Website : www.chem-tox-ecotox.org/ScientificReviews 8 February 2019

SCIENTIFIC REVIEWS

Dietary Recommentations for Fat Intake and Chronic Diseases

Consensus and controversy on advice from the 1980s to reduce consumption of total and saturated fat among other dietary goals

Athanasios Valavanidis

Department of Chemistry, National and Kapodistrian University of Athens, University Campus Zografou, 15784 Athens , Greece
E-mail : valavanidis@chem.uoa.gr

Abstract

Epidemiological and clinical studies have found that consumption of saturated animal fats (butter, cream, cheese, red meat, processed meat foods, etc) and higher risk for heart diseases are connected. Medical practitioners in the USA were the first to raise the issues of dietary recommendations for fats 40 years ago. But in the last decades numerous prospective epidemiological investigations of dietary fat and coronary disease have been inconsistent. Some investigations showed significant positive association between saturated fat and disease, but some other studies did not. Lately, large prospective epidemiological studies offered new evidence. Studies showed that elevated levels of lipids [total cholesterol, low-density lipoprotein cholesterol (LDL), and triglycerides] are widely recognized as risk factors for cardiovascular diseases (CVDs). Oxidized LDL and lipid peroxidation mechanisms are associated with oxidative stress and endothelial dysfunction leading to atherosclerotic damage. Atherosclerosis is a form of chronic inflammation resulting from interaction between oxidized lipoproteins, monocyte-derived macrophages, T cells, and the normal cellular elements of the arterial wall. This review examines the latest studies and reviews investigating the association between dietary fat intake, especially saturated fats, and increased risk to cardiovascular diseases (CVDs), various types of cancer, obesity and type 2 diabetes. The review explores the dietary guidelines that have almost universally advocated reducing the intake of total and saturated fat, and replacement with Polyunsatuerated fatty acids (PUFAs) and Monounsaturated fatty acids (MUFAs). Also, contains critical presentations of the medical literature that is still full of articles arguing opposing positions and data of studies where consumption of total fats were not associated with risk of CVDs, or myocardial infarction. Lately, some experts insist on the inherent complexity of human diets, methodological considerations, and the role of bias and confounding. While latest studies argued that it is the type of saturated fats we eat that can affect our risk of chronic diseases.

Introduction: Dietary advice on the consumption of fat

For many decades saturated animal fats and cholesterol are considered the most important group of nutrients in the human diet which are associated with risk of chronic diseases, such as heart diseases, cancer, type 2 diabetes and obesity, leading killers in idustrial developed countries. Many studies found that consumption of saturated animal fats (butter, cream, cheese, red meat, processed mea foods, etc) and higher risk from heart diseases are connected. Dietary investigations showed a positive association of high serum cholesterol levels with increased risk of having a heart attack and the culprits are diets with high levels of saturated animal fatty acids. Also, other studies observed association between dietary fat consumption and risk of cancer, especially colon, breast, prostate, and ovary cancer. These findings have been debated for years among medical researchers and nutritional specialists.[1-4]

A number of prospective epidemiologic investigations of dietary fat and coronary disease have been inconsistent. Some investigations found significant positive association between saturated fat and disease, but some other studies did not show this association. But large prospective epidemiological studies in the last decade offered new evidence. The relation of dietary fat to risk of coronary heart disease (CHD) has been studied extensively using many approaches, including controlled feeding studies with surrogate end-points such as plasma lipids, limited randomized trials and large cohort studies. All lines of evidence indicate that specific dietary fatty acids play important roles in the cause and the prevention of CHD, but total fat as a percent of energy is unimportant. [5-9]

At the same time dietary nutrition scientists advised people to reduce high intake of fat (especially saturated animal fats) for reducing heart diseases, obesity and type 2 diabetes. Most health and human services organizations in the USA (such as the American Medical Association) and in other developed countries followed similar advise for reduction in fat consumptio for preventing chronic diseases. In the last decades this has become the central principle of dietary guidelines from western governments and from the majority of nutrition- and health-related professional organizations. It must be emphasized that in the 1970s and 1980s the studies of diet-related chronic diseases were in their infancy and only the modern epidemiological

and clinical observations gave a clearer picture of the broad situation involving fat consumption, types of fats and “bad” and “good” lipoproteins and the role of cholesterol.[10-13]

Recommendations on saturated and unsaturated fat consumption

The latest dietary advice from the National Health Service (NHS) in the United Kingdom for the consumption of fat is specific. It recommends that men should have no more than 30 g of saturated fat per day, women should have no more than 20g and children should have less. Lower consumption of saturated fats is beneficial because it can raise cholesterol in the blood increasing the risk of heart disease. Also, the American Heart Association (AHA) recommends a healthy dietary pattern that achieves only 5% to 6% of calories from saturated fat. All types of fat are important and give to the human body high energy (9 kcal of energy compared 4kcal for carbohydrate and protein) and help the body to absorb fat soluble vitamins A, D and E and minerals. All public medical institutions recommend for humans to reduce the amount of fat as an essentila part of healthy and balanced diet. But at the same time state fat in moderation is needed to build cell membranes, is essential for blood clotting, muscle movement and inflammation. Fats that are considered healthy for humans are the monounsaturated (one double bond in their fatty acids) and polyunsaturated fats (with more than two double bonds). Fats that are considered unhealhty include industrial-made trans-fats. In the case of saturated fats some are healthy (when consumed in moderation) and some cause health problems, especially animal fats, in excess conusmption (meat, cheese, butter).[14,15]

Concerns among medical practitioners were raised with the first dietary recommendations in the USA 40 years ago. The 1977 Dietary Goals for Americans included recommendions by the Dietary Guidelines Advisory Committee on various food items and fat consumption (DGAC, Senate Select Committee on Nutrition and Human Needs, led by Senator George McGovern and a brochure was issued by Dpt of Health and Human Services and the US Department of Agriculture).[15]

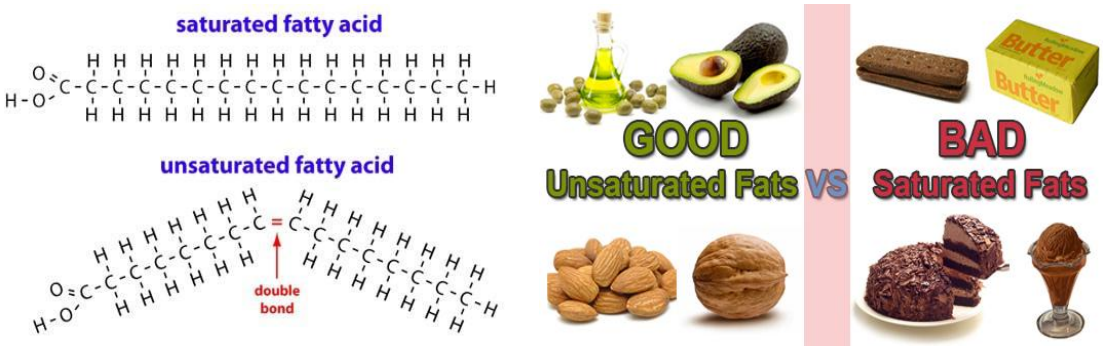


Figure 1. Medical experts recommend less than 30% of human diet being fat. That includes the 10% from saturated fats and 20% from unsaturated fats in balanced diet (in terms of calories consumed daily only 5-6 % from saturated fats)

The committee proposed increases in carbohydrate intake and decreases in fat (especially saturated fat, cholesterol, and salt consumption). The same recommendations were carried further in the 2010 Dietary Guidelines Advisory Committee Report. Important aspects of these recommendations remain unproven, yet a dietary shift in this direction has already taken place even as overweight/obesity and diabeteshave increased. Although appealing to an evidence-based methodology, the DGAC Report demonstrates several critical weaknesses, including use of an incomplete body of relevant science.[16,17]

In the 1970s and 1980s scientists did not differentiate among the two basic types of holesterol and the common practice was to measured "total" cholesterol in the blood (blood test), which includes cholesterol within both Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL). The LDL is considered the “bad” cholesterol, because it contributes to fatty buildups in arteries, whereas HDL cholesterol is thought as the “good” cholesterol, because HDL acts as a scavenger, carrying LDL (“bad”) cholesterol away from the arteries and back to the liver, where the LDL is broken down and passed away from the human body. But to complicate matters more, HDL cholesterol does not completely eliminate LDL cholesterol. Only 30%-25% of blood cholesterol is carried away by HDL. Later studies showed that while LDL was linked to increased risk of chronic diseases and especially cardiovascular diseases (CVDs), HDL was linked to reduced risk.[18-22]

The fats in the human body are in the form of triclycerides which store excess energy from the daily diet of humans. It has been found that a high triglyceride level

combined with high level of LDL cholesterol (“bad’) or low HDL cholesterol (“good”) is linked with fatty buildups inside the blood artery walls, thus increasing risk for CVD. Also, the human body makes triglycerides from the carbohydrates in the diet and send them to fat cells where they are stored for energy. So, dietary advice in western societies is to increase consumption of mono- and poly- unsaturated fats and reduce saturated fats. Trans-fats are formed in the industrial process of hydrogenation to produce margarins. Their chemical structures make them hard to metabolize into smaller compounds and they accumulate in the tissues. Trans-fats have been found to raise LDL (“bad”) and lower HDL (“good”). Trans-fats are more likely to increase type 2 diabetes and increase the risk for heart attacks. Many years ago, oil, butter and food manufacturers have reduced substantially the formation of trans-fats for the majority of processed foods. The FDA in the USA and other dietary advise organisations began a campaign requiring food manufacturers to list the amount of trans-fats per serving on the food package labeling in 2006.[23]

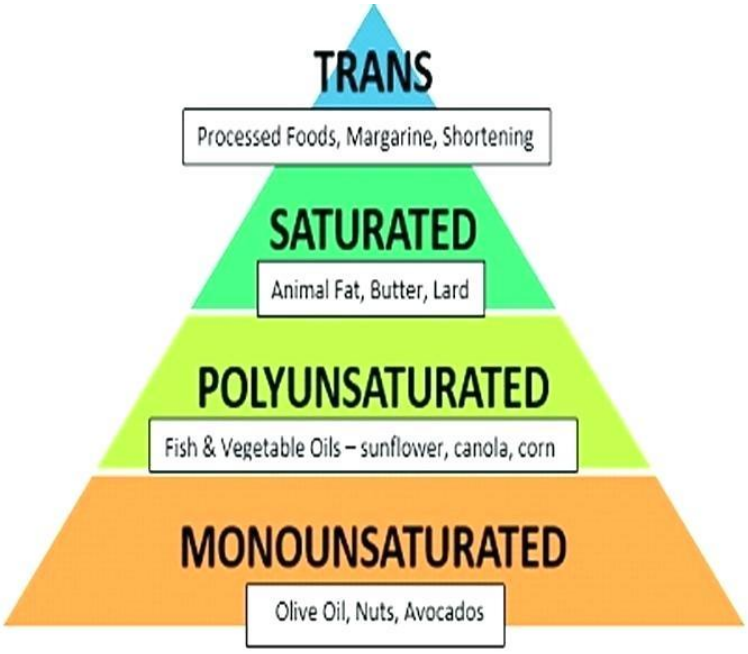


Figure. 2. The diet pyramid of fat consumption. In the same way of the Mediterranean diet, vegetable oils with monounsaturated fatty acids (olive oil, nuts and avocado) are highly recommended, then is polyunsaturated fats (fish, other vegetable oils, sunflower, canola, corn), reduced intake is recommended for animal fats, butter and lard with saturated fatty acids and avoidance of fats with trans-fatty acids. Scientific dietary studies found that there is a strong association between diets high in saturated fatty acids and a high incidence of heart attacks.

Mechanisms of lipid peroxidation leading to atherosclerosis

Elevated levels of lipids, such as total cholesterol, low-density lipoprotein cholesterol (LDL), and triglycerides, are widely recognized as risk factors for cardiovascular disease (CVD). Scientific reasearch on oxidized LDL (ox-LDL) and lipid peroxidation in general, has been an interesting area associated with oxidative stress and endothelial dysfunction, which is implicated in the progression of CVDs. Increased lipid peroxidation and decreased antioxidants with ageing indicate that peroxidative damage further increases with higher blood pressure and the ageing process. Extensive epidemiological research evidence has been gathered on the role of ox-LDL in the progression of atherosclerosis.[24]

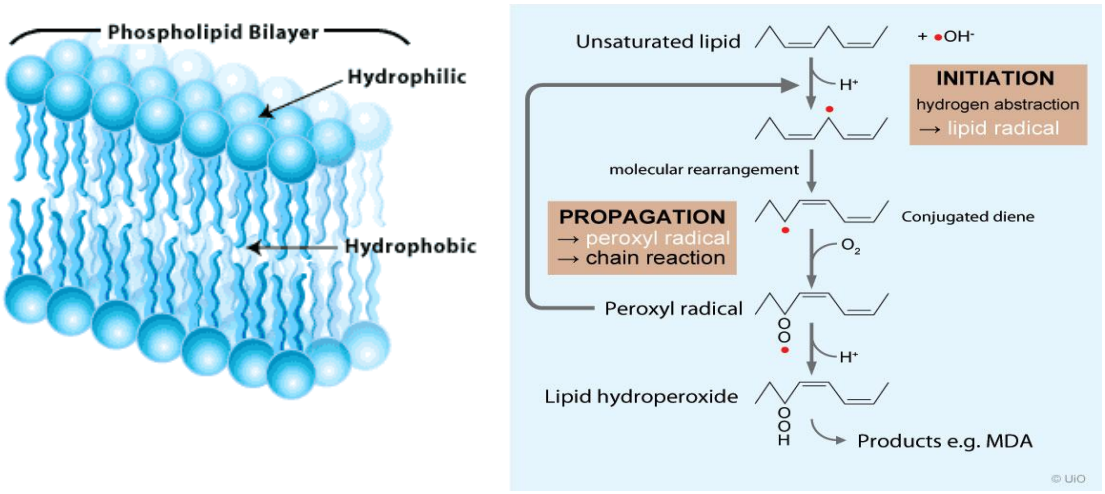


Figure 3. A picture of the phospholipid bilayer, the main component of cell membranes with the hydrophilic layer outside. On the right the pathways of free radical mediated lipid peroxidation, proceeding by a chain mechanism. The initiation takes place by free radical ($X^{\bullet}$) which can oxidize lipid molecules. Chain progression is carried by lipid peroxyl radicals independent of the type of chain-initiating free radicals. [Niki E. Free radicals in the 1900's: from *in vitro to in vivo*. *Free Radic Res*. 33:693–704, 2000].

Scientific evidence demonstrated that reactive oxygen species (ROS) and oxidative stress are important features of cardiovascular diseases (CVDs), including atherosclerosis, hypertension, and congestive heart failure.[25] Endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis. The impaired endothelium-dependent vasodilation is found in the forearm, coronary, and renal vasculature in patients with CVD.[26]

Cholesterole is a fatty substance known as a lipid, which is made by the liver but also can be found in some foods (saturated fat, meat, cheese, cured meat, cream, etc). Cholesterol is vital for the normal functioning of the human body, but excess or high levels of lipids in the blood (called hyperlipidemia) have negative effects on the human health. High cholesterol itself does not usually cause any adverse symptoms but increases the risk for various diseases. Clinical and nutritional studies provide strong evidense that high cholesterol in the blood can produce: narrowing of the arteries (called atherosclerosis), leading to peripheral arterial disease, transient ischaemic attack (called "mini stroke"), heart atttacks and stroke. The molecules of cholesterol can be oxidized, its structure changes into foam cell formations which stick to the artery's wall (blood clot). The build up of clots in the blood arteries restricts the flow of blood to the heart, brain and the rest of the human body leading to chronic CVDs. It is established fact, that increased plasma concentrations of cholesterol-rich apolipoprotein-B (apoB)-containing lipoproteins are causatively linked to atherosclerotic diseases and that lowering plasma LDL concentrations reduces cardiovascular events in humans.[27,28]

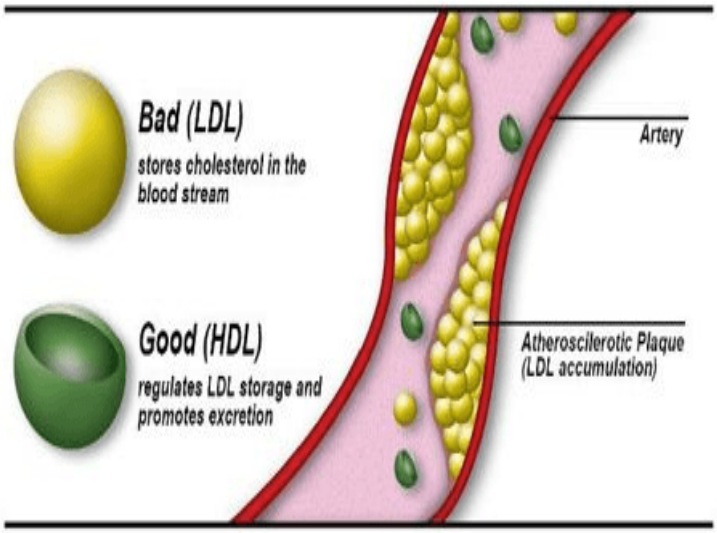
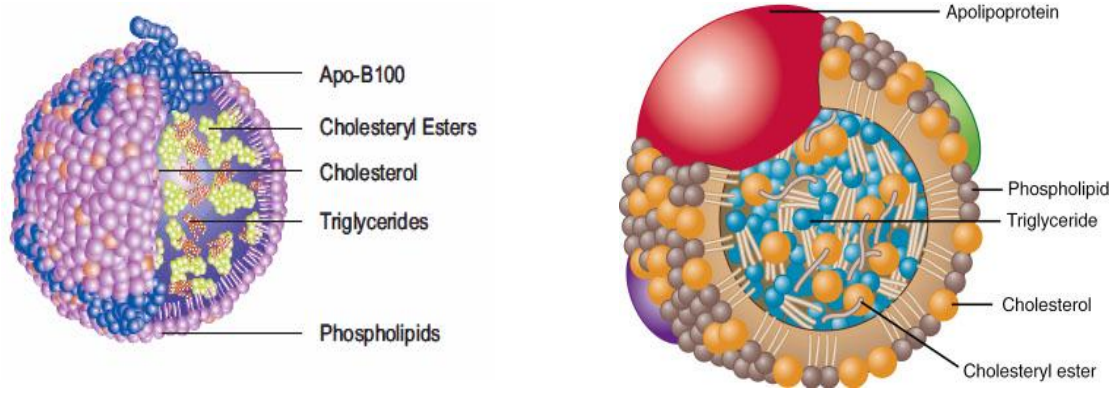


Figure 4. The LDL stores cholesterol in the blood stream of the arteries, whereas the HDL regulates LDL storage and promotes excretion of cholesterol to the liver. The accumulation of foam cell LDL in the arteries by sticking together initiate atherosclerotic plaques restricting the flow of blood.

Fats are insoluble in water (including cholesterol) resulting in a difficult transportation by the extracellular water of the blood. The human body's solution to this problem is to bind cholesterol to certain proteins that function as transport

vehicles carrying different types of fats such as cholesterol, triglycerides and phospholipids. These combinations of fats and protein are termed lipoproteins. Low-density lipoprotein (LDL, it is called also the "bad" cholesterol) is one of the body's lipoproteins and an important carrier of cholesterol. LDL is considered "bad" because it deposits excess cholesterol in blood vessel walls, contributing to heart diseases. Unlike for high-density lipoproteins (HDL**)** which is called the "good" cholesterol because it carries cholesterol from other parts of your body back to the human body's liver.[29,30]



Low Density Liporpotein (LDL) **High Density Lipoprotein (HDL)**

Figure 5. Chemical structures and composition of LDL and HDL. The lipoproteins are composed of an outer shell of phospholipid, which renders the particle soluble in water. These lipoproteins are characterized by their density: high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL).

The atherogenic properties of oxidized LDL (ox-LDL) are very different from the original or native LDL. The ox-LDL enhances the uptake by macrophages leading to foam cell formation. The products of ox-LDL are chemotractic for monocytes (macrophages are important cells of the immune system that are formed in response to an infection) and T cells (T cell, or T lymphocyte, is a subtype of white blood cell that plays a central role in cell-mediated immunity) as well as inhibit the motility of tissue mactophages. The oxidized sterols (also known as steroid alcohols, subgroup of the steroids, important class of organic molecules occuring naturally in plants, animals, and fung), which are products of ox-LDL are cytotoxic and can induce apoptosis. Also, ox-LDL is mitogenic (triggering mitosis, which can influence the mutation or cell division) for smooth cells and mactophages. Ox-LDL can alter gene expression of vascular cells, such as induction of MCP-1 (Monocyte Chemoattractant

Protein-1, one of the key chemokines that regulate migration and infiltration of monocytes/macrophages), interleukins and adhesion molecules. Ox-LDL and its products can induce pro-inflammatoryu genes. Finally, products of ox-LDL can lead to plaque disruption, thus being involved in acute coronary syndromes.[31]

Oxidized lipoproteins and coronary plaque formation in arteries leading to atherosclerosis

Studies showed that atherosclerosis causes more premature deaths and long-term disability than any other disorder in industrialized societies. Coronary artery disease (CAD) arising from atherosclerosis is a leading cause of death and morbidity worldwide. Atherosclerosis can be considered to be a form of chronic inflammation resulting from interaction between modified lipoproteins (oxidized), monocyte-derived macrophages, T cells, and the normal cellular elements of the arterial wall. This inflammatory process can ultimately lead to the development of complex lesions, or plaques, that protrude into the arterial lumen. Plaque rupture and thrombosis results in the acute clinical complications of myocardial infarction and stroke.[32,33]

The underlying pathogenesis of atherogenesis involves an imbalanced lipid metabolism (oxidation of LDL through a complex process) leading to a maladaptive immune response entailing a chronic inflammation of the arterial wall.[34,35]

Scientists studying the stages of formation of atherosclerotic lesions in the 1980s investigated the initiation in association with the concept of oxidative stress and the oxidation and degradation of low-density lipoprotein (LDL). This theory originated over 25 years ago and proved to have a fundamental role in atherosclerosis. Experiments showed that oxidatively modified LDL play a potential role in recruitment and retention of monocyte/macrophages during atherogenesis.[36-38]

The concept of oxidation of lipoproteins originated from experimental observation that *in vitro* incubation of macrophages with oxidized LDL and not with native LDL led to cholesterol ester accumulation.[39] Research observed that oxidation of LDL is a complex process during which both the protein and the lipids undergo

oxidative changes and form complex products. Non-enzymatic oxidative changes in protein amino acids as well as proteolysis and cross-links of apoprotein B (apo B) takes place during this process, and result is substanctial alteration in the protein composition and structure. Additionally, the peroxidized lipids decompose generating both free and core aldehydes and ketones that covalently modify *ε*-amino groups of lysine residues of the protein moiety.[40]

The decomposition of Low density lipoproteins inevitably intitiates the scavenging and degradative action of mactophages (are a type of white blood cell, of the immune system, that engulfs and digests cellular debris, foreign substances, microbes, cancer cells, and anything else that does not have the type of proteins specific to healthy body). This oxidative modifications cover also other lipoproteins with accompanying changes in their pro- or anti-atherogenic behaviour. [41-43]

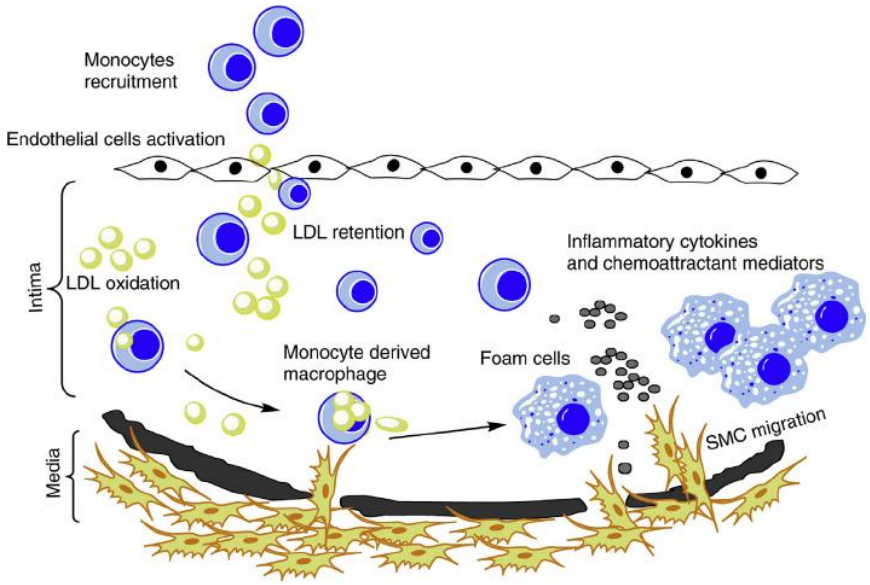


Figure 6. The initial steps of atherosclerosis include recruitment of blood monocytes to the activated endothelial monolayer of the normal artery wall by the action of chemo- attractants and adhesion molecules. (monocytes are a type of white blood cell, can differentiate into macrophages and myeloid lineage dendritic cells). Monocytes can differentiate into macrophages, which engulf oxidized LDL and form foam cells. The macrophages secrete inflammatory cytokines that attract additional monocytes. Oxidized LDL stimulates vascular smooth muscle cells migration and proliferation, which also contribute to intimal thickening and plaque formation. (SMC, smooth muscle cells). [Othman RA, Myrie SB. Non-cholesterol sterols and cholesterol metabolism in sitosterolemia. *Atherosclerosi*s 231(2):291-299, 2013].

In the pre-atherosclerotic stage, a large number of blood cells, predominantly monocytes and T-cells, become attached to the luminal endothelium in atherosclerosis-predisposed areas of the arteries. The continuously increasing adhesion of monocytes and T-cells to the luminal endothelium is a hallmark of the early stages of the development of atherosclerotic lesions.[44]

In the subendothelial space enriched with atherogenic lipoproteins, most macrophages transform into foam cells. Foam cells aggregate to form the atheromatous core and as this process progresses, the atheromatous centres of plaques become necrotic, consisting of lipids, cholesterol crystals and cell debris.[45]

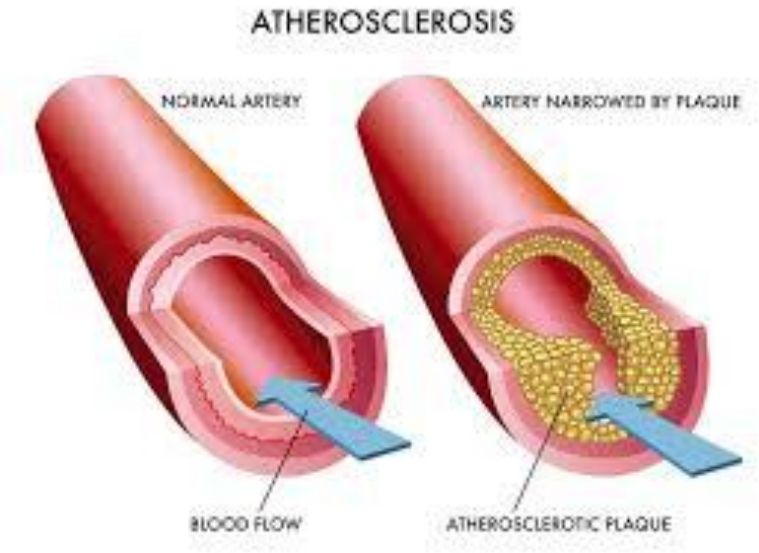


Figure 7. Buildup of cholesterol in arteries is the process that produces atherosclerotic plaques narrowing the arteries and restricting blood flow. Different types of cholesterol-containing lipid particles are found in atherosclerotic lesions. Foam cells aggregate to form the atheromatous core and as this process progresses, the atheromatous centres of plaques become necrotic (Kruth HS. Mactophage foam cells and atherosclerosis. *Front Biosci* 6:429-455, 2001).

In atherosclerosis, the accumulation of apolipoprotein B-lipoproteins in the matrix beneath the endothelial cell layer of blood vessels leads to the recruitment of monocytes, the cells of the immune system that give rise to macrophages and dendritic cells. Macrophages derived from these recruited monocytes participate in a maladaptive, nonresolving inflammatory response that expands the subendothelial layer due to the accumulation of cells, lipid, and matrix. Some lesions subsequently form a necrotic core, triggering acute thrombotic vascular disease, including

myocardial infarction, stroke, and sudden cardiac death. Macrophages on these stages play a central role of disease progress and pathogenesis.[46]

The evidence that dietary fats increase the risk of cardiovascular diseases (CVDs)

Fom the 1960s there was evidence on the association of dietary fats and cardiovascular diseases (CVDs) that was based on comparisons of incidence and mortality rates across geographical areas, and on knowledge of the effects of dietary fats on blood cholesterol levels.[47]

In the Seven Countries Study disease data were compared from 7 countries (US-New York, Finland, Netherlands, Italy, Yugoslavia, Japan, Greece). The results established the benefits of the Mediterranean diet, in association with per capita intake of saturated fat, but not total fat. The results showed strongly correlated rates of CVD with fat consumption. Although results were potentially confounded by other variables, this provided a strong incentive to understand the major geographical variation in CVD rates.[48]

Although early studies showed that saturated fat diets with very low levels of polyunsaturates (PUFAs) increase serum cholesterol, other studies showed high serum cholesterol increased the risk of coronary artery disease (CAD). The evidence of dietary saturate fats causing premature death was weak. But over the years, data revealed that dietary saturated fatty acids (SAFA) are not associated with CAD and other adverse health effects or at worst are weakly associated in some analyses when other contributing factors may be overlooked. Several recent analyses indicate that SAFAs, particularly in dairy products and coconut oil, can improve health. The evidence of ω-6 polyunsaturated fatty acids (omega-6 PUFAs) promoting inflammation and augmenting many diseases continues to grow, whereas ω-3 PUFAs seem to counter these adverse effects. The replacement of saturated fats in the diet with carbohydrates, especially sugars, has resulted in increased obesity and its associated health complications. Well-established mechanisms have been proposed for the adverse health effects of some alternative or replacement nutrients, such as simple carbohydrates and PUFAs. The focus on dietary manipulation of serum

cholesterol may be moot in view of numerous other factors that increase the risk of heart disease. A recent review calls for a rational reevaluation of existing dietary recommendations that focus on minimizing dietary SAFAs, for which mechanisms for adverse health effects are lacking.[49] Also, recent meta-analyses of prospective observational studies reported that SAFA intake was associated with neither coronary heart disease (CHD) nor stroke mortality nor myocardial infarction.[50,51]

Some studies showed that when reduced SAFA intake was replaced by *cis*-polyunsaturated fatty acids (cis-PUFA) there was an association with a 17% lower risk of cardiovascular events.[52] Similar results were confirmed by randomized controlled diet trials. The lack of association of dietary SAFA with increased CHD risk could imply that the potential contribution is probably comparable to that of all other macronutrients together, under isoenergetic conditions [53] In comparative studies the SAFA consumption significantly increases the plasma concentration of low-density lipoprotein cholesterol (LDL-C) compared with mixed carbohydrates and *cis*-unsaturated fatty acids potentially increasing the risk of CHD.[54,55]

The results of all these contrasting dietary findings have challenged current saturated fats dietary recommendations by the medical sector and at the same time contributed to a vigorous debate among doctors and dieticians. Public social media, newspapers and TV programmers reproduced the arguments with highly controversial titles that led to consumer confusion on dietary advice.[56,57]

It was apparent in the scientific community that an update of the evidence was needed. In this respect, a large group of nternational experts on dietary fat and health convened to update and discuss the scientific evidence linking saturated fat comsumption and CHD risk and ischemnic stroke.The meeting took place in Leiden (Netherlands) in 2015, as guests of the International Expert Movement to Improve Dietary Fat Quality (www.theiem.org), The Netherlands Oils and Fats Industry (www.mvo.nl) and the European Palm Oil Alliance (www.palmoilandfood.eu). The summary and conclusions of this meeting was: “ Saturated fatty acids have important metabolic functions, but their consumption is not essential because they can be synthesized de novo. However, their functional properties make them virtually indispensable for the production of fat-containing foods. Dietary saturated fats, when compared to carbohydrates and *cis*-unsaturated fatty acids, raise plasma

LDL-C, a causal risk factor for CHD. Individual SAFAs affect plasma lipoprotein levels differently, with each major dietary SAFA except stearic acid resulting in higher levels of LDL- and HDL-C and lower levels of triglycerides (TG). In prospective observational studies and randomized controlled trials, higher total saturated fat intakes were not associated with higher incident of CHD events or mortality, but replacement nutrients were not taken into account. The effect of reducing dietary SAFA is most strongly affected by the macronutrients that replace them. The greatest reduction in CHD risk occurs when *cis*-PUFA replace dietary SAFA. In intervention studies replacement of 10% E (energy intake) from SAFA by *cis*-PUFA reduced CVD events by 27% and the replacement of 5%E from SAFA by *cis*-PUFA decreased CHD risk by 10%...." [58]

The American Heart Association (AHA, National Center, 7272 Greenville Avenue Dallas, TX, USA) in 2017 issued a dietary advice on fat consumption and its association with increased risk of CVDs. AHA reached that conclusion by reviewing the most recent studies on the effects of dietary saturated fat intake and its replacement by other types of fats and carbohydrates. AHA scientists conlcuded that randomized controlled trials (RCTs) showed that lowered intake of dietary saturated fat and replaced with PUFAs of vegetable oil reduced CVD by ~30%. This reduction is similar to the reduction achieved by taking statins. Prospective observational studies in many worldwide populations showed that lower intake of saturated fat coupled with higher intake of PUFA and MUFA dietary fat is associated with lower rates of CVDs and all-cause mortality. Also, studies established that replacement of saturated with unsaturated fats lowers LDL cholesterol, a cause of atherosclerosis, linking biological evidence with incidence of CVD in populations and in clinical trials. The American Heart Association strongly recemmends lowering intake of saturated fat and replacing it with PUFAs, to achieve lower incidence of CVDs, but this shift should occur simultaneously in an overall healthful dietary pattern such as DASH (Dietary Approaches to Stop Hypertension) or the Mediterranean diet.[59]

Results for dairy intake and risks for cardiovascular diseases (CVD, CHD, stroke) were recently published in a systematic review and meta-analysis of 31 prospective cohort studies. The results reported significant inverse relative risks for intakes of total dairy, cheese, and calcium from dairy foods and total stroke, but

dose-response associations did not hold after adjustment for within-study covariance.[60] Another meta-analysis of prospective studies reported a significant inverse association between stroke and low-fat dairy and cheese consumption.[61]

Recent examples of dietary guidelines supported by the experimental results are from the Nordic Council of Ministers (Finland, Norway, Sweden, Denmark) for nutrition recommendations in 2014. Dietary guidelines focus on foods and dietary patterns to improve consumer understanding of more healthful food choices and to acknowledge food matrix and nutrient interactions. [62]

Also, new dietary guideline were issued in 2015 by the U.S. Dept of Health and Human Services and the U.S. Dept of Agriculture, in their *2015-2020 Dietary Guidelines for Americans*. 8th Edition, Dec. 2015 [Available at https://health.gov/dietaryguidelines/2015/ guidelines/]. The recommendation is to reduce saturated fats, and increase fat-free and low-fat (1%) dairy products which provide the same nutrients but less fatty acids (and thus, fewer calories) than higher (>2%) fat options. Fat-free or low-fat milk and yogurt, in comparison to cheese, contain less saturated fats and sodium (Na) and more potassium (K), vitamin A, and vitamin D. Additionally, the U.S. DHHS & DA recommend increasing the proportion of dairy intake that is fat-free or low-fat milk, low fat yogurt and cheese. [63]

The Dutch Committee on dietary guidelines concluded that foods rich in *cis*-unsaturated fatty acids, such as soft margarines or vegetable oils, convincingly reduce the risk of CHD compared with foods rich in saturated fatty acids such as butter and hard margarines. The results of the Randomized Controlled Trials (RCTs) showed a reduction in LDL-cholesterol when butter was replaced by soft margarine and when saturated fatty acids was replaced by unsaturated fatty acids.[64.65] Also, replacement of saturated fatty acids by polyunsaturated fatty acids (PUFA) reduced the risk of CHD.[66]

A recent review (2018) by scientists in the Department of Epidemiology and Nutrition, Harvard T.H. Chan School of Public Health, examined evidence of the association of dairy fatty acids and cardiovascular diseases. Dairy products compose about 10% of the calories in a typical American diet (fluid milk, cheese, and yogurt). Most meta-analyses report no or weak inverse association between dairy intake with cardiovascular diseases (CVDs). The group concluded that current evidence suggests

null or weak inverse association between consumption of dairy products and risk of CVDs. However, replacing dairy fat (saturated) with polyunsaturated fat (PUFA), especially from plant-based foods, may confer health benefits. More research is needed to examine health effects of different types of dairy products in diverse populations.[67]

Is fish consumption and daily fish oil supplements beneficial to health?

Many years ago epidemiological studies have indicated that Inuit populations in Greenland, whose diets contain a high level of fish oils (rich in ω-3 PUFAs), have low incidences of CVDs and rheumatoid arthritis, conditions with a significant inflammatory etiology. For many decades cod oil and other fish oils were recommended worldwide as supplements for children to boost their immune system and protect the development of their brain in infancy.[68]

Fish oils are rich sources of long-chain ω-3 PUFAs, in particularly fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which get incorporated into the cell membrane phospholipids throughout the body, especially in the heart and brain. Experimental studies and clinical trials have documented the benefits of fish oil supplementation in decreasing the incidence and progression of atherosclerosis, myocardial infarction and stroke. Possible mechanisms include reduction in triglycerides, alteration in membrane fluidity, modulation of cardiac ion channels, and anti-inflammatory, anti-thrombotic, and anti-arrhythmic effects. Current guidelines recommend the consumption of 1-2 servings of oily fish per week or daily fish oil supplements, around 1 g of omega-3 PUFAs per day, in adults. However, recent large-scale studies have failed to demonstrate any benefit of fish oil supplements on cardiovascular outcomes and mortality.[69]

A recent 2019 study by the National Institutes of Health (NIH, USA) found that supplementation with fish n-3 fatty acids [there are three names, omega−3 fatty acids, also called ω−3 fatty acids or n−3 fatty acids, are polyunsaturated fatty or PUFA] did not result in a lower incidence of major cardiovascular events or risk from cancer than placebo. The study was funded by the National Institutes of Health and others; VITAL ClinicalTrials. Government number, NCT01169259.[70]

Consumption of dietary fat and associated risk of certain cancers

In ther last decades growing epidemiological and clinical evidence points to certain dietary habits responsible for increasing cancer risk in humans. Also, some types of foods have been proved to prevent premature cancers at younger ages. In general, nutrition has been established to play an important role in certain inflammatory processes and carcinogenic mechanisms. Studies showed that diet rich in red meat, saturated fat, processed meat, salt, fried food at high temperature and low consumption of fruit, vegetables, pulses, wholegrain cereals and olive oil, contribute in increasing cancer risk. Diet is connected with certain types of cancer: colortectal, breast, prostate, gastrointestinal or gastric cancer, pacreatic, lung and ovarian cancer.[71-73]

The scientific literature has numerous epidemiological studies investigating the high dietary saturated fat consumption and its association with increased risk of certain cancers.

Some studies identified dietary total fat and certain fatty acids with increased rik of **prostate cancer** in men. A recent study (2018) enrolled 1903 men in a prospective cohort from 2000-2010 (San Antonio Biomarkers of Risk clinical validation site for the National Cancer Institute Early Detection Research Network, USA). A total of 229 men were diagnosed with Prostate cancer (Pca) by prostate biopsy. Among all nutrients, increased risk of PCa was associated with intake of dietary fat scaled by the total caloric intake, particularly saturated fatty acid (SFA).[74]

In 2015 a group of scientists reviewed evidence for the association of increased risk of prostate cancer with high intake of fat in human diet. In the last 50 years the average global supply of fat has increased by 20 g per capita per day but the results of epidemiological studies remained equivocal. The review indentified 14 cohort stuides with 37,349 cases and a total of 751,030 participants but there is no evidence of a non-linear association between fat intake and the risk for prostate cancer. The reviewers concluded that there is little evidence from published cohort studies supporting that total fat, saturated fat or unsaturated fat intake increase the risk for prostate cancer.[75]

Gastrointestinal (include esophageal, stomach **) or gastric** (lining of stomach) **cancer** are types of cancer which have been linked to increased consumption of saturated and total fats, but the results of epidemiological studies until now remained inconsistent. A meta-analysis in 2015 collected relevant observational studies with evidence regarding the dietary association with gastrointestinal cancer. Searching for articles 22 studies were included in the meta-analysis. The summary of relative risk (SRR) for gastric cancer was 1.18 (18% increase) for individuals with highest intake versus lowest intake of total fat. The meta-analysis found a positive association between saturated fat intake (SRR = 1.31) and gastric cancer risk. Also, the results showed an inverse association between polyunsaturated fat (PUFA) intake (SRR = 0.77), and vegetable fat intake (SRR = 0.55) with gastric cancer risk. The meta-analysis found no association between monounsaturated fat (MUFA) intake (SRR = 1.00). Researchers concluded that intake of total fat is potentially positively associated with increased gastric cancer risk.[76]

The consumption of fish containing PUFAs were supposed to decrease the risk for gastric cancer. In the past decades epidemiological studies that were aiming to examine the possible association between fish consumption and gastric cancer reached to inconclusive results. In 2012 a group of researchers contacted a more systematic review and meta-analysis to examine the association between fish intake and the vrisk of gastric cancer. Researchers indentified 17 epidemiological studies (for the period 1991-2009). Data were collected using standardized data forms. Relative Risks (RRs) or Odds Ratios (ORs) for the highest versus non/lowest fish consumption levels were calculated using random-effects model. The study included 5,323 cases of gastric cancer and over 130,000 non-cases. The combined results from all studies indicated that the association between high fish consumption and reduced gastric cancer risk was not statistically insignificant (RR = 0.87). Researchers concluded that current evidence for association between fish consumption and risk of gastric cancer remained unclear.[77]

Observational and prospective studies assessing the association of dietary fat and risk mof **epithelial ovarian cancer** are incosistent and yield discrepant results.
A review collected 16 independent case-control and 9 cohort studies on dietary fat intake (with approx. 900,000 subjects in total). The review pooled the Relative Risks

(RRs) with 95% confidence intervals using a random effects model. The results showed a significant increase of ovarian cancer risk with high consumptionof total, saturated-, and trans-fats, while serous ovarian cancer was more susceptible to dietary fat consumption. No evidence of positive association between dietary fat intake and ovarian cancer risk was provided by cohort studies. Reesarchers concluded that the meta-analysis findings indicate that high consumption of total, saturated and trans-fats increase ovarian cancer risk.[78]

Scientists of the project European Prospective Investigation into Cancer and Nutrition published their results in 2014 on the associtionof epithelial ovarian cancer risk and dietary fat intake. The study included 1,095 cases of epithelial ovarian canercr incident invasive and 96 cases of borderline. Cox proportional hazards regression was used to estimate hazard ratios (HRs). The results showed that there is no (increased risk) association with consumption of total fat, animal or plant fat, saturated fat, cholesterol, monounsaturated fat, or fatty fish. There was, however, an increased risk of invasive epithelial ovarian cancer in the highest category of intake (Quartile 4 vs. Quartile 1) of polyunsaturated fat (HR=1.22). Scienstis concluded that there is no support for an etiological role of total fat intake in relation to epithelial ovarian cancer risk.[79]

Lung cancer risk and dietary fat consumption is another area of research in the last decade because of the importance of this type of cancer in human morbidity and mortality worldwide. Also, the fact that polyunsaturated fatty acids (PUFAs) have been proved to have antineoplastic and anti-inflammatory properties was an interesting area of research. Scientists investigated the replacement of saturated fat with PUFAs in animal studies to indentify reduction of lung cancer. Results of epidemiologic studies were inconclusive. Two population-based cohort studies, the Shanghai Women's Health Study (SWHS) and the Shanghai Men's Health Study (SMHS) with a total of 121,970 study participants (i.e., 65,076 women and 56,894 men) were used to investigate the hypothesis. Total, saturated and monounsaturated fattyacid (MUFAs) intakes were not significantly associated with lung cancer risk. But total PUFAs intake was inversely associated with lung cancer risk [Hazard ratios, HRs, ~0.84).The ratio of n-6 PUFAs to n-3 PUFAs was inversely associated with lung cancer risk, particularly among never-smokers and

adenocarcinoma patients. This study highlighted the importance of public health impact of PUFA intakes toward intervention/prevention programs of lung cancer.[80]

Colorectal cancer (CRC) is another one of the most common cancers in both men and women worldwide. Among factors associated with CRC in the last decade were physical activity, smoking, alcohol drinking, and diet. Some epidemiological studies showed that fat intake is associated with the incidence of colorectal cancer but the results were controversial.[81,82]

Scientists in the Dpt of Food and Nutrition of Yeungnam University, Gyeongsan, South Korea in 2018 conducted a systematic search of PubMed, Web of Science, and the Cochrane library for articles related to dietary fat and the risk of colorectal cancer. A total of 18 scientific papers were identified as relevant on the subject. The pooled relative risk (RR) for the risk of CRC were 1.00 (total fat), 0.99 (saturated fatty acids), 1.08 (MUFAs), and 0.99 (PUFAs) respectively. No significant associations were found in subgroup analyses.[83]

As the number of **breast cancer** survivors increases worldwide there is growing interest among cancer specialists for the potential effect of dietary and lifestyle behaviours on overall prognosis. In the last decades randomized controlled trials (RCTs) multiple observational studies and meta-analyses have reported increased breast cancer risk with increased dietary fat intake.[84,85]

Although there are many studies on breast cancer mortality the sublect remains largely understudied. In 2017 a group of scientists searched for relevant papers on breast-cancer-specific death associated with total and saturated fat intake. The study indentified 15 prospective cohort studies. The results of meta-analyses showed that there was no difference in risk of breast-cancer-specific death for women in the highest versus lowest category of total fat intake. But, the results for breast-cancer-specific death was higher for women in the highest versus lowest category of saturated fat intake.[86]

In 2018, a French research team (Sorbonne Paris Cité Epidemiology and Statistics Research Center, and INRA, TOXALIM-Research Center in Food Toxicology, Université de Toulouse, France) investigated by a prospective study the association between lipid intakes and cancer risk, and their potential modulation by vitamin C, vitamin E, dietary fibre and fruit and vegetable intakes. The prospective study

included 44,039 participants aged ≥ 45 years from the NutriNet-Santé cohort (2009-2017). Dietary data were collected using dietary records. Multivariable Cox models were performed to characterize associations. The results found that saturated fatty acids (SFAs) intake was associated with increased overall cancer risk and breast cancer risk. Researchers concluded that SFA intake could increase overall breast cancer risks.[87]

Consumption of dietary fats and increased risk for obesity and type 2 diabetes

Another very interesting area of clinical investigation in the last decades focused on dietary fats and their association with increased risk for obesity and type 2 diabetes in obese humans. Overweight among children and young people is considered the most important public health problem of our times. Obesity is considered a condition associated with an increased risk of metabolic disorders (hypertension, dyslipidaemia, and insulin resistance), and in particular of type-2 diabetes. The treatment and prevention of overweight individuals and associated metabolic disorders present great medicalfinancial challenges worldwide for therapeutic treatments.[88]

Although in the last decades, numerous meta-analyses and systematic reviews revealed that the saturated fat intake and CVDs are not supported by the evidence, saturated fat has been shown in some cases to have an inverse relationship with obesity-related type 2 diabetes. Also, it is known that diabetes is associated with overweight or obesity and insulin resistance. Therefore, maintaining a healthy weight is a core part of clinical management of type 2 diabetes. Weight loss is also linked to improvements in glycaemia, blood pressure, and lipids and as result can delay or prevent complications, particularly cardiovascular events.[89]

In 2017 a prospective cohort analysis of 3,349 individuals in Spain, who were free of diabetes at baseline but were at high cardiovascular risk , were investigated in a special project [PREvención con DIeta MEDiterránea (PREDIMED), funded by Instituto de Salud Carlos III, Spanish Ministry of Health] study was conducted. Researchers examined the associations between total fat, subtypes of dietary fat (PUFAs, MUFAS), and food sources rich in saturated fatty acids and the incidence of

type 2 diabetes. Detailed dietary information was assessed yearly during the follow-up using a food frequency questionnaire**.** During the follow-up 266 incident cases were recorded (4.3 years). The consumption of 1 serving of butter and cheese was associated with a higher risk of diabetes, whereas whole-fat yogurt intake was associated with a lower risk. The results showed tha high intake of saturated and animal fat had a higher risk of diabetes than the lowest quartile. Scientists concluded that intake of saturated and animal fat had a higher risk of type 2 diabetes than the lowest quartile (Higher Risk, HR=2.19).[90]

Inflammatory response connencted to dietary fat intakes is an important field of research. Scientists are interested to investigate especially the role of dietary fatty acids and animal fats in inflammatory response both acutely and chronically. There were numerous RCTs (2010 to 2016) regarding the effects of dietary fat intake on levels of inflammatory markers in overweight humans. In 2017, a review collected 37 relevent articles (interventions with dairy products, vegetable oils, or nuts with effects on inflammatory markers). The most consistent inflammatory-mediating effects were found in intervention with whole diets, which suggests that many components of the diet reduce inflammation synergistically. Furthermore, interventions with weight reduction and different fatty acids did not clearly show beneficial effects on inflammatory markers. The reviewers found that most interventions studies showed either no or minor effects of dietary fatintake on inflammatory markers in overweight and obese subjects.[91]

A systematic review was published in 2013 for nutritional data (observational studies) on the relationship between the consumption of dairy fat and high-fat dairy foods, obesity, and cardiometabolic disease (diabetes, insulin resistance, impaired glucose tolerance, dyslipidemia, hypertension, and central adiposity). In 11 of 16 studies reviewed, high-fat dairy intake was inversely associated with measures of adiposity. Also, the relationship between high-fat dairy consumption and metabolic health reported either an inverse or no association. Studies investigating the connection between high-fat dairy intake and diabetes or CVDs incidence were inconsistent. Reviewers concluded that observational evidence does not support the hypothesis that dairy fat or high-fat dairy foods contribute to obesity or

cardiometabolic risk, but suggest that high-fat dairy consumption is inversely associated with obesity risk.[92]

Latest studies put emphasis on the type of saturated fats we eat can affect our risk of cardiovascular diseases

Medical experts were facing for decades two contrasting sides of the fat consumption. Fats are essential for human body energy and cell growth, protect organs and help the human body to absorb nutrients and produce important hormones. But also dietary studies showed that diets high in saturated fatty acids (SAFAs) were linked to elevated "bad" LDL cholesterol and increased risk for cardiovascular diseases (CVDs). Now, new studies have put emphasis on the types of saturated fats and chronic disease risk. Extensive epidemiological studies and Random Controlled Trials (RCTs) have raised a series of questions about what was considered established evidence. Inconsistent findings have pointed to the possibility that different types of saturated fats have different effects on cholesterol levels and the development of coronary heart disease.[93]

In 2018 a new study investigating the association of total fat and CVDs recommended caution before changing dietary guidelines. Investigators proposed that the type of saturated fats we eat can affect our risk of CVDs, according to a study published in the *International Journal of Cardiology using data from EPIC studies in England and Denmark* (on line 2018, printed 2019).[94]

The study used data from 22,050 and 53,375 participants from EPIC-Norfolk (UK) and EPIC-Denmark, respectively. Baseline SFA intakes were assessed through validated, country-specific food frequency questionnaires. Cox regression analysis was used to estimate associations between intakes of individual saturated fatty acids (AFAs) and myocardial infarction (MI) risk, for each cohort separately. The results showed that people whose diets contain relatively little palmitic and stearic acid – saturated fats composed of 16 or more carbon atoms (longer-chain saturated fats) that are typically found in meats – and eat plant-based proteins instead have decreased chances of myocardial infarction. Moreover, individuals who eat more saturated fats with 14 or fewer carbon atoms (shorter-chain saturated fats) that are

typically found in dairy products have lower risk of myocardial infarction. “…..Our analysis of the diets of large groups of individuals in two countries over time shows that the type of saturated fats we consume could affect our cardiovascular heath…..,” explained lead investigator Ivonne Sluijs (Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Netherlands).[94]

Comments on the importance of the results of this study came from Dr. Li and Dr. Sun (both at the Harvard T.H. Chan School of Public Health, Boston, MA, USA) in an accompanying editorial of the same journal . "…….The study is applaudable for its large size, prospective cohort study design, and detailed assessment of diet and lifestyle factors. In addition, it is among the few studies that specifically examined individual saturated fatty acids (SAFAs) in relation to coronary heart disease (CHD) risk and compared with different macronutrients….”. The two experienced scientists also noted a few limitations of the study and called for cautious interpretation of the overall null results for the primary saturated fatty acids. Their advice is that shifts in fat intake should align with the recommended healthy dietary patterns (for many years the main dietary proposals in western countries), which emphasize limited intakes of red and processed meat and added sugars, lower salt intake, replacement of refined grains with whole grains, and higher consumption of fruits and vegetables.[95]

The Times magazin and other popular newspapers commended to the announcement of the article in *Int J of Cardiology*. “….The type of saturated fats we consume can put us at risk of a heart attack, according to a new study suggesting that eating plant-based proteins can decrease chances of the disease instead. The study showed that people whose diets contain relatively little palmitic and stearic acid -- saturated fats composed of 16 or more carbon atoms (longer-chain saturated fats) that are typically found in meats can affect our risk of a myocardial infarction or heart attack…”.[96]

A roundtable discussion on dietary fats was inspired by a recent Presidential Advisory from the American Heart Association (2018) giving recommendations about dietary fats for prevention of atherosclerotic cardiovascular disease. The Advisory clarifies a long-held position that saturated fat should be reduced in the American diet. The Advisory adds a crucial clarification based primarily on 4 randomized

controlled diet trials, each conducted over 4 to 8 years during the 1960s extending to the 1970s. In each trial, saturated fat was reduced and replaced by vegetable oil rich in PUFA. Meta-analysis of the results showed 29% reduction in major coronary events in the participants' groups receiving PUFAs. RCTs provide the best kind of evidence. Replacing saturated fat with PUFA reduces cardiovascular events. Replacing saturated fats with carbohydrates or *trans*-fats does not reduce cardiovascular events. Cardiovascular risk reduction has been seen in RCTs with MUFAs in the context of whole food diets, mostly plant based (Mediterranean diets).[97]

Conclusions

Dietary guidelines in the past decades have almost universally advocated reducing the intake of total and saturated fat, and replacement with PUFAs and MUFAs. These recommendations and their risk for CVDs, various cancers, obesity and type 2 diabetes have been among the most vexed issues in public health, but also caused numerous controversies among medical and dietary health scientists. The medical literature is still full of articles arguing opposing positions. Prestigious health authorities in the industrial countries strongly endorsed lowering intake of SAFAs and replacement with polyunsaturated and monounsaturated fats. But some others argued that total fats were not associated with risk of CVDs, myocardial infarction, or CVDs mortality. Some will argue that the devil, as always, is in the detail, including the inherent complexity of human diets, methodological considerations, and the role of bias and confounding. But the majority of medical experts, after 50 years of research, agree that there is an established link among saturated fat diet, atherosclerosis and cardiovascular events. A clear example is the studies on the Mediterranean diet as an effective and feasible dietary practice to prevent CVDs by different mechanisms including reductions in blood pressure, body mass index, oxidative stress, low-grade inflammatory status and improvements in lipid profiles.

References

1. Hegsted DM, Ausman LM, Johnson JA, Dallal GE. Dietary fat and serum lipids: an evaluation of the experimental data. *Am J Clin Nutr* 57: 875-883, 1993.
2. Kullermd LH. Dietary fat and chronic diseasess: Epidemiologic overview. *J Am Diet Assoc* 97(7, Suppl): S9-S15, 1997.
3. Lichtenstein AH, Kennedy E, Barrier P, et al. Dietary fat consumption and health. *Nutr Reviews* 56(5):3-19, 1998.
4. WHO/FAO. Report of WHO/FAO Expert Consultation. *Diet, Nutrition and the Prevention of Chronic Diseases*. WHO publs, Geneva, 2003.
5. Hu FB, Stampfer MJ, Manson JAE, Rimm E, et al. Dietary fat intake and the risk of coronary heart disease in women. *New Engl J Med* 337:1491-1499, 1997.
6. Hu FB, Manson JE, Willett WC. Types of dietary fat and risk of coronary heart disease: A critical review. *J Am Coll Nutr 20*: 5–19, 2001.
7. Willett WC. Diet and coronary heart diseases. In: Willett WC. *Nutritional Epidemiology*. 2nd edition, Oxford, University Press, New York, 1998, pp. 414-466.
8. Aschertio A. Epidemiologic studies on dietary fats and coronary heart disease. *Am J Med* 113(9): 9-12, 2002.
9. Willett WC. Dietary fats and coronary heart disease. *J Intern Med* 272(1): 13-24, 2012.
10. Ludwig DS, Willett WC, Volek JS, Neuhouser ML. Dietary fat: From foe to friend? *Science* 362 (No 6416): 764-770, 2018.
11. Mozaffarian D, Ludwig DS. Dietary guidelines in the 21st century—a time for food. *J Am Med Assoc* (JAMA) 30**4**: 681–682, 2010.
12. McGinnis JM, Nestle M. The Surgeon General's Report on Nutrition and Health: Policy implications and implementation strategies. *Am J Clin Nutr* 49: 23–28, 1989.
13. Public Health Service, U.S. Department of Health and Human Services, *Healthy People 2000: National Health Promotion and Disease Prevention Objective*, Washington DC, 1991.
14. National Health Service (NHS), UK. Fat: the facts. [https://www.nhs.uk/live-well/eat-well/different-fats-nutrition/].
15. American Heart Association (AHA). Healthy Living. Saturated fat [https://www.heart.org/en/healthy-living/healthy-eating/eat-smart/fats/saturated-fats]. Last review 2015, Dallas-Texas, USA.
16. Appendix G-5: History of the Dietary Guidelines for Americans [https://health.gov/dietaryguidelines/dga2005/report/PDF/G5_History.pdf].
17. Hite AH, Feinman RD, Guzman GE, et al. In the face of contradictory evidence: Report of the *Dietary Guidelines for Americans* Committee. *Nutrition* 26(10):915-924, 2010.
18. Health Line. Gunnars K. Saturated Fat: Good or Bad? [https://www.healthline.com/nutrition/saturated-fat-good-or-bad].
19. Weinberg SL. The diet–heart hypothesis: a critique. *J Am Coll Cardiol* 43(5):731-733, 2004.

20. Gordon T, Kannel WB, Castelli WP, Dawber TR. Lipoproteins, cardiovascular disease, and death. The Framingham Study. *Arch Intern Med* 141(9):1128-1131, 1981.
21. Totth PP. The "Good Cholesterol". High-density lipoprotein. *Circulation* 111(5):e89-e91, 2005.
22. Gordon DJ, Probsfield JL, Neaton JD, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* 79:8-15, 1989.
23. Ascherio A, Willett WC. Health effects of trans fatty acids. *Am J Clin Nutrit* 66(4):1006S-1010S, 1997.
24. Gao S, Liu J. Association between circulating oxidized low-density lipoprotein and atherosclerotic cardiovascular disease. *Chronic Dis Tranl Med* 3(2):89-94, 2017.
25. Sugamura K, Keaney JF. Reactive oxygen species in cardiovascular disease. *Free Radic Biol Med* 51(5):978–992, 2011.
26. Higashi Y, Noma K, Yoshizumi M, Kihara Y. Endothelial function and oxidative stress in cardiovascular diseases. *Circul J* 73(3):411–418, 2009.
27. Boren J, Williams KJ, The central role of arterial retention of cholesterol-rich apolipoprotein-B-containing lipoproteins in the pathogenesis of atherosclerosis: a triumph of simplicity. *Curr Opin Lipidology* 27(5):173-483, 2016.
28. Tabas I, Garcia-Cardena G, Owens GK. Recent insights into the cellular biology of atherosclerosis. *J Cell Biol* 209(1):13-20, 2015.
29. Tripkovic A, Resanovic I, Stanimirovic J, et al. Oxidized low-density lipoprotein as a biomarker of cardiovascular diseases. *Crit Revs Clin Labor Sci* 52(2):70-85, 2015.
30. Rader DJ. Spotlight on HDL biology: new insights in metabolism, function, and translation. *Cardiovasc Res* 103:337-34, 2014.
31. Bourassa MG, Tardif JC (Eds). *Antioxidants and Cardiovascular Disease,* 2nd edn. Springer Science and Business Media, Berlin, 2006.
32. Ross R. Atherosclerosis-an inflammation disease. *N Engl J Med* 340:115-126, 1999.
33. Glass CK, Witztum JL. Atherosclerosis: the road ahead. *Cell* 104(4):503-516, 2001.
34. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 352: 1655-1695, 2005.
35. Weber C, Nopels H. Atherosclerosis: current pathogenesis and therapeutic options. *Nature Med* 17:1410-1422, 2011.
36. Steinbrecher UP, Parthasarathy S, Leake DS, Witztum JL, Steinberg D. Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. *Proc Natl Acad Sci USA* 81(12):3883-3887, 1984.
37. Quinn MT, Parthasarathy S, Fong LG, Steinberg D. Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during atherogenesis. *Proc Natl Acad Sci USA*. 84:2995–2998, 1987.

38. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 320:915–924, 1989.
39. Parthasarathy S, Quinn MT, Steinberg D. Is oxidized low density lipoprotein involved in the recruitment and retention of monocyte/macrophages in the artery wall during the initiation of atherosclerosis? *Basic Life Sci*.49:375–380, 1988.
40. Fruebis J, Parthasarathy S, Steinberg D. Evidence for a concerted reaction between lipid hydroperoxides and polypeptides. *Proc Natl Acad Sci* USA 89:10588–10592, 1992.
41. Parthasarathy S, Quinn MT, Schwenke DC, Carew TE, Steinberg D. Oxidative modification of beta-very low density lipoprotein. Potential role in monocyte recruitment and foam cell formation. *Arteriosclerosi*s 9:398–404, 1989.
42. Nagano Y, Arai H, Kita T. High density lipoprotein loses its effect to stimulate efflux of cholesterol from foam cells after oxidative modification. *Proc Natl Acad Sci USA* 88:6457–6461, 1991.
43. Navab M, Ananthramaiah GM, Reddy ST, Van Lenten BJ, Ansell BJ, Fonarow GC, et al. The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL. *J Lipid Res* 45:993–1000, 2004.
44. Bobryshev YV, Lord RS. S-100 positive cells in human arterial intima and in atherosclerotic lesions. *Cardiovasc Res* 29(5): 689-696, 1995.
45. Bobryshev YV. Monocyte recruitment and foam cell formation in atherosclerosis. *Micron* 37(3):208-222, 2006.
46. Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. *Cell* 145(3):341-355, 2011.
47. Kannel WB, Dawber TR, Kagan A, Revotskie N, Stokes J. Factors of risk in the development of coronary heart disease: six-year follow-up experience. *Ann Intern Med. 55: 33-50, 1961.*
48. Keys A. Mediterranean diet and public health: personal reflections. *Am J Clin Nutr* 61: 1321S-1323S, 1995.
49. Lawrence GD. Dietary fats and health: Dietary recommendations in the context of scientific evidence. *Advan Nutrition* 4**(**3**):** 294–302, 2013.
50. Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. *Am J Clin Nutr* 91(3):535-546, 2010.
51. de Souza RJ, Mente A, Maroleanu A, Cozma AI, Ha V, et al. Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. *Brit Med J* 351: 3978-, 2015.
52. Jakobsen MU, O'Reilly EJ, Heitmann BL, Pereira MA, Bälter K, et al. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. *Am J Clin Nutr.* 89(5):1425-143*2,* 2009.
53. Mozaffarian D, Micha R, Wallace S. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. *PLoS Med.* 7(3):e1000252, 2010.

54. Mensink RP. *Effects of Saturated Fatty Acids on Serum Lipids and Lipoproteins: A Systematic Review and Regression Analysis*. World Health Organization (WHO), Geneva; 2016.
55. Ridker PM. LDL cholesterol: controversies and future therapeutic directions. *Lancet* 384:607–617, 2014.
56. DiNicolantonio JJ. The cardiometabolic consequences of replacing saturated fats with carbohydrates or ω-6 polyunsaturated fats: do the dietary guidelines have it wrong? *Open Heart* 2014;1: e000032, 2014.
57. Hoenselaar R. Saturated fat and cardiovascular disease: the discrepancy between the scientific literature and dietary advice. *Nutrition* 28:118–123, 2012.
58. Nettleton JA, Brouwer IA, Geleijnse JM, Homstra G. Saturated fat consumption and risk of coronary heart disease and ischemic stroke: A science update. *Ann Nutr Meta*b 70(1):26-33, 2017.
59. Sacks FM, Lichtenstein AH, Wu JHY, Appel LJ , et al. Dietary fats and cardiovascular disease: A presidential advisory from the American Heart Association. *Circulation* 136(3): e1-e23, 2017.
60. Alexander DD, Bylsma LC, Vargas AJ, Cohen SS, et al. Dairy consumption and CVD: a systematic review and meta-analysis. *Br J Nutr* 115:737–750, 2016.
61. Qin LQ, Xu JY, Han SF, Zhang ZL, et al. Dairy consumption and risk of cardiovascular disease: an updated meta-analysis of prospective cohort studies. *Asia Pac J Clin Nutr* 24:90–100, 2015.
62. Nordic Council of Ministers. Integrating nutrition and physical activity. Copenhagen: Nordic Council of Ministers; 2014. Nordic Nutrition Recommendations 2012, 2014
63. US Department of Health and Human -Services and US Department of Agriculture, 2015-2020 Dietary Guidelines for Americans. 8th Edition. Washington DC, US Department of Health and Human Services; 2015. [https://health.gov/dietaryguidelines/2015/guidelines/chapter-1/a-closer-look-inside-healthy-eating-patterns/].
64. Zock PL, Katan MB. Butter, margarine and serum lipoproteins. *Atherosclerosis* 131 (1):7-16, 1997.
65. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr* 77(5): 1146-155, 2003.
66. Kromhout D, Spaaij CJ, de Goede J, Weggemans RM. The 2015 Dutch food-based dietary guidelines. *Eur J Clin Nutr* 70:869–878, 2016.
67. Yu E, Hu FB. Dairy products, dairy fatty acids, and the prevention of cardiometabolic disease: a review of recent evidence. *Curr Atheroscler Rep* 20(5):24, 2018.
68. Dyerberg J. Epidemiology of n-3 fatty acids and disease. In: De Caterina R, Endres S, Kristensen SD, Schmidt EB (Eds). *Fatty Acids and Vascular Disease*. Springer-Verlag, London, 1993.
69. Goel A, Pothineni NV, Singhal M , Paydak H, et al. Fish, fish oils and cardioprotection: Promise or fish Ttle? *Int J Mol Sci* 19(12): pii: E3703, 2018.

70. Manson J.E., Cook N.R., Lee I.M., Christen W., Bassuk S.S., Mora S., Gibson H., Albert C.M., Gordon D., Copeland T., et al. Marine n−3 fatty acids and prevention of cardiovascular disease and cancer. *N Engl J Med* 380 (1):23-32, 2018.
71. Bouvard V, Loomis D , Guyton KZ , Grosse Y , Ghissassi FE, et al. …and IARC on Cancer Monograph Working group. Carcinogenicity of consumption of red and processed meat. *Lancet Oncol*. 16(16):1599-600, 2015.
72. Sugimura T, Wakabayashi K, Nakagama H, Nagao M. Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish. *Cancer Sci* 95(4):290-299, 2004.
73. Pettersson A, Kasperzyk JL, Kenfield SA, Richman EL, et al. Milk and dairy consumption among men with prostate cancer and risk of metastases and prostate cancer death. *Cancer Epidemiol Biomarkers Prev* 21(3):428-436, 2012.
74. Liss MA, Al-Bayati O, Gelfond J, Goros M, et al. Higher baseline dietary fat and fatty acid intake is associated with increased risk of incident prostate cancer in the SABOR study. *Prostate Cancer Prostatic Dis*. 2018 Nov 1. doi: 10.1038/s41391-018-0105-2.
75. Xu C, Han FF , Zeng XT, Liu TZ, et al. Fat intakeis not linked to prostate cancer: A systematic review and dose-response meta-analysis. *PLoS One* 17; 10(7): e0131747, 2015.
76. Han J, Jiang Y, Liu X, Meng Q, Xi Q, et al. Dietary fat intake and riskof gastric cancer: A meta-analysis of observational studies. *PLoS One* 10(9):e0138580, 2015.
77. Wu S , Liang J, Zhang L, Zhu X, Liu X, Miao D.. Fish consumption and the risk of gastric cancer: systematic review and meta-analysis. *BMC Cancer*. 20: 11:26, 2011.
78. Qiu W, Lu H, Qi L, Wang X. Dietary fat intake and ovarian cancer risk: a meta-analysis of epidemiological studies. *Oncotarget* 7(24):37390-37406, 2016.
79. Merritt MA, Riboli E, Weiderpass E , Tsilidis KK, Overvad K, et al. Dietary fat intake and risk of epithelial ovarian cancer in the European Prospective Investigation into Cancer and Nutrition. *Cancer Epidemiol* 38(5):528-537, 2014.
80. Luu HN, Cai H, Murff HJ, Xiang YB, et al. A prospective study of dietary polyunsaturated fatty acids intake and lung cancer risk. *Int J Cancer* 143(9):2225-2237, 2018.
81. Reddy B.S. Types and amount of dietary fat and colon cancer **ris**k: Prevention by omega-3 fatty acid-rich diets. *Environ Health Prev Med* 7:95–102, 2002.
82. Haggar F.A., Boushey R.P. Colorectal cancer epidemiology: Incidence, mortality, survival, and risk factors. *Clin Colon Rectal Surg* 22:191–197, 2009.
83. Kim M, Park K. Dietary fat intake and Risk of colorectal cancer: A systematic review and meta-analysis of prospective studies. *Nutrients* 10(12): pii:E 1963, 2018.
84. Rohan T E, Negassa A, Caan B, Chlebowski RT, et al. Low-fat dietary pattern and risk of benign proliferative breast disease: A randomized, controlled dietary modification trial. *Cancer Prev Res* **1**(4):275–284, 2008.
85. Boyd N, Stone F, Vogt J. Dietary fat and breast cancer risk revisited: A meta-analysis of the published literature. *Brit J Cancer* 89:1672–1685, 2003.

86. Brennan SF, Woodside JV, Lunny PM, Cardwell CR, Cantwell MM. Dietary fat and breast cancer mortality: A systematic review and meta-analysis. *Crit Rev Food Sci Nutr* 57(10):1999-2008, 2017.
87. Sellem L, Srour B, Guéraud F, Pierre F, et al. Saturated, mono- and polyunsaturated fatty acid intake and cancer risk: results from the French prospective cohort NutriNet-Santé. *Europ J Nutr* 2018, pp.1-13, Apr 3, doi: 10.1007/s00394-018-1682-5. [Epub ahead of print].
88. Lawlor DA, Chaturvedi N. Treatment and prevention of obesity—are there critical periods for intervention? *Int J Epidemiol* 35(1):3-9, 2006.
89. Forouhi N, Misra A, Mohan V, Taylor R, Yancy W. Dietary and nutritional approaches for prevention and management of type 2 diabetes. *Brit Med J* 361:k2234-, 2018.
90. Guasch-Ferré M, Becerra-Tomás N, Ruiz-Canela M, Corella D, et al. Total and subtypes of dietary fat intake and risk of type 2 diabetes mellitus in the Prevención con Dieta Mediterránea (PREDIMED) study. *Am J Clin Nutr* 105(3):723-735, 2017.
91. Telle-Hansen VH, Christensen JJ, Ulven SM, Holven KB. Does dietary fat affect inflammatory markers in overweight and obese individuals?-A review of randomized controlled trials from 2010 to 2016. *Genes Nutr* 12:26-, 2017.
92. Kratz M, Baars T, Guyenet S. The relationship between high-fat dairy consumption and obesity, cardiovascular, and metabolic disease**.** *Eur J Nutr* 52(1):1-24, 2013.
93. Forouhi NG, Krauss RM, Taubes G, Willett W. Dietary fat and cardiometabolic health: evidence, controversies, and consensus for guidance. *Brit Med J* 2018, 361:k2139, 2018.
94. Jaike Praagman, Linda E.T. Vissers , Angela A. Mulligan, Anne Sofie Dam Laursen et al. Consumption of individual saturated fatty acids and the risk of myocardial infarction in a UK and a Danish cohort. *Int J Cardiology* 279:18-26, 2019.
95. Li J, Sun Q. Consumption of saturated fatty acids and coronary heart disease risk. Editorial. *Int J Cardiol* 279:27-28, 15/3/2019.
96. Timesnownews. Health.These saturated fats can put your heart at risk, 30 January 2019, [https://www.timesnownews.com/health/article/these-saturated-fats-can-put-your-heart-at-risk/356796].
97. Severson T, Kris-Etherton PM, Robinson JG, Guyton JR. Roundtable discussion: Dietary fats in prevention of atherosclerotic cardiovascular disease. *J Clin Lipidology* 12(3):574-582, 2018.