PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 10 (8): 773-780, 2011
ISSN 1680-5194

Consumption of Corn or Olive Oil Have Protective Effects Due to Production of Pro-inflammatory Cytokines; Immunological Responses to Dietary Oil

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Abstract: Consumption of unsaturated fatty acids could affect the immune response to oxidative or bacterial challenge. To evaluate the protective effect of dietary oils on immune system, female rats were fed for 6 weeks either with 25 or 210 mg/kg of corn or olive oils, followed by acute lipopolysaccharide (100 and 200 μg/kg) challenge. The cytokines (IL-4, IL-10 and IFN-γ) productions were evaluated and the values were compared with the corresponding results obtained for the untreated rats. Following low dose of LPS challenge, production of IL-4 and IL-10 were higher among the animals consumed oils ($p<0.05$) while production of IFN-γ was lower following septic shock ($p<0.05$). These observations may suggest consumption of corn or olive oil could have protective effects by production of pro-inflammatory cytokines. Furthermore, use of unsaturated fatty acids presented in these oils might be useful therapies in acute or chronic inflammatory diseases.

Key words: Corn oil, olive oil, atherogenic compounds, fatty component, cardio vascular disease (CVD), fatty acids, high saturated fatty acids, health benefits, pathogen-derived molecules, immune system, bacterial sepsis

INTRODUCTION

It is well documented that diet and lifestyle directly affect the state of health, diseases or even death rate of different population (Nakamura, 2008; Parekh *et al*., 2008; Ilich *et al*., 2009; Lopez *et al*., 2009). Improving diet and lifestyle is a critical component of the American Heart Association's (AHA's) strategy for preventing Cardiovascular Disease (CVD), which is the leading cause of morbidity and mortality in Americans (Lichtenstein *et al*., 2006). AHA recommended besides focusing in the balance diet, individuals should aim to improve their whole or overall diet and consume a variety of fruits, vegetables and grain products, especially whole grains (Lichtenstein *et al*., 2006). The reasons for such recommendations are the results of many studies that showed reduction of high saturated fatty acids or animal fat in the diets (atherogenic compounds) could reduce the risk of CVD, while consumption of different vegetables could reduce the chance of developing different acute or chronic diseases (Kuller *et al*., 2006; Kris-Etherton, 2007). That is why there is a most impressive effort is going on by many different groups to communicate nutrition messages to many target populations.

It is well documented that almost all the vegetables and their oil have some poly-unsaturated fatty acids. These fatty acids not only could be used as structural components of cells that provide an adequate fluidity to the biological membranes, but also have role in diminishing the hazard of lipid peroxidation by reducing compounds that could neutral oxidizing agents (free radicals) developed in the body during normal life and inflammation or chronic diseases (De Lorgeril *et al*., 2002; Rasheed *et al*., 2009). That is why Tunisian traditionally uses olive oils as a medicinal food for chronic inflammation (Yamada *et al*., 2008), without knowing its mechanism.

Olive oil which is the main fatty component of the Mediterranean diet, characterized by consisting of monounsaturated fatty acids as well as elevated content of antioxidant agents. This oil exhibits numerous biological functions which are beneficial for the state of health (Alarcon De La Lastra *et al*., 2001; Bitler *et al*., 2005; Yamada *et al*., 2008). For these reasons, extra-virgin olive oil was considered as a middle road between food and medicine, while there is growing evidence that its health benefits include reduction of CVD risk factor, prevention of several types of cancer and the modification of immune and inflammatory responses (Caramia, 2006).

Previous researcher showed addition of different dilutions of olive oil to the cell line established from a patient with chronic myelogenous leukemia, dose-dependently inhibited the production of tumor necrosis

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factor-alpha (TNF- α), interleukin-4 (IL-4) and histamine (Marquez-Martin *et al*., 2006; Rasheed *et al*., 2009). Though these in-vitro results showed direct effect of olive oil on the immune cells, but it is far to extend these results to in-vivo, especially due the possible changes of oil during its passage through digestive and lymphatic systems. On the other hands, there are some discrepancy between the researches that studied the *in-vivo* effect of olive oil on immune system (Yaqoob *et al*., 1998; Gawecka *et al*., 2008), particularly during inflammation.

Furthermore, bacterial sepsis or septic shock results from the overproduction of inflammatory mediators as consequence of the interaction between the host immune system and bacteria or bacterial wall components. One of the most known pathogen-derived molecules is Lipopolysaccharide (LPS), which is the main glycolipids component of the outer membrane of gram-negative bacteria. It was shown that Corn oil supplements increases the production of proinflammatory cytokine (TNF-α and Eicosanoids) by LPS-stimulated BALF cells (Hall *et al*., 2004), while in another *in-vivo* study corn oil diminished the production of proinflammatory cytokines of rats following a LPS challenge (Sadeghi *et al*., 1999). On the other hand, mice fed with high corn oil diet had significantly reduced interferon-gamma (IFN-γ) and IL-10 levels, while mice fed with poly unsaturated fatty acid have produced greater levels of IFN-γ than the corn oil fed animals (Ly *et al*., 2005).

Although previous studies showed exposure to physiological concentrations of dietary polyunsaturated fatty acids can trigger inflammatory pathways and leads to the up-regulation of inflammatory cytokines, still many questions remain to be answered about the amount of oil consume or its composition and their consequences in health and diseases. Furthermore, while nutritional manipulation is helpful in endotoxemia, contradictory result were reported in previous studies. Finally, lack of a long term oil consumption on immune responses in health and diseases or its consequences on circulating glucose level or lipid profile at the same time, made us to design this study to determine *in vivo* role of T cel-derived cytokines (IL-10, IL-4 and INF-γ) before and after 6 weeks oil consumption. These cytokines were selected because IL-10 acts as a potent anti-inflammatory cytokine by conditioning the activation and function of innate and antigen-specific immune cells (Bazzoni *et al*., 2010), while IL-4 is pro-inflammatory cytokine that has role in the pathogenesis of immune system (Njoku, 2010). Furthermore, both IL-10 and IL-4 are produced by T-helper 2 (Th2) lymphocytes and the balance between Th2 and Th1 could be assess by a cytokine produced by Th1 such as INF-γ (Liu *et al*., 2009). The protocol was followed by the induction of infection by LPS or production of septic shock.

MATERIALS AND METHODS

Research approval and ethic committees of Kerman University of Medical Sciences approved the protocols for this study, which is in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) or the US guidelines (NIH publication No. 85-23, revised in 1985).

Forty- six female Sprague-Dawley rats weighing 250-300 g were randomly divided to 6 different groups (8 heads per group). The groups were untreated control group, vehicle treated group that received distilled water by gavage (sham) and four treated groups of low (25 mg/kg) and high (210 mg/kg) doses of olive oil or corn oil, respectively. All the animals were weighted initially and housed in an air conditioned animal house at 23±2° C with an alternating light/dark cycle of 12 h and free access to pelleted diet and water. The animals received human care in compliance before handling and gavage feeding. Body weights of all the groups were measured weekly, throughout the experimental protocol. The experimental animals were fed with appropriated doses of corn or olive oil by gavage daily, for six weeks. At the end of six weeks of experimental protocol, a blood sample was collected from all animals.

To produce infection, each member of the respective experimental groups was injected intrapretoneally with a dose of 100 μg/kg (called low dose) of LPS (*Escherichia coli* serotype 055: B5, Sigma Chemical, St. Louis, MO) (Sadeghi *et al*., 1999) and another blood sample was collected from each member of all groups. To produce septic shock, 24 h after first LPS challenge; animals received another dose of LPS (200 μg/kg) injection.

Blood collection and analysis: The initial blood sample was collected via tail in anesthetized condition and after six weeks of oil consumption. The blood samples were kept in room temperature for 10-15 min for the formation of clot and then centrifuged (800 g for 10 min) to separate sera. Twenty four hours post LPS challenge (low dose) another sample was collected, while the last sample was collected 4 h after high dose of LPS injection. All the serum samples collected from each rat at different times were labeled and stored at -70^{0}C until analysis.

Blood glucose (end point colorimetric glucose oxidase method), cholesterol (cholesterol esterase enzymatic colorimetric method) and triglyceride (lipoprotein lipase enzymatic colorimetric) of all animals were measured, using an automated analyzer (RA-1000, Technicon, USA). Cytokines (interleukin 4, interleukin 10 and IFN-γ) were evaluated for all samples collected, following the protocols provided by the manufacture of ELISA (Bender MedSystems GmbH, Austria) kits.

Data analysis: Data were analyzed using the SPSS (Version 14.0) program. The statistical model included the effects of diet for six weeks (normal rats pellet diet or sham group versus addition of corn oil or olive oil) and LPS challenges (saline versus LPS). Differences between treatments were analyzed using student pair T-test, while for comparison of effects among different groups ANOVA (Post Hoc model of Bonferroni) was used. The data in the text and table are presented as mean ± Standard Deviation (SD) and in the figures present as mean ± Standard Error of Means (SEM). P values of less than 0.05 were considered as statistical significance.

RESULTS

Except the sham group, body weights of all other groups gradually increased during the experimental protocol. These increases were significant ($p<0.05$). Statically, comparison of weight gained among different groups showed only corn oil consumed groups had a significant weight gain compared to the sham group ($p<0.001$), while neither of groups had a significant increase in the weight, when compared with control groups.

Evaluation of serum chemistries showed consumption of different doses of olive oil or corn oil did not affected circulating glucose level of animals, but cholesterol and triglyceride of animals on corn oil (both groups) increased significantly ($p<0.01$) after 6 weeks. Finally, evaluated circulating cytokines showed that the housing of all animals for 6 weeks did increase ($p<0.01$) the level of IFN-γ irrespective of different treatment that they received. On the other hand, neither the housing nor different treatments could affect circulating levels of IL-10 or IL-4 (Table 1).

Twenty-four hours post injection of animals with low dose of LPS (100 μg/kg), circulating level of IL-4 increased significantly ($p<0.01$) among the oil consumed groups, while it did not changed (significantly) among the control or sham groups. In addition, 4 h after production of septic shock (injection with 200 μg/kg) IL-4 raised significantly ($p<0.01$) among the group fed with the high dose of olive oil, while a non-significant rise was also detected for the low olive oil group. Septic shock could not further increase the level of IL-4 that were increased after production of infection among corn oil fed rats (Fig. 1).

Following low dose of LPS injection, production of IL-10 significantly ($p<0.001$) raised among all experimental groups (compared with their baseline), but with the dissimilarity as shown in Fig. 2. When these responses were compared with the results obtained for the sham groups, increases detected for olive oil fed groups were significantly higher ($p<0.05$), while the differences for the corn oil fed groups were not significant. IL-10 productions after septic shock were also different among different experimental groups. While IL-10

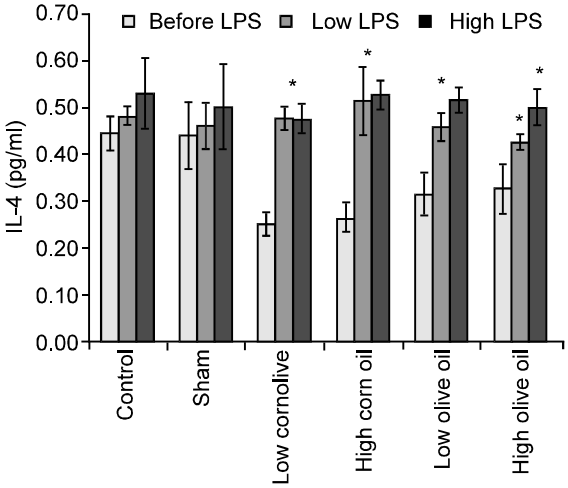

Fig. 1: Circulating IL-4 (pg/ml) levels determined for the different animal groups after 6 weeks primary treatments (before LPS), 24h subsequent to receiving 100 μg/kg (low LPS) LPS intrapretoneally and 4h following producing septic shock by injection of 200 μg/kg of LPS to the animals (high LPS). The (*) symbol shows significant (at least $p<0.05$) differences between self-baseline and post LPS injection or septic shock results

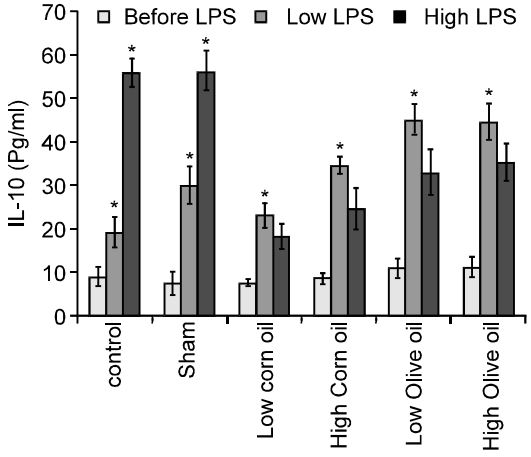

Fig. 2: Circulating IL-10 (pg/ml) levels determined for the different animal groups after 6 weeks primary treatments (before LPS), 24h subsequent to receiving 100 μg/kg (low LPS) LPS intrapretoneally and 4h following producing septic shock by injection of 200 μg/kg of LPS to the animals (high LPS). The (*) symbol shows significant (at least $p<0.05$) differences between self-baseline and post LPS injection or septic shock results

Table 1: Results of different parameters evaluated for the different experimental groups

	Control	Sham	Low corn oil	High corn oil	Low olive oil	High olive oil
Weight (gm) before	211.14 (15.61)	206.63 (8.57)	214.75 (11.94)	211.63 (10.09)	203.00 (11.63)	208.00 (12.68)
Weight (gm) after	227.57 (16.81)*	214.13 (11.24)	241.88 (18.60)*	241.75 (14.49)*	221.71 (20.62)*	228.25 (21.52)*
Glucose (mg/dl) before	139.43 (12.95)	135.75 (7.97)	135.38 (6.36)	138.38 (13.27)	129.57 (10.24)	132.88 (9.43)
Glucose (mg/dl) after	143.57 (10.58)	141.38 (7.91)	148.63 (14.47)	147.88 (23.05)	125.86 (13.01)	143.88 (20.15)
Cholesterol (mg/dl) before	62.71 (11.17)	54.12 (8.02)	55.87 (7.79)	63.00 (4.78)	57.14 (6.54)	56.87 (9.23)
Cholesterol (mg/dl) after	58.85 (6.22)	57.62 (6.52)	63.75 (3.69)*	69.87 (5.43)*	56.13 (6.64)	57.37 (5.82)
Triglyceride (mg/dl) before	88.42 (17.98)	88.12 (20.69)	69.00 (11.23)	72.00 (21.46)	75.42 (27.04)	70.50 (28.57)
Triglyceride (mg/dl) after	87.42 (14.42)	85.50 (21.45)	103.25 (10.08)*	103.00 (17.63)*	77.71 (26.45)	73.50 (19.39)
IL-10 (pg/ml) before	8.63 (4.94)	8.83 (1.90)	8.01 (1.43)	9.06 (1.61)	10.61 (6.72)	7.54 (3.41)
IL-10 (pg/ml) after	8.89 (3.02)	7.55 (2.96)	7.63 (2.06)	8.56 (3.52)	10.93 (5.95)	11.19 (6.36)
IL-4 (pg/ml) before	0.34 (0.08)	0.34 (0.09)	0.25 (0.08)	0.26 (0.05)	0.23 (0.05)	0.28 (0.05)
IL-4 (pg/ml) after	0.44 (0.10)	0.44 (0.20)	0.26 (0.05)	0.25 (0.07)	0.31 (0.12)	0.33 (0.15)
INF-γ (pg/ml) before	19.84 (7.85)	16.89 (4.25)	13.69 (5.50)	12.69 (2.90)	15.36 (5.13)	15.45 (3.11)
INF-γ (pg/ml) after	30.31 (10.85)*	47.24 (15.71)*	37.80 (15.74)*	35.95 (13.15)*	41.43 (14.57)*	35.14 (6.79)*

Female rats (n = 8 in each group) were fed with normal rats' pellet (control group) or by gavage method with normal saline (sham), low (25mg/kg) and high (210mg/kg) doses of olive or corn oils for six weeks. Significant differences (at least $p<0.05$) between pre and post experimental protocol are shown by*

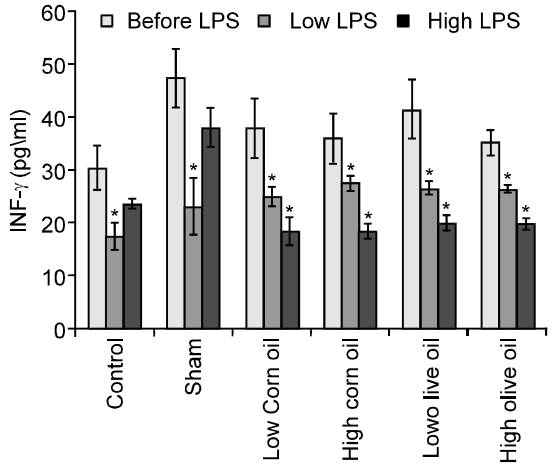

Fig. 3: Circulating INF-γ (pg/ml) levels determined for the different animal groups after 6 weeks primary treatments (before LPS), 24 h subsequent to receiving 100 μg/kg (low LPS) LPS intrapretoneally and 4 h following producing septic shock by injection of 200 μg/kg of LPS to the animals (high LPS). The (*) symbol shows significant (at least $p<0.05$) differences between self-baseline and post LPS injection or septic shock results

production were rises for the animals in the control and sham groups ($p<0.001$),none significantly declines were observed for the oil fed animals (Fig. 2).

As mentioned above, housing of animals for six week increased the circulating level of IFN-γ, but 24 h following low dose of LPS challenge, circulating level of this cytokine decreased for all the groups. Compared to the respective self baseline, all the decreases were significant ($p<0.01$). These decreases obtained for the oil fed groups were not significant when compared with the values obtained for the sham group. On the other hand, after producing septic shock circulating IFN-γ increased for the control and sham groups, while the level of this cytokine decreases among oil fed animals. The increases detected for the control and sham groups were not different from the corresponding values for their baseline or post-infection results of these groups. Finally, the results evaluated for the post septic shock samples of oil fed groups showed significant decreases ($p<0.01$) compared with the self baseline or post-infection results (Fig. 3).

DISCUSSION

Numerous investigations have revealed that nutritional status is a crucial factor that contributes to the modulation of the host immune response (Roebothan and Chandra, 1996; De Pablo *et al*., 2002; Puertollano *et al*., 2004; Puertollano *et al*., 2007). Predominant fatty acid of olive oil is oleic acid ($18:1^{\Delta 9}$) and corn oil contains about 50% linoleic acid ($18:2^{\Delta 9,12}$) as triglycerides (Hennig *et al*., 2005), both of these unsaturated fatty acid are essential for mammals.

While there is a report showing corn oil is well tolerated by mice but tolerance may decrease with excessive ingestion. When given a choice, animal will continue to overeat corn oil and over the long term they induce excessive caloric intake and obesity (Takeda *et al*., 2001). For this reason we restricted the amount of oil by gavage feeding only for six weeks(Wallace *et al*., 2001; Leite *et al*., 2005). Although we carried out all the necessary precaution, still all animals fed with the oils (olive or corn oil) developed obesity when compared with the sham groups, but this overeating could not affect the glucose levels of them. Furthermore, corn oil consumption caused mild increases in the circulating cholesterol level of the animals that were associated with a significant rise of their triglyceride. On the other hand, lipids profiles of olive oil fed animals did not increase during the experimental course (Table 1).

The relationship between dietary lipids and their role in the reduction of the host immune defense against infectious agents is of substantial importance. Our

results showed neither circulating IL-10 nor IL-4 changed after 6 weeks of oil consumption, but the production of INF-γ among all animals including control group increased significantly (nearly doubled). Interferons are cytokines that play a complex and central role in the resistance of mammalian hosts to pathogens (Boehm *et al*., 1997). Although originally defined as an agent with direct antiviral activity, the properties of IFN-γ include regulation of several aspects of the immune response, stimulation of bactericidal activity of phagocytes, stimulation of antigen presentation through class 1 and class 2 Major Histocompatibility Complex (MHC) molecules, orchestration of leukocyte-endothelium interactions (Boehm *et al*., 1997). These increases detected among our animals could be the responses of immune cells (natural killer) to housing of animals and resistance to the pathogen (Boehm *et al*., 1997).

Endotoxin (lipopolysaccharide) is considered to be a central mediator in the pathogenesis of gram-negative sepsis. Since intravenous injection of LPS into healthy animals initiates a cascade of inflammatory pathways, we used LPS to determine the effects of excess dietary fats on immune responses. Results of these set of experiment showed IL-4 of all oil fed animals did increased 24 h after a low dose of LPS (100 μg/kg) injection, but the increases detected for sham or control group did not reach significant level, due to higher level of their IL-4, as shown in Fig. 1. On the other hands, responses to septic shock (4 h after injection of 200 μg/kg LPS) were different for dissimilar oil consumed groups. The level of IL-4 production by corn oil fed groups, remain the same as the one produced 24 h post infection, but the olive oil fed animals produced more IL-4 than the 24 h infection (Fig. 1). Our results also revealed production of IL-10 in response to the infection and septic shock were higher among the control or sham groups, compared to oil fed animals as shown in Fig. 2. Furthermore, comparing the responses among the oil fed animals showed that production of IL-10 was higher among the olive oil consumed animals. Finally, production of infection or septic shock decreased the circulating level of IFN-γ with similar pattern for oil fed animals and dissimilarity for the control or sham groups (Fig. 3). Since our experimental design is established for the first time, we were unable to compare our data with others.

Previous studies of events occurring during the infectious processes contributes to establishment of the action of dietary lipids on the immune system in the course of an infection (Flora *et al*., 2002). similar to our results, other studies also suggest that oleic acid (olive oil) has little effect or even can decrease an inflammatory response, by the alteration in secretion of different cytokines (Calder, 1998; De Pablo and Alvarez De Cienfuegos, 2000; Wallace *et al*., 2001). In the present study, we shown dietary fat could modulate responses to LPS as seen in the cases of septic shock of oil fed animals and changes in the patterns of cytokines production. These results are also similar to pervious reported data that shown the ability of nutrients to modulate the production of cytokines (Carrick *et al*., 1994; Yaqoob and Calder, 1995; Yaqoob *et al*., 1998) that influence the severity of sepsis (Clouva-Molyvdas *et al*., 1992; Lowry, 1993; Utsunomiya *et al*., 1994). Epidemiologic studies also suggest that diets high in olive oil or oleic acid have a direct vascular athero-protective effect and therefore are complementary to, drug-based therapy (Massaro *et al*., 2002a; Massaro *et al*., 2002b; Massaro and De Caterina, 2002).

T-helper (Th) cells can be divided at least into two groups of Th1 and Th2 cells and after stimulation can be distinguished by the pattern of cytokine production (Mosmann and Sad, 1996). A Th1 mediated response is known to enhance cell-mediated immunity, while a Th2 mediated response is associated with humoral immunity (Abbas *et al*., 1996). In vivo administration of LPS with mice resulted in a reduced ability of splenocytes to produce the T-cell cytokines IL-2, IL-4 and IFN-γ (Castro *et al*., 1998) while during systemic infection, a shift toward a Th2 cytokine response has occurred that results in a defect in cell-mediated immunity. In addition, since IFN-γ is a major activator of monocyte functions (Boehm *et al*., 1997), the reduced capacity of T cells to produce IFN-γ may contribute to the diminished monocyte responsiveness during endotoxemia and sepsis (Docke *et al*., 1997). On the other hand, the capacity of T cells to secrete IL-10 is well documented, in particular for Th2 cells and some T cell subsets with regulatory function. T regulatory cells have been shown to suppress immune responses by IL-10 dependent or IL-10 independent mechanisms in various experimental systems (Muraille and Leo, 1998). Also several experimental observations appear that Th1 cells do not produce their own growth factor; but both Th1 and Th2 cells can promote inflammatory responses. IL-10 can inhibit inflammatory responses in a Th1/Th2-independent fashion and the Th1/Th2 balance plays a critical role in the outcome of several infectious diseases (Muraille and Leo, 1998). Here we found that post administration of LPS after 6 weeks consumption of either corn or olive oil resulted in a markedly decreased production of the Th1 cytokines IFN-γ, while the production of the Th2 cytokines IL-4 and IL-10 was increased and the Th1/Th2 balance was shifted towards a Th2 cytokine response.

In conclusion our findings for the first time provides that in female rats consumption of diets containing corn or olive oil can produce anti-inflammatory properties through an enhanced production of IL-4 and hence could be particularly associated with an increase in resistance against infection and inflammation. Since the

modulation of other cytokines by the action of fatty acids may constitute a critical factor directly involving the capacity of immune cells to produce various cytokines a further extension of our studies will be require to identify the soluble factors and intracellular pathways and also the cell type specific activation of IL-4 receptor in post-LPS challenge after dietary fat consumption.

ACKNOWLEDGEMENTS

This study was carried out in Physiology Research Center of Kerman University of Medical Sciences. The funding of this study was provided by research deputy of Kerman University of Medical Sciences, Kerman, Iran (86/34). Research approval and ethic committees of Kerman University approved the protocols for this study (K/87/19).

REFERENCES

Abbas, A.K., K.M. Murphy and A. Sher, 1996. Functional diversity of helper t lymphocytes. Nature, 383: 787-793.

Alarcon De La Lastra, C., M.D. Barranco, V. Motilva and J.M. Herrerias, 2001. Mediterranean diet and health: Biological importance of olive oil. Curr. Pharma. Des., 7: 933-950.

Bazzoni, F., N. Tamassia, M. Rossato and M.A. Cassatella, 2010. Understanding the molecular mechanisms of the multifaceted il-10-mediated anti-inflammatory response: Lessons from neutrophils. Eur. J. Immunol., 40: 2360-2368.

Bitler, C.M., T.M. Viale, B. Damaj and R. Crea, 2005. Hydrolyzed olive vegetation water in mice has anti-inflammatory activity. The J. Nutr., 135: 1475-1479.

Boehm, U., T. Klamp, M. Groot and J.C. Howard, 1997. Cellular responses to interferon-gamma. Annu. Rev. Immunol., 15: 749-795.

Calder, P.C., 1998. Fat chance of immunomodulation. Immunol. Today, 19: 244-247

Caramia, G., 2006. Virgin olive oil. From legend to scientific knowledge of the nutraceutical aspects. Pediatr. Med. Chir., 28: 9-23.

Carrick, J.B., R.G. Schnellmann and J.N. Moore, 1994. Dietary source of omega-3 fatty acids affects endotoxin-induced peritoneal macrophage tumor necrosis factor and eicosanoid synthesis. Shock, Augusta, Ga, 2: 421-426.

Castro, A., V. Bemer, A. Nobrega, A. Coutinho and P. Truffa-Bachi, 1998. Administration to mouse of endotoxin from gram-negative bacteria leads to activation and apoptosis of t lymphocytes. Eur. J. Immunol., 28: 488-495.

Clouva-Molyvdas, P., M.D. Peck and J.W. Alexander, 1992. Short-term dietary lipid manipulation does not affect survival in two models of murine sepsis. Jpen, 16: 343-347.

De Lorgeril, M., P. Salen, F. Paillard, F. Laporte, F. Boucher and J. De Leiris, 2002. Mediterranean diet and the french paradox: Two distinct biogeographic concepts for one consolidated scientific theory on the role of nutrition in coronary heart disease. Cardiovascular Res., 54: 503-515.

De Pablo, M.A. and G. Alvarez De Cienfuegos, 2000. Modulatory effects of dietary lipids on immune system functions. Immunol. Cell Biol., 78: 31-39.

De Pablo, M.A., M.A. Puertollano and G. Alvarez De Cienfuegos, 2002. Biological and clinical significance of lipids as modulators of immune system functions. Clin. Diagnostic Lab. Immunol., 9: 945-950.

Docke, W.D., F. Randow, U. Syrbe, D. Krausch, K. Asadullah, P. Reinke, H.D. Volk and W. Kox, 1997. Monocyte deactivation in septic patients: Restoration by ifn-gamma treatment. Nature Med., 3: 678-681.

Flora, G., Y.W. Lee, A. Nath, W. Maragos, B. Hennig and M. Toborek, 2002. Methamphetamine-induced tnf-alpha gene expression and activation of ap-1 in discrete regions of mouse brain: Potential role of reactive oxygen intermediates and lipid peroxidation. Neuromolecular Med., 2: 71-85.

Gawecka, A., J. Michalkiewicz, M.K. Kornacka, B. Luckiewicz and I. Kubiszewska, 2008. Immunologic properties differ in preterm infants fed olive oil vs soy-based lipid emulsions during parenteral nutrition. Jpen, 32: 448-453.

Hall, J.A., R.J. Van Saun, S.J. Tornquist, J.L. Gradin, E.G. Pearson and R.C. Wander, 2004. Effect of type of dietary polyunsaturated fatty acid supplement (corn oil or fish oil) on immune responses in healthy horses. J. Vet. Internal Med./Am. Coll. Vet. Internal Med., 18: 880-886.

Hennig, B., G. Reiterer, M. Toborek, S.V. Matveev, A. Daugherty, E. Smart and L.W. Robertson, 2005. Dietary fat interacts with pcbs to induce changes in lipid metabolism in mice deficient in low-density lipoprotein receptor. Environ. Health Perspectives, 113: 83-87.

Ilich, J.Z., S. Cvijetic, I.C. Baric, I. Cecic, M. Saric, Z. Crncevic-Orlic, M. Blanusa and M. Korsic, 2009. Nutrition and lifestyle in relation to bone health and body weight in croatian postmenopausal women. Int. J. Food Sci. Nutr., 60: 319-332.

Kris-Etherton, P.M., 2007. The minnesota heart survey: Nutrition successes pave the way for strategic opportunities for food and nutrition professionals. J. Am. Diet. Assoc., 107: 209-212.

Kuller, L.H., L.S. Kinzel, K.K. Pettee, A.M. Kriska, L.R. Simkin-Silverman, M.B. Conroy, F. Averbach, W.S. Pappert and B.D. Johnson, 2006. Lifestyle intervention and coronary heart disease risk factor changes over 18 months in postmenopausal women: The women on the move through activity and nutrition (woman study) clinical trial. J. Women's Health 2002, 15: 962-974.

Leite, M.S., P. Pacheco, R.N. Gomes, A.T. Guedes, H.C. Castro-Faria-Neto, P.T. Bozza and V.L. Koatz, 2005. Mechanisms of increased survival after lipopolysaccharide-induced endotoxic shock in mice consuming olive oil-enriched diet. Shock, Augusta, Ga, 23: 173-178.

Lichtenstein, A.H., L.J. Appel, M. Brands, M. Carnethon, S. Daniels, H.A. Franch, B. Franklin, P. Kris-Etherton, W.S. Harris, B. Howard, N. Karanja, M. Lefevre, L. Rudel, F. Sacks, L. Van Horn, M. Winston and J. Wylie-Rosett, 2006. Diet and lifestyle recommendations revision 2006: A scientific statement from the American heart association nutrition committee. Circulation, 114: 82-96.

Liu, C.T., H.M. Su, C.K. Lii and L.Y. Sheen, 2009. Effect of supplementation with garlic oil on activity of th1 and th2 lymphocytes from rats. Planta Medica, 75: 205-210.

Lopez, L., E.F. Cook, M.S. Horng and L.S. Hicks, 2009. Lifestyle modification counseling for hypertensive patients: Results from the national health and nutrition examination survey 1999-2004. Am. J. Hypertension, 22: 325-331.

Lowry, S.F., 1993. Cytokine mediators of immunity and inflammation. Arch Surg., 128: 1235-1241.

Ly, L.H., R. Smith, R.S. Chapkin and D.N. McMurray, 2005. Dietary n-3 polyunsaturated fatty acids suppress splenic cd4(+) t cell function in interleukin (il)-10(-/-) mice. Clin. Exp. Immunol., 139: 202-209.

Marquez-Martin, A., R. De La Puerta, A. Fernandez-Arche, V. Ruiz-Gutierrez and P. Yaqoob, 2006. Modulation of cytokine secretion by pentacyclic triterpenes from olive pomace oil in human mononuclear cells. Cytokine, 36: 211-217.

Massaro, M. and R. De Caterina, 2002. Vasculoprotective effects of oleic acid: Epidemiological background and direct vascular antiatherogenic properties. Nutr. Metab. Cardiovasc. Dis., 12: 42-51.

Massaro, M., G. Basta, G. Lazzerini, M.A. Carluccio, F. Bosetti, G. Solaini, F. Visioli, A. Paolicchi and R. De Caterina, 2002a. Quenching of intracellular ros generation as a mechanism for oleate-induced reduction of endothelial activation and early atherogenesis. Thrombosis Haemostasis, 88: 335-344.

Massaro, M., M.A. Carluccio, A. Paolicchi, F. Bosetti, G. Solaini and R. De Caterina, 2002b. Mechanisms for reduction of endothelial activation by oleate: Inhibition of nuclear factor-kappab through antioxidant effects. Prostaglandins, Leukotrienes and Essential Fatty Acids, 67: 175-181.

Mosmann, T.R. and S. Sad, 1996. The expanding universe of t-cell subsets: Th1, th2 and more. Immunol. Today, 17: 138-146.

Muraille, E. and O. Leo, 1998. Revisiting the th1/th2 paradigm. Scandinavian J. Immunol., 47: 1-9.

Nakamura, T., 2008. The integration of school nutrition program into health promotion and prevention of lifestyle-related diseases in japan. Asia Pacific J. Clin. Nutr., 17 Suppl 1: 349-351.

Njoku, D.B., 2010. Suppressive and pro-inflammatory roles for il-4 in the pathogenesis of experimental drug-induced liver injury: A review. Expert Opinion Drug Metab. Toxicol., 6: 519-531.

Parekh, N., Y. Lin, S. Marcella, A.K. Kant and G. Lu-Yao, 2008. Associations of lifestyle and physiologic factors with prostate-specific antigen concentrations: Evidence from the national health and nutrition examination survey (2001-2004). Cancer Epidemiol. Biomarkers Prev., 17: 2467-2472.

Puertollano, M.A., E. Puertollano, G. Alvarez De Cienfuegos and M.A. De Pablo, 2007. Significance of olive oil in the host immune resistance to infection. The Br. J. Nutr., 98 Suppl 1: S54-58.

Puertollano, M.A., E. Puertollano, A. Ruiz-Bravo, M. Jimenez-Valera, M.A. De Pablo and G.A. De Cienfuegos, 2004. Changes in the immune functions and susceptibility to listeria monocyto-genes infection in mice fed dietary lipids. Immunol. Cell Biol., 82: 370-376.

Rasheed, Z., N. Akhtar, A.N. Anbazhagan, S. Ramamurthy, M. Shukla and T.M. Haqqi, 2009. Polyphenol-rich pomegranate fruit extract (pomx) suppresses pmaci-induced expression of pro-inflammatory cytokines by inhibiting the activation of map kinases and nf-kappab in human ku812 cells. J. Inflammation (London, England), 6: 1.

Roebothan, B.V. and R.K. Chandra, 1996. The contribution of dietary iron to iron status in a group of elderly subjects. International journal for vitamin and nutrition research. Internationale Zeitschrift fur Vitamin-und Ernahrungsforschung, 66: 66-70.

Sadeghi, S., F.A. Wallace and P.C. Calder, 1999. Dietary lipids modify the cytokine response to bacterial lipopolysaccharide in mice. Immunology, 96: 404-410.

Takeda, M., M. Imaizumi, S. Sawano, Y. Manabe and T. Fushiki, 2001. Long-term optional ingestion of corn oil induces excessive caloric intake and obesity in mice. Nutrition (Burbank, Los Angeles County, Calif), 17: 117-120.

Utsunomiya, T., S.R. Chavali, W.W. Zhong and R.A. Forse, 1994. Effects of continuous tube feeding of dietary fat emulsions on eicosanoid production and on fatty acid composition during an acute septic shock in rats. Biochimica et Biophysica Acta, 1214: 333-339.

Wallace, F.A., E.A. Miles, C. Evans, T.E. Stock, P. Yaqoob and P.C. Calder, 2001. Dietary fatty acids influence the production of th1- but not th2-type cytokines. J. Leukocyte Biol., 69: 449-457.

Yamada, P., M. Zarrouk, K. Kawasaki and H. Isoda, 2008. Inhibitory effect of various tunisian olive oils on chemical mediator release and cytokine production by basophilic cells. J. Ethnopharmacol., 116: 279-287.

Yaqoob, P. and P.C. Calder, 1995. The effects of dietary lipid manipulation on the production of murine t cell-derived cytokines. Cytokine, 7: 548-553.

Yaqoob, P., J.A. Knapper, D.H. Webb, C.M. Williams, E.A. Newsholme and P.C. Calder, 1998. Effect of olive oil on immune function in middle-aged men. The Am. J. Clin. Nutr., 67: 129-135.