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Proceeding Paper

Immunonutritional Benefits of *Chenopodium quinoa*'s Ingredients Preventing Obesity-Derived Metabolic Imbalances †

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Abstract: Over 1.6 billion people (aged 15 years and above) worldwide are currently either overweight or obese, and this number is predicted to increase to 2.3 billion by 2050 (WHO). Excessive or impaired energy storage in the liver incurs a high risk of liver dysfunction and development of obesity, lipodystrophy, or cachexia, and impairs organismal homeostasis. Chenopodium quinoa seeds constitute a good source of immunonutritional compounds, enabling the selective functional differentiation and function of intrahepatic monocyte-derived macrophages. The latter play a key role in controlling adiposity associated with innate lymphoid cells (ILCs), which determine the induction of diet-induced obesity (DIO). Herein, two immune-conditioned mouse models—Rag2$^{-/-}$ and Rag2$^{-/-}$IL2$^{-/-}$—were used to examine the influences preventing DIO with a protein-rich fraction (PRF) and oil obtained from *C. quinoa* seeds. Variations in myeloid cells and precursors of ILCs were evaluated by FACS analyses as well as the hepatosomatic index to estimate liver inflammation. Only the administration of *C. quinoa* PRF prevented alterations in the liver/body weight ratio, both in animals carrying ILCs (i.e., Rag2$^{-/-}$) and not (Rag2$^{-/-}$IL2$^{-/-}$). These effects were associated with significantly decreased variations in the hepatic triglyceride content. FACS revealed that PRF from *C. quinoa* favors the hepatic infiltration of myeloid and enables the selective functional differentiation and function of intrahepatic monocyte-derived macrophages, preserving tissue integrity and function.

Keywords: obesity; macrophages; serine-type protease inhibitors; quinoa; food

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1. Introduction

Over 1.6 billion people (aged 15 years and above) worldwide are currently either overweight or obese, and this number is predicted to increase to 2.3 billion by 2050 (WHO). Recent research has highlighted the key role of innate and adaptive lymphocytes to operate sequentially and in distinct ways during normal development to establish tissue lipid homeostasis [1]. Moreover, innate lymphoid cells (ILCs) have been identified as determinants in the induction of diet-induced obesity [2]. Non-alcoholic fatty liver disease (NAFLD) has become the most common liver pathology worldwide, affecting an estimated 15–30% of most populations due to dramatic increases in risk factors such as obesity, sedentary lifestyle, and altered food supplies and preferences. Between 10% and 20% of subjects with NAFLD will have a severe variant of non-alcoholic steatohepatitis (NASH), where the fatty liver has progressed to massive hepatocyte apoptosis (mitochondrial dysfunction and lipoapoptosis due to an excess of free fatty acids and uncontrolled oxidative processes), hepatic inflammation, and the development of liver fibrosis. Fibrosis progresses to cirrhosis

in 10–20% of patients with persistent NASH, with high liver-related morbidity and mortality, part of which is due to the development of hepatocellular carcinoma (HCC) [3]. Here, macrophage-dependent mechanisms appear to control energy storage into the liver [4].

Previous research evidenced the potential role of ingredients from *C. quinoa* to modulate insulin resistance as well as hepatic triglyceride accumulation [5]. Immunonutritional bioactive compounds from *C. quinoa* positively preserved alterations in peripheral myeloid populations [6]. However, to the best of our knowledge, there is no information about the influence of *C. quinoa* on the maturation of ILCs and their association with the selective functional differentiation of monocyte-derived macrophage.

In this respect, this study explored the impact of the administration of *C. quinoa*'s ingredients in the differentiation of innate immune effectors key in liver fat mobilization and energy storage.

2. Materials and Methods

2.1. Ingredients

C. quinoa germs were obtained by cold pressing and from oil by wet milling [7]. A protein-rich fraction from the germ was obtained by vigorous vortexing with phosphate-buffered solution (PBS); 1 h/25 °C.

2.2. Experimental Model and Subject

All animal experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Spanish High Research Council of Research (CSIC) and regional government of Madrid (Spain) (Proex 298.0/21). Mice used in this study were on a C57BL/6 background and were maintained in the CIB-CSIC Center under pathogen-free conditions. $Rag2^{-/-}$ and $Rag2^{-/-}IL2^{-/-}$ mice were kindly donated by Dr. Balbino Alarcón (CBM-CSIC). Mice (6 weeks old) were placed on a 42 kcal% HFD (IN93G mod, irradiated, Ssniff spezialdiäten gmbh) for 15 days and received the protein-rich fraction (PRF) (10 µg) and oil (0.8 µL) from *C. quinoa* 3 times per week. The diet was exchanged twice a week, and body weights were measured every 2 days. Liver weight as a percentage of the body weight (hepatosomatic index) was calculated. Liver samples were collected and stored at −80 °C until analysis.

2.3. Cell Preparation for Flow Cytometry and the Gating Strategy

Cells in the liver tissue were prepared as previously described, with a few modifications [2]. Briefly, the liver was cut into 1 cm pieces and stirred in a 100 mL conical flask containing 5 mL of 1 mM EDTA in PBS with a stir bar at 37 °C and 500 rpm for 20 min. The pieces were shaken vigorously in 20 mL PBS, minced into 1–2 mm pieces with scissors, and stirred in 10 mL of 2% (*v*/*v*) FCS in RPMI medium containing Trypsin-EDTA solution, 0.25%, sterile-filtered, suitable for cell culture, 2.5 g porcine trypsin and 0.2 g EDTA, 4Na per liter of Hanks' Balanced Salt Solution (HBSS) with phenol red with a stir bar at 37 °C and 500 rpm for 60 min. Finally, samples were filtered through a 40 µm strainer into a new 50 mL tube. The tubes were centrifuged at room temperature and 500× *g* for 10 min, and the pellets were then used for subsequent analyses. Prepared cells were suspended in 2% FCS in HBSS containing 0.02% NaN_3, and propidium iodide was added to gate out dead cells immediately before analysis. Flow cytometry was performed using FACS SORP apparatus (BD Biosciences, Franklin Lakes, NJ, USA). Data were analyzed using FlowJo Software (FlowJo LLC, Ashland, OR, USA). Based on the surface expression levels of NKp46, CD56, and killer cell lectin-like receptor G1 (KLRG1), four subsets of immature ILCs could be distinguished [8]: (i) one subset consisted of $CD117^+NKp46^-CD56^-KLRG1^-$ cells that could be multipotential; (ii) two immature $CD117^+NKp46^+$ and $CD117^+NKp46^+CD56^+$ subsets were biased to mature into group 3 ILCs (ILC3s); and (iii) an immature $CD117^+$ $KLRG1^+$ subset was biased to mature into group 2 ILCs (ILC2s). For analysis of macrophages, $PI\text{-}CD68^+F4/80^+$ cells were identified as M1 macrophages.

2.4. Statistical Analyses

Statistical analysis between the different groups of treatment within the same experimental model was conducted using one-way analysis of variance (ANOVA) and the Kruskal–Wallis post hoc test by ranks. Analyses were performed with Statgraphics Centurion XVI software (Statgraphics Technologies, Inc., The Plains, VA, USA), and significance was established at $p < 0.05$ for all comparisons.

3. Results

Animals administered with PRF displayed downward trends in body weight gain (Figure 1A–C) rates in relation to controls. The administration of *C. quinoa*'s ingredients prevented increased values in the hepatosomatic index (Figure 1D) in both animal models.

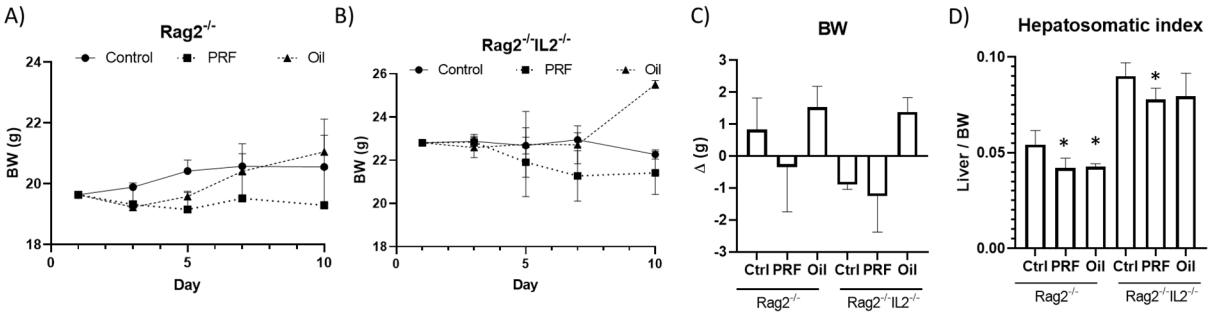

Figure 1. Morphometric measurements. Daily (**A**,**B**) and total (**C**) body weight gain (BW), and hepatosomatic index (**D**) of Rag2$^{-/-}$ and Rag2$^{-/-}$Il2$^{-/-}$ mice fed a high-fat diet and administered with a protein-rich fraction (PRF) or oil from *C. quinoa*. Results are expressed as the mean ± standard error (SEM) (n = 6). * Indicates statistical differences in relation to the respective controls.

Animals receiving either PRF or oil exhibited increased proportions of infiltrated monocytes (Figure 2A) to a different extent. These values were associated with mirage trends in the proportion of immature ILC2s (Figure 2B). Notably, only administration of PRF enabled decreased contents of hepatic triglycerides (Figure 2C), whereas both PRF and oil promoted upward amounts of saturated triglycerides, although only in animals carrying ILCs (Rag2$^{-/-}$).

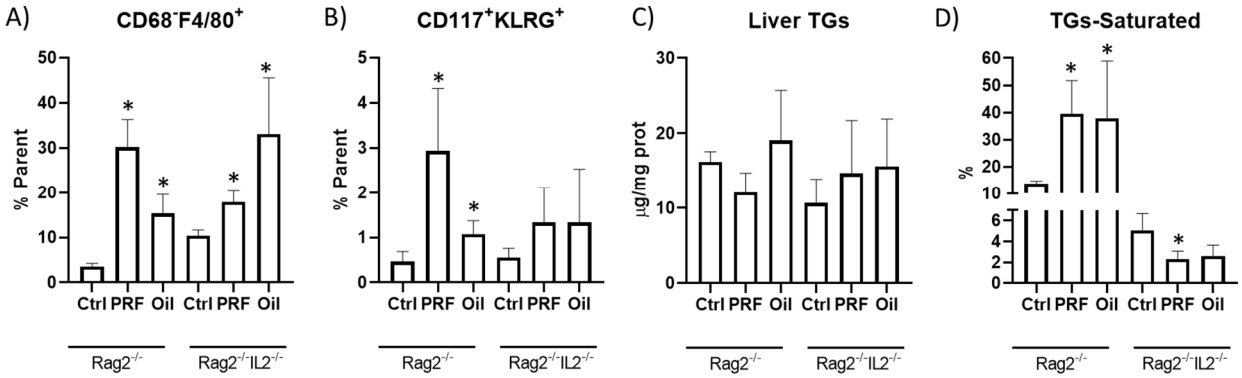

Figure 2. Immunonutritional measurements. Changes in hepatic infiltrated myeloid (**A**) and immature innate lymphoid cells type 2 (i-ILC2s) (**B**), hepatic triglycerides (TGs) (**C**) and saturated TGs (**D**) in Rag2$^{-/-}$ and Rag2$^{-/-}$Il2$^{-/-}$ mice fed a high-fat diet and administered with a protein-rich fraction (PRF) or oil from *C. quinoa*. Results are expressed as the mean ± standard error (SEM) (n = 6). * Indicates statistical differences in relation to the respective controls.

4. Discussion

This study investigated the influence of the independent administration of quinoa's ingredients on hepatic homeostasis in animals fed an HFD. The administration of *C. quinoa*'s ingredients boosted immunonutritional hepatic features, which was reflected in decreased hepatosomatic index values. Here, triglyceride breakdown from hepatic tissue seems to regulate inflammation. Monocyte-derived macrophages have been shown to play key roles coupling energy intake with fat accumulation [4]. Thus, by promoting hepatic TGs mobilization, infiltrated macrophages appear to regulate the systemic availability of lipids in animals under an HFD.

ILC2s and ILC3s are involved in the progression of NAFLD. The influence of PRF, enabling the proliferation of immature ILC2s in an apparent independent fashion from inflammation, could have important consequences in vivo, limiting obesity and insulin resistance and controlling hepatic metabolic homeostasis. ILC2s also interact with other immune cells through newly identified pathways in the adipose tissue. Overall, it can be hypothesized that beneficial effects of the IL-33/ILC2s axis during early stages of diet-induced obesity could result in a potential deleterious role of endogenous IL-33 in late stages of the disease.

5. Conclusions

C. quinoa's ingredients display a different potential to induce relevant innate immune myeloid and lymphoid populations. These changes evidence the clear impact that lipid homeostasis has on diet-induced low-grade inflammation in obesity.

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Institutional Review Board Statement: Animal experiments were carried out in strict accordance-with the recommendations in the Guide for the Care and Use of Laboratory Animals of CSIC (Consejo Superior de Investigaciones Científicas), and the protocol was approved by their ethic committee and the regional government (Ethic code, Proex 220/17, approved 21/01/2018).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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

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