biology and life sciences forum

MDPI

Proceedings Paper

Mediterranean-like diet may modulate acute inflammation in Wistar rats†

Casanova-Crespo, S [1,2], Ceballos-Sánchez, D [1,2], Rodríguez-Lagunas, M.J. [1,2], Massot-Cladera, M [1,2], Castell, M. [1,2,3], Pérez-Cano and F.J. [1,2*]

[1] Physiology Section, Department of Biochemistry and Physiology, Faculty of Pharmacy and Food Science, University of Barcelona (UB), 08028 Barcelona, Spain; danielaceballos@ub.edu (D.C-S.); sergi.casanova@ub.edu (S.C-C); mjrodriguez@ub.edu (M.J.R.-L.) margaridacastell@ub.edu (M.C.); malen.massot@ub.edu (M.M.-C.); franciscoperez@ub.edu (F.J.P-C)
[2] Nutrition and Food Safety Research Institute (INSA-UB), 08921 Santa Coloma de Gramenet, Spain
[3] Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Instituto de Salud Carlos III, 28029 Madrid, Spain
* Correspondence: franciscoperez@ub.edu
† Presented at the 3rd International Electronic Conference on Nutrients – The Role of Gut Microbiota in Precision Nutrition, 1-15 November 2023. .

Abstract: Mediterranean Diet (MD) is very rich in bioactive and immunomodulatory components. Some of these have demonstrated their protective activity in inflammation. The objective of this study was to evaluate the impact of a diet rich in fiber and polyphenols on an inflammatory process. The intervention was performed in two groups of 7-week-old rats, one receiving an experimental MD-like diet and another fed a reference diet (REF). At the end of the study, a local inflammation was induced by injecting the rat's paw with carrageenan. A lower paw volume was observed in the rats from the MD-like diet group.

Keywords: Mediterranean diet; fiber; polyphenols; inflammation; rat

Citation: Lastname, F.; Lastname, F.; Lastname, F. Title. *Biol. Life Sci. Forum* **2022**, *2*, x. https://doi.org/10.3390/xxxxx

Academic Editor: Firstname Lastname

Published: date

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1. Introduction

1.1. Polyphenols

Polyphenols are substances derived from plant metabolism characterized by the presence of several phenolic rings in their molecular structure [1]. These compounds are involved in the plant defense against various forms of aggression, including pathogens and other biotic stress factors [2]. Polyphenols can be classified according to the number of phenolic rings they contain and the structural elements that link these rings together. Based on these criteria, polyphenols can be phenolic acids, stilbenes, lignans, and flavonoids. The latter group has been described to possess anti-inflammatory and immunomodulatory effects [3]. In the present study, isolated flavonoids such as catechin and epicatechin, hesperidin and naringenin, and quercetin have been used.

1.2. Dietary Fiber

Dietary fiber (DF) refers to the edible part of plants or carbohydrates that resists digestion and absorption in the small intestine, undergoing complete or partial fermentation by the microbiota in the large intestine. The term DF primarily encompasses polysaccharides, oligosaccharides, and lignin. DF provides health benefits due to two key characteristics: solubility and fermentation [4]. Regarding solubility, soluble fiber absorbs water and thicken gastrointestinal contents. This leads to distension of the gastrointestinal walls, ultimately stimulating reflexes such as the sensation of satiety. In contrast, insoluble or poorly soluble fibers can retain water within their structural matrix, forming mixtures with low viscosity that increase fecal content, which speeds up intestinal transit [4]. The fermentability of DF is directly related to its solubility. Fermentation promotes the proliferation of intestinal microbiota and the production of short-chain fatty acids (SCFAs). These SCFAs are found in high concentrations in the cecum and proximal colon and can serve as energy sources by colonocytes [5].

In the current study, two types of DF have been used: pectin (found in citrus and sweet fruits) and inulin (found in fruits and cereals). Inulin has demonstrated benefits related to lipid metabolism, weight loss, blood sugar reduction, and alleviation of inflammation [6-7]. Pectin, on the other hand, is of nutritional interest due to its solubility, le rapid fermentation and promotion of the growth of beneficial bacteria [4,8].

1.3. Inflammation

Inflammation is a natural and protective response of the body in which vascularized tissues transport leukocytes and host defense molecules from the bloodstream to sites of infection and cellular injury to eliminate harmful agents [9-10]. During the inflammatory process, circulating proteins are released into the affected tissues, and both recruited and resident cells are activated to eliminate unwanted substances [9]. The last inflammatory phase involves the regulation of its response and finally, tissue repair [10-11]. The predominant leukocyte recruited to areas with acute inflammation is the neutrophil, but blood monocytes, which will differentiate into macrophages within the target tissue over time, can become the dominant population in certain reactions. Once activated, they generate tissue damage and prolong the inflammatory response [9].

2. Objective

The objective of this study was to evaluate the impact of a diet rich in fiber and polyphenols on a model of acute inflammatory response. Specifically, we ascertain how 9-weeks of this diet influenced the development of a local inflammation and whether the diet had also an impact on fecal features and plasma immunoglobulin (Ig) levels.

3. Material and Methods

3.1. Animals

Eight six-week-old female Wistar rats from Janvier Labs (Saint-Berthevin, France) were housed individually in the experimental animal facility at the Diagonal-Campus within the Faculty of Pharmacy and Food Science at the University of Barcelona (UB). One-week acclimatization period passed before the project's initiation. The animals were maintained under controlled environmental conditions, including humidity (50–55%), temperature (21 ± 2 ºC), 12-hour light-dark cycles, and *ad libitum* access to food and water. All procedures adhered to the ethical guidelines and were approved by the Ethics Committee for Animal Experimentation of the University of Barcelona (Ref. 240/19) and the Generalitat de Catalunya (Ref.10933).

3.2. Diet and Experimental Design

Two experimental diets were acquired from Envigo® - Teklad Diets (Madison, WI, USA). The MD-like diet contained 8% inulin from chicory roots, 1% pectin, and 0.5% of a mixture of polyphenols, including catechin, epicatechin, hesperidin, naringenin, and quercetin (Sigma-Aldrich®, Madrid, Spain). The standard AIN-93G diet was used as the reference diet (REF). The animals were distributed into two groups according to their diet: MD and REF groups (n=4/group). Animals from both groups received the diet for 9 weeks. Body weight, water and food intake, and fecal pH and humidity were monitored throughout the entire period.

3.3. Paw Edema Induction and Evaluation

To evaluate the impact of the MD-like diet, carrageenan-λ (Sigma-Aldrich®) was injected into the animal's right paw (2 mg/kg animal weight), while the vehicle (NaCl 0.9%) was injected into the left paw. Prior to carrageenan-λ injection, a baseline measurement of the animal's paw was taken at time 0. Paw edema was measured in a blinded manner by determining hind-paw volume using a water plethysmometer (7140; Ugo Basile, Comerio, Italy). This procedure was carried out for up to 4 h.

Following that, the animals were anesthetized using ketamine (90 mg/kg, Merial Laboratories S.A., Barcelona), and xylazine (10 mg/kg, (Bayer A.G., Leverkusen, Germany). Blood samples were collected via intracardiac puncture for plasma analysis

3.4. Plasma Immunoglobulins Quantification

The Ig concentrations of IgA, IgM and IgG, as well as its isotypes (IgG1, IgG2a, IgG 2b, and IgG2c) were quantified in plasma at the end of the study using the ProcartaPlex™ Multiplex immunoassay (Thermo Fisher Scientific, Vienna, Austria), as previously described [12].

3.5. Statistical Analysis

The Student-T test was used for statistical analysis. Significant differences were established at $p < 0.05$.

4. Results and Discussion

4.1. MD Effect on Acute Inflammation

All animals displayed an increase in paw volume over time in their right paw (R) with respect to their non-injected left paw (L) ($p < 0.05$). The inflammatory process varied among individual rats, independently of the group. Although the volume of the inflamed paw in animals following the MD-like diet for 9 weeks was of about 2.5 mL, and in those from the REF group, it was around 2 mL, there was not statistical difference between the two groups. Unlike these results, other have reported polyphenol derived protection in a similar model [13-14]. Although this lack of clear effect in the paw volume, the study of additional inflammatory markers would shed light on these results. Additionally, further studies with a higher number of animals per group must be carried out to confirm the protective effect of the MD diet.

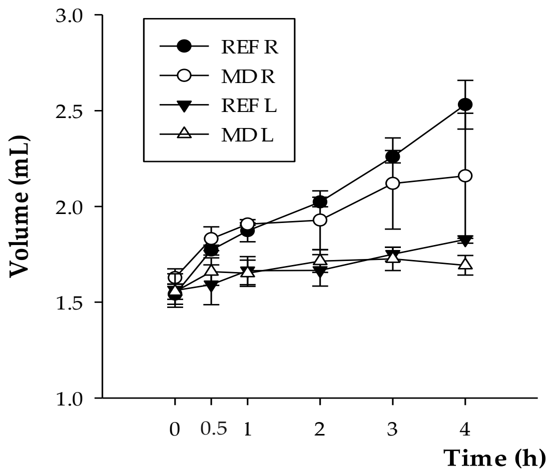

Figure 1. Volume (ml) of paw edema of reference group right paw (REF R) and left paw (REF L) and MD group right paw (MD R) and left paw (MD L) until 4 h. Data is expressed as mean ± S.E.M. (n=4/group).

4.2. MD Impact on Plasma Immunoglobulin Levels

The plasma levels of IgM, IgA and IgM were similar to those reported in previous studies [15-16]. The diet did not significantly affect the plasma concentrations in MD-fed animals (Table 1). It was expected to observe an effect on the concentration of these Ig due to some existing literature showing this type of influence due to polyphenols intake [17]. A deeper study focused on IgG isotypes and those associated with Th1/Th2 balance could help to ascertain whether existed an impact in the Ig profile or not.

Table 1. Immunoglobulin levels in plasma after 9 weeks of treatment.

	IgM (µg/mL)	IgA (µg/mL)	IgG (mg/mL)
REF	197.8 ± 51.7	20.8 ± 0.7	5.6 ± 8.1
MD	266.7 ± 173.4	19.9 ± 3.0	11.7 ± 4.8

Data shown is expressed as mean ± S.E.M. (n=4/group).

On the contrary, the intestinal impact of the MD diet was clearly observed by changes in the fecal pH and humidity (Figure 2). Fecal pH of MD like diet significantly decreases 1.5-2 units ($p < 0.05$) from the first week of treatment until the end of the study. Feacal humidity instead it is increased in MD fed diet almost 15% from the first week until the ninth week ($p < 0.05$). These results align with other articles with similar supplementation approaches [17]. These results suggest an intestinal impact of the diet, that seems not to imply a clear influence at systemic level. More studies are necessary to evaluate the specific mechanisms of this process to gain a better understanding.

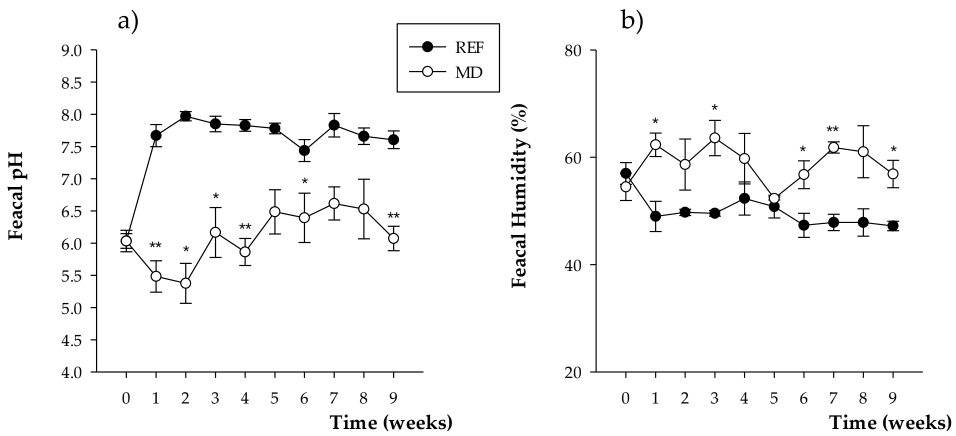

Figure 2. Feacal pH (a) and humidity (b) from week 0 to week 9 of reference group (REF) and MD like diet group (MD). Results are expressed as mean ± S.E.M. (n=4). Statistical analysis: p < 0.05 * and p < 0.01 ** MD *vs*. REF.

5. Conclusion

In conclusion, a Mediterranean diet, which is characterized by being enriched in polyphenols and fiber, have a direct intestinal impact that does not imply a significant systemic anti-inflammatory and immunomodulatory effect.

Author Contributions: S.C.-C., D.C.-S., M.M.-C., M.C., M.J.R.-L. and F.J.P.-C. were involved in the design and/or execution of the experiments. S.C.-C., D.C.-S., M.M.-C, M.C., and F.J.P.-C. analyzed and interpreted the results and drafted the paper. All authors have read and agreed to the published version of the manuscript.

Funding: The author thanks the project PID2020-119602RB-I00 funded by the MCIN/AEI /10.13039/501100011033 and the INSA Maria de Maeztu Unit of Excellence grant (CEX2021-001234-M) funded by MICIN/AEI/FEDER, UE.

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Ethics Committee of the University of Barcelona (protocol code 240/19 approved on 11/03/20.

Informed Consent Statement: Not applicable.

Acknowledgments: The authors would like to thank the members of the Animal Facility of the Faculty of Pharmacy and Food Science of the University for their assessment of the animal work.

Conflicts of Interest: The authors declare no conflict of interest.

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