biology and life sciences forum

MDPI

Proceeding Paper

Mediterranean-like Diet May Modulate Acute Inflammation in Wistar Rats †

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† Presented at the 3rd International Electronic Conference on Nutrients, 1–15 November 2023; Available online: https://iecn2023.sciforum.net/.

Abstract: The 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, local inflammation was induced by injecting the rat's paw with carrageenan. A lower paw volume was observed in = rats from the MD-like diet group.

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

Citation: Casanova-Crespo, S.; Ceballos-Sánchez, D.; Rodríguez-Lagunas, M.J.; Massot-Cladera, M.; Castell, M.; Pérez-Cano, F.J. Mediterranean-like Diet May Modulate Acute Inflammation in Wistar Rats. *Biol. Life Sci. Forum* **2023**, *29*, 26. https://doi.org/10.3390/IECN2023-15796

Academic Editor: Maria-Luz Fernandez

Published: 1 November 2023

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 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, were 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 thickens 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 via colonocytes [5].

In the current study, two types of DF were 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, the 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 local inflammation and whether the diet also had 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 (Le Genest-Saint-Isle, 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 °C), 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, WA, 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) ($p < 0.05$) (Figure 1). 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 about 2.5 mL, and in those from the REF group, it was around 2 mL, and there was no statistical difference between the two groups. Unlike these results, others have reported polyphenol-derived protection in a similar model [13,14]. Despite this lack of clear effect in 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.

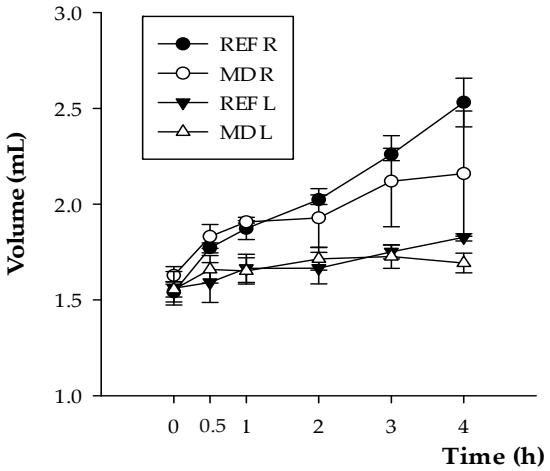

Figure 1. Volume (mL) of paw edema of reference group right paw (REF R) and left paw (REF L) and MD group right paw (MD R) and left paw (MD L) until 4 h. Data are expressed as mean ± S.E.M. (n = 4/group).

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). We expected to observe an effect on the concentration of these Igs due to some existing literature showing this type of influence due to polyphenol intake [17]. A

deeper study focused on IgG isotypes, and those associated with Th1/Th2 balance could help to ascertain whether there was an impact on 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 ± 0.9
MD	266.7 ± 173.4	19.9 ± 3.0	11.7 ± 4.8

Data shown are expressed as mean ± S.E.M of thetwo experimental groups: reference (REF) and Mediterranean diet (MD) (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 the MD-like diet significantly decreased by 1.5–2 units ($p < 0.05$) from the first week of treatment until the end of the study. Fecal humidity instead was increased in the MD 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 the systemic level. More studies are necessary to evaluate the specific mechanisms of this process to gain a better understanding.

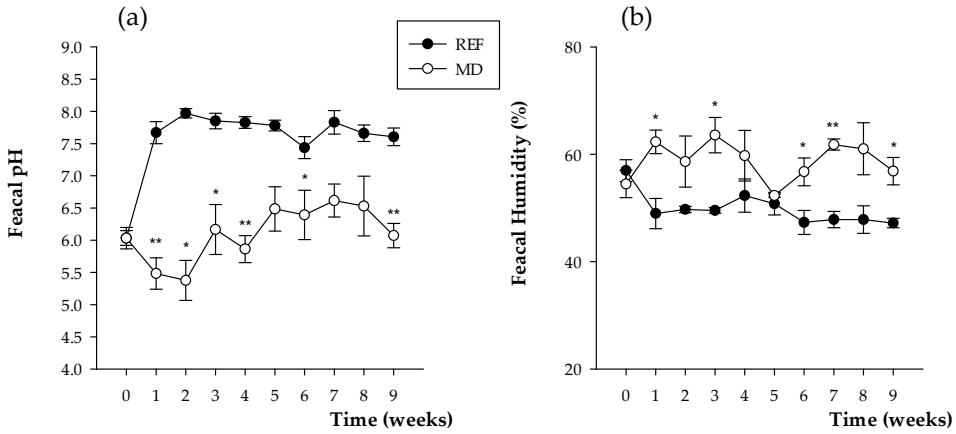

Figure 2. Fecal pH (**a**) and humidity (**b**) from week 0 to week 9 of reference group (REF) and MD like diet group (MD). Results are expressed as mean ± S.E.M. (n = 4). Statistical analysis: $p < 0.05$ * and $p < 0.01$ ** MD vs. REF.

5. Conclusions

In conclusion, a Mediterranean diet, which is characterized by being enriched in polyphenols and fiber, has 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 is thankful for 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: All procedures adhered to the ethical guidelines and were approved by the Ethics Committee for Animal Experimentation of the University of Barcelona (protocol code 240/19 approved on 3 November 2020) and the Generalitat de Catalunya (Ref. 10933).

Informed Consent Statement: Not applicable.

Data Availability Statement: Dataset available on request from the authors.

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 conflicts of interest.

References

1. Quiñones, M.; Miguel, M. Los polifenoles, compuestos de origen natural con efectos saludables sobre el sistema cardiovascular. *Nutr. Hosp.* **2012**, *27*, 76–89. [PubMed]
2. Shen, N.; Wang, T. Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. *Food Chem.* **2022**, *383*, 132531. [CrossRef] [PubMed]
3. Pérez-Cano, F.J.; Castell, M. Flavonoids, Inflammation and Immune System. *Nutrients* **2016**, *8*, 659. [CrossRef] [PubMed]
4. Gil, A. *Tratado de Nutrición*, 3rd ed.; Panamericana: Madrid, Spain, 2017; pp. 88–90.
5. Koh, A.; De Vadder, F. From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell* **2016**, *165*, 1332–1345. [CrossRef] [PubMed]
6. Massot-Cladera, M.; Azagra-Boronat, I. Gut Health-Promoting Benefits of a Dietary Supplement of Vitamins with Inulin and Acacia Fibers in Rats. *Nutrients* **2020**, *12*, 2196. [CrossRef] [PubMed]
7. Massot-Cladera, M.; Franch, À. Cocoa and cocoa fibre differentially modulate IgA and IgM production at mucosal sites. *Br. J. Nutr.* **2016**, *115*, 1539–1546. [CrossRef] [PubMed]
8. Qin, Y.Q.; Wang, L.Y. Inulin: Properties and health benefits. *Food Funct.* **2023**, *14*, 2948–2968. [CrossRef] [PubMed]
9. Kumar, V.; Abbas, A. *Robbins y Cotran. Patología Estructural y Funcional*, 10th ed.; Elsevier: Barcelona, Spain, 2021; pp. 71–113.
10. Lisset, M.; Regal, L. Respuesta inflamatoria aguda. Consideraciones bioquímicas. *Finley* **2015**, *5*, 47–62.
11. Abbas, A.; Litchman, A. *Inmunologia Celular y Molecular*, 8th ed.; Elsevier: Barcelona, España, 2015; pp. 1–35.
12. Morales-Ferré, C.; Franch, À. Staphylococcus epidermidis: Overload during Suckling Impacts the Immune Development in Rats. *Front Nutr.* **2022**, *9*, 916690. [CrossRef] [PubMed]
13. Ramos-Romero, S.; Pérez-Cano, F. Effect of a cocoa flavonoid-enriched diet on experimental autoimmune arthritis. *Br. J. Nutr.* **2012**, *107*, 523–532. [CrossRef] [PubMed]
14. Cordaro, M.; Siracusa, R. Cashew (*Anacardium occidentale* L.) Nuts Counteract Oxidative Stress and Inflammation in an Acute Experimental Model of Carrageenan-Induced Paw Edema. *Antioxidants* **2020**, *9*, 660. [CrossRef] [PubMed]
15. Grases-Pintó, B.; Abril-Gil, M. Rat Milk and Plasma Immunological Profile throughout Lactation. *Nutrients* **2021**, *13*, 1257. [CrossRef] [PubMed]
16. Massot-Cladera, M.; Franch, A. Cocoa flavonoid-enriched diet modulates systemic and intestinal immunoglobulin synthesis in adult Lewis rats. *Nutrients* **2013**, *5*, 3272–3286. [CrossRef] [PubMed]
17. Morales-Ferré, C.; Azagra-Boronat, I. Effects of a Postbiotic and Prebiotic Mixture on Suckling Rats Microbiota and Immunity. *Nutrients* **2021**, *13*, 2975. [CrossRef] [PubMed]