REVIEW-THEMED ISSUE

Mediterranean diet, dietary polyphenols and low grade inflammation: results from the MOLI-SANI study

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Low grade inflammation is characterized by raised concentrations of inflammatory markers in the absence of any overt symptoms and is recognized as a risk factor for a number of chronic diseases including cancer, cardiovascular, cerebrovascular and neurodegenerative diseases. Many studies suggest that low grade inflammation is mitigated by health promoting behaviours such as healthy eating patterns, physical activity, body weight maintenance and tobacco cessation. To date, large scale studies were mainly focused on circulating markers and little evidence is available on cellular biomarkers. The MOLI-SANI study is a prospective cohort study that has recruited 24 325 men and women aged ≥35 years from the general population of the Molise Region, a Southern Italian area, with the purpose of investigating genetic and environmental risk/protection factors for cardiovascular and cerebrovascular disease and cancer. Within this cohort, a composite score of low grade inflammation based on the use of plasmatic (C-reactive protein) and cellular (leukocyte and platelet counts and granulocyte : lymphocyte ratio) biomarkers has been proposed and validated. This score accounts for all possible synergistic effects of such inflammatory markers, thus overcoming any potential bias linked to the multi-collinearity of these variables. Of notice, the MOLI-SANI study was the first to address the relationship between the traditional Mediterranean diet and platelet and leucocyte counts as emerging cellular biomarkers of low grade inflammation. The present review paper will discuss the main findings derived from the MOLI-SANI study on the association of low grade inflammation with a Mediterranean eating pattern, with a particular emphasis on the associated dietary polyphenols.

Introduction

To date, a number of circulating (e.g. C-reactive protein, fibrinogen) or cellular biomarkers (e.g. white blood cell and platelet counts) have been used to assess a low grade inflammation [1, 2], a condition not yet consistently defined or measured.

This subclinical disorder has been recognized as a risk factor for a number of chronic diseases including cancer, cardiovascular and neurodegenerative disease [3–6] and has been found to increase the risk of insulin resistance and type 2 diabetes [7].

Pioneering large scale studies were focused on circulating fibrinogen, C-reactive protein (CRP) and white blood cell (WBC) counts as reliable inflammatory biomarkers mostly in relation to cardiovascular events [6, 8–11]. Lately, a pro-inflammatory action of blood platelets has also been proposed [12, 13], whereas the neutrophil : lymphocyte ratio better expresses an early inflammatory cellular response [14].

More recently, the MOLI-SANI study has proposed and validated a composite score of low grade inflammation based on the use of plasmatic and cellular biomarkers relatively easy to measure also at the epidemiological level. A significant

inverse association of this composite low grade inflammation score with dietary polyphenol intake has been observed in the MOLI-SANI cohort, with higher dietary polyphenol intake being related to decreased low grade inflammation [15].

Many studies suggest that low grade inflammation is mitigated by healthy dietary habits that are associated with lower circulating concentrations of inflammatory markers [16] whilst Western type or meat-based patterns are positively associated with low grade inflammation [1]. Among the components of a healthy diet, whole grains, vegetables and fruits and fish are all associated with lower inflammation [17] and a limited number of observational studies have suggested a pro-inflammatory action of diets rich in saturated fatty acids (SFA) or trans-MUFA [17].

Among other health-related behaviours, the association of adiposity with inflammation is largely documented [18]. While smoking was found independently involved in the pathophysiology of low grade inflammation [19], physical activity was recognized as an effective mean to control low grade systemic inflammation [20].

The purpose of this review paper is to provide an overview of the relationship between low grade inflammation and dietary behaviours as observed within the cohort of the MOLI-SANI study, a large Mediterranean population-based cohort study.

The MOLI-SANI study

The MOLI-SANI study is a prospective cohort study that has recruited 24 325 men and women aged ≥35 years from the general population of the Molise Region, a Southern Italian area, with the purpose of investigating genetic and environmental risk/protection factors for cardiovascular and cerebrovascular disease and cancer [21]. The recruitment period was from March 2005 to April 2010 and the first follow-up was completed in December 2011. In this study, the Italian version of the EPIC food frequency questionnaire [22, 23] was administered to all subjects, allowing analyses of diet and dietary components association with a number of risk factors, biomarkers and incidence of diseases at follow-up.

The MOLI-SANI study complies with the Declaration of Helsinki and was approved by the Ethics Committee of the Catholic University of Rome, Italy. All participants provided written informed consent.

Dietary intake ascertainment

The EPIC food frequency questionnaire includes 188 food items, later classified into 45 predefined food groups on the basis of similar nutrient characteristics or culinary usage.

The a priori Mediterranean diet adherence score

Adherence to the traditional Mediterranean diet (MD) is commonly appraised by using the Mediterranean diet score developed by Trichopoulou *et al.* [24]. This approach is called *a priori* and is oriented to test whether the eating behaviour of people does reflect a pre-defined ideal way of eating.

Scoring is calculated in the MOLI-SANI population free from cardiovascular disease, cancer or diabetes and is based on the intake of the following nine items: vegetables, legumes, fruit and nuts, dairy products, cereals, meat and meat products, fish, alcohol and monounsaturated : saturated fat ratio. For most items, consumption above the study median received 1 point. All other intakes received 0 points. For dairy products, meat and meat products, consumption below the median received 1 point. Medians are gender specific. For ethanol, men who consumed 10–50 g day^{-1} and women who consumed 5–25 g day^{-1} received 1 point. Otherwise, the score was 0. The possible scores ranged between 0 and 9, the latter reflecting the maximal adherence to the MD.

The a posteriori principal factor analysis

An alternative way to measure dietary habits is by using the principal factor analysis (PFA) conducted on the correlation matrix of 45 food groups [16, 25]. This approach is called *a posteriori* and, different from the *a priori* one, is a data driven methodology which basically allows a list of specific food groups to be put together in such a way that reflects the actual dietary habits of the population under study.

Within the MOLI-SANI cohort, the PFA allows one to obtain three major dietary patterns: the pattern identified as 'olive oil and vegetables' is characterized by high positive loadings for olive oil, vegetables, legumes, soups, fruits and fish and closely resembles a Mediterranean diet, the 'pasta and meat' is characterized by high positive loadings for pasta, cooked tomatoes, red meat, animal fats and alcoholic beverages, and negative loadings of breakfast cereals and yogurt, resembling a Western diet pattern, while the 'eggs and sweets' pattern is characterized by high positive loadings for eggs, margarines, processed meat, sugar and sweets.

More recently, the MOLI-SANI study went beyond dietary patterns by focusing on nutrient content of diets as well. Dietary antioxidant and polyphenol intake was calculated through composite scores to provide a comprehensive understanding of the role of such healthy compounds largely present in the Mediterranean diet [15, 26].

A score to assess low grade inflammation

To assess overall a low grade inflammation condition in the cohort of the MOLI-SANI study an index INFLA score was constructed [15]. The development of this score contributes to the evaluation of a possible synergetic effect of inflammation biomarkers not taking into account the variability presented by the differences in units and biological actions. In addition, inflammation biomarkers are usually strongly autocorrelated and that may produce multi-collinearity when these data are simultaneously studied in a regression model. This source of biased estimations is limited by the use of an index [15].

The 10 tiles of all biomarkers level (i.e. CRP, WBC, PLT, granulocyte : lymphocyte ratio) were generated. The four components of the INFLA score were derived by scoring the four different 10 tiles of biomarkers level. For all components, higher levels (i.e > Q6) scored increasingly positively while lower levels (i.e. < Q5) got negative scoring. The INFLA score ranged between −16 and 16 and came up as the sum of the four components. An increase in score indicates an increase in the total low grade inflammation condition.

Diet and low grade inflammation: the contribution of the MOLI-SANI study

Healthy diets rich in functional foods, such as the Mediterranean diet, have been shown to have cardioprotective effects in the primary and secondary prevention of cardiovascular disease [13]. Part of the beneficial effects has been ascribed to the modulation of key players in the pathogenesis of atherosclerosis, including amelioration in the lipid profile and vascular function and a decrease in oxidative stress and inflammation. Natural dietary compounds have also been demonstrated to exert antiplatelet activities, further contributing to the reduction in thrombotic risk [13].

On the contrary, Western type or meat-based eating patterns, largely based on high intakes of refined grains, meat and meat products, saturated fats and poor in vegetables and fruits, have been linked to an increase in inflammatory biomarkers such as CRP, IL-6 and fibrinogen [1].

The Mediterranean diet

There is plenty of evidence on the relationship between a Mediterranean diet and low grade inflammation both in observational and intervention studies.

A recent systematic review and meta-analysis of randomized controlled trials showed that a Mediterranean dietary pattern decreases inflammation and improves endothelial function [27]. A 12 month study on subjects at high risk for cardiovascular disease joining the PREDIMED trial showed that inflammatory biomarkers related to plaque instability, such as C-reactive protein and interleukin-6, were reduced in the groups assigned to an MD compared with the low fat diet group [28].

In patients at high risk of cardiovascular disease, an MD was found to induce significant reductions in the plasma concentrations of TNFR, IL-6 and ICAM-1 [29].

A number of pathways have been proposed to explain how an MD is capable of providing health advantages, especially for cardiovascular health. Such a diet has been suggested to reduce the post-prandial inflammatory response in mononuclear cells compared with the saturated fatty acid rich and high carbohydrate diet enriched in n-3 PUFA diets in elderly people and this may be partly responsible for the lower CVD risk found in populations with a high adherence to the MD [30].

Adherence to the Mediterranean diet was associated with an increase in adiponectin concentrations that may partially explain the beneficial effects of this traditional healthy diet on the cardiovascular system [31]. An intervention study in apparently healthy subjects has shown that the concomitant intake of orange juice, which is rich in polyphenols, may prevent the low grade inflammatory reaction induced by a fatty meal, at cellular and possibly at vascular function levels [32]. The same study group has also demonstrated that cellular responses to a fatty meal, in particular platelet P-selectin expression and neutrophil generation and leucocyte degranulation, were attenuated in subjects at a higher degree of cardiovascular risk, who had not suffered any cardiovascular event [33].

It has been argued that the direct link between adipose tissue and chronic low grade inflammation makes it unclear how concurrent reductions in body weight frequently seen with the MD modulate its effects on CVD risk factors, including inflammation [34]. A recent intervention trial in men with metabolic syndrome has found that an MD significantly reduces inflammation even in the absence of weight loss although the degree of waist circumference reduction with weight loss magnifies the impact of the MD on other markers of inflammation associated with metabolic syndrome [34].

Results from the MOLI-SANI study

In the study by Centritto et al. [16] conducted in over 7000 healthy subjects, a Mediterranean dietary pattern derived from principal factor analysis was closely associated with relatively lower values of glucose, lipids, C-reactive protein, blood pressure and cardiovascular risk. Conversely, subjects adhering most to the pasta and meat pattern, close to the Western-type diet, showed higher glucose, lipids, C-reactive protein and cardiovascular risk score [16].

The MOLI-SANI study was the first to address the relationship between adherence to the traditional MD and platelet count starting from the hypothesis that a diet rich in healthy compounds could favourably influence this cellular biomarker of low grade inflammation in subjects free from any overt chronic or haematological disease [12].

Platelet count was set according to age-sex defined ranges recently established for the Italian population [35]. Results clearly showed that a higher adherence to an MD was significantly associated with reduced platelet and white blood cell (WBC) counts, independently of other inflammatory markers as CRP. In addition, subjects having a higher adherence to this eating pattern reported lower odds of being in the highest platelet count group as compared with those with lower adherence. Similarly, maximum adherence to the MD was also linked to greater chances of having a lower WBC count. The associations observed between diet and these two inflammatory cell markers were partially explained by the high content of both antioxidants and fibre typical of a Mediterranean-style behaviour. The findings are in agreement with the results of an intervention trial showing that a MD eating pattern was directly linked to a lowering in some markers of inflammation, including platelets and WBC [36].

The favourable accounting of antioxidants found in that context is supported by the inverse association of antioxidants and oxidative stress, an important intermediate mechanism of disease.

Additional evidence on the health-promoting action of dietary antioxidants on inflammation was provided by analyzing the consumption of healthy foods with high rather than low content in antioxidant vitamins and phytochemicals in relation on health status. Findings showed that an increase in the food antioxidant content, which represents an increased consumption of high antioxidant content with respect to low antioxidant content foods, was associated with reduced systolic and diastolic blood pressure and CRP concentrations in men [26].

Besides the Mediterranean diet as a whole, research has recently focused also on single food groups or functional foods. A study from the MOLI-SANI cohort [37] has shown the beneficial properties of regular intake of dark chocolate against inflammation, defined as high plasma concentrations

of CRP. A J-shaped relationship between dark chocolate consumption and serum CRP was observed. Consumers of up to 1 serving (~20 g) of dark chocolate every 3 days had serum CRP concentrations significantly lower than either non-consumers or higher consumers [37]. In addition, nut eaters were found to have lower inflammatory markers independently from adherence to the MD, although this was not on the pathway of the inverse association with mortality [38].

Dietary polyphenols

Polyphenols are secondary metabolites of plants and are generally involved in defence against ultraviolet radiation or aggression by pathogens and found largely in the fruits, vegetables, cereals and beverages [38].

Epidemiological evidence strongly suggests that long term consumption of diets rich in plant polyphenols is capable of offering protection against the development of major chronic and neurodegenerative diseases [40, 41].

The consumption of polyphenol rich food such as fruits, vegetables, dark chocolate [37], tea, coffee and wine has been shown to having an effect in modulating low grade inflammation [42–44].

Polyphenols have been reported to reduce inflammation by (a) acting as an antioxidant or increasing antioxidant gene or protein expression, (b) attenuating endoplasmic reticulum stress signalling, (c) blocking pro-inflammatory cytokines or endotoxin-mediated kinases and transcription factors involved in metabolic disease, (d) suppressing inflammatory or inducing metabolic gene expression via increasing histone deacetylase activity or (e) activating transcription factors that antagonize chronic inflammation [45].

Several short term intervention studies have shown favourable effects on biomarkers of inflammation by intake of polyphenols and polyphenol rich food [46–49] although few large scale epidemiological studies have evaluated the association between the extensive range of polyphenols and biomarkers of low grade inflammation [50, 51].

As already pointed out for the association of dietary patterns and low grade inflammation, the relationship between low grade inflammation and dietary polyphenols has been mainly addressed by the use of single biomarkers of inflammation, with all the limitations inherent to such an approach [15].

To overcome any potential bias, the MOLI-SANI study has investigated the association of flavonoid and lignan intake with low grade inflammation by testing the association of the INFLA score with the polyphenol content of diet as measured by a novel PAC score [15]. Such a score was constructed on the basis of the intake of polyphenol subclasses (flavonol, flavone, flavanone, flavanol, anthocyanin, isoflavone and lignan) calculated using Eurofir eBASIS to assess finally the total content of diet in these nutrients [15]. The PAC score ranged from −28 to +28 with higher values indicating increased dietary polyphenols.

This newly developed methodology allows the accounting of all possible synergistic effects of polyphenols and biomarkers and likely overcomes gender differences in the association between individual components [15].

In the present study, when the low grade inflammation was analyzed as single biomarkers negative associations with polyphenol intake showed mostly in women. However, when the overall inflammation status was considered through the construction of the INFLA score, negative associations with polyphenol content of diet was observed for both genders in regression analyses. In addition, we found that a 10 units increase in PAC score was associated with a 5–8 % decrease on the likelihood of higher low grade inflammation status [15].

The variation of INFLA score explained by polyphenol content of diet measured by the PAC score was 16.7 % in women and 9.1 % in men. These numbers are relatively high taking into consideration that other dietary or environmental or clinical factors could also affect low grade inflammation status in humans and explain a proportion of its variation.

Conclusions

To date, there are plenty of studies showing that healthy eating patterns are inversely related to low grade inflammation, a condition in close connection with a number of chronic diseases and health conditions. Dietary behaviours may act by modulating the degrees of several inflammatory markers, from the most well-known such as C-reactive protein, interleukin-6 or leukocyte count, to novel biomarkers such as platelet count. Besides the single biomarker approach, a composite measure of low grade inflammation, as tested within the cohort of the MOLI-SANI population was also associated with dietary modifications.

There is consensus on the favourable effect of Mediterranean type eating, rich in antioxidants, fibre and healthy fats, on reducing these biomarkers. At variance, Western-like or meat-based diets are associated with detrimental health outcomes possibly due to the high content of pro-inflammatory foods and nutrients.

The prominent role of health-related behaviours in mitigating the degree of low grade inflammation should lead to the encouragement of health-promoting interventions for reducing the risk of major adverse health outcomes.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

Contributors

MB wrote the manuscript, GP, CC, MBD, LI and GdG critically reviewed the manuscript.

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Appendix 1

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