538 bp |
| VEGF | 5′-GCCACGGGAGGTGTGTATAG-3′<br>5′- TATTGCAGCAACCCCCACAT -3′ | 58°C | 107 bp |

### *RNA Isolation, Reverse Transcription, and PCR*

Total RNA were extracted and reverse transcribed using the kit from Bioteker (Beijing, China). Primers for polymerase chain reaction (PCR) quantification of expression Akt, mammalian target of rapamycin (mTOR) and vascular endothelial growth factor (VEGF) mRNAs were purchased from Invitrogen (Shanghai, China), with expression of β-actin serving as endogenous control. Primer sequences are presented in Table I. PCR products were elecrophoresed on a 1.5%-agarose gel (100 volt for 40 min), and then analyzed using gel imaging analysis system.

### *Statistical Analysis*

The experiments were repeated three times. All statistical analyses were performed using the SPSS13 (SPSS Inc., Chicago, IL, USA). The data are presented are mean ± SEM of three experiments. The values were compared using the Student's *t* or ANOVA tests. The *p* value of < 0.05 was considered as statistically significant.

## Results

### *Effects of Different Ratios of PUFAs on Growth of Ishikawa Cells*

Compared with cells in control group, n-6 PUFAs and 10:1 n-6/n-3PUFAs significantly facilitated cell proliferation ($p < 0.05$ vs. control group; Figure 1). By contrast, n-3 PUFAs and 1:1 n-6/n-3 PUFAs greatly suppressed cell proliferation ($p < 0.05$ vs. control group; Figure 1).

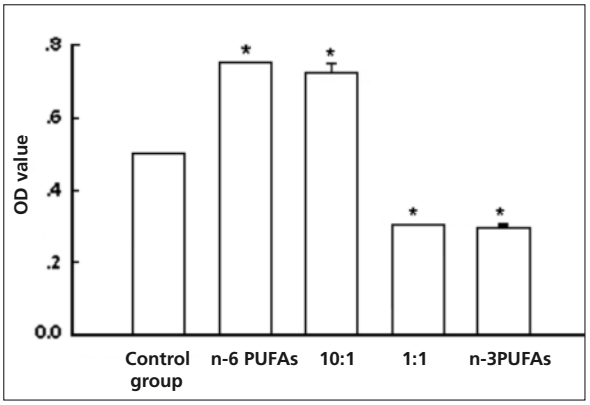


**Figure 1.** Cell viability. *$p < 0.05$ vs. control group.

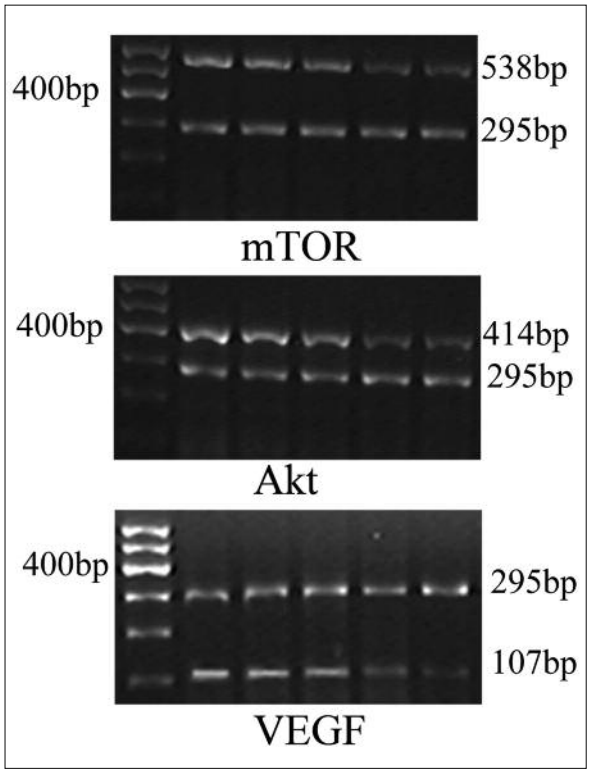


**Figure 2.** Expression of Akt, mTOR and VEGF mRNAs.

**Table II.** Expression of Akt, mTOR and VEGF mRNAs.

| Groups | Akt | mTOR | VEGF |
|---|---|---|---|
| n-6 | 0.903 ± 0.03 | 1.133 ± 0.03 | 0.767 ± 0.02* |
| 10:1 | 0.894 ± 0.05 | 1.122 ± 0.03 | 0.733 ± 0.03* |
| Control | 0.867 ± 0.09 | 1.089 ± 0.04 | 0.667 ± 0.04 |
| 1:1 | 0.575 ± 0.02 | 0.611 ± 0.02 | 0.417 ± 0.02* |
| n-3 | 0.531 ± 0.04 | 0.556 ± 0.02 | 0.367 ± 0.02* |

*Footnote:* Results are presented as mean ± SEM of three experiments. *$p < 0.05$ vs. control group.

### Expression of Akt, mTOR and VEGF mRNAs

We observed that n-6 PUFAs and 10:1 n-6/n-3PUFAs markedly up-regulated expression of Akt, mTOR and VEGF mRNAs ($p < 0.05$ vs. control group; Figure 2 and Table II). In a marked contrast, n-3 PUFAs and 1:1 n-6/n-3 PUFAs significantly down-regulated the levels of these mRNAs ($p < 0.05$ vs. control group; Figure 2 and Table II).

## Discussion

PUFAs, uptaken with food, are necessary fatty acids. Levels of n-3 PFUAs in cancer patients are less 50% than those in healthy individuals. The incidence of cancer inversely correlates with the n-3 PUFAs intake[6-8]. Supporting the role of these fatty acids, our study demonstrates that proliferation of Ishikawa carcinoma cells is facilitated by n-6PUFAs and 10:1 n-6/n-3 PUFAs, while n-3PUFAs and 1:1 n-6/n-3 PUFAs markedly suppressed cell proliferation.

The phosphatidylinositol 3'-kinase (*PI3K*)/Akt pathway regulates cell proliferation, growth, survival, and apoptosis. One of the major consequences of PI3k/Akt activation is phosphorylation of mTOR, a PI3k regulated protein kinase, that responds to external environment[9]. VEGF is also one of the most important factors in indicating the prognosis of malignant tumor[1,10]. When the mTOR is phosphorylated by PI3k/Akt information, it auto-phosphorylates, then activates and releases eIF4E, up-regulating several growth factors, including VEGF, to promote cell proliferation[11,12]. It has been reported that the PI3k/Akt/mTOR pathways is the most frequent abnormal signaling pathway in endometrial carcinoma[13]. Specifically, when estrogen receptor is overexpressed in endometrial carcinoma, it binds to the p85 subunit of PI3K which leads to activation of the PI3K/Akt/mTOR pathway and uncontrolled tumor cell proliferation[14]. We, therefore, decided to test Ishikawa cells which overexpress estrogen receptor as a model for endometrial carcinoma. In these cells n-6 PUFAs and 10:1 n-6/n-3PUFAs markedly up-regulated the levels of Akt, mTOR and VEGF mRNAs, while the effects n-3 PUFAs and 1:1 n-6/n-3 PUFAs were exactly the opposite. Therefore, it can be concluded that different ratios of n-6/n-3PUFAs exhibit differential effects on the endometrial carcinoma Ishikawa cells through the PI3k/Akt/mTOR signalling mechanism and regulation of VEGF expression.

The present study examined one pertinent pathway that is modulated by PUFAs. It is possible that other pathways can be affected as well. It is necessary to study in a greater detail the beneficial effects of n-3PUFAs as anticancer agents or as adjuvants to conventional anticancer therapies for endometrial carcinoma.

## Conclusions

Our results confirm the anti-proliferative and anti-tumorigenic efficacy of n-3PUFAs.

### Conflict of Interest

The Authors declare that there are no conflicts of interest.

## References

1) Dobrzycka B, Terlikowski SJ, Kwiatkowski M, Garbowicz M, Kinalski M, Chyczewski L. Prognostic significance of VEGF and its receptors in endometrioid endometrial cancer. Ginekol Pol 2010; 81: 422-425.

2) Buchanan EM, Weinstein LC, Hillson C. Endometrial cancer. Am Fam Physician 2009; 80: 1075-1080.

3) Simopoulos AP. Genetic variants in the metabolism of omega-6 and omega-3 fatty acids: their role in the determination of nutritional requirements and chronic disease risk. Exp Biol Med (Maywood) 2010; 235: 785-795.

4) Colomer R, Moreno-Nogueira JM, Garcia-Luna PP, Garcia-Peris P, Garcia-de-Lorenzo A, Zarazaga A, Quecedo L, del Llano J, Usan L, Casimiro C. N-3 fatty acids, cancer and cachexia: a systematic review of the literature. Br J Nutr 2007; 97: 823-831.

5) Puska P. The North Karelia Project: 30 years successfully preventing chronic diseases. Diabetes Voice 2008; 53: 26-29 Special Issue.

6) Murphy RA, Mourtzakis M, Mazurak VC. n-3 polyunsaturated fatty acids: the potential role for supplementation in cancer. Curr Opin Clin Nutr Metab Care 2012; 15: 246-251.

7) Gerber M. Omega-3 fatty acids and cancers: a systematic update review of epidemiological studies. Br J Nutr 2012; 107(Suppl 2): S228-239.

8) Gerber M. Background review paper on total fat, fatty acid intake and cancers. Ann Nutr Metab 2009; 55: 140-161.

9) Coutte L, Dreyer C, Sablin MP, Faivre S, Raymond E. [PI3K-AKT-mTOR pathway and cancer]. Bull Cancer 2012; 99: 173-180. in French.

10) Smerdel MP, Waldstrom M, Brandslund I, Steffensen KD, Andersen RF, Jakobsen A. Prognostic importance of vascular endothelial growth factor-A expression and vascular endothelial growth factor polymorphisms in epithelial ovarian cancer. Int J Gynecol Cancer 2009; 19: 578-584.

11) Baldo P, Cecco S, Giacomin E, Lazzarini R, Ros B, Marastoni S. mTOR pathway and mTOR inhibitors as agents for cancer therapy. Curr Cancer Drug Targets 2008; 8: 647-665.

12) Richter JD, Sonenberg N. Regulation of cap-dependent translation by eIF4E inhibitory proteins. Nature 2005; 433: 477-480.

13) Dedes KJ, Wetterskog D, Ashworth A, Kaye SB, Reis-Filho JS. Emerging therapeutic targets in endometrial cancer. Nat Rev Clin Oncol 2011; 8: 261-271.

14) O'Regan R, Hawk NN. mTOR inhibition in breast cancer: unraveling the complex mechanisms of mTOR signal transduction and its clinical implications in therapy. Expert Opin Ther Targets 2011; 15: 859-872.