# TABLE OF CONTENTS

## Mini Review
1. Impact of Dietary Oxidized Lipids on Energy Metabolism  
   – Marc Pignitter* and Veronika Somoza  
   76-81

## Opinion
2. Perspectives on Foodborne Parasites  
   – Rodrigo Labello Barbosa  
   82-83

## Editorial
3. Essential Oil Nanoemulsions and Food Applications  
   – Daniel Mathias Fuzette Amaral and Kanika Bhargava  
   84-87

## Research
4. Association between Smoking and Anthropometric Characteristics, Biochemical Markers, and Dietary Intake of Pakistani Male Adult Population  
   – Parvez I. Paracha*, Tariq Khan, Frank Vriesekoop, Momin Salahuddin, Saima I. Paracha, Zia Uddin, Niamat Ullah and Yasmin Asif  
   88-96

## Review
5. Ginger and its Effects on Inflammatory Diseases  
   – Naheed Aryaeian* and Hajar Tavakkoli  
   97-101
Impact of Dietary Oxidized Lipids on Energy Metabolism

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ABSTRACT

Consumption of dietary fat is known to influence metabolic rate and metabolic pathways. Dietary intake of unoxidized polyunsaturated fatty acids was shown to lead to an increased metabolic rate. Identification of the underlying mechanism revealed that modifications of the energy metabolism are associated with modifications of membrane lipid composition leading to the membrane pacemaker theory of metabolism. Mitochondrial membranes were shown to adapt their lipids to the dietary fat composition. Dietary fat is commonly prepared by applying heat treatment to increase palatability. Heat treatment of food lipids result in the formation of oxidized lipids. Intake of oxidized lipids might affect energy metabolism in a different way than their corresponding unoxidized lipids. However, scientific literature of the effects of individual oxidized lipids found in heat-treated dietary fats on the energy metabolism relevant for metabolic syndrome, diabetes and obesity research is scarce. This review comprises current knowledge of the impact of unoxidized and oxidized lipids on the energy metabolism.

KEYWORDS: Oxidized lipids; Energy metabolism; Membrane pacemaker theory of metabolism.

ABBREVIATIONS: ATP: Adenosine triphosphate; DHA: Docosahexaenoic acid; EPA: Eicosapentenoic acid; GIT: Gastro Intestinal Tract; LDLR: Low Density Lipoprotein Receptor; PUFA: Polyunsaturated fatty acids; ROS: Reactive Oxygen Species; TAG: Triacylglycerol.

ORIGINS OF DIETARY OXIDIZED LIPIDS

Lipids are macronutrients which predominantly serve as constituents of all membranes, provide energy, and are involved in cellular signaling. The most abundant dietary lipids are Triacylglycerols (TAGs), comprising approximately 80-95% of all dietary lipids.1 Other main dietary lipids are phospholipids and sterols. One of the most prominent representatives of sterols is cholesterol. Besides playing a key role in the physical characteristics of membranes, cholesterol is the precursor for steroid hormones and bile acids. Cholesterol is a monounsaturated lipid, which makes it prone to oxidation comparable to other mono- and polyunsaturated fatty acyl chains in TAGs and phospholipids. The susceptibility of fatty acids to oxidation strongly depends on the degree of unsaturation. A high number of double bonds decreases the energy required for detachment of the bis-allylic hydrogen. While abstraction of the allylic hydrogen atom in oleic acid requires 322 kJ/mol, it only needs 171 kJ/mol in linoleic acid.2 Once lipid oxidation is initiated, lipid radicals are rearranged to form conjugated diene radicals, which, in the presence of molecular oxygen, form peroxy radicals. By generating hydroperoxy lipids, autoxidation propagates. Fatty acid hydroperoxides can be further decomposed to volatile short-chain aldehydes, ketones or alcohols via scission of the carbon chain. Degradation of fatty acid hydroperoxides without scission of the carbon chain leads to the formation of triacylglycericdies with keto, epoxy, hydroxyl and aldehyde groups, the so called oxidized monomers. Fatty acid hydroperoxides can also undergo condensation reactions resulting in the production of oxidized dimers and oligomers. Due to cyclization reactions and isomerizations cyclic fatty acid monomers and trans fatty acids could be identified as degradation products of fatty acid hydroperoxides. For
cholesterol hydroperoxide, which is generated by the abstraction of allylic C-7 hydrogen, the most predominant decomposition products are 7-ketosterone, 25-hydroxycholesterol, 7α-hydroxycholesterol, 7β-hydroxycholesterol, cholesterol-5α,6α-epoxide, cholesterol-5β,6β-epoxide and cholesterol-3,5,6-triol. The amount of lipid oxidation products formed in foods depends on environmental factors, such as temperature, irradiation, oxygen availability, presence of (anti)oxidants, metals and enzymes (lipoxigenases).

AMOUNT OF DIETARY OXIDIZED LIPIDS

Considerable amounts of dietary oxidized lipids have been quantified in Western diet due to processing of food. The amount of cholesterol oxidation products varies from 0.1 µg/g beef to 18.7 µg/g mortadella in meat and can reach up to 33.6 µg/g anchovies as in sea food. In butter, the content of oxysterols ranges from 13.7 to 27.3 µg/g. Formation of lipid hydroperoxides in corn oil heated at 100 °C for 36 h in the presence of air was calculated to be 243 mmol/L, while in the untreated oil solely 0.9 mmol/L lipid hydroperoxides could be detected. Thus, heating promotes lipid oxidation. Heating of safflower oil by frying potato chips seven times for 10 min with one hour storage at room temperature between each frying resulted in an approximately 15-fold increase of the peroxide value. Cholesterol oxidation products were also shown to be elevated in roasted salmon treated at 200 °C for 30 min yielding 7.38 µg/g compared to fried samples treated at 180 °C for 3 min in olive oil yielding solely 2.98 µg/g. Besides elevated temperatures, cold fluorescent light was shown to induce lipid oxidation. Recently, we could demonstrate an increase of the peroxide value by 1473±1.79% (p<0.001) after household-representative storage of soybean oil in the presence of cold fluorescent light for 56 days. During the household-representative storage of the study oil an increasing oxygen-containing headspace was considered to mimic consumer handling. Due to the multiple environmental factors determining the kind and amount of lipid oxidation products formed during food processing quantitative exposure of lipid oxidation products to humans is hard to generalize. However, under defined processing conditions the susceptibility of each lipid-containing food product to oxidation can be determined, leading to quantifiable amounts of ingested lipids.

IMPACT OF (OXIDIZED) LIPIPS ON ENERGY METABOLISM

Once absorbed fatty acids can be stored as triglyceride in adipose tissue, transferred to extra-hepatic tissue within lipoproteins and used for energy supply in any tissue containing mitochondria with oxygen availability. As mitochondria are the site of β-oxidation of fatty acids and ATP production, it plays a key role in energy supply. Total energy expenditure at rest is measured as basal metabolic rate in humans and animals. Several animal studies reported a correlation between dietary fatty acid profile and the basal metabolic rate in animals. It could be shown that feeding omega-3 or omega-6 enriched diets to rats led to an increase of the metabolic rate compared to rats fed a saturated fat diet. Human studies confirmed that increasing PUFA content in the diet was associated with an elevated metabolic rate. In a human cross-over study six healthy volunteers received a control diet ad libitum for 3 weeks and after a break of 10-12 weeks the subjects were administered the same control diet where 6 g visible fat per day was replaced by 6 g fish oil per day for 3 weeks. Dietary intake of fish oil significantly reduced the body fat mass by -0.88±0.16 kg compared to the intake of visible fat which led to an alteration of the body fat mass by -0.3±0.34 kg. In addition, an increase of the rate of lipid oxidation to 1.06±0.17 mg/kg·min⁻¹ was obtained when fish oil was consumed compared to the intake of the visible fat (0.87±0.13 mg/kg·min⁻¹).

A recent study with LDLR-/- mice fed a Western diet for 16 weeks revealed that liver metabolites associated with lipid
and amino acid pathways were chiefly affected by the diet as determined by a non-targeted metabolomic approach. Supplementation of the Western diet with EPA or DHA reduced the Western diet-induced effects, whereby feeding the mice with a DHA-supplemented Western diet reversed the Western diet-induced effects more pronouncedly. Hepatic C<sub>30:2</sub>, omega 3 fatty acids and their oxidation products were demonstrated to be enhanced after administration of DHA-supplemented Western diet, whereas monounsaturates, omega 6 fatty acids and its corresponding oxidation products were significantly decreased. Metabolomic analyses identified, for instance, 18-hydroxy-5Z, 8Z,11Z,14Z,16E-eicosapentaenoic acid and 17,18-dihydroxy-eicosa-5,8,11,14-tetraenoic acid, two omega 3 fatty acids-derived oxidation products. DHA-supplemented diet was shown to affect the hepatic lipid metabolism, explaining the protective effect of DHA against Western-diet-induced nonalcoholic steatohepatitis in mice.

So, dietary fat has an impact on the energy metabolism by modifying the metabolic rate and the metabolic pathways. However, the impact of processed food-derived lipid oxidation products on energy metabolism has not yet been addressed, despite the fact that many foods undergo processes before consumption to increase palatability (Figure 1).

The molecular mechanisms explaining the role of dietary (unoxidized) fat on energy metabolism have been under thorough investigations. The energy metabolism has been suggested to be associated with membrane lipid composition. In the field of comparative biology it could be demonstrated that species with high metabolic rates (endotherms) have highly polyunsaturated membranes while ectotherms with low metabolic rates are linked to cellular membranes which consists of more monounsaturated fatty acid acyl chains. This finding led to the development of the so-called membrane pacemaker theory of metabolism. In particular, membrane composition affects Na<sup>+</sup>/K<sup>+</sup> antiporter activity, which accounts for 10-60% of the resting metabolic rate. The activity of the membrane-bound Na<sup>+</sup>/K<sup>+</sup>-ATPase was strongly correlated with the DHA content of the surrounding phospholipids. It was suggested that a decrease in the degree of membrane lipid polyunsaturation might reduce energy-consuming processes such as the activity of ion transporters. The membrane fatty acid composition might affect membrane-bound proteins, thereby modifying intracellular signaling. One of the integral membrane proteins, the glucose transporter 1, for instance, covers an area of approximately 17 molecules of a phosphatidylethanolamine bilayer consisting of saturated fatty acid chains. Thus, high membrane fluidity is required for the insertion of the glucose transporter into the membrane. Membrane fluidity is primarily determined by the membrane composition. Unsaturated hydrocarbon tails cause a greater surface of the cross-section of the cylindrical hydrocarbon part of the phospholipid molecule compared to saturated tails. As a consequence, the interaction energy between the two unsaturated fatty acid chains is reduced, leading to an enhanced membrane fluidity. The mechanism of fatty acid uptake was found to be similar to the mechanism of glucose uptake. The membrane-located fatty acid transporters were reported to regulate lipid metabolism. As for the activity of the glucose transporter, an impact of the membrane flexibility, and thus degree of membrane lipid polyunsaturation, on the activity of the fatty acid transporters might be conceivable. The impact of oxidized lipids on the fatty acid and glucose uptake and any correlation to the membrane flexibility has not yet been studied.

The extent to which dietary lipids are incorporated into cellular membrane has been investigated previously. The physiological conformer-regulator paradigm was applied to quantify the incorporation of dietary lipids into the membrane, whereby the membrane lipids were plotted against the dietary lipids. Even though dietary lipid composition was changed this change could not be reflected in plasma membrane (average slope 0.07). Membranes are, thus, homeostatically regulated independent of the dietary fatty acids. However, a conforming response to dietary fat was obtained when the PUFA balance of the diet was below 10% of the membrane composition. An average slope of the relationship between dietary fats and membrane lipids was determined to be 0.95 for membrane lipids from heart, liver, muscle, brain and red blood cells.

More specifically, mitochondrial membrane phospho-
lipids were shown to conform to dietary fatty acids. Hepatic mitochondrial membrane lipid composition of rats fed a rapeseed oil-rich diet for 11, 22 and 33 days was changed compared to the mitochondrial membrane of rats fed a standard diet. The modified diet induced a decrease in the saturated to unsaturated molar ratio and an increased incorporation of oleic acid in the major mitochondrial tetra-acyl phospholipid, cardiolipin. It was reported that cardiolipin, which comprises 10-20% of total mitochondrial phospholipids, is essential for mitochondrial ATP formation. Several studies showed that dietary lipids can modify mitochondrial respiration and ROS formation. Polysaturated fatty acids as well as lipid oxidation products are known to activate uncoupling proteins leading to proton leak across the inner mitochondrial membrane without using the electrochemical gradient for ATP production. Brookes, et al. demonstrated that membrane unsaturation was positively correlated with proton permeability and metabolic rate suggesting that mitochondrial fatty acid composition might affect mitochondrial inner membrane proteins. Battino, et al. investigated the effect of feeding fried oil to rats on their liver mitochondrial respiratory proteins. Intake of fried extra virgin olive oil rich in polar lipid oxidation products enhanced the hydroperoxide and the thiobarbituric acid reactive substances contents of mitochondrial membranes. In addition, it induced a stimulatory effect on the cytochrome c oxidase activity and increased the cytochrome c+c1 and cytochrome a+a3 content compared to the administration of non-fried extra virgin olive oil.

CONCLUSION

The impact of dietary oxidized fatty acids on the energy metabolism has been under thorough investigation. However, the bioenergetic effect of oxidized lipids still needs to be elucidated in mechanistic studies. So far, there is only little evidence that oxidized lipids might exert differential effects on mitochondrial respiratory chain. A systematic approach of the impact of lipid oxidation products from differently processed dietary fats on the bioenergetic pathways would be of great importance for the general public and especially for patients suffering from metabolic disorders.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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Opinion

Perspectives on Foodborne Parasites

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Recently, the emergence of a global food market, the growth of organic food produced by organic farming, and changes in eating habits have aroused an interest in foodborne illnesses, which constitute an important public health problem around the world. The World Health Organization (WHO) and Food and Agriculture Organization (FAO) of the United Nations estimate that parasites such as helminths (e.g., Anisakidae, Cysticercus spp, Taenia saginata, T. solium and Trichinella spiralis) and protozoa (e.g., Cryptosporidium spp., Cyclospora cayetanensis, Entamoeba histolytica, Giardia duodenalis, Toxoplasma gondii and Trypanosoma cruzi), which have consequences on human and animal health, are currently responsible for some foodborne diseases.1-3

The research provides various information regarding the foodborne by these parasites. Several parasites detected in humans can be transmitted by water, fruit juices and other raw or undercooked foods, however, a large portion of outbreaks are caused by unknown or unidentified foods; and data about the percentage of foodborne illnesses caused by helminths or protozoa are variable worldwide. Unlike bacteria, fungi and virus, helminths and protozoa are multicellular eukaryotic organisms and have a complex life cycle that generally includes several intermediate forms, and invertebrate and vertebrate hosts.

Moreover, because foodborne illnesses such as acute Chagas’ disease, cysticercosis, taeniasis or toxoplasmosis is frequently overlooked – and by transmission is one of the reasons why these are called neglected diseases – research is still faced with difficulties, mainly due to no notification of or the underreporting of human cases.

Consequently, parasitology research in the context of food science is a newly recognized and expanding area, and thus will become a major challenge during the upcoming decades both in developed countries and in developing countries, given economic importance and in public health about foodborne diseases, which can affect source of income based on fresh foods and costs with medical treatment and hospitalization.

Accordingly, the formation of multidisciplinary research teams composed of professionals involved in parasitology, food science and technology, genetics and laboratory animal science, coming together in an integrated fashion and with common goals, is fundamentally important. Rapprochement between university, industry, official food control laboratories and other research institutions and technology centers, public or private, is also necessary, especially in countries where such cooperation occurs infrequently.

To summarize ecological and epidemiological investigations, in general, analysis of foodborne outbreaks from biological contamination shows that occurrence of cases of disease can be caused by different developmental forms of parasitic agents, especially oocysts and cysts of protozoa or helminth eggs.

In humans, these investigations are primarily based on associations or correlations between consumption of a food possibly contaminated; secondly, the clinical conditions and/or laboratory diagnosis of human parasitosis, as the parasitological certification (e.g., “parasite-free”) in food and beverages as prophylaxis is still incipient, mainly with respect to protozoa.
Biological contamination of food occurs accidentally, but a careful assessment must also consider that the occurrence of foodborne parasitic diseases may become tendency or endemic in some regions over the years, if left uncontrolled. Imagine the number of asymptomatic hosts that are not known in such cycles.

For prophylactic methods of foodborne parasitic illnesses to be well known, they must be present in public policy development and be constantly diffused in a high-quality fashion in basic education. This is important as these methods aim to easily reduce or eliminate biological hazards in food and include the use of simple hygienic and health measures.

Among the proposed effective control measures, those primarily suggested are as follows: the consumption and/or use of filtered or boiled water for food preparation and proper hand washing after using the toilet and before preparing food (both by the general population and by food handlers). In addition; the proper washing or cooking of fruit and vegetables before contact with work surfaces (in the home or commercial kitchen); and lastly, as the proper cleaning of instruments and work surfaces between preparing different food products. All of these above steps can help to prevent cross contamination. Besides proper handling and preparation of food, there are many factors and difficulties to consider with regards to implementation of an anti-parasitic and prophylactic food safety system.

Nowadays, these factors include the possibility of obtaining samples of water and food, possibly contaminated at the time of occurrence of outbreaks; transport and correct storage of samples; knowledge about the composition and the complexity of the food matrix; the standardization of new analytical methods of detecting parasites in food in contrast to modern molecular biology technologies; the training of human resources, mainly microscopy experts; the inclusion and the speed of clinical and laboratory diagnosis of parasitic illness in non-endemic regions; and advances in legislation.

Hence, advances in these factors are indispensable in order to ensure safe food provision for populations and will minimize other difficulties to be overcome so that foodborne outbreak investigations are efficient and, in fact, conclusive.

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Essential Oil Nanoemulsions and Food Applications

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INTRODUCTION

Food quality, food preservation, and food safety are the most significant concerns in the food industry currently. Moreover, consumers demand for minimally processed foods and ready-to-eat fruits and vegetables because of its convenience since they do not need to process the product later. However, minimally processed foods are known for high chances of microbial contamination and high enzymatic activity. When the product is cut, the nutrients inside the fruits and vegetables are exposed to enzymes and microorganisms that will provide decrease of the shelf life. Therefore, microbial and enzymatic activity is a leading concern in the food industry in regards to proving food safety and convenience for the consumers. Due to this challenge, food industries have been using chemical substances to inactivate some of the enzymes, and specially, to reduce the microbial population. Nevertheless, these kinds of chemicals are most of the time corrosive, ineffective in the presence of high organic loads, may form organochlorides and may have long-term toxicological implications. Because of this issue, new technologies are being developed in order to find out new alternatives for replacement of chemical treatments.

Currently, there is a growing interest in the utilization of new preservative methods that are from natural origin. Essential Oils (EOs) are natural compounds that have been shown promising treatment for food application because of its strong antifungal, antiviral, and antibacterial activities. EOs present photo-chemicals, such as 1,8-cineole, carvacrol, eugenol, cinnamaldehyde, carvone, citral, estragole, geraniol, perillaldehyde, terpineol, thymol, and vanillin which are able to extend shelf life of processed food products by preventing lipid oxidation and antimicrobial properties. Moreover, EOs also have been proved to have other diverse beneficial functions, such as anti-diabetic, antiradical, and antioxidant effects. The antimicrobial properties of EOs is associated with the dissolving of the cytoplasmic membrane of bacterial cells in the hydrophobic domain. Previous studies have shown that EOs were able to inhibit Bacillus cereus, Zygosaccharomyces bailii, Listeria monocytogenes, and Staphylococcus aureus. Pandit and Shelef have applied rosemary oil in pork liver sausage and verified that it was effective against Listeria monocytogenes. Another study evaluated the use of carvacrol and cinnamaldehyde in kiwifruit and melon by dipping the food products in solution. The study showed that the natural flora of the product was reduced significantly after the EOs application.

Although essential oils have been shown to be promising alternative to chemical preservatives against foodborne pathogens, they present special limitations that preclude its use in food products. Low water solubility, high volatility, and strong odor of EOs are the main properties that make it difficult for food application. It is also a big challenge to incorporate...
NANOEMULSIONS AS ANTIMICROBIAL DELIVERY SYSTEM

Nanoemulsions differ appreciably from conventional emulsions in their functional activity due to the decreased size. For example, these emulsions may not scatter light strongly in the visible region and can thus be transparent. Nanoemulsions have the advantages of high surface area and kinetic stability against coalescence or creaming. As a result, nanoemulsions are important vehicles for hydrophobic bioactive substances through the formation of nano-dispersions. Hence, these systems permit the application of essential oils in food since they present solubilization in the water phase through molecular dispersion.

Nanoemulsions can be formulated using low-energy methods or high-energy emulsification methods. High-energy methods include high-pressure homogenization, microfluidization and sonication. In this case, energy is necessary to provide intense disruptive forces and minimized droplet size. Low-energy methods include Spontaneous Emulsification (SE) method, Emulsion Inversion Point (EIP) method, Phase Inversion Composition (PIC) method and Phase Inversion Temperature (PIT) method. In these methods, emulsion is formed spontaneously by mixing the ingredients together. The droplet size can be reduced by varying the composition and altering the environmental factors. Ultrasonic emulsification is one of the methods, which has been showing promising properties for application of EOs in foods. It is a high-energy method of formulation nanoemulsions that can decrease the size of droplets in the emulsion, which can promote very small droplet diameter, high physical stability, high bioavailability, and optical transparency. Because of the small size of the droplets, gravitational separation, flocculation, and coalescence often occur at a reduced rate in nanoemulsions. Nanoemulsion presents a very interesting application in certain food and beverage since the small droplet size promotes transparency or only slightly turbid in the food product.

In nanoemulsions, the choice of surfactants is very critical since emulsifiers have to rapidly cover the many new surfaces that are formed. Generally, in food emulsions two classes of surface-active species are used: (1) small-molecule surfactants such as monoglycerides, sucrose esters, and others and (2) macro-molecular emulsifiers such as protein or modified starch. EOs have to be associated or combined with surfactants in order to enhance the antimicrobial activities by increasing the solubility of EOs in the aqueous phase. Tween 80 has a high hydrophilic and lipophilic balance. Tween 80 is non-ionic in nature and stabilizes emulsion droplets by stearic stabilization. Moreover, being a low molecular weight surfactant, it is efficient in minimizing droplet size better than polymeric surfactants.

Nanoemulsion based delivery system could be characterized by dynamic light scattering, zeta potential, thermodynamic stability studies, pH, refractive index and viscosity. Dynamic light scattering is used to determine the size distribution profile of particles in nanoemulsions and zeta potential indicate stability of emulsions. Imaging techniques such as transmission electron microscopy, scanning electron microscopy and atomic force microscopy is used to confirm diameter of particles and understand distribution of nanoparticles.

APPLICATIONS IN FOOD MODELS

Recently, few studies have applied EOs in food systems, which makes these studies even more exciting and needed. Table 1 summarizes the most recent studies that apply EOs and nanoemulsions in food systems.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Target Microorganism</th>
<th>Food System</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oregano oil</td>
<td><em>Listeria monocytogenes, Salmonella Typhimurium, E.coli O157:H7</em></td>
<td>Lettuce</td>
<td>28</td>
</tr>
<tr>
<td>Carvacrol</td>
<td><em>Salmonella enteric Enteritidis E. coli</em></td>
<td>Broccoli and Radish seed</td>
<td>29</td>
</tr>
<tr>
<td>Carvacrol</td>
<td><em>Salmonella enteric Enteritidis E. coli</em></td>
<td>Mung bean and Alfalfa seeds</td>
<td>30</td>
</tr>
<tr>
<td>Mandarin oil</td>
<td><em>Listeria innocua</em></td>
<td>Green beans</td>
<td>31</td>
</tr>
<tr>
<td>Mandarin oil</td>
<td><em>Listeria innocua</em></td>
<td>Green beans</td>
<td>32</td>
</tr>
<tr>
<td>Lemongrass oil</td>
<td><em>Salmonella Typhimurium E. coli</em></td>
<td>Plums</td>
<td>33</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td><em>Lactobacillus delbrueckii Saccharomyces cerevisiae Escherichia coli</em></td>
<td>Apple and Pear Juice</td>
<td>34</td>
</tr>
<tr>
<td>Eugenol</td>
<td><em>Escherichia coli O157:H7 Listeria monocytogenes</em></td>
<td>Fruit Juice</td>
<td>35</td>
</tr>
</tbody>
</table>

Table 1: Food model research studies on delivery system for natural antimicrobials.
Bhargava, et al. applied oregano oil nanoemulsion to control the foodborne on fresh lettuce.\textsuperscript{24} The food product was evaluated against \textit{Listeria monocytogenes}, \textit{Salmonella Typhimurium} and \textit{Escherichia coli} O157:H7. The data suggested that applying oregano oil nanoemulsion to fresh produce may be an effective antimicrobial control strategy. Another similar study evaluated the effectiveness of carvacrol nanoemulsion against \textit{Salmonella} enterica Enteritidis and \textit{E. coli} on broccoli, radish seed,\textsuperscript{29} mung bean, and alfalfa seeds.\textsuperscript{29} The experiments have shown that the nanoemulsion is effective on radish seed, mung beans, and alfalfa seed but not effective on broccoli seeds. The antibacterial and physical effects of modified chitosan base-coating containing nanoemulsion of mandarin essential oil on green beans is recently being analyzed.\textsuperscript{31,32} The experiments were associated with different non-thermal treatments against \textit{Listeria innocua} and the results have shown promising application of this type of nano-emulsion in food products.

A very interesting food application of EOs nanoemulsion has been observed in plums. Recently, lemongrass oil nanoemulsion was used to evaluate antimicrobial properties, physical, and chemical changes in plums.\textsuperscript{33,34} The nanoemulsion was able to inhibit \textit{Salmonella} and \textit{E. coli} population without changing flavor, fracturability, and glossiness of the product. It was also able to reduce the ethylene production and retard changes in lightness and concentration of phenolic compounds.

Essential oil-based delivery system is emerging as viable solution to control growth of foodborne pathogens on food. However, limited studies are performed on food model and there exist several challenges in application of this system in complex food matrices such as meat and meat products. Application of nanoemulsions in food models will also offer challenges to government and industry.\textsuperscript{35} Food industry has to build consumer confidence on acceptance on nano food ingredients such as antimicrobial essential oil nanoemulsions. On the other side, regulatory agencies such as FDA should ensure safety of these antimicrobial delivery systems.

**CONFLICTS OF INTEREST**

The authors declare that they have no conflicts of interest.

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Association between Smoking and Anthropometric Characteristics, Biochemical Markers, and Dietary Intake of Pakistani Male Adult Population

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ABSTRACT

Background/Objectives: A community-based study was conducted to compare the nutritional status between smokers and non-smokers in association with dietary, biochemical and socio-economic characteristics.

Methods: A convenient sampling method was used to enroll 100 smokers and 99 non-smokers aged between 46 and 78 years from the urban and semi-urban areas of district Peshawar, Pakistan. Weight, height, waist and hip circumferences of the subjects were taken while body composition was determined by employing a Bodystat Analyzer. A blood sample was taken from each subject for the determination of serum vitamin A and zinc levels. Subjects were interviewed for a 24-hr dietary recall and demographic and socio-economic characteristics. Student’s t-test and bivariate analysis were conducted to compare the mean differences and examine the association between different variables of smoker and non-smoker groups.

Results: The results revealed that there was no significant (p>0.05) difference between the mean age, weight, height and body mass index of smokers and non smokers. However, the mean body fat, waist and hip circumference of the smokers were significantly (p<0.05) lower than the non-smokers. Conversely, the mean serum vitamin A (32.30±15.99 µg/dl) of smokers was significantly (p<0.05) higher than non-smokers (26.50±20.44 µg/dl) but the mean serum zinc concentration of smokers (99.76±27.42 µg/dl) was significantly lower than the non-smokers (108.25±32.20 µg/dl).

Conclusions: The study concludes that anthropometric (body mass index), biochemical (vitamin A and zinc status), dietary (energy intake) and socio-economic (income, profession) characteristics failed to establish an association with smoking as most of the indicators of smokers are comparable to non-smokers.

KEYWORDS: Smoking; Anthropometry; Vitamin A; Zinc, Dietary intake; Pakistan.

INTRODUCTION

Smoking has long been implicated as a risk factor for many chronic diseases, including cardiovascular, respiratory and gastrointestinal diseases and a variety of cancers.¹⁻³ Tobacco smoke contains many oxidants and free radicals that can cause damage to lipids, proteins, DNA, carbohydrates and other bio-molecules.⁴⁻⁵ It also contains numerous pro-oxidants capable of producing free radicals and enhancing the oxidative stress in vivo.⁶ Each puff of tobacco contains approximately 1014 oxidant molecules in the tar phase and approximately 1015 in the gas phase including oxygen and nitrogen derived free radicals.⁷ These free radicals are consid-
ered to be the major patho-physiological factors responsible for the development of many chronic diseases.8

There is a growing body of evidence that oxidants such as reactive oxygen species are involved in the development of cerebrovascular degenerative diseases, hypertension, increased oxidative stress, impaired nitric oxide bioavailability and endothelial dysfunction.9,10 Production of reactive oxygen species in quantities that overwhelm the endogenous antioxidant defense system is referred to as oxidative stress and involves the oxidation of molecules in ways that impair cellular function.11 Numerous epidemiological studies have shown that cigarette smoking causes oxidative stress, impaired antioxidant blood levels, increased risk of cancer, cardiovascular diseases, diabetes, pulmonary hypertension, stroke and premature deaths.12,13 It has also been estimated that tobacco smoking accounts for 33% of cancer related deaths in male and 10% in women.14 Cigarette smoking alone has been attributed to annually cause about 5 million deaths worldwide; while the number of the deaths is expected to increase to 10 million by the year 2030 with 70% of deaths in low to middle income countries.15

In Pakistan, the prevalence of cigarette smoking has been estimated to be 19.4% among the population aged over 14 years causing serious health, economic and social challenges to the society at large.16 Cigarette smoking, with no minimum age with regards to the legal purchase of tobacco and related products has multiplied the human sufferings by the overwhelming burden of chronic diseases, disabilities and premature deaths. While the increased vulnerability of smokers to chronic diseases and premature deaths have been partly attributed to elevated oxidative stress and reduced blood antioxidant levels; it remains unclear whether lower antioxidant levels are due to decreased dietary intake of antioxidant rich foods or the depletion of circulating antioxidants through chronic smoke exposure.17

The damage that can be caused by free radicals could potentially be minimized by the regular intake of dietary nutrients that are an integral part of enzymatic and non-enzymatic antioxidant systems. Important antioxidant enzymes include copper-zinc superoxide dismutase, manganese superoxide dismutase, ceruloplasmin, selenium glutathione peroxidase, glutathione reductase and catalase.18 Non-enzymatic antioxidants include vitamin E (alpha-tocopherol), vitamin C (ascorbic acid), vitamin A (retinal), pro-vitamin A (carotenoids) and urate.19

No study related to nutritional status and dietary intake of smokers and non-smokers was found in Pakistan which is important from a public health perspective to assess their nutritional status, develop appropriate policies and plan of actions to improve their nutrition well being, combat tobacco smoke and mitigate subsequent health hazards. Considering the deleterious effects of smoking on human health, this study was designed to assess and compare the anthropometric and biochemical characteristics, dietary intake of smokers with non-smokers and examine the relationship between anthropometry, biochemical, dietary and demographic and socio-economic characteristics.

**MATERIALS AND METHODS**

A community-based study was carried out among male adult smokers and non-smokers in the urban and semi-urban areas of district Peshawar, Pakistan. A non-probability convenience sampling procedure was followed to enroll 100 smokers and 100 non-smokers from two different localities. One of the participants from the non-smoking group migrated in the middle of the study so the sample size was reduced to 99. Inclusion criteria for enrollment of smokers: (i). were male adults aged ≥40 years; (ii). had been smoking two or more cigarettes per day for the last five years; and (iii). were free from all types of infectious and chronic diseases. The inclusion criteria for non-smokers were similar to the smokers except that they were non-smokers. Subjects fulfilling the inclusion criteria were informed about the purpose of the study and informed consent was obtained. The study was approved by the Board of Studies, University of Agriculture, Peshawar, Pakistan.

Participants were interviewed for their demographic and socio economic characteristics and their systolic and diastolic blood pressures were measured in the supine position by a sphygmomanometer. Weight and height of the subjects were taken by following the WHO recommended procedures.20 Body Mass Index (BMI) of the subjects was calculated using the formula (weight (kg)/height (m²)). Waist circumference was measured at the midpoint between the lower rib and the iliac crest; while hip circumference was measured as the maximum circumference around the buttocks and recorded to the nearest 0.1 cm to assess abdominal and central obesity.21 Waist to hip ratio (WHR) was determined by applying the equation: waist circumference (cm)/hip circumference (cm). The prevalence of abdominal obesity in smokers and non-smokers was assessed by following the recommended waist circumference cut-off value of (>85 cm) and WHR (>0.90) for Asian adult males.22 The anthropometric data were compared with the corresponding age reference population, i.e. National Centre for Health Statistic data to generate weight-for-age, weight-for-height and height-for-age Z-score of adults.23 The subjects were categorized as underweight, normal, overweight, and obese according to the WHO cut off values.24

Body composition (body fat; lean body mass; water) and body energy requirements were estimated using a Body Stat Analyzer (BSA 1500) (Bodystat LTD, Douglas, Isle of Man). A 24-hr dietary recall was used to interview the subjects for all the foods and beverages that they had consumed during the last 24-hrs. Dietary energy, carbohydrate, protein, fat, vitamin A and zinc intakes of the smokers and non-smokers were calculated using the Food Composition Table of Pakistan.25

Blood retinol and zinc levels were determined by taking about 8 ml of blood from subject’s antecubital vein follow-
ing an overnight fast. Blood samples were taken by employing a Vacuette blood collection system (Greiner Bio-One, Monroe, NC). The blood samples were immediately transported to the Department of Human Nutrition Laboratory, the University of Agriculture, Peshawar, Pakistan) where they were centrifuged (Hermle Z 200 A, Wehingen, Germany) for the separation of serum at 3000 rpm for 10 minutes, which was then stored at -80 °C until analysis. All the chemical and analytical procedures were carried out in a dim light environment.

Serum vitamin A was determined by following the method of Bieri, et al.26 on a Perkin Elmer Series 200 HPLC fitted with a UV/V is 200 at 325 nm, and a Hypersil C18 column (250 mm x 4.6 mm ID, 5 µm). The mobile phase was an isocratic mixture of methanol (95%) and water (5%), applied at a flow rate of 1 ml/minute. The standards used were all-trans retinol and retinyl acetate as internal standard (Sigma Aldrich, St. Louis USA). All chemicals and solvents were ultra-purified HPLC grade. Extraction of retinol from the samples was carried out by taking 100 µl serum in a tube to which 100 µl ethanol containing the internal standard was added and the content was mixed in a vortex mixer. The lipid components were extracted by adding 200 µl n-hexane, followed by vortexing for 45 seconds and centrifugation at 3000 rpm for 2 minutes. The solvent layer was transferred and dried under a gentle stream of nitrogen gas at 60 °C. The dried sample was dissolved in 100 µl methanol for injection into the HPLC.

The prevalence of vitamin A deficiency was determined using the WHO recommended cut-off values.27 Serum zinc was determined by employing an atomic absorption spectrophotometer (Shimadzu, AA 6300, Kyoto, Japan).28 One ml serum was transferred into a centrifuge tube and 0.4 ml of 24% Trichloroacetic acid (TCA) was added to it and mixed in a vortex for 30 seconds and then 1.0 ml deionized water was added to it. The sample was centrifuged at 3000 rpm for 10 minutes to remove blood proteins, after which the aqueous solution was analyzed on the atomic absorption spectrophotometer. The prevalence of zinc deficiency was assessed by using standard zinc cut-off value of <12 µmol/L.29

Data regarding demographic characteristics, socio-economic status, anthropometric measurements, body composition, biochemical assessment and dietary intake were analyzed using SAS (The SAS Institute, Inc., Cary, NC, USA). Simple statistics and bivariate analysis were carried out on the continuous and ordinal data to examine the mean differences in anthropometric measurements, body composition, biochemical and dietary data at 5% level of significance between the smokers and non-smokers. Pearson product-moment correlation coefficients were calculated to determine the relationship between different variables.

RESULTS

General characteristics of smokers and non-smokers presented in Table 1 indicate that there was no significant (p>0.05) difference in the mean age between the smokers and non-smokers. The smokers had an average smoking history of ~19 years and smoked an average of 13 cigarettes per day. All the subjects were married: 49% of the smokers lived in a joint family structure; while the remaining 51% lived in a nuclear family structure. No significant difference (p>0.05) was found between smokers and non-smokers with regards to the mean family size; number of children; monthly income; the subject’s education; and profession (Table 1). However, a significantly (p<0.05) higher percentage of non-smoker’s spouses were uneducated compared to the smoker’s spouses. Conversely, there was no significant difference (p>0.05) between the spouse’s professions in the two groups but a higher percentage (4%) of smokers’ spouses were employed as compared to the spouses (1%) of non-smokers. This suggests that smoking by males with employed spouses is perceived to be a symbol of higher social standing, signifying an improved socio-economic status and enhanced quality of life of the family.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Smokers (n=100) Mean±SD</th>
<th>Non-smokers (n=99) Mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject Age (Yrs)</td>
<td>45.88±7.29</td>
<td>46.93 ± 8.39</td>
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</tr>
<tr>
<td>Smoking Period (Yrs)</td>
<td>19.37±7.80</td>
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<td>0.35</td>
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<td>Cigarettes per day</td>
<td>12.59±7.62</td>
<td>None</td>
<td>0.35</td>
</tr>
<tr>
<td>Family Type</td>
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<tr>
<td>Joint</td>
<td>49%</td>
<td>37%</td>
<td>0.35</td>
</tr>
<tr>
<td>Nuclear</td>
<td>51%</td>
<td>62%</td>
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<tr>
<td>Marital Status</td>
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<tr>
<td>Married</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Family Size</td>
<td>8.05±3.14</td>
<td>8.32±4.98</td>
<td>0.65</td>
</tr>
<tr>
<td>Number of Children</td>
<td>4.07±2.03</td>
<td>4.16±2.44</td>
<td>0.77</td>
</tr>
<tr>
<td>Family Income (rupees)/month*</td>
<td>18680.00±13466</td>
<td>21011.11±22948</td>
<td>0.38</td>
</tr>
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</table>
Anthropometric data as shown in Table 2 indicate that there was no significant (p>0.05) difference in the mean weight, height, weight-for-age, height-for-age, weight-for-height Z-scores, body mass index and waist-to-hip ratio between the smokers and non-smokers. However, the mean waist and hip circumferences of smokers were significantly (p<0.05) lower than the non-smokers. Data on body composition revealed that smokers had significantly (p<0.05) lower mean body fat and lean body mass but there was no significant difference in the mean basal metabolic energy requirements, total energy requirements and systolic and diastolic blood pressure values between the smokers and non-smokers (Table 2).

The mean serum vitamin A and zinc levels revealed that smokers had a significantly (p<0.05) higher mean serum vitamin A level but a significantly (p<0.05) lower mean serum zinc level than the non-smokers (Table 3). The prevalence of vitamin A deficiency among smokers (21%) was found to be significantly (p<0.05) lower than in the non-smokers (41%). However, no significant (p>0.05) difference was observed in the prevalence of zinc deficiency between the smokers and non-smokers. The correlation analysis also failed to reveal any significant (p<0.05) association between serum vitamin A and zinc levels and the number of cigarettes smoked per day or the duration of smoking.

Dietary nutrients intake results showed that the mean energy and carbohydrate intakes of smokers were significantly (p<0.05) higher than the non-smokers (Table 4). Conversely, mean protein and fat intakes of smokers were significantly (p<0.05) lower than the non-smokers. Though, the mean dietary vitamin A intake of smokers was slightly higher and dietary zinc intake lower than the non-smokers but the differences were non-significant (p>0.05).

Correlation coefficients revealed that there was a significant relationship between weight, height, BMI, waist and hip circumferences, energy, carbohydrates, protein, fat, systolic and diastolic blood pressure but there was a lack of association between the biochemical, anthropometric and dietary intake variables.

DISCUSSION

The hypothesis that smoking adversely affects the nutritional status could not be verified on the basis of the results obtained from anthropometric measurements, serum vitamin A levels and dietary intake of smokers. The lack of significant differences in majority of the physical growth indicators i.e., weight, height, body mass index, weight-for-age, height-for-age and weight-for-height Z-scores between the smokers and non-smokers indicate the insensitivity of anthropometry to detect the adverse effects of smoking. The results are in fair agreement to those of Stolzenberg-Solomon, et al. who reported a non significant difference in the mean weight and height between the

<table>
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<tr>
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<td>Matric</td>
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<td>Graduate</td>
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<td>Postgraduate</td>
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<th>Subject Profession</th>
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<td>Public Sector</td>
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<tr>
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<tr>
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<tr>
<td>Matric</td>
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<td>Postgraduate</td>
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<tr>
<th>Spouse Profession</th>
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<tbody>
<tr>
<td>Housewife</td>
<td>96</td>
<td>98</td>
</tr>
<tr>
<td>Working</td>
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*1 US $≈=100 Pakistani rupees as per (February 2015).

Table 1: General characteristics of smokers and non-smokers.
smokers and non-smokers. The results are also consistent with those of Bradley, et al.\textsuperscript{31} who also found no significant difference in the mean body mass index between the male smokers and non-smokers. Similarly, Chopra, et al.\textsuperscript{32} and AL-Riyami and Afifi\textsuperscript{33} also reported no significant difference in the mean BMI between the smokers and non-smokers. Conversely, others\textsuperscript{34,35} reported an inverse association between nicotine intake and body weight; while another group of researchers noted a positive association between obesity and number of cigarettes smoked per day.

Interestingly, the markers of overweight and abdominal obesity such as percent body fat, waist circumference and hip circumference of smokers were significantly lower than the non-smokers. The reasons of lower adiposity in smokers may be attributed to their increased energy expenditure, physical activ-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Smokers (n=100) Mean±SD</th>
<th>Non-smokers (n=99) Mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>73.12±13.70</td>
<td>76.17±13.23</td>
<td>0.11</td>
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<tr>
<td>Height (cm)</td>
<td>168.35±6.15</td>
<td>168.84±6.73</td>
<td>0.59</td>
</tr>
<tr>
<td>Weight-for-age Z-score</td>
<td>-0.44±1.24</td>
<td>-0.15±1.20</td>
<td>0.10</td>
</tr>
<tr>
<td>Height-for-age Z-score</td>
<td>-1.01±0.90</td>
<td>-0.91±0.99</td>
<td>0.43</td>
</tr>
<tr>
<td>Weight-for-height Z-score</td>
<td>0.24±1.40</td>
<td>0.53±1.29</td>
<td>0.13</td>
</tr>
<tr>
<td>Body mass index</td>
<td>25.77±4.48</td>
<td>26.68±4.17</td>
<td>0.14</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>84.87±11.60</td>
<td>88.61±11.32</td>
<td>0.02</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>94.44±9.49</td>
<td>97.96±8.85</td>
<td>0.00</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.89±0.06</td>
<td>0.90±0.07</td>
<td>0.48</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>23.00±4.76</td>
<td>24.90±4.63</td>
<td>0.00</td>
</tr>
<tr>
<td>Lean body mass (%)</td>
<td>76.99±4.76</td>
<td>75.20±4.70</td>
<td>0.00</td>
</tr>
<tr>
<td>Basal metabolic energy require-</td>
<td>1665.32±245.31</td>
<td>1718.70±236.46</td>
<td>0.12</td>
</tr>
<tr>
<td>ments (Kcal)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Total energy requirements (Kcal)</td>
<td>2717.32±346.73</td>
<td>2733.30±483.25</td>
<td>0.79</td>
</tr>
</tbody>
</table>

**Table 2:** Anthropometric measurements of smokers and non-smokers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Smokers (n=100) Mean±SD</th>
<th>Non-smokers (n=99) Mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (Kcal)</td>
<td>3127.73±389.43</td>
<td>2993.10±448.14</td>
<td>0.02</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>418.96±61.83</td>
<td>374.88±66.14</td>
<td>0.00</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>126.96±19.47</td>
<td>141.03±22.43</td>
<td>0.00</td>
</tr>
<tr>
<td>Fats (g)</td>
<td>75.89±14.41</td>
<td>94.21±15.65</td>
<td>0.00</td>
</tr>
<tr>
<td>Vitamin A (µg)</td>
<td>1407.76±14</td>
<td>1378.80±12</td>
<td>0.06</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>10.76±6.43</td>
<td>11.59±5.82</td>
<td>0.12</td>
</tr>
</tbody>
</table>

**Table 4:** Dietary nutrients intake of smokers and non-smokers.
ity or their increased participation in manual jobs which were not measured in this study. The results are somewhat similar to others\textsuperscript{36} who also reported a lower mean waist circumference for smokers. The smokers appear to be advantageous to have lower central obesity which may serve as a barrier against cardiovascular and other chronic diseases. Similarly, no significant difference in the waist-to-hip ratio between the smokers and non-smokers also indicates a non increasing risk of metabolic syndrome for smokers. The results are corroborated by Agarwall\textsuperscript{37} and others\textsuperscript{38} who reported a non significant difference in the waist-to-hip ratio between the smokers and non smokers.

In general, the Pakistani male adult population is confronted with challenges of poor dietary practices and physical inactivity that have lead to increased prevalence of overweight and obesity. The recent cross sectional studies around the country have revealed that about one thirds of the adult population is overweight and obese with increasing vulnerability to chronic diseases and premature deaths.\textsuperscript{38,39} Cereals constitute a staple of Pakistani diet and serve as a major source of carbohydrates, energy, proteins, iron, zinc and other nutrients while animal products, vegetables, fruits and dairy products are less frequently consumed, the imbalanced and less diversified dietary patterns result in micro-nutrient deficiencies which are exacerbated by unhealthy lifestyle practices including smoking and physical inactivity.

No significant difference in the systolic and diastolic blood pressure between the smokers and non-smokers also suggests that smoking alone may not be a causative factor for hypertension rather it may exacerbate the risk by working synergistically with other potential risk factors. Our results are supported by Stolzenberg-Solomon, et al.\textsuperscript{40} who reported a non significant difference in the mean systolic and diastolic blood pressures between the smokers and non smokers. But the results are contrary to the generally established fact that smoking causes oxidative stress and is responsible for increasing the risk of coronary artery disease, stroke and cerebrovascular diseases, hypertension and premature deaths.\textsuperscript{12,13}

A significantly higher mean serum vitamin A level of smokers than non-smokers also demonstrates that smokers are not at an increasing risk of clinical vitamin A deficiency or for the development of abnormal degenerative changes in eyes such as age-related macular degeneration. Our results on vitamin A levels of smokers are similar to those of Chiu et al.\textsuperscript{42} and Liu et al.\textsuperscript{43} who reported a higher mean serum vitamin A level for smokers, however, Faure, et al.\textsuperscript{41} reported no significant effect of smoking on serum retinol level in male and female French participants of SU.VI.MAX study. These results are somewhat different than those of Hawkins, et al.\textsuperscript{42} who reported that smoking doubles the risk of age-related macular degeneration which could be attributed to oxidative stress rather than the vitamin A levels of smokers. The results on vitamin A status of smokers also suggest that either serum vitamin A levels are unaffected by smoking or vitamin A level may not have any potential role in fighting against oxidative stress, suppressing metabolic disorders or degenerative diseases.

Lower mean serum zinc level of smokers but a non-significant difference in the mean dietary intake between the smokers and non-smokers suggest that toxic compounds of tobacco may alter zinc metabolism to the extent of changing the serum zinc level. Decreased serum zinc level of smokers may also be attributed to decreased dietary zinc intake which was lower than the recommended dietary zinc intake of 15 mg/day for adults.\textsuperscript{43} The argument of lower dietary zinc intake by smokers was further substantiated by others\textsuperscript{44} who reported that smokers had a significantly lower fruits and vegetables consumption as compared to their non-smoking counterparts.

Zinc being an integral component of more than 200 enzymes, catalyzes various oxidation-reduction reactions, its deficiency may affect the metabolism of other nutrients as well as increase the risk of metabolic diseases in smokers by depressing antioxidant enzymes like superoxide dismutase activity.\textsuperscript{29} Our results on zinc status of smokers and non-smokers correspond to those of Uz, et al.\textsuperscript{45} and Anetor, et al.\textsuperscript{46} who also reported significantly lower serum zinc levels in smokers in comparison to non-smokers. The lower serum zinc concentration of smokers compared to non-smokers could also be due to the influence of cadmium, an essential constituent of cigarette smoke that may act as an antagonist to zinc bioavailability.\textsuperscript{47,48}

The results on dietary nutrients intake showed that smokers had a significantly higher mean energy and carbohydrates intake than non-smokers. The higher intake of energy and carbohydrates by smokers is somewhat contrary to the generally perceived notion that smoking depresses appetite. It has been reported\textsuperscript{49} that nicotine increases energy expenditure and that could lead to increased energy intake while others\textsuperscript{50} suggested that nicotine increases serotonin and dopamine levels in the brain that decrease the demand for energy intake and suppress appetite. Our results on energy and carbohydrates intake are in agreement with those of Cade and Margetts\textsuperscript{51} who reported a significantly higher intake of energy and carbohydrates in smokers, on the other hand the results are contrary to those of English, et al.\textsuperscript{52} who reported that smokers had a lower mean energy and carbohydrates intake than non-smokers.

The lower mean dietary protein and fat intake of smokers could be due to economic or personal reasons or due to decreased appetite caused by nicotine and other toxic compounds of the cigarettes smoke. However, the results do not correspond to those of Faruque, et al.\textsuperscript{52} who reported a non significant difference in the dietary protein and fat intake between smokers and non-smokers. Similarly, no significant difference in the mean dietary vitamin A intake between smokers and non-smokers suggest that smoking did not have any significant affect on the dietary vitamin A intake of smokers. No significant difference in
the mean dietary zinc intake between smokers and non-smokers suggests that dietary habits in terms of zinc intake are almost similar. The results of the study are inconsistent with the generally perceived hypothesis that smoking adversely affects nutritional status, the reasons for inconsistent association between indicators of nutritional status and smoking could be attributed to different degree of indicators’ sensitivity owing to their different characteristics. We as authors are satisfied with the results of the study and feel that smoking may have more devastating health implications on the general health than on nutritional status of individuals as indicated by the study’s results. A larger similar epidemiological case-control study with an increased number of anthropometric, body composition, biochemical and clinical indicators needs to be conducted to prove or refute the results of the current study.

The study concludes that anthropometric, biochemical, dietary, demographic and socio-economic characteristics are insensitive to the adverse effects of smoking as most of the indicators of smokers are comparable to non-smokers. Due to funding constraints, the sample size of smokers and non-smokers was relatively small that could have limited the statistical power of the study. In addition, all variables of interest such as morbidity and mortality associated with smoking could not be included and that could be one of the limiting factors in this study. Keeping in view the above stated limitations, the results may be used with caution, further studies are needed in the area to augment the study findings.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ACKNOWLEDGMENTS

The researchers are grateful to the University of Agriculture Peshawar, for financially supporting this study. Thanks are extended to all subjects for their volunteer participation in the study.

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Ginger and its Effects on Inflammatory Diseases

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1Department of Nutrition, School of Public Health, Iran University of Medical Sciences Tehran, Iran
2Department of Cellular and Molecular Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran

ABSTRACT

Today, Ginger is used as a spice all around the world. In the past, Ginger was consumed for the treatment of various diseases, including osteo-arthritis, neurological diseases, vomiting, asthma, and so on. It seems that Ginger can reduce inflammation in those diseases. We searched the following keywords in PubMed, Google scholar, and Scopus database until 2015: inflammation and Ginger, Ginger and diseases. Clinical trials, animal studies, and human studies were included in the results of this search. Ginger extract with the antioxidant and anti-inflammatory ingredients such as 6 Gingerols, 6-Shogoals, Zhingerol, etc can reduce inflammatory mediators such as inflammatory cytokines and chemokines due to their effects on NF-κB activation, cyclooxygenase 2 reduction and serotonin receptors inhibition. It increases reducing antioxidant enzymes so it can be useful in inflammatory diseases improvement and their complications prevention. In conclusion, Ginger can help in the treatment of inflammatory chronic diseases such as Fatty Liver, Asthma, Cancer and Arthritis through anti-inflammatory, immunoregulatory and antioxidative mechanisms.

KEYWORDS: Ginger; Zingiber officinale; Inflammation; Diseases.

ABBREVIATIONS: NF-κB: Nuclear factor κB; BMI: Body mass index; SLM: Soft Lean Mass; IgE: Immunoglobulin E; COX-2: Cyclooxygenase2; AchE: Acetylcholinesterase; PPARδ: Peroxisome proliferator-activated receptor δ.

INTRODUCTION

Today, Ginger, in both fresh and dried forms, is used as a spice all around the world. In the past, Ginger was consumed in the treatment of various diseases, including arthritis, neurological diseases, vomiting, and so on. More than 50 types of antioxidants have been extracted from Ginger rhizome. The major pharmacological activity of Ginger, with scientific name of “Zingiber officinalis”, is related to its active ingredients such as 2 and 6-Gingerol, 6-Shogoals, Zhingerol, etc can reduce inflammatory mediators such as inflammatory cytokines and chemokines due to their effects on NF-κB activation, cyclooxygenase 2 reduction and serotonin receptors inhibition. It increases reducing antioxidant enzymes so it can be useful in inflammatory diseases improvement and their complications prevention. In conclusion, Ginger can help in the treatment of inflammatory chronic diseases such as Fatty Liver, Asthma, Cancer and Arthritis through anti-inflammatory, immunoregulatory and antioxidative mechanisms.

MATERIALS AND METHODS

We searched in PubMed, Google scholar, and Scopus database until 2015 and the key words, inflammation and Ginger, Clinical trials, animal studies, and human studies were included in our search.
RESULTS

Our review of recent studies showed that Ginger, due to its anti-inflammatory, anti-carcinogenic, and antioxidative properties, can reduce inflammation in the body and improve related diseases. Below important effects are mentioned:

Ginger and Body Composition

Ginger may reduce the rate of weight gain, Body Mass Index (BMI). It can improve body composition by decreasing body fat levels and increasing Soft Lean Mass (SLM). In addition, some enzymes such as Acetyl-coenzyme A, acyltransferase I and enoyl-CoA hydratase, which participate in the β-oxidation of fatty acids, have increased by consumption of Ginger. Moreover, Ginger extract prevents high-fat diet-induced obesity in mice via activation of the Peroxisome proliferator-activated receptor δ (PPARδ) pathway.

Besides, ginger tends to reduce lipid metabolism related-proteins mRNA expression levels in liver and visceral fat in hyperlipidemia and may also improve lipid metabolism. The aqueous extract of Z. officinale Roscoe might inhibit the intestinal absorption of dietary fat by inhibiting its hydrolysis.

Therefore, Ginger seems to improve body composition via its effects on liver enzymes, by reducing fat absorption, by increasing beta-oxidation of fats and energy expenditure.

Ginger and Reduction of Airway Inflammation

Ginger can reduce airway inflammation in mice by enhancing the Th1 response and ameliorates ovalbumin-induced Th2 responses, and by reducing level of IL4, IL5, eosinax, and Immunoglobulin E (IgE). It can also improve the symptoms of asthma by relaxing the airway smooth muscle due to the regulation of calcium channels function.

Ginger and Kidney Function

Gingerol fraction from Zingiber officinale prevents gentamycin-induced nephrotoxicity. It improves kidney functions, reduces lipid peroxidation, and decreases nitrosative stress. In addition, Ginger extract diminishes chronic fructose consumption-induced kidney injury by suppression of renal over expression of pro-inflammatory cytokines in rats.

Ginger and Liver Function

Dried Ginger (Zingiber officinale) inhibits inflammation in a mouse model, improves Pathological changes, and reduces level of INFγ and IL6. It can also decrease liver Pro-inflammatory responses, TNFα, IL-6, and other inflammatory cytokines levels via inhibition of NFkB activation.

Ginger and Improvement of the Neurological Degenerative Diseases

6-Shogaol, an active constituent of Ginger, attenuates neuro-inflammation and cognitive deficits in animal models of dementia. Consequently, it plays an important role in the improvement of symptoms in patients who suffer from Alzheimer and other neurological diseases. It improves memory by inhibiting the activity of glial cells in animal models of dementia and also by reducing memory corruption. Besides, Ginger decreases activity of NF-xB, iNOS, and Cyclooxygenase2 (COX-2). It protects HaCaT cells and C57BL/6 mice from ultraviolet B-induced inflammation. Ginger also has an inhibitory effect on melanogenesis in B16F10 melanoma cells and as a result can protect skin from darkening.

Ginger and Diabetes

Ginger consumption in patients who suffer from type 2 diabetes mellitus affects glycemic status, insulin sensitivity, lipid profiles, and other metabolic disorders. It improves them by decreasing inflammatory factors like CRP, IL6, TNFα, CRP, IL6, TNFα, CRP. It shows antagonistic activity against serotonin receptors. Moreover, it inhibits the activity of intestinal glucosidase and amylase, resulting in the reduction of glucose absorption.

Neuroprotective effect of Ginger on the brain of streptozotocin-induced diabetic rats, may also be due to adjustment of astrocyte damage response, decreasing the expression of Acetylholines-terase (AchE), and improving the construction of neurons.

Ginger and Rheumatic Disorders

Ginger has protective effects on joint inflammation, arthritis, and musculoskeletal disorders via its anti-inflammatory, antioxidant, and anti-serotonin influences. It inhibits Cyclooxygenase-2 and 5-Lipoxygenase pathways. Ginger induces T-helper-2, and anti-inflammatory cytokines such as IL-4 and IL-10 production, increases glutathione level, and activity of the antioxidant enzyme like superoxide dismutase, inhibits the release of substance P (mediator of inflammation and pain), and decreases TNFα, IL1β, IL6, IL2, and prostaglandins levels. One Study shows Ginger is more effective than indomethacin in reducing the pain associated with inflammation and oxidative stress. Moreover, Ginger can decrease muscle pain caused by severe exercise.

Ginger and Chemo-preventive Effects

Some active constituent of Ginger like [6]-Gingerol, and [6]-paradol have chemo-preventive and anti-tumor effects. Ginger extract is effective in decreasing the gastric inflammation. Besides, it prevents gastric, colon, and lung carcinogenesis through bacterial reduction load. It also suppresses acute and chronic inflammation. In addition, Ginger can inhibit COX-2, NFkB, IL1β, IL8, and IL6 pathways. Shogaol can suppress
cancer cell invasion and inflammation, and displays cytoprotective effects through modulation of NF-κB and Nrf2-Keap1 signaling pathways. Moreover, it induces NAD(P)H, heme-oxidoreductase genes, and oxidoreductase genes. 6-Gingerol exerts anti-cancer activities via its effects on cell cycle regulation, cytotoxic activity, and angiogenesis inhibition.

**DISCUSSION**

Briefly, Ginger can be useful in the treatment of patients who suffer from inflammatory chronic diseases, due to its anti-inflammatory and anti-oxidative properties. The anti-inflammatory and anti-oxidative activities of Ginger are caused by its inhibitory influence on COX-2, lipoxigenase, NFkB and TNFα activity, likewise they are caused by reduction of inflammatory factors such as IL-1β, IL6, and IL2. The inhibitory effects of 6-Gingerol on the arachidonic acid metabolites include reduction of platelet aggregation, formation of thromboxan B2, and Prostaglandin 2D. Ginger’s anti-inflammatory effects are due to SOD activity induction, glutathione enhancement, and ROS reduction. Moreover, Ginger has an inhibitory effect on xanthine oxidase system which is responsible for the production of reactive oxygen species like superoxide anion. Besides, Ginger is a serotonin blocker and inhibits the release of substance P. Several compounds have found in Ginger may act as a blocker of serotonin receptors. Laboratory studies have also shown the inhibition of serotonin receptors which is associated with the reduction of TNFα, IL1β, IL6, IL2, and prostaglandins. In addition Ginger have thermogenic properties and increases energy expenditure by enhancing the thermic effect of food.

**CONCLUSIONS**

To conclude, It seems Ginger has anti-inflammatory effects. It can improve the symptoms of inflammatory disease. However, more clinical trial studies are needed to approve its effects and mechanisms of such effects.

**CONFLICTS OF INTEREST**

The authors declare that they have no conflicts of interest.

**REFERENCES**


