

Georgia State University

ScholarWorks @ Georgia State University

Nutrition Theses

Department of Nutrition

Spring 4-25-2011

Oxidized Lipid and its Association with Markers of Adiposity NHANES-2005-06

Payal Arora

Follow this and additional works at: https://scholarworks.gsu.edu/nutrition_theses



Part of the [Nutrition Commons](#)

Recommended Citation

Arora, Payal, "Oxidized Lipid and its Association with Markers of Adiposity NHANES-2005-06." Thesis, Georgia State University, 2011.

https://scholarworks.gsu.edu/nutrition_theses/16

This Thesis is brought to you for free and open access by the Department of Nutrition at ScholarWorks @ Georgia State University. It has been accepted for inclusion in Nutrition Theses by an authorized administrator of ScholarWorks @ Georgia State University. For more information, please contact scholarworks@gsu.edu.

ACCEPTANCE

This thesis, Consumption of Oxidized lipid and its Association with Markers of Adiposity NHANES-2005-06, by Payal Arora was prepared under the direction of the Master's Thesis Advisory Committee. It is accepted by the committee members in partial fulfillment of the requirements for the degree Master of Science in the College of Health and Human Sciences, Georgia State University.

The Master's Thesis Advisory Committee, as representatives of the faculty, certify that this thesis has met all standards of excellence and scholarship as determined by the faculty.

Dr Meera Penumetcha PhD
Committee Chair

Dr Vijay Ganji PhD
Committee Member

Dr Frances McCarty PhD
Committee Member

04/25/2011
Date

AUTHOR'S STATEMENT

In presenting this thesis as a partial fulfillment of the requirements for the advanced degree from Georgia State University, I agree that the library of Georgia State University shall make it available for inspection and circulation in accordance with its regulations governing materials of this type. I agree that permission to quote, to copy from, or to publish this thesis may be granted by the professor under whose direction it was written, by the College of Health and Human Sciences director of graduate studies and research, or by me. Such quoting, copying, or publishing must be solely for scholarly purposes and will not involve potential financial gain. It is understood that any copying from or publication of this thesis which involves potential financial gain will not be allowed without my written permission.

Payal Arora
Signature of Author

NOTICE TO BORROWERS

All theses deposited in the Georgia State University library must be used in accordance with the stipulations prescribed by the author in the preceding statement. The author of this thesis is:

Payal Arora
4867 Ashford Dunwoody Rd,
Apt # 10220
Dunwoody, GA 30338

The director of this thesis is:

Dr. Meera Penumetcha
Department of Nutrition
College of Health and Human Sciences
Georgia State University
Atlanta, Georgia 30303-3083

VITA
Payal Arora

ADDRESS: 4867 Ashford Dunwoody Rd,
Apt # 10220
Dunwoody, GA 30338

EDUCATION:

M.S. 2011 Georgia State University
Health Sciences

PGD 2003 SNDT University
Nutrition and Food Technology

BSC 2001 Calcutta University
Clinical Nutrition and Dietetics

PROFESSIONAL EXPERIENCE:

Jan 2005- Oct.2007	Area Sales Manager JP Morgan Asset Management, Kolkata, India
Jan 2004-Jan 2005	Junior Processing Officer E Serve international, Mumbai, India
June. 2003 – Dec. 2003	Research Intern (R&D Department) Cadbury's India Pvt Ltd, Mumbai, India
June.2001-Aug.2002	Dietitian Body Clinic, Kolkata, India

PROFESSIONAL SOCIETIES AND ORGANIZATIONS:

2008-present American Dietetic Association
2011 Georgia Nutrition Council
2008-present Greater Atlanta Dietetics Association

PRESENTATIONS AND PUBLICATIONS:

Presentation of Estimation of the dietary intake of oxidized lipids in commonly consumed foods using NHANES 2005-2006 data at Georgia Nutrition Council March 2011

ABSTRACT

Background: Polyunsaturated fatty acids (PUFA) are found in nuts and seeds, salad dressings and vegetable oil and are prone to oxidation during storage and food preparation. Evidence supports that consumption of oxidized lipids promotes atherosclerosis and glucose intolerance in animal models. However there is a dearth of evidence with regard to the amount of oxidized lipids consumed and its association with parameters of adiposity and glucose homeostasis in humans.

Objective: The objective of this study is to estimate the amount of oxidized lipids in common foods and the oxidized lipid consumption in the US population using the data from National Health and Nutrition Examination Survey (NHANES) 2005-06. The second objective of this study is to investigate if there is an association between consumption of oxidized lipids with markers of adiposity and glucose tolerance.

Methods- Foods with possible high oxidized lipid content were selected from the NHANES food frequency questionnaire. Oxidized lipid content /Peroxide Values (PV) of these foods were determined from published values in the literature. Oxidized lipid consumption was stratified into tertiles to determine the relationship between consumption of oxidized lipids and markers of adiposity. Regression analysis was used to explore to the extent to which body fat % and HOMA- IR scores could be attributed to oxidized lipid intake.

Results- The estimated mean daily consumption of oxidized lipids was 0.625 meq/kg of fat for the US population. Estimated mean consumption of oxidized lipids was significantly greater in men compared to women, in children compared to adults and

among African Americans compared to other races. In both men and women it was observed that the markers of adiposity like body fat%, waist circumference, triceps skinfold decreased significantly with increased consumption of oxidized lipids. However in women (below 18 years) there was a significant increase in HOMA-IR with increased consumption of oxidized lipids.

Conclusion- Increased consumption of oxidized lipids is associated with decreased fat mass but increased glucose intolerance in women, but not in men.

Consumption of Oxidized lipid and its Association with
Markers of Adiposity NHANES-2005-06

By
Payal Arora

A Thesis

Submitted to the Graduate Faculty of Georgia State University in Partial
Fulfillment of Requirements for the Degree of

Master of Science in Health Sciences
College of Health and Human Sciences
Division of Nutrition
Georgia State University

Atlanta, Georgia
2011

ACKNOWLEDGEMENTS

I would like to thank my thesis committee: Dr Meera Penumetcha (chair), Dr Vijay Ganji and Dr Frances McCarty. I have learned a great deal from each of them. Dr Penumetcha has been a wonderful guiding force and mentor. I appreciate her patience and perseverance for making this journey successful. I would like to thank Dr Vijay Ganji for his guidance and support towards writing the thesis. I would like to thank Dr Frances McCarty for helping me with the statistical calculations. I would like to thank my fellow student Joanna Skinner who was a part of this incredible journey as we spend so much time in the lab together. All the faculty members in the Division of Nutrition have been an inspiration especially Dr Mildred Cody. Finally I would like to thank my family especially my husband without whom this journey would be impossible and my daughter who was the foremost inspiration.

Table of Contents

	Page
List of Tables.....	iv
Abbreviations.....	v
Chapter	
I. INTRODUCTION.....	1
II. REVIEW OF THE LITERATURE.....	3
III. METHODOLOGY.....	10
IV. RESULTS.....	15
V. DISCUSSION.....	21
References.....	26

List of Tables	page
Table 1.....	14
Table 2.....	17
Table 3.....	18
Table 4.....	19
Table 5.....	20

Abbreviations

α Linolenic Acid - ALA

Body Mass Index- BMI

Docosahexaenoic Acid -DHA

Eicosapentanoic acid - EPA

Food Frequency Questionnaire -FFQ

High-Performance Liquid Chromatography- HPLC

Homeostasis Model Assessment - HOMA-IR

Malondialdehyde - MDA

Mobile Examination Centers -MEC.

National Health and Nutrition Examination Survey - NHANES

Peroxide Values -PV

Peroxisome proliferator-activated receptor-gamma - PPAR γ

Polyunsaturated Fatty Acid - PUFA

Thiazolidinediones - TZD

Thiobarbituric acid - TBA

Ultra Violet -UV

Chapter I

Introduction

Studies indicate that high intake of energy from fat contributes to the development of obesity which increases the risk of chronic diseases such as, type 2 diabetes, atherosclerosis, and cancer (1) . On the other hand, metabolic studies reveal that polyunsaturated fatty acids (PUFAs) such as n-3 and n-6 fatty acids are linked to reduced risk of type 2 diabetes, atherosclerosis, and cancer (2). Foods rich in n-3 PUFA are cold water oily fish like salmon, herring, and mackerel. While food sources of n-6 fatty acids are nuts and oils from soybean, rapeseed, and sunflower. In addition, certain foods are enriched in PUFAs such as, α linolenic acid (ALA), eicosapentanoic acid (EPA), and docosahexaenoic acid (DHA) because of their role in improving cognitive function and decreasing cardiovascular risk (2). Through effective marketing strategies the intake of fish oil supplements (containing EPA and DHA) is on the rise among older Americans. In 2002, 7% of the American adults reported taking fish oil supplements. According to the latest update from the agricultural industry the consumption of vegetable is was estimated to rise by about 10 % in 2010 – 2011 (3).

Because PUFAS are naturally present in our daily diet, it is important to note that these fats are prone to autoxidation generating a complex mixture of lipid hydroperoxides, chain-cleavage products, and polymeric compounds (3).

However there is paucity of research on the health risks associated with the consumption of oxidized lipids in humans. A few studies in animals confirm that consumption of lipid-oxidation products may increase the risk of atherosclerosis and glucose intolerance. (4).

In PUFA, the number of double bonds present is the determinant factor for the number of positional isomeric monohydroperoxides that are generated and equals to $(2n-2)$ where n stands for number of carbon atoms). The process of lipid oxidation is initiated by the attack on a fatty acid or fatty acyl side chain which has reasonable reactivity to a hydrogen atom from a methylene carbon in the side chain. The more double bonds in a fatty acid side chain, the easier is the removal of a hydrogen atom. This is the reason why PUFAs are prone to oxidation. With the increase of temperature, storage, and processing of food, the rate of autoxidation increases (5). Studies conducted especially in animal models have concluded that the biological effects of consuming lipid oxidation products may be relevant in various diseases. Oxidized lipids have been implicated in atherosclerosis as they are absorbed by the intestine and incorporated into lipoproteins as demonstrated by an *in vitro* study by Stapran et al (6). Consumption of oxidized lipids has also shown to cause cancer by generating lipid peroxy radicals that have DNA- cleaving activity in mice (7). There are studies which have shown that oxidized lipid consumption may cause decreased adiposity but increased glucose intolerance in mice (8).

However there is a scarcity of studies which have reported the amount of oxidized lipids being consumed by the general population. There are also no reported studies which indicate whether chronic intake of smaller amounts of oxidized lipids presents any health hazard or influence obesity (4).

Therefore the purposes of this study were to quantify the consumption of oxidized lipids and to investigate if there is any association between the consumption of oxidized lipids and markers of adiposity.

We hypothesize that there will be a positive association between the consumption of oxidized lipids and markers of adiposity in the US population.

Chapter II

Literature Review

Obesity is a significant public health issue in the United States. Clinically, obesity is defined as a body mass index ≥ 30 m/kg². Other measures such as waist circumference (9), triceps skinfold and body fat % are also used as indicators of obesity (10). According to NHANES 2007-2008, the prevalence of obesity has plateaued since the last decade and the current rate of obesity is 32.2% among adult men and 35.5% among adult women (11). Obesity is directly related with higher risk of overall morbidity and mortality. Obese individuals are at a higher risk of developing diabetes, hypertension, dyslipidemia, insulin resistance, dyspnea, and apnea (12). The Western diet is characterized with elevated intake of high-fat red-meat, processed and fried foods. The Western diet is high in saturated fats which has been implicated in increasing the risk for cardiovascular disease (13), cancer, and other chronic diseases.

Indeed PUFAs are classified as n-3 or n-6 based on their chemical structure. These are present in vegetable oils, nuts, and fish. Consumption of these fats has been found to have a number of beneficial health effects. Metabolic studies have demonstrated that n-3 and n-6 PUFA have cholesterol-lowering effects (14). Additionally results of nutritional intervention trials indicate that diets high in PUFA were more successful in lowering total serum cholesterol than diets low in fat and high in carbohydrate (2). In the prospective Nurses Health Study results have indicated

that elevated consumption of n-6 PUFA was associated with significantly lower incidence of type 2 diabetes by increasing insulin sensitivity (15). Consumption of fish (n-3 PUFA) was associated with decreased cardiovascular risk in various populations especially Alaskan Native Americans (16), Greenland Eskimos (17) and people residing in Japanese living fishing villages (18). Subsequent prospective cohort studies have also found an inverse association between fish consumption and risk of cardiovascular mortality in diverse populations (19). During frying, storage, and other process such as preparation of fish oil supplements, PUFAs undergo oxidation. Lipid oxidation is a major degradative process in food, which not only causes changes in flavor, color, and texture but also results in the production of cytotoxic and genotoxic compounds (20). The process of lipid peroxidation can be categorized into 3 phases. These are initiation, propagation, and termination.(5). In PUFA, the number of double bonds present is the determinant factor for the number of positional isomeric monohydroperoxides that are generated and equals to $(2n-2)$ where n stands for number of carbon atoms. Therefore in oxidation of linoleic acid (18:2) 2 monohydroperoxides with the hydroperoxy groups are formed.(21).

Measurement of Oxidized lipids in Common Foods

Food rich in PUFA are more prone to oxidation. Therefore there may be conflicting conclusions on the benefits of consuming food rich in PUFA. For example there may be decreased therapeutic benefits of consuming fish oil supplements as they are more prone to oxidation. Studies indicate that the rate of

oxidation in over the counter commercial fish oil supplements is about 20-30% (22). It is essential to consider that food cooked in oil that has been heated and reused also has higher amount of oxidized lipids. Common examples of such foods are French fries, chicken nuggets, and fried seafood. Because hydroperoxides are the primary products of lipid oxidation, therefore, usually the oxidized lipids in foods are measured as peroxide value (PV meq/Kg of fat). The extent of lipid oxidation may be measured by various methods. High-performance liquid chromatography (HPLC) is a common and accurate technique for measuring the peroxides and cytotoxic aldehydes in foods (23) . However this method is cumbersome and requires expensive equipments. Another common and popular method for measuring primary products of lipid oxidation is to measure the conjugated dienes, by measuring the absorbance at ultra violet (UV) light in the wavelength range 230-235 nm. Thiobarbituric acid (TBA) test is another popular and simple test to measure secondary products of lipid oxidation in food. In this test the sample is heated with TBA at low pH, and the resultant Malondialdehyde (MDA) is measured by its absorbance at 532 nm (24) . For our study we have considered reported baseline PV values. PV of foods like French fries and chicken nuggets increase with increased exposure to heated oil. In a study by Hazuka et al the PV for oil extracted from the potato fritters increased from 10.5 meq/ kg to 11.5 meq/kg from day 1 to day 5 of frying. In the same study the results indicated that the PV of the vegetable oil used for frying the potato fritters increased from 1meq/kg to 3meq/kg (25). Thus it becomes essential

to investigate the effect of consuming food that are rich in PUFA but are more prone to oxidation.

Role of dietary Oxidized Lipids in chronic disease

Various studies conducted *in vivo* (22) and *in vitro* (23, 24) suggest that consumption of oxidized lipids may cause atherosclerosis. Evidence from studies suggests that oxidized lipoproteins play a significant role in atherosclerosis. In both human and animal studies oxidized lipoproteins have been observed in atherosclerotic lesions (25-27). Studies indicate that these atherosclerotic lesions may occur locally in the artery wall or that circulating oxidized lipoproteins are incorporated in the lesions. Although the exact mechanism of the origin of development of such lesions is still not clear some studies suggest that oxidized lipids are absorbed in the intestines which are incorporated into serum lipoproteins which are potentially atherogenic (28).

Animal Studies

A study by Staprans et al (6) established that when oxidized linoleic acid, oxidized with Copper Sulphate is administered to rodents, they are absorbed and incorporated into chylomicrons and transported to the liver in the remnant particles. In a study by Merchant et al (26) LDL receptor knockout mice were fed linoleic acid that was oxidized enzymatically with soybean lipoxidase along with a high fat diet (21/% fat), Consumption of a high fat diet containing oxidized linoleic acid increased aortic lesions, plasma cholesterol and oxidative stress

compared to mice fed a high fat diet with unoxidized linoleic acid. The amount of oxidized lipids fed to the mice models was 1.3% of the total fat consumption. Studies in animal models have also demonstrated that oxidized lipid consumption may cause increased glucose intolerance. In a study when rats were fed oxidized oil (soybean oil used to fry flour dough for 6hours/day for 4 days) there was decreased tissue mass, cell size and lipid/DNA ratio in the fat pads of the rats consuming the oxidized oil in comparison to the rats fed fresh fish oil. This suggests that consumption of heated oil decreased fat pad mass as compared to a diet containing unheated oil. However the group consuming the oxidized oil had higher blood glucose measured by oral glucose tolerance test in comparison to the group consuming fresh soybean oil and fresh fish oil (8).

Human Studies

A few studies that have been conducted to study the effect of the consumption of oxidized lipids in humans have been short term and postprandial studies in small cohort. Staprans et al (27) examined if oxidized lipids (vitamin E depleted corn oil after storage of 6-8 weeks with a PV value of 66nmol/mg of oil) are absorbed and incorporated into postprandial serum chylomicrons in humans. The results from this study concluded that in humans oxidized lipids are incorporated in chylomicrons into the circulation and act as a contributor of total body pool of oxidized lipid. In a study by Sutherland et al men (n=12) were given a meal of used fat (the fat used for frying had a PV value 4 times higher than the recommended by the American Oil Chemists Association) in comparison to a meal containing unused fat.

The group consuming the meal with the used fat had a reduced level of paraoxonase activity in comparison to the control group. The enzyme paraoxonase present in HDL is important in prevention of atherogenesis as it hydrolyzes oxidized phospholipids and inhibits LDL (29). In a study conducted in the same cohort, the researchers found that the meal with used fat also caused impaired arterial endothelial function. Impaired endothelial dysfunction is observed in the first stages of atherogenesis and is also associated with other health conditions like hypercholesterolemia and diabetes mellitus (30) .

Although there have been a number of studies conducted on the effect of oxidized lipids on atherosclerosis the method of oxidizing lipids in different studies vary and this may cause variability in the amount of oxidation as well as the outcome. In conclusion, there is some evidence that consumption of oxidized lipids can promote atherosclerosis in animal models and could increase risk factors for cardiovascular disease in humans.

Relationship of PPAR gamma with Markers of Adiposity

Peroxisome proliferator-activated receptor-gamma (PPAR γ) is a ligand activated transcription factor, which plays a key role in the differentiation of adipocytes. It is a member of the PPAR family of nuclear receptors. The PPAR nuclear receptors are predominantly expressed in adipose tissue, adrenal gland and spleen. Various studies have shown that PPAR γ is essential in regulation of adipocyte differentiation and glucose homeostasis (31). In type 2 diabetes, insulin resistance occurs in the peripheral organs such as the muscle by which there is a decreased uptake and

utilization of glucose. Thiazolidinediones (TZD), drugs used in the treatment of type 2 diabetes, are pharmacological ligands for PPAR γ . It is hypothesized that the activation of PPAR gamma by the TZD improves the ability of adipose tissue to store triglycerides (TG) thereby removing those from the liver and muscle. Thereby, the action of the drug may cause slight weight gain but may result in better insulin sensitivity. Studies conducted in mice which are heterozygous *Pparg*^{+/-} (indicates only 50% of PPAR γ) report that these mice have increased insulin sensitivity and resistance to obesity by high fat diet. The mechanism for this phenomenon cannot be fully explained (32). Therefore it can be concluded that both activation and partial loss of PPAR γ cause increased insulin sensitivity. However mice with PPAR γ knocked out in the adipose tissue have elevated plasma glucose and insulin levels, and decreased adiposity. (33). In summary, the level and the activation/inactivation of PPAR γ seem to dictate the adiposity and glucose homeostasis. Another important reason that we are interested in PPAR γ is because oxidized lipids have to shown to be strong ligands of this transcription factor. According to studies, bioactive metabolites generated during lipid oxidation of linoleic acid, 9-HODE and 13-HODE act as endogenous activators and ligands of PPAR gamma (31). Thus we hypothesize that dietary oxidized lipids might influence the adiposity of an animal and thus influence glucose homeostasis. A significant number of studies have demonstrated the effect of dietary oxidized lipids on disease risk in animals. However, the few studies that have looked at the effect of consumption of oxidized lipids in humans have been on a small cohort of people. To our knowledge, there are no data that estimates the consumption of

oxidized lipids in humans. Therefore, our current investigation will estimate the consumption of oxidized lipids in the US population. Next we plan to determine if this consumption of oxidized lipids is associated with markers of adiposity and glucose tolerance.

Chapter III

Methods

NHANES population:

The population for the study is derived from the NHANES, a nationwide survey designed to evaluate the health and nutritional status of adults and children in the US. The NHANES program started in 1960. At the beginning of 1999 the survey became a continuous program that included a range of health and nutrition measurements. We chose NHANES 2005-06 to estimate oxidized lipid consumption because the survey includes more than 5000 people representing the US population. The sample population is identified across different counties nationwide. Sampled persons were interviewed and underwent physical examination in the Mobile Examination Centers (MEC).

NHANES 2005–2006 was a stratified, multistage probability sample of noninstitutionalized civilians in the US population. The data were collected by trained bilingual interviewers by using standardized questionnaires and physical examinations. The data were collected from participant homes or at mobile examination centers. The Food Frequency Questionnaire (FFQ) was used to collect information on the frequency of consumption of foods and food groups during the previous 12 months. This was usually mailed to respondents after the completion of the second 24-hour recall. Although the FFQ does not mention any portion sizes it was assumed that the respondent referred to a single portion size when they answered the FFQ.

The 2005-2006 NHANES also includes anthropometric examination data.

Measurements like height, weight were assessed at mobile examination centers to the nearest 0.1 kg and 0.1 cm, respectively, by using standardized equipment and procedures. Waist circumference was measured to the nearest 0.1 cm and triceps skinfold measured to the nearest 0.1 mm. The BMI was calculated by the formula $BMI = \text{weight (kg)}/\text{height (m)}^2$. The laboratory data on fasting blood glucose levels were reported as mmol/l and insulin levels were reported as pmol/l.

Apart from the above measures reported in NHANES 2005-06, we added two additional markers, body fat % and homeostasis model assessment (HOMA-IR) scores, as parameters for measuring adiposity and glucose homeostasis. The body fat % was calculated by the formula in males = $0.353 \text{ waist (cm)} + 0.756 \text{ triceps (mm)} + 0.235 \text{ age (y)} - 26.4$; for women = $0.232 \text{ waist (cm)} + 0.657 \text{ triceps (mm)} + 0.215 \text{ age (y)} - 5.5$ (10). As insulin resistance is the primary cause of type 2 diabetes and can therefore be deemed a risk factor for other chronic diseases HOMA scores were included as an outcome variable. HOMA scores were calculated by the formula: $\text{fasting serum insulin } (\mu\text{U/ml}) \times \text{fasting plasma glucose (mmol/l)}/22.5$ (37).

Estimation of oxidized lipid consumption (PV, meq/kg of fat):

As a first step, foods rich in PUFA which are likely to have elevated oxidized lipids were identified from the FFQ. This list included French fries, chicken nuggets, and fried fish. Such foods were chosen because usually vegetable oil, rich in PUFA, is used to fry these foods and these oils are heated repeatedly to fry

the foods thus incorporating the peroxides into the food. Some of the popular fast food restaurants have reported using canola oil for frying foods like French fries, chicken nuggets and fried fish(38). Other foods included from the FFQ were nuts as they are a good source of PUFA. Other foods like pizza, and oil used for cooking were also included. The PV for the above foods were determined from published studies. The estimated consumption of oxidized lipids is reported as mean \pm standard error of mean. **Table 1** summarizes the list of foods and the studies from which oxidized lipid amount for each food was determined. The PV was expressed as (meq/kg of fat). In order to determine the relationship between oxidized lipid intake and markers of adiposity the PV were divided into tertiles and trend of mean values for BMI, triceps, waist circumference, body fat % and HOMA IR across the tertiles was determined by SPSS one way ANOVA (but the complex sample weight was accounted for in the calculation in complex module of SPSS version 18).

The data were analyzed using SPSS version (9.2) and SAS version (18). The SAS software was used as it accounts for sample design and sample weights to calculate appropriate estimates of variance for the data in NHANES 2005-2006.

The data related to the estimated consumption of oxidized lipids (as PV) were checked for normal distribution. The mean intake of oxidized lipids was reported as peroxide consumption /day and peroxide consumption /day/gm of PUFA.

Because the data were found not to be normally distributed it was logarithmically transformed. The data related to markers for adiposity like waist circumference, triceps skinfold, BMI and body fat %, fasting blood glucose, insulin, and HOMA-IR were also checked for normal distribution. Apart from waist circumference , triceps skinfold, and body fat%, all the other parameters were found not to be

normally distributed and therefore logarithmically transformed with the exception of BMI which was transformed by square root. Blood glucose was not to be normally distributed even after log transformation, therefore it was decided to include only subjects with a blood glucose ≤ 200 mmol/l which was then log transformed.

Indeed the data for oxidized lipid consumption was reported by gender, age, race and education in adults. Peroxide consumption was stratified into tertiles to determine the relationship between consumption of oxidized lipids with markers adiposity like waist circumference, Body Mass Index (BMI), triceps skinfold, body fat%, fasting glucose, insulin and HOMA-IR score. The general linear model procedure included in the complex samples module of SPSS (version 18) was used to obtain p-value for tests for linear trend. Comparisons were considered statistically significant at a $p \leq 0.05$. Regression analysis was used to explore to the extent to which body fat % and HOMA- IR scores could be attributed to oxidized lipid intake. Regression coefficients and standard errors were estimated after multivariate adjustment for the following covariates: age, sex, and energy intake.

Table 1

Food	PV Value	Reference
French Fries	1.98 meq/kg	Lake RS (1997) (39), Jonnalagadda PR (2001) (40), Hazuka Z(2000) (25)
Fried Fish	2.2 meq/kg	Lake PR (1997) (39), Olayinka S(2009), O’Leary(2000) (41)
Pizza	2.45 meq/kg	Singh P(2010) (42), O’Leary (2000) (41)
Nuts	1.20 meq/kg	LS Maguire (2004) (43), A Kaijser (2000) (44), GP Savage (1999) (45), E Ryan (2010) (46), V Nepote (2010) (47)
Cooking Oil	PV Baseline in oil x Frequency x Type of Oil *	Naaz S (2004) (48), Chapman (2009) (49), Rastogi (2006) (50),

* The calculation of oil was done by multiplying the PV value for the type of oil (Olive/Corn/Canola/Other) reported, with the frequency of consumption/day

Chapter IV

Results

The mean oxidized lipid consumption (measured as PV) in a day was estimated in the sample population (n= 5971) and was found to be 0.625 ± 0.024 meq/kg* of fat. The mean oxidized lipid consumption calculated per gm of PUFA for (n= 5638) was $0.0286 \pm 0.031^*$ meq/kg of fat. **Table 2** provides the estimated mean daily oxidized lipid consumption by gender, age, race and education levels in adults. The results indicate that children (≤ 18 yrs) have a higher peroxide consumption in comparison to adults (≥ 18 yrs). Men consumed more oxidized lipids than women (0.695 ± 0.02 Vs 0.569 ± 0.03). In addition African Americans had the highest consumption which was significantly different from the people of other races. There was no significant difference in the mean consumption based on education.

As shown in **Table 3**, mean values of BMI, body fat% and waist circumference decreased with increasing consumption of oxidized lipids among young men (≤ 18 years). In adult men **Table 3** body fat%, triceps skinfolds decreased with increased consumption of oxidized lipids in adult men (≥ 18 years) ($p \leq 0.10$). We observed a trend of decreased HOMA-IR score with increased consumption of oxidized lipid.

Among women below 18 years of age mean body fat % and waist circumference decreased with increasing oxidized lipid consumption. This trend was significant for both variables with p values of 0.009 and 0.007, respectively **Table 4**.

*The reported means are antilog values.

However there was an increase in mean insulin concentration and HOMA-IR with increasing consumption of oxidized lipids. Among adult women **Table 4** there was no significant relation between markers of adiposity and oxidized lipid but we observed a trend of an increase in mean triceps skinfolds and HOMA-IR with increasing oxidized lipid.

The results of multiple regression analyses for body fat % and log transformed HOMA- IR score are presented in **Table 5** for men for women. We decided to perform regression analysis separately for men and women as there are clear physiological differences in both the groups. There was a significant inverse relationship ($p \leq 0.05$) between body fat % and oxidized lipid consumption/gm of PUFA in both men and women. There were no significant relationship between log transformed HOMA-IR score and oxidized lipid consumption/gm of PUFA in men. There was a positive relation between ($p \leq 0.05$) log transformed HOMA-IR score and oxidized lipid consumption/gm of PUFA in women.

Table 2 Estimated mean intake of oxidized lipids based on gender, age, race and education level

Parameter	Mean (meq/kg of fat)	SEM	p value
Gender			
Men	0.695*	0.02	Sig
Women	0.569*	0.03	
Age			
≤ 18 yrs	0.788*	0.03	Sig
≥ 18 yrs	0.584*	0.02	
Race/ Ethnicity			
Mexican American	0.556*	0.05	Sig
Other Hispanics	0.547*	0.08	
Caucasian	0.633*	0.03	
African American	0.712*	0.03	
Others	0.530*	0.06	
Education			
Upto High School	0.562*	0.02	N Sig
Some College	0.596*	0.04	
College Degree	0.580*	0.04	

Intake of oxidized lipids expressed as (PV consumption/day) meq/ kg of fat are reported by gender, age, race and education above. *The reported means are antilog values. Sig –Statistically significant ($p \leq 0.05$). N Sig- Statistically not Significant ($p \geq 0.05$)

Table3: Relationship between estimated consumption of oxidized lipids and markers of adiposity in men

Category	Parameter	Tertile 1 (≤ 0.0214 meq/kg of fat)	Tertile 2 ($0.0215-0.0477$ meq/kg of fat)	Tertile 3 (≥ 0.0478 meq/kg of fat)	P for trend
Male \leq 18 yr	Fasting Blood Glucose (mmol/l)	94.44 \pm 0.01	94.16 \pm 0.01	94.82 \pm 0.01	0.76
	BMI (kg/m ²)	19.99 \pm 0.04	19.35 \pm 0.03	18.71 \pm 0.03	0.006*
	Body Fat%	10.44 \pm 0.76	8.83 \pm 0.81	7.33 \pm 0.64	0.002*
	Triceps Skinfold (mm)	11.72 \pm 0.51	12.03 \pm 0.48	11.50 \pm 0.33	0.712
	Waist Circumference (cm)	71.75 \pm 1.2	68.18 \pm 1.17	65.12 \pm 1.02	0.001*
	Insulin (pmol/l)	51.47 \pm 0.10	54.16 \pm 0.12	55.53 \pm 0.12	0.574
	HOMA-IR	2.00 \pm 0.11	2.10 \pm 0.38	2.17 \pm 0.10	0.561
Male \geq 18 yr	Fasting Blood Glucose (mmol/l)	101.9 \pm 0.01	100.99 \pm 0.01	94.82 \pm 0.02	0.264
	BMI (kg/m ²)	28.57 \pm 0.03	28.26 \pm 0.04	27.97 \pm 0.04	0.152
	Body Fat%	32.57 \pm 0.03	31.28 \pm 0.68	30.67 \pm 0.96	0.01*
	Triceps Skinfold (mm)	15.54 \pm 0.39	14.81 \pm 0.34	14.38 \pm 0.31	0.001*
	Waist Circumference (cm)	102.61 \pm 1.03	101.44 \pm 1.14	100.85 \pm 1.55	0.176
	Insulin (pmol/l)	54.98 \pm 0.04	52.35 \pm 0.06	49.06 \pm 0.07	0.149
	HOMA-IR	2.34 \pm 0.04	2.18 \pm 0.07	2.02 \pm 0.08	0.09

Intake of oxidized lipids measured as (oxidized lipid consumption/day/gm of PUFA) meq/kg was divided into tertiles and the mean markers of adiposity in males are reported above. * Statistically significant at $p \leq 0.05$, the means for fasting blood glucose, insulin and HOMA are expressed as the antilog of the actual log transformed value. The mean BMI are expressed as square of the square root value

Table4: Relationship between estimated consumption of oxidized lipids and markers of adiposity in women

Category	Parameter	Tertile 1 (≤ 0.0214 meq/kg of fat)	Tertile 2 ($0.0215-0.0477$ meq/kg of fat)	Tertile 3 (≥ 0.0478 meq/kg of fat)	P for trend
Female \leq 18 yr	Fasting Blood Glucose (mmol/l)	90.11 \pm 0.01	90.2 \pm 0.01	92.2 \pm 0.012	0.264
	BMI (kg/m ²)	20.46 \pm 0.04	19.25 \pm 0.03	19.58 \pm 0.05	0.123
	Body Fat%	24.14 \pm 0.65	22.01 \pm 0.30	21.69 \pm 0.67	0.009*
	Triceps Skinfold (mm)	16.00 \pm 0.69	14.87 \pm 0.28	14.66 \pm 0.50	0.128
	Waist Circumference (cm)	72.19 \pm 1.14	67.68 \pm 0.74	67.72 \pm 1.26	0.007*
	Insulin (pmol/l)	54.71 \pm 0.10	55.7 \pm 0.05	72.82 \pm 0.08	0.029*
	HOMA-IR	2.04 \pm 0.10	2.07 \pm 0.04	2.77 \pm 0.09	0.03*
Female \geq 18 yr	Fasting Blood Glucose (mmol/l)	96.54 \pm 0.01	96.16 \pm 0.01	98.49 \pm 0.01	0.351
	BMI (kg/m ²)	27.98 \pm 0.03	28.04 \pm 0.04	28.33 \pm 0.05	0.564
	Body Fat%	41.43 \pm 0.42	41.48 \pm 0.61	41.43 \pm 0.65	0.1
	Triceps Skinfold (mm)	23.07 \pm 0.30	23.91 \pm 0.42	23.80 \pm 0.420	0.086
	Waist Circumference (cm)	94.25 \pm 0.89	93.69 \pm 1.05	94.69 \pm 1.18	0.735
	Insulin (pmol/l)	46.11 \pm 0.05	44.97 \pm 0.07	52.72 \pm 0.07	0.134
	HOMA-IR	1.86 \pm 0.06	1.81 \pm 0.08	2.21 \pm 0.07	0.081

Intake of oxidized lipids measured as (oxidized lipid consumption/day/gm of PUFA) meq/kg was divided into tertiles and the mean markers of adiposity in females are reported above. * Statistically significant at $p \leq 0.05$, the means for fasting blood glucose, insulin and HOMA are expressed as the antilog of the actual log transformed value. The mean BMI are expressed as square of the square root value

Table 5: Regression Analysis of Body Fat % and HOMA-IR in Men and Women

Gender	Outcome Variable	Predictor Variable	β Coefficients	P Value
Male	Body Fat % ¹	Age	0.593 \pm 0.041	\leq 0.001 *
		PV/gm of PUFA	-1.586 \pm 0.451	0.003 *
		PV /gm of PUFA * Age	0.027 \pm 0.011	0.029 *
		Total Kcal/day	0.001 \pm 0 .00	0.024 *
	HOMA- IR ²	Age	0.004 \pm 0.008	0.589
		PV n/gm of PUFA	-0.129 \pm 0.101	0.22
		PV /gm of PUFA * Age	0.001 \pm 0.002	0.683
		Total Kcal/day	0.001 \pm 0.00	0.002 *
Female	Body Fat % ¹	Age	0.598 \pm 0.035	\leq 0.001 *
		PV n/gm of PUFA	-2.007 \pm 0.382	\leq 0.001*
		PV /gm of PUFA * Age	0.057 \pm 0.008	\leq 0.001 *
		Total Kcal/day	0.001 \pm 0.001	0.371
	HOMA- IR ²	Age	-0.003 \pm 0.005	0.607
		PV n/gm of PUFA	0.166 \pm 0.07	0.051*
		PV /gm of PUFA * Age	-0.001 \pm 0.001	0.412
		Total Kcal/day	0.001 \pm 0.001	0.227

Above table contains the results of regression analysis to predict the extent to which body fat % and HOMA- IR scores could be attributed to oxidized lipid intake. * Statistically significant at $p \leq 0.05$

1. Model: Body Fat%= (Intercept) + Age +estimated oxidized lipid consumption/gm of PUFA + Age* estimated oxidized lipid consumption/gm of PUFA +Total Kcal consumed/day.
2. Model: HOMA-IR = (Intercept) + Age +estimated oxidized lipid consumption/gm of PUFA + Age* estimated oxidized lipid consumption/gm of PUFA +Total Kcal consumed/day

Chapter V

Discussion

In both animal and human studies, consumption of oxidized lipids has been associated with detrimental health effects like, increased risk of atherosclerosis (32, 51) , insulin resistance (8) and cancer (52). In order to understand the implication of the effect of oxidized lipids in humans, the necessary first step is to estimate the amount of oxidized lipid consumed by the general population. There are no considerable studies which report the consumption of oxidized lipids in the US population and how it relates to markers of adiposity like BMI, waist circumference, body fat%. The first aim of this study, therefore, was to estimate the oxidized lipid consumption of the representative US population from NHANES 2005-06. The second aim was to see if the estimated oxidized lipid consumption is associated with markers of adiposity and glucose tolerance.

The results from our study indicate that the estimated consumption of oxidized lipids is significantly higher in children (≤ 18 years) as compared to adults **Table 2**. This may be indicative of the fact that children are consuming more fried and processed food as compared to adults. In fact Paeratakul et al reported that, consumption of fast food is higher in children (47%) compared to adults (37%) (53). Our results also indicated that men had a higher consumption of oxidized lipids in comparison to women. The results indicate that the consumption of oxidized lipids was highest in African Americans and lowest in the other races. A study by Block et al reported that African American neighborhoods tend to have higher number of fast food restaurants /sq km compared to other neighborhoods. The result from the study indicated that

there were approximately 2.4 fast food restaurants in predominantly African American neighborhoods compared to 1.5 fast food restaurants in predominantly white neighborhoods.(54). The predominance of fast food restaurants in neighborhood with high African American population reflects that the consumption of fried and processed food is higher among this race.

The results in the study demonstrate that with increased consumption of oxidized lipids there was a decrease in the markers of adiposity like waist circumference, body fat% and triceps skinfolds. The reported results were surprising as we had anticipated that with increased consumption of oxidized lipids there would be an increase in adiposity. We made this assumption because of the evidence that suggests that PPAR γ ligands (oxidized lipids) increase adipose mass. The lack of an increase in adipose mass with increasing consumption of oxidized lipids suggests just the opposite; that oxidized lipids are perhaps acting as antagonists of PPAR γ . We also noticed that in women (≤ 18 years) the HOMA- IR scores increased significantly with increased consumption of oxidized lipids /gm of PUFA. However, markers of adiposity decreased. There was no significant increase in HOMA-IR scores in women ≥ 18 years with increased consumption of oxidized lipids, although we noticed that there was an increased trend ($p=0.081$). This result was also seen in a mouse study (55). In that study there was decreased adiposity in mice fed oxidized lipids but there was increased glucose intolerance in comparison to the control mice that were fed unoxidized fat. Together, these results may therefore suggest that oxidized lipids in females act as an antagonist for PPAR γ , thereby decreasing the triglyceride content of white adipose tissue (WAT) but increasing the fat deposition in the liver and muscle

which causes an increase in insulin resistance. This phenomenon of action is directly opposite of the agonist action of the drug Thiazolidinediones (TZD) on PPAR γ . In patients with diabetes, TZDs act by stimulating differentiation in adipocytes thereby causing a reduction of free fatty acids, and tumor necrosis factor α causing improved insulin resistance but overall weight gain (56). However the same trend is not observed in men although with increased intake of oxidized lipids there was a significant decrease in the markers of adiposity for both the groups. There was no significant relationship between the HOMA-IR score and peroxide consumption/gm of PUFA in men ≤ 18 years.

The regression analysis indicated that in both men and women body fat% could be predicted based on log transformed peroxide consumption/gm of PUFA when controlled for age, gender, and energy intake. In women, we observed that the HOMA-IR score could be significantly predicted based on log transformed peroxide consumption/gm of PUFA, however the same was not observed in men. The above results may be indicative of metabolic obesity where the accumulation of fat may be around abdominal viscera and inside intraabdominal solid organs and may not be measured by the conventional measures of adiposity like BMI, waist circumference, and body fat %. Studies indicate that the effect of fat accumulation is dependent on the anatomical location (57). Accumulation of fat in and around visceral organs causes increased production of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6) and decreased adiponectin thereby causing increased insulin resistance. In another study by Rodriquez et al (58), in rodents, it was observed that the fat pad mass was dependent on the expression of

PPAR γ . The expression of PPAR γ differed in male versus female mice. The accumulation of fat in and around the visceral organs also differs based on gender, and ethnicity. This may be the reason why we do not see the same relationship of peroxide consumption/gm of PUFA and HOMA-IR scores in men and women.

There are many strengths as well as limitations to our study. The strength of the study is that it's an initial attempt in estimating the amount of oxidized lipid consumption in the US population. It is also one of the first attempts to estimate if there is an association between consumption of oxidized lipid and markers of adiposity in a large population. The limitation of our study is that it is cross sectional and therefore it is difficult to differentiate cause and effect from simple association. Also the confounding factors may not be equally distributed in the groups that are being compared and therefore may contribute to bias based on unequal distribution. Another limitation of our study is that the reported consumption of oxidized lipids is the estimated mean and may be different from the usual or the actual intake in the population. As our estimation of consumption of oxidized lipids is based on the qualitative FFQ and the portion sizes are not measured therefore the amount reported may not be the actual amount being consumed. The oxidized lipid consumption in our study is just based on consumption of the few foods identified from the NHANES FFQ. There are other foods which may be contributing to the total consumption of oxidized lipids that have not been identified by us.

Summary and Conclusion

In this study based on NHANES 2005-06 data, we observed that in men and women markers of adiposity like body fat%, triceps skinfold and waist circumference decreased with increased consumption of oxidized lipids. In women we observed that HOMA-IR scores increased significantly with increased consumption of oxidized lipids. However the same trend was not observed in men. This may indicate that oxidized lipids act as antagonists of PPAR γ which could lead to an increased deposition of fat in the visceral organs and decreased subcutaneous fat thereby causing increased insulin resistance in women. However this effect is not true in case of men.

Further research needs to be done in assessing other risk factors with oxidized lipid consumption like LDL levels, C Reactive Protein which may be implicated in diseases like atherosclerosis and cancer

References

1. Hossain P, Kavar B, El Nahas M. Obesity and diabetes in the developing world-- a growing challenge. *N Engl J Med.* 2007;356:213-215.
2. Sacks F. Dietary fats and coronary heart disease. *J Cardiovasc Risk.* 1994;1:3-8.
3. Vegetable Oil World Production & Consumption, Vegetable oil Major Importers & Exporters http://www.agricommodityprices.com/futures_prices.php?id=244 Updated, 2010. Accessed 3/29/2011.
4. Esterbauer H. Cytotoxicity and genotoxicity of lipid-oxidation products. *Am J Clin Nutr.* 1993;57:779S-785S; discussion 785S-786S.
5. Schneider C. An update on products and mechanisms of lipid peroxidation. *Mol Nutr Food Res.* 2009;53:315-321.
6. Staprans I, Rapp JH, Pan XM, Feingold KR. Oxidized lipids in the diet are incorporated by the liver into very low density lipoprotein in rats. *J Lipid Res.* 1996;37:420-430.
7. Sawa T, Akaike T, Kida K, Fukushima Y, Takagi K, Maeda H. Lipid peroxy radicals from oxidized oils and heme-iron: implication of a high-fat diet in colon carcinogenesis. *Cancer Epidemiol Biomarkers Prev.* 1998;7:1007-1012.
8. Chao PM, Huang HL, Liao CH, Huang ST, Huang CJ. A high oxidised frying oil content diet is less adipogenic, but induces glucose intolerance in rodents. *Br J Nutr.* 2007;98:63-71.
9. Zhu S, Wang Z, Heshka S, Heo M, Faith MS, Heymsfield SB. Waist circumference and obesity-associated risk factors among whites in the third

- National Health and Nutrition Examination Survey: clinical action thresholds. *Am J Clin Nutr.* 2002;76:743-749.
10. Lean ME, Han TS, Deurenberg P. Predicting body composition by densitometry from simple anthropometric measurements. *Am J Clin Nutr.* 1996;63:4-14.
 11. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999-2008. *JAMA.* 303:235-241.
 12. Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. *Int J Obes (Lond).* 2006;30:400-418.
 13. Menotti A, Blackburn H, Kromhout D, Nissinen A, Adachi H, Lanti M. Cardiovascular risk factors as determinants of 25-year all-cause mortality in the seven countries study. *Eur J Epidemiol.* 2001;17:337-346.
 14. Diniz YS, Cicogna AC, Padovani CR, Santana LS, Faine LA, Novelli EL. Diets rich in saturated and polyunsaturated fatty acids: metabolic shifting and cardiac health. *Nutrition.* 2004;20:230-234.
 15. Salmeron J, Hu FB, Manson JE, Stampfer MJ, Colditz GA, Rimm EB, Willett WC. Dietary fat intake and risk of type 2 diabetes in women. *Am J Clin Nutr.* 2001;73:1019-1026.
 16. Newman WP, Middaugh JP, Propst MT, Rogers DR. Atherosclerosis in Alaska Natives and non-natives. *Lancet.* 1993;341:1056-1057.
 17. Kromann N, Green A. Epidemiological studies in the Upernavik district, Greenland. Incidence of some chronic diseases 1950-1974. *Acta Med Scand.* 1980;208:401-406.

18. Hirai A, Hamazaki T, Terano T, Nishikawa T, Tamura Y, Kamugai A, Jajiki J. Eicosapentaenoic acid and platelet function in Japanese. *Lancet*. 1980;2:1132-1133.
19. Kromhout D, Bosschieter EB, de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N Engl J Med*. 1985;312:1205-1209.
20. Addis PB. Occurrence of lipid oxidation products in foods. *Food Chem Toxicol*. 1986;24:1021-1030.
21. Gray J. Measurement of Lipid Oxidation" A Review. *Jl of the Am Oil Chem Society*. 1978;55.
22. Shukla VK, Perkins EG. The presence of oxidative polymeric materials in encapsulated fish oils. *Lipids*. 1991;26:23-26.
23. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med*. 1991;11:81-128.
24. Halliwell B, Chirico S. Lipid peroxidation: its mechanism, measurement, and significance. *Am J Clin Nutr*. 1993;57:715S-724S; discussion 724S-725S.
25. Hazuka Z, Pawlowicz R, Tynek M, Drozdowski B. Correlation of quality of frying oil and oil extracted from potato fritters fried in liquid and partially hydrogenated rapeseed oil. *J Food Lpds*. 2000;7:225-236.
26. Emerit I, Khan SH, Esterbauer H. Hydroxynonenal, a component of clastogenic factors? *Free Radic Biol Med*. 1991;10:371-377.

27. Radu A, Moldovan N. 4-Hydroxynonenal reduces junctional communication between endothelial cells in culture. *Exp Cell Res.* 1991;196:121-126.
28. Steinberg D. Antioxidants and atherosclerosis. A current assessment. *Circulation.* 1991;84:1420-1425.
29. Khan-Merchant N, Penumetcha M, Meilhac O, Parthasarathy S. Oxidized fatty acids promote atherosclerosis only in the presence of dietary cholesterol in low-density lipoprotein receptor knockout mice. *J Nutr.* 2002;132:3256-3262.
30. Staprans I, Rapp JH, Pan XM, Kim KY, Feingold KR. Oxidized lipids in the diet are a source of oxidized lipid in chylomicrons of human serum. *Arterioscler Thromb.* 1994;14:1900-1905.
31. Palinski W, Rosenfeld ME, Yla-Herttuala S, Gurtner GC, Socher SS, Butler SW, Parthasarathy S, Carew TE, Steinberg D, Witztum JL. Low density lipoprotein undergoes oxidative modification in vivo. *Proc Natl Acad Sci U S A.* 1989;86:1372-1376.
32. Sutherland WH, Walker RJ, de Jong SA, van Rij AM, Phillips V, Walker HL. Reduced postprandial serum paraoxonase activity after a meal rich in used cooking fat. *Arterioscler Thromb Vasc Biol.* 1999;19:1340-1347.
33. Williams MJ, Sutherland WH, McCormick MP, de Jong SA, Walker RJ, Wilkins GT. Impaired endothelial function following a meal rich in used cooking fat. *J Am Coll Cardiol.* 1999;33:1050-1055.
34. Kubota N, Terauchi Y, Miki H, Tamemoto H, Yamauchi T, Komeda K, Satoh S, Nakano R, Ishii C, Sugiyama T, Eto K, Tsubamoto Y, Okuno A, Murakami K, Sekihara H, Hasegawa G, Naito M, Toyoshima Y, Tanaka S, Shiota K, Kitamura

- T, Fujita T, Ezaki O, Aizawa S, Kadowaki T, et al. PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol Cell*. 1999;4:597-609.
35. Miles PD, Barak Y, He W, Evans RM, Olefsky JM. Improved insulin-sensitivity in mice heterozygous for PPAR-gamma deficiency. *J Clin Invest*. 2000;105:287-292.
36. Gavrilova O, Haluzik M, Matsusue K, Cutson JJ, Johnson L, Dietz KR, Nicol CJ, Vinson C, Gonzalez FJ, Reitman ML. Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass. *J Biol Chem*. 2003;278:34268-34276.
37. Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, Monauni T, Muggeo M. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care*. 2000;23:57-63.
38. The type of oil used by McDonald's to fry of French fries, chicken nuggets. http://www.mcdonalds.com/us/en/food/food_quality/see_what_we_are_made_of/your_questions_answered/canola_blend_oil.html?DCSext.destination=http://www.mcdonalds.com/us/en/food/food_quality/see_what_we_are_made_of/your_questions_answered/canola_blend_oil.html Updated-2010, Accessed- 04/19/2011, 2011.
39. Lake RS, P. Quality and consumption of oxidized lipids from deep-frying fats and oils in New Zealand. *J Am Oil Chem*. 1997;31:1065-1068.

40. Jonnalagadda PR, Bhat RV, Sudershan RV, Nadamuni Naidu A. Suitability of chemical parameters in setting quality standards for deep-fried snacks. *Food Quality and Preference*. 2001;12:223-228.
41. O'Leary E, Gormley TR, Butler F, Shilton N. The Effect of Freeze-chilling on the Quality of Ready-meal Components. *Lebensmittel-Wissenschaft und-Technologie*. 2000;33:217-224.
42. Goyal PSGK. Combined Effect of Refrigeration and Modified Atmosphere Packaging on the Shelf Life of Ready-to-Serve Pizza: Biochemical and Sensory Attributes. *American Journal of Food Technology*. 2011;6:202-214.
43. Maguire LS, O'Sullivan SM, Galvin K, O'Connor TP, O'Brien NM. Fatty acid profile, tocopherol, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut. *Int J Food Sci Nutr*. 2004;55:171-178.
44. Kaijser AD, P; Savage G. Oxidative stability and lipid composition of macadamia nuts grown in New Zealand. *J Food Chem*. 2000;71:67-70.
45. G. P. Savage PCD, D. L. McNeil Fatty acid and tocopherol contents and oxidative stability of walnut oils. *Je Am Oil Chem*. 1999;76:1059-1063.
46. Ryan E, Galvin K, O'Connor TP, Maguire AR, O'Brien NM. Fatty acid profile, tocopherol, squalene and phytosterol content of brazil, pecan, pine, pistachio and cashew nuts. *Int J Food Sci Nutr*. 2006;57:219-228.
47. Nepote V, Olmedo RH, Mestrallet MG, Grosso NR. A study of the relationships among consumer acceptance, oxidation chemical indicators, and sensory attributes in high-oleic and normal peanuts. *J Food Sci*. 2009;74:S1-8.

48. Naz S, Sheikh H, Siddiqi R, Asad Sayeed S. Oxidative stability of olive, corn and soybean oil under different conditions. *Food Chemistry*. 2004;88:253-259.
49. Chapman TM, Kim HJ, Min DB. Prooxidant Activity of Oxidized α -Tocopherol in Vegetable Oils.
50. Rastogi PM, Beena; Rastogi, Shweta; Gupta, V.P.; Gupta, Rajeev. Fatty acid oxidation and other biochemical changes induced by cooking in commonly used Indian fats and oils. *Nutrition & Food Science*. 2006;36:407-413.
51. Cohn JS. Oxidized fat in the diet, postprandial lipaemia and cardiovascular disease. *Curr Opin Lipidol*. 2002;13:19-24.
52. Bull AW, Steffensen KR, Leers J, Rafter JJ. Activation of PPAR gamma in colon tumor cell lines by oxidized metabolites of linoleic acid, endogenous ligands for PPAR gamma. *Carcinogenesis*. 2003;24:1717-1722.
53. Paeratakul S, Ferdinand DP, Champagne CM, Ryan DH, Bray GA. Fast-food consumption among US adults and children: dietary and nutrient intake profile. *J Am Diet Assoc*. 2003;103:1332-1338.
54. Block JP, Scribner RA, DeSalvo KB. Fast food, race/ethnicity, and income: a geographic analysis. *Am J Prev Med*. 2004;27:211-217.
55. Liao CH, Shaw HM, Chao PM. Impairment of glucose metabolism in mice induced by dietary oxidized frying oil is different from that induced by conjugated linoleic acid. *Nutrition*. 2008;24:744-752.
56. Yamauchi T, Kamon J, Waki H, Murakami K, Motojima K, Komeda K, Ide T, Kubota N, Terauchi Y, Tobe K, Miki H, Tsuchida A, Akanuma Y, Nagai R, Kimura S, Kadowaki T. The mechanisms by which both heterozygous

peroxisome proliferator-activated receptor gamma (PPARgamma) deficiency and PPARgamma agonist improve insulin resistance. *J Biol Chem.* 2001;276:41245-41254.

57. Hamdy O, Porramatikul S, Al-Ozairi E. Metabolic obesity: the paradox between visceral and subcutaneous fat. *Curr Diabetes Rev.* 2006;2:367-373.
58. Rodríguez E, Ribot J, Rodríguez AM, Palou A. PPAR-gamma2 expression in response to cafeteria diet: gender- and depot-specific effects. *Obes Res.* 2004 ;12(9):1455-63.