

Inflammation, obesity and comorbidities: the role of diet

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Abstract

The adipocyte metabolism has been shown to change during the fat enlargement process associated to obesity. Several procoagulant proteins such as plasminogen activator inhibitor type 1, tissue factor or factor VII and also inducible nitric oxide synthase show higher expression in adipose tissue of obese people in comparison to lean. This overexpression could explain at least a part of the atherogenic and cardiovascular risk associated with obesity.

In addition to cytokine secretion, many other features have been observed to be common to adipocyte and monocyte/macrophage lines: for example, phagocytic and microbicidal activities, and possibly a cellular plasticity of adipose precursors.

Overweight and obesity are associated with an increased risk of such metabolic abnormalities as dyslipidemia, hypertension or type 2 diabetes mellitus and cardiovascular diseases, common features of the metabolic syndrome. Initially, insulin resistance or hyperinsulinemia was suggested as the origin of these abnormalities. More recent studies indicate that adipokynes have an important role in obesity-associated metabolic complications, and suggest that chronically elevated local or systemic concentrations of adipokynes contribute to the development of complications associated with obesity and metabolic syndrome.

Considering all the evidence relating to diet and inflammation, the best diet for protecting against the metabolic derangements associated with obesity and metabolic syndrome would be high in fibre-rich cereals, fruit, vegetables, fish, virgin olive oil and nuts; moderate in wine; and low in meat, processed meat foods and trans-fatty acids.

Keywords
Inflammation
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Obesity is considered to be an epidemic of the 21st century. Its prevalence is increasing and its importance to public health is considerable because it is associated with a higher risk of type 2 diabetes, coronary heart disease and certain cancers, and a shorter life expectancy. Although the mechanism underlying the development of obesity and its comorbidities are not well established, it has been recognised that these clusters of disorders are associated to chronic mild inflammation in which the metabolism of fat tissue plays an important role^{1,2}.

In recent decades, fat was considered to have a passive role in the development of obesity and was simply stored in the adipocytes. More recently, the adipocyte metabolism has been shown to change during the fat enlargement process associated to obesity. Within these mechanisms, a greater release of free fatty acids (FFA) and glycerol from adipocytes has been described in obese people than in lean individuals, probably promoting insulin resistance and type 2 diabetes through its specific

actions that block insulin signal transduction³. Several procoagulant proteins such as plasminogen activator inhibitor type 1, tissue factor or factor VII and also inducible nitric oxide synthase (iNOS) show higher expression in adipose tissue of obese people in comparison to lean people^{4–7}. This overexpression could explain at least a part of the atherogenic and cardiovascular risk associated obesity. White adipose tissue is also specially characterised by the production and secretion of more than 50 adipokines, such as leptin, tumour necrosis factor (TNF) or interleukin-6 (IL6) with characterised local and systemic pro-inflammatory effects^{2,8,9}. Several of these molecules are over released into the circulation in obese subjects leading to a low grade of chronic systemic inflammation. Recent data indicate that white adipose tissue in obesity is infiltrated by macrophages, where they apparently function to scavenge moribund adipocytes^{10,11}. Nevertheless, infiltrated macrophages could be the major source of pro-inflammatory cytokine production

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in obese adipose tissue. Interestingly, weight loss is associated with an improvement in the circulating pro-inflammatory profile and a reduction in the macrophage infiltration of white adipose tissue¹².

Although knowledge about the interplay of inflammation and obesity is still being gained, the available evidence indicates that dietary intervention will become an integral part of future approaches to prevent and treat obesity, the metabolic derangements associated with obesity, the metabolic syndrome and ultimately the cardiovascular disease associated with the atherosclerotic processes. For this reason, in this review we analyse the importance of a healthy diet if these metabolic alterations are to be prevented.

Adipose tissue and inflammation

Since the discovery of leptin in 1994, the perspective on the physiological role of white adipose tissue has dramatically changed and it is now considered to be a major endocrine organ with an important role in the regulation of energy intake and metabolism. Indeed, as explained above, adipose tissue secretes a multiplicity of factors commonly termed adipokines with different protein structures and functions: for example, cytokines or related-proteins (leptin, TNF, IL-6, neurotrophins), chemo-attractant proteins (monocyte chemoattractant protein-1 (MCP-1)), proteins of the complement system (adipsin), proteins involved in the regulation of blood pressure, vascular haemostasis or angiogenesis (angiotensinogen, plasminogen activator inhibitor-1 (PAI-1), vascular endothelial growth factor (VEGF)), and molecules involved in the glucose and lipid metabolism (adiponectin, resistin, visfatin, cholesteryl ester transfer protein (CETP)). The functional homology between adipocytes and cells from the immune system reinforces the previously observed relationship between adipose tissue and immunity¹³. In addition to cytokine secretion, many other features have been observed to be common to adipocyte and monocyte/macrophage lines: for example, phagocytic and microbicidal activities¹⁴, and possibly a cellular plasticity of adipose precursors¹⁵.

The theoretical role of adipose tissue in the development of local or systemic inflammatory status, especially in obesity, could be complicated by its heterogeneity at the cellular level. Mature adipocytes are not more than half of the total cell content of white fat. The remaining cell components contain pre-adipocytes, fibroblast, endothelial cells and macrophages¹⁰. It is well-documented that during adipose tissue growth, for example in obesity, there is an increase in the size and number of mature adipocytes differentiated from progenitor cells like preadipocytes present in the stroma-vascular fraction. However, this phenomenon seems to be reversible during adult life and the relation between the number of mature

adipocytes and preadipocytes can change¹⁶. Moreover, infiltration of macrophages from peripheral blood has been reported during fat mass expansion in obesity^{10,17}. Although the molecular mechanisms responsible for these microphagic infiltrations have not yet been elucidated, some adipokines such as MCP-1 and leptin can favour the diapedesis of macrophages from circulation to adipose tissue¹⁸. Macrophages that infiltrate adipose tissue seem to be responsible for almost the total amount of TNF α and a significant part of IL-6 produced by this tissue¹⁰. Factors secreted by infiltrated macrophages inhibit the differentiation of preadipocytes into mature adipocytes probably because the extracellular matrix is remodelled¹⁹. So, the real capacity of adipose tissue to produce and release inflammatory proteins may be related to its cellular composition, and depends on the degree of obesity.

Obesity, metabolic syndrome and inflammation

Overweight and obesity are associated with an increased risk of such metabolic abnormalities as dyslipidemia, hypertension or type 2 diabetes mellitus and cardiovascular diseases – common features of the metabolic syndrome. Initially, insulin resistance or hyperinsulinemia was suggested as the origin of these abnormalities²⁰. Nowadays, more recent studies indicate that adipokines have an important role in obesity-associated metabolic complications, and suggest that chronically elevated local or systemic concentrations of adipokines contribute to the development of complications associated with obesity and metabolic syndrome.

TNF α is a potent cytokine that induces the production of IL-6⁹, which is the major determinant of the acute-phase response^{16,17} and is also produced by adipose tissue. Plasma levels of TNF are persistently elevated in obese or type 2 diabetic patients and among patients at increased risk of recurrent coronary events^{9,21}. Its role favoring insulin resistance is mediated by the increase in adipocyte lipolysis²² and through its role on insulin receptor substrate-1 phosphorylation and modulating glucose transporter type 4 (GLUT4) activity²³. Because TNF is the first step in the inflammatory cascade, it is not surprising that plasma levels of IL-6 are high in obese or diabetic subjects⁸. Like TNF, IL-6 concentrations correlate with insulin resistance^{24,25} and increase lipolysis and fat oxidation in humans²⁵. Therefore, it is not surprising that elevated levels of IL-6 have been associated with an increased risk of type 2 diabetes²⁶ and future myocardial infarction in healthy men²⁷.

One of the most important effects of IL-6 is that it controls hepatic C-reactive protein (CRP) production, one of the most important inflammatory markers in humans, also positively associated with degree of obesity, fasting glucose and insulin, blood pressure and lipid profile²⁶.

CRP induces the synthesis of other cytokines, cell adhesion molecules such as vascular cell adhesion molecule (VCAM) or intercellular adhesion molecule (ICAM), and tissue factor, and provides a link between inflammation and atherosclerosis²⁸. Elevated CRP levels have been described as an independent predictor of risk of type 2 diabetes, myocardial infarction, stroke and peripheral arterial disease even in healthy subjects^{26,29}. Other inflammatory markers, such as blood cell count, fibrinogen, ESR, inflammation-associated haemostatic variables and low serum albumin, have also been associated with obesity and its metabolic derangements³⁰.

In obesity, the decrease in production and expression of adiponectin, a molecule with an anti-inflammatory action, exacerbates the important role of inflammation in the obese state^{31,32}. Plasma adiponectin concentrations are lower in patients who have type 2 diabetes or coronary heart disease than in controls, even when body mass index or age are matched^{33,34}. Adiponectin may affect insulin sensitivity because it acts on muscle fatty acid oxidation and hormone-sensitivity lipase^{2,35}. The stimulation of nitric oxide production and the reduction in the expression of adhesion molecules in endothelial cells explains the anti-hypertensive and anti-atherogenic properties of adiponectin.

Several clinical studies have demonstrated that it is abdominal fat distribution, not the amount of fat deposition, which is better associated with multiple metabolic risk factors. In fact, epidemiological studies have suggested that abdominal fat distribution is a significant predictor for coronary artery disease independent of body mass index³⁶. Quantitative and qualitative changes in serum lipids and lipoproteins such as small dense low-density lipoproteins (LDL), hypertriglyceridemia or the hyperinsulinemic state are more closely related to visceral than subcutaneous fat. Furthermore, differences in adipokine expression and production between visceral and subcutaneous fat depots have also been reported. For example, TNF is significantly overproduced in visceral adipose tissue. Because this cytokine is a strong inhibitor of adiponectin promoter activity³⁷, it may explain the negative correlation observed between visceral adiposity and adiponectin levels. It has been speculated that approximately 20% of all genes in subcutaneous adipose tissue encode secretory proteins, and increase visceral fat by about 30%³⁸. Recently, a visceral-fat-specific adipocytokine, visfatin, was described³⁹. Plasma visfatin levels strongly correlated with the amount of visceral adipose tissue estimated by computed tomography³⁹. Higher visfatin plasma levels have also been observed in patients with type 2 diabetes mellitus⁴⁰ or in those with rheumatoid arthritis⁴¹. Although its physiological significance is not fully understood, visfatin mimics the effect of insulin and has a potent activity on adipogenesis.

Although knowledge about the relationship between inflammation and obesity is increasing, whether the

inflammatory state is the cause or the consequence of obesity remains to be elucidated. It has been recently proposed that local hypoxia during white adipose tissue mass expansion is the first sign that the production of inflammatory adipokines and related substances is being stimulated. Hypertrophic adipocyte expansion during the development of obesity is associated with low oxygen tension and metabolic adaptation to hypoxia^{42,43}. Within the adaptive mechanism, the overexpression of hypoxia-inducible genes such as VEGF, erythropoietin (EPO) or hypoxia-inducible factor (HIF-1) has been described in the adipose tissue of human obese subjects. This overexpression decreases after weight loss¹². It has been suggested that these molecules may stimulate the release of inflammatory cytokines and other inflammatory substances^{44,45}.

Inflammation and weight loss

Evidence for a connection between obesity and inflammation has also been found in clinical weight loss studies. Since adipose tissue from obese patients overexpresses adipokines, it seems reasonable that a reduction in adipose mass after weight loss will restore inflammatory levels because adipose tissue expression and secretion will decrease, and insulin resistance and other cardiovascular risk factors will improve. Several studies of weight loss due to dietary intervention or exercise, report a decrease in circulating levels of several peripheral inflammatory markers^{46,47}. Weight loss due to bariatric surgery or fat removal by liposuction is also associated with an improvement in the inflammatory state⁴⁸. However, there are some discrepancies in terms of adipose tissue adipokine expression after weight loss. While some studies describe a decrease in TNF or IL-6 adipose tissue expression, others have found an increase or no changes^{49,50}. As has been previously suggested by our group, these discrepancies may be due to the sign of the energy balance at the moment of the study⁵¹. While most of the studies describing a decrease in adipokine expression were made a long time after weight loss, and the weight loss had stabilised, in our study we evaluated subcutaneous adipose tissue adipokine expression in negative energy balance conditions when lipid metabolism is stimulated. Because several pro-inflammatory cytokines have lipolytic activity, our results were not surprising.

The restoration of inflammatory levels could also be explained by the decrease in the infiltration of macrophage adipose tissue after weight loss¹⁰ and changes in its distribution¹². Although the mechanisms responsible for macrophage recruitment in white adipose tissue are unknown, such chemotactic factors as MCP-1 showed decreased expression after weight loss. Tissue and cellular hypoxia is a well-known cause of macrophage

attraction and retention, particularly in tumours and atherosclerotic plaque⁵². The overexpression of HIF-1 α in morbid obesity and its lower expression after weight loss also indicate that local white adipose tissue hypoxia contributes to macrophage movement, as suggested⁵³.

The role of diet in inflammatory modulation

Inflammation is a normal response to tissue injury or infection. However, several studies suggest that when a source of chronic inflammation exposure persists, a low-grade inflammation response develops, thus increasing the risk of obesity, insulin resistance, diabetes, metabolic syndrome, cardiovascular disease or cancer^{26,54–56}. Dietary habits are probably one of the key determinants of the balance that influences the overall inflammatory process in chronic conditions. Genetic polymorphisms at multiple sites may alter the ability of bioactive food components to influence the overall process by modulating pro- and/or anti-inflammatory mediators. The modulation of this inflammatory response by diet would be fundamental if such metabolic derangements associated with obesity such as insulin resistance, type 2 diabetes, hypertension, atherogenic dyslipidaemia and metabolic syndrome are to be prevented and treated.

The inflammatory process is initiated by the synthesis and secretion of pro-inflammatory cytokines in response to an inflammatory insult (for example, a non-healthy diet). The increased production of cytokines and the subsequent increase in reactive oxygen and nitrogen species are recognised hallmarks of inflammation. This process is regulated by a negative feedback mechanism and is closely followed by the secretion of anti-inflammatory cytokines to reduce the accumulation of reactive species. The cellular anti-oxidant defence system is also activated to limit the development of chronic inflammation in which the risk of metabolic syndrome and subsequently atherosclerosis is much higher than normal.

The binding of pro-inflammatory cytokines to their receptors triggers the mitogen-activated protein kinase (MAPK) pathway that ultimately results in the activation of two redox-sensitive transcription factors: nuclear factor kappa B (NF κ B) and the c-Jun part of activating protein-1 (AP-1). These transcription factors activate the expression of a wide variety of genes including cytokines, chemokines, adhesion molecules, and inducible effector enzymes such as iNOS and cyclooxygenase-2 (COX-2).

There is evidence to suggest that such dietary components as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), butyrate, curcumin, resveratrol, lutein, quercetin and oleuropein can influence the inflammatory process at various sites and thus modulate the balance within the process. For example, the production of IL-6 or IL-1 β in THP-1 cell lines was reduced after incubation with DHA or ALA⁵⁷. Several phenolic compounds have also

been identified as anti-inflammatory compounds; however, phenolic compounds from extra virgin olive oil have been shown to have different anti-inflammatory effects in human whole blood cultures⁵⁸. Likewise, a number of dietary components, including resveratrol in red grapes, butyrate generated through the microbial metabolism of dietary fibre in colon, and curcumin in curry spice, have been reported to suppress the TNF- α -induced activation of NF- κ B and COX-2 expression *in vitro* and *in vivo*. The mechanisms by which these bioactive components influence inflammation are not known with any certainty, but may involve transport, activation or inactivation processes. Resveratrol has been shown to inhibit the translocation of NF- κ B from the cytoplasm to the nucleus by modulating the I κ B kinase (IKK) activity in various cell types (for example, myeloid, lymphoid and epithelial cells)⁵⁹.

Cereals, fibre and glycaemic response

Fasting and postprandial hyperglycaemia are very frequently observed in obesity. They can reduce the availability of nitric oxide⁶⁰, and increase the free radical production, which also activates inflammation by modulating protein kinase C (PKC) and NF- κ B function⁶¹. Refined starches and sugars cause a rapid increase in blood glucose and insulin levels, and a subsequent decrease in glycaemia, which leads to hunger and a decrease in fat oxidation. For this mechanism, the intake of foods with a high glycaemic index is associated with hyperglycaemia being a major stimulus for inflammation.

A cross-sectional study performed in 720 diabetic patients between 40 and 75 years old from the Health Professionals' Follow-up study supports this hypothesis. In this study, subjects with diets low in glycemic load and high in dietary fibre have higher plasma levels of adiponectin, a molecule with anti-inflammatory properties that protects against diabetes and atherosclerosis⁶². Also recently, in two more cross-sectional studies, plasma levels of CRP have been observed to be negatively related to the total fibre intake in 524 healthy adult subjects⁶³ and in patients with diabetes, hypertension or obesity from the NHANES study⁶⁴. These associations were maintained when variables were adjusted for other confounding dietary and non-dietary factors. Some interventional studies support the hypothesis that fibre can improve inflammation⁴⁶. In fact, recent epidemiologic studies suggest that a high consumption of dietary fibre or cereals decreases the risk of diabetes or atherosclerosis and is associated with a decrease in weight gain^{65–67}.

Fish and omega-3 fatty acids

One of the most commonly studied dietary compounds is omega-3 (ω 3) fatty acids. ω 3 fatty acids (especially EPA

and DHA) decrease the arachidonic acid content of cell membranes, which results in the synthesis of eicosanoids that have fewer inflammatory properties than those synthesised from omega-6 ($\omega 6$) fatty acids. Several studies support the idea that $\omega 3$ may play a major role in modulating inflammation associated with diseases and situations.

Several cross-sectional studies support the idea that $\omega 3$ fatty acids have anti-inflammatory properties. In a sample of individuals from the Nurses' Health Study II and the Health Professionals Follow-up Study, Pischon *et al.*⁶⁸ observed that a high intake of $\omega 3$ (EPA and DHA) and a low intake of $\omega 6$ (linoleic acid) was associated with lower levels of soluble TNF receptors 1 and 2, indicating a low level of inflammation. This anti-inflammatory effect of EPA and DHA has also been observed in other cross-sectional studies performed in healthy individuals from the Nurses' Health Study⁶⁹ and the Attica Study⁷⁰ or in patients with established coronary artery disease⁷¹. Some interventional studies also support the anti-inflammatory effect of the $\omega 3$ in humans. For example, dietary fish oil has been observed to decrease CRP and IL-6 in post-menopausal women⁷². The anti-inflammatory effects of the α -linolenic acid have also been observed in several interventional studies^{73–75}.

Trans- and saturated fatty acids

Some observational and interventional studies in humans suggest that diets rich in trans- or saturated fatty acids are more closely associated with inflammation than vegetarian diets, which usually contain fewer of these compounds. For example, subjects consuming a diet rich in trans-fatty acids showed higher levels of CRP, IL-6, sTNFR2, E-selectin, sICAM-1 and sVCAM-1 than those in the low quintile of trans-fatty acid consumption⁷⁶. In a randomised cross-over study, CRP and IL-6 increased when 9% of fatty acids were replaced with trans-fatty acid or stearic acid⁷⁷. However, some studies show that intake of trans-fatty acid has no effect on inflammatory parameters. CRP levels increase with trans-fatty acid substitution in a high fat diet in healthy subjects, whereas 6% substitution of trans-fatty acids in a standard fat diet (30% fat) showed no effects on CRP in moderately hypercholesterolemic subjects although TNF and IL-6 levels increased⁷⁸.

Virgin olive oil and nuts

Other important foods with anti-inflammatory properties are virgin olive oil and nuts. Virgin olive oil is a rich source of MUFA, and retains all the lipophilic components of the olive fruit, especially the phenolic compounds with strong anti-oxidant and anti-inflammatory properties^{79,80}.

The administration of olive oil with a high phenolic content has been shown to protect against inflammation⁸¹. In addition, phenolic compounds derived from extra virgin oil were recently shown to decrease the production of inflammatory mediators in human whole-blood cultures⁵⁸ and to inhibit endothelial adhesion molecule expression *in vitro*⁸². These processes may at least partly explain why the Mediterranean-type pattern of food intake is thought to protect against atherosclerosis.

Nuts are rich in unsaturated fatty acids⁸³ and, besides having favourable fatty acid profiles, they are a good source of bioactive compounds with potential benefits against metabolic syndrome and cardiovascular disease. Nuts are rich in fibre⁸⁴, phenolic compounds and other anti-inflammatory and anti-oxidant molecules⁸⁵. Also, nuts contain sizeable amounts of L-arginine, a precursor of the endogenous vasodilator nitric oxide^{86,87}. Walnuts differ from all other nuts in that they have a high α -linolenic acid content^{88,89} which also has anti-inflammatory properties. A high consumption of nuts and seeds has recently been shown to be associated with lower concentrations of inflammatory markers in the MESA atherosclerosis study⁹⁰. Also, clinical trials of nut consumption have reported decreases in inflammatory markers⁷⁵ and improvements in the endothelial function⁹¹. This mechanism has been proposed as a potential explanation for the established cardio-protective effect of frequent nut intake⁹².

Fruit and vegetables

An increase in the consumption of fruit and vegetables has also been advocated for the prevention of strokes and other cardiovascular events⁹³. Fruit and vegetables are very rich in anti-oxidants and other anti-inflammatory phytochemicals. Plasma CRP and homocysteine concentrations have been related to frequent fruit and vegetable intake in the Massachusetts Hispanic Elders Cross-sectional Study after adjusting for confounding variables. Moreover, a significant relationship between vitamin C or β -carotene and CRP levels has been observed in healthy subjects⁹⁴. Several interventional studies have demonstrated an anti-inflammatory effect of fruit and vegetable or some of their individual compounds^{95–97}.

Wine and alcohol

Both epidemiological and experimental studies have proposed that moderate consumption of red wine can contribute to promote health and prevent disease probably because of their content of resveratrol or other polyphenolic compounds and flavonoids, and their effect

on the modulation of inflammation. A 4-week consumption of 30 g day⁻¹ of red wine led to a significant decrease in the serum concentration of CRP, VCAM and ICAM in healthy adult men⁹⁸. Lower levels of circulating CRP have also been observed associated to a moderate consumption of red wine in a cross-sectional study performed on more than 2800 men and women of the PRINCE study⁹⁹ or in subjects from the NHANES III study¹⁰⁰. Some of the mechanisms that explain the biological activities of resveratrol involving downregulation of the inflammatory response are the inhibition of synthesis and release of pro-inflammatory cytokines, modification of eicosanoid synthesis, inhibition of activated immune cells, or inhibition of iNOS and COX-2 via the inhibitory effects on NFκB or the activator protein-1 (AP-1)¹⁰¹. The effect on NFκB may explain the suppression of the ex vivo production of TNF, IL-1 and IL-6 by mononuclear blood cells observed after the incubation with trans-resveratrol¹⁰². Flavonoids are another of the bioactive compounds of red wine with anti-oxidant and anti-inflammatory properties. Studies performed in cell cultures show that the expression of endothelial adhesion molecules is inhibited, which leads to a reduction in atherosclerotic lesion formation¹⁰³.

Moderate alcohol intake has also been associated with beneficial effects on markers of inflammation, independently of the bioactive components of grapes¹⁰⁴. This effect, however, is controversial¹⁰⁵.

Healthy dietary pattern

Considering all the evidence relating to diet and inflammation, the best diet for protecting against the metabolic derangements associated with obesity and metabolic syndrome would be high in fibre-rich cereals, fruit, vegetables, fish, virgin olive oil and nuts; moderate in wine; and low in meat, processed meat foods and trans-fatty acids. In fact, in a cross-sectional study from the Nurse's Health Study I Cohort, a prudent pattern was associated to low levels of some inflammatory and endothelial function markers in comparison to the results from a Western dietary pattern⁶⁹. Furthermore, the traditional Mediterranean-type diet has been associated to a low risk of cardiovascular disease¹⁰⁶ probably mediated by inflammation related factors^{107,108} although some of the results are controversial¹⁰⁹. Some unpublished results from our group show lower serum concentrations of inflammatory markers, especially those related to endothelial function, in those subjects who consume more fruit, cereals, olive oil and nuts. However, controlled trials analysing the effects of Mediterranean-type diets on inflammation parameters are required if the cross-talk between diet compounds and the modulation of inflammatory state associated with obesity and metabolic syndrome is to be elucidated.

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