INSTITUTIONAL PROTOCOL NO. 211247

PROJECT TITLE: The effect of a plant-based diet on active rheumatoid arthritis activity.

INTERNATIONAL REGISTER: YES [ ] No [x] Specify _____

THE GOALS ARE:

PUBLISHED WORK [x] **THESIS** [X]

PATENT [ ]

TRAINING OF HUMAN RESOURCES

BACHELOR'S [ ] **DEGREE SPECIALTY** [ ]

MASTER [X] **PHD** [ ]

DEPARTAMENT RESPONSIBLE: Immunology

NAME AND Y SIGNATURE OF THE DEPARTAMENT HEAD: Dr. Luis Manuel Amezcua Guerra

PARTICIPANTS:

Participation	Appointment
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APPROXIMATE DURATION OF THE PROJECT:

BEGINNING		TERMINATION	
Month	**Year**	**Month**	**Year**
August	2021	April	2024

INVESTIGATION TYPE:

Basic	X	Clinic		Sociomedic		Technology and Development	

GENERATES:

Scientific Knowledge	x	Technology Application	

OBJECTIVE:

Comprehension	x	Prevention		Diagnostic		Treatment		Rehabilitation	

RESEARCH LINE TO WHICH IT BELONGS:

- [ ] Coronary disease
- [ ] Metabolic syndrome
- [ ] Primary systemic/pulmonary arterial hypertension
- [ ] Valve disease
- [ ] Cardiomyopathies and Chagas disease
- [ ] Biological systems: cellular, molecular and energy production
- [ ] Congenital heart disease
- [ ] Nephropathies
- [ ] Fabrication of intracardiac devices
- [ ] Environment and sociomedicine
- [x] **Other (Specify):** Rheumatoid arthritis

CERTIFICATION ACCORDING TO PROJECT REQUIREMENTS. THE STUDY REQUIRES:

Subject Human	x	**Animals (species, strain)**		**Components Electronics/Mechanical**		**Other**	

Human cells		**Bioassays**		**Radioactive material**	
Consent Informed	x			**Assent of Minors**	

ABSTRACT:

The recent increase in autoimmune diseases in Western countries has led to the consideration of environmental factors as important disease triggers since the genetic basis has remained apparently constant in affected populations. For several years, diet has been seen as a modifying factor in rheumatoid arthritis (RA).

A high-fat diet changes the microbiome's structure even when there is an absence of obesity. However, it is also considered an important factor that promotes excessive accumulation of white adipose tissue (WAT) and systemic inflammation. For several decades, the participation of the intestinal microbiome in particular diet components' metabolism has been evaluated, and it has the potential to modify pro-inflammatory mediators or circulants anti-inflammatories. In the context of RA patients, the bacterium Provotella copri is found in a higher proportion in newly diagnosed patients. This bacterium generates a pro-inflammatory metabolite called trimethylamine N-oxide, derived from choline and carnitine, which is present in red meat, eggs, and dairy products. On the other hand, fruits and vegetables can modulate the intestinal microbiota through dietary fiber. The proportion of anti-inflammatory bacteria, such as Faecalibacterium prausnitzii, is found higher in vegetarian diets. It has been demonstrated that these bacteria are beneficial in RA patients associated with decreased inflammation.

For several years, it has been reported that the consumption of certain foods is associated with an increase or improvement in the symptoms of RA. However, a diet that combines several suggested strategies to reduce inflammation may represent a more significant intervention than simply including specific foods in the diet. Currently, the role of the plant-based diet in RA activity has not been fully elucidated.

Based on this evidence, this study aims to determine the decrease in RA activity associated with modifications of the plant-based diet. To carry out this protocol, we will evaluate women and men who are 18 years old or older with low, moderate, or severe RA activity. For the dietary intervention, at the beginning of the treatment, the RA patients will be evaluated anthropometrically (weight, height, body mass index, waist and hip circumference, waist-hip index, percentage of total fat, visceral fat, and percentage of muscle) and will have a baseline blood sample taken. The kilocalories consumed regularly will be calculated through a 24-hour recall to establish their personalized meal plan. A diet synthesis table will be created with the following macronutrient distribution: carbohydrates 57%, protein 18%, and lipids 25%. Subsequently, a distribution of the food will be made in an equivalent distribution table, complying with the kilocalories and the established percentage of macronutrients. The consumption of animal-origin foods will be reduced, replacing this protein with protein of vegetable origin. The intervention will be carried out for 15 days, and the menu will be modified after seven days of intervention following the same specifications.

At the end of the treatment, the patients will be evaluated under the same anthropometric conditions, and a blood sample will be collected for laboratory analysis. The DAS (Disease Activity Score) index, a scale that measures RA activity, will be used to determine the severity of the pathology. This scale takes into account the count of swollen and painful joints over 28 joints, the erythrocyte sedimentation rate (ESR), and the evaluation of the disease by the patient. The scale has a rating of 0.0 to 9.4.

PROJECT OBJECTIVES:

Primary(s):	**Secondary(s):**
To determine the decrease in rheumatoid arthritis (RA) activity associated with plant-based diet modifications	• To analyze the factors associated with changes in disease activity. • To evaluate changes in acute phase reactants. • To identify the adipokines related to the decrease or increase in disease activity tumor necrosis factor-alpha (TNF-α), Interleukin 6 (IL-6), Leptin, and C-reactive protein (CRP).

1. TITLE.

The Effect of a Plant-based Diet on the Active Rheumatoid Arthritis Activity.

2. BACKGROUND.

The recent increase in autoimmune diseases in Western countries has been attributed to environmental factors, as the genetic basis of affected populations has remained constant. For several years, diet has been considered a modifying factor in rheumatoid arthritis (RA).

A high-fat diet can alter the microbiome structure, even in the absence of obesity. However, excessive accumulation of white adipose tissue (WAT) and systemic inflammation are also important factors that can alter the microbiome. WAT is considered an endocrine organ that releases a large number of pro-inflammatory mediators, such as TNF-α, IL-6, leptin, resistin, and CRP, which can have a major impact on T-cell responses and directly influence the inflammatory activity of autoimmune diseases (Manzel et al., 2014). The imbalance of these adipokines could explain the low-grade chronic systemic inflammation in obese subjects. Furthermore, upregulation of these factors leads to a chronic inflammatory state and contributes to metabolic dysfunction (Ouchi Noriyuki et al., 2011).

For several decades, the role of the intestinal microbiome in the metabolism of some diet components and its potential to modify circulating pro-inflammatory or anti-inflammatory mediators has been evaluated. In RA patients, the bacterium Provotellacopri is found in higher proportions in newly diagnosed patients. This bacterium produces a pro-inflammatory metabolite called trimethylamine N-oxide derived from choline and carnitine present in red meat, eggs, and dairy products (Scher et al., 2013). On the other hand, fruits and vegetables can modulate the intestinal microbiota through dietary fiber. The proportion of anti-inflammatory bacteria, such as Faecalibacteriumprausnitzii, is higher in vegetarian diets. In the fecal flora, these bacteria have been found to be beneficial in RA patients associated with decreased inflammation (Haghighatdoost et al., 2017).

Early changes in the microbiota have been associated with changes in adipocytokine levels. Various studies have investigated the association between adiponectin levels and pro-inflammatory markers in several diseases. In RA patients, elevated local and systemic adiponectin levels have been observed in the presence of chronic inflammation. In vitro studies indicate that adiponectin has pro-inflammatory effects on chondrocytes and synovial fibroblasts (Fantuzzi, 2008). Furthermore, adiponectin production by adipocytes can be inhibited by pro-inflammatory factors, such as TNF and IL-6 (Berg & Scherer, 2005). Also, a correlation between high adiponectin and reduced inflammation has been observed in RA patients.

3. PROBLEM STATEMENT.

For several years, it has been suggested that certain foods may be associated with an increase or improvement in RA symptoms. However, combining several dietary strategies to reduce inflammation may represent a more effective intervention than simply incorporating specific foods. Currently, the role of a plant-based diet in RA activity has not been fully elucidated.

3.1. JUSTIFICATION.

In a clinical trial that aimed to examine the role of fecal flora in diet-induced improvement in RA patients, the findings showed an association between intestinal flora and disease activity, which may have implications for our understanding of how the diet can affect RA (Peltonen et al., 1994). On the other hand, a meta-analysis showed that individuals who followed a vegetarian diet for at least two years had low serum concentrations of C-reactive protein (CRP) and high concentrations of IL-6 compared to omnivores (Haghighatdoost et al., 2017). Therefore, evaluating in the short term how dietary modifications could influence disease activity could serve as an adjunct to standard therapy in the care of patients with disease activity, even those who are difficult to control.

3.2. RESEARCH QUESTION.

What is the influence of changing from a dietary regime daily to a plant-based diet for 14 days on active RA disease activity?

4. HYPOTHESIS.

- A plant-based diet modifies disease activity in patients with active RA.

5. METHOD.

The study population will consist of women or men aged 18 years or older with rheumatoid arthritis, including those with low, moderate, or severe disease activity.

5.1 Preparation of the plant-based diet

Diet:

The food plan will take into consideration all the data obtained during the nutritional intervention. The total number of calories required by each patient will be estimated using the average calories obtained from the 24-hour recall and the Harris-Benedict formula (appointment). A synthetic diet chart will be created with the following macronutrient distribution: carbohydrates 57%, protein 18%, and lipids 25%. The food will then be distributed in an equivalent distribution table, complying with the established percentage of macronutrients and calorie requirements. In this protocol, the consumption of animal-based foods will be reduced and substituted with plant-based protein. The intervention will be carried out for 15 days. After seven days of intervention, the menu will be modified following the same specifications, resulting in two weekly menus. Patients will keep a food diary to track the food consumed (servings) on a daily basis. The diary will be reported daily to the responsible nutritionists by telephone and/or text message.

Diet overview:

The plant-based diet will consist of a normal diet with a macronutrient distribution of carbohydrates 57%, protein 18%, and lipids 25%. The diet will be divided into four meals: breakfast, morning or evening snack, lunch, and dinner, and will be consumed orally.

5.2. STUDY DESIGN

● Type and general design of the study

● RA patients with disease activity and modification with dietary intervention in the determined time of 14 days.

● According to the interference of the variables to be analyzed: Intervention

● According to the information capture period: Prospective

● According to the evolution of the study: Longitudinal

● According to the comparison of the populations: Analytical

5.3. DESCRIPTION OF THE STUDY POPULATION:

● 5.3.1. Target population:

● RA patients with disease activity to any degree.

● 5.3.2. Eligible population

● Patients attending the Rheumatology consultation at the National Institute of Cardiology Ignacio Chávez

● 5.3.3. Inclusion criteria

● People ≥18 years old

● Established diagnosis of RA according to the criteria ACR 1987 or ACR/EULAR 2010

● Disease activity according to DAS 28 (defined as everyone >2.6).

5.3.4. Exclusion criteria

● Systemic autoimmune overlap syndrome (with the exception of: fibromyalgia, thyroid disease, DM1, Sjögren's syndrome, and antiphospholipid syndrome)

● Patients treated with coumarins.

● Cancer patients, chronic hepatitis, HIV, pregnancy

● Weight changes associated with diet in the last 6 months

5.3.5. Elimination criteria

● Patients who have died in the process before finishing the sampling.

● Patients who have suspended the intervention

● Lack of adherence to diet <80%

● Loss to follow-up

● COVID infection requiring hospitalization (moderate-severe)

5.4. SAMPLE SIZE.

Convenience sampling will be carried out on patients at the National Institute of Cardiology who agree to participate, with a total n of 28.

5.5. SPECIFICATION OF DEPENDENT AND INDEPENDENT VARIABLE (S).

Variable	Measurement Level	Concept
DAS28 initial and post intervention	Continuous quantitative	Remission: <or equal to 2.6 (assess valid only in post-intervention), mild:> 2.6- <3.2, moderate:> or equal to 3.2- <or = 5.1, severe:> 5.1
CDAIinitial and post intervention	Continuous quantitative	<or equal 2.8, remission,> 2.8-10: mild activity, moderate> 10-22: moderate activity,> 22 serious activity
SDAI initial and post intervention	Continuous quantitative	<or equal to 3.3: remission, 3.3-11 mild activity,> 11-26 moderate activity,> 26 serious activity
PCR	Continuous quantitative	
Globular Sedimentation Volume (GSV)	Continuous quantitative	
IL-6	Continuous quantitative	
Adiponectin	Continuous quantitative	Protein produced mainly by adipocytes that belongs to the adipokine family.
Aggregate comorbidities (DM, HAS, Hypothyroidismsm, Dandslipidemia, smoking)	Nominal qualitativ.	Concomitant diseases added to patients, determined as present or not.
Age of diagnosis	Discrete quantitative.	Determined in completed years
Age of the patient	Discrete quantitative.	Determined in completed yearss
Gender	Nominal qualitativ.	Dichotomous in feminine or masculine
BMI	Continuous quantitative	Determination estimated according to weight and height, standardized by degrees in overweight (25-29.9), obesity grade I (30-34.9), grade II (35-40) and morbid obesity (> 40)
% total body fat	Continuous quantitative	Measurement established by bioimpedance determined by weighing machine TANITA
% visceral fat	Continuous quantitative	Measurement established by bioimpedance determined by weighing machine TANITA
% muscle / water	Continuous quantitative	Measurement established by bioimpedance determined by weighing machine TANITA
Morning stiffness	Nominal qualitativ.	Patient's perception of gelling phenomenon that improves with physical activity over a period of time.
#Swollen joints	Discrete quantitative.	Assessment established by the physician upon examination of the patients, without dependent relationship with painful joints.
#Painful joints	Discrete quantitative.	Patient referency to palpation on physical examination of pain joints
Fatigue	Qualitative ordinal.	Determinant as mild, moderate or severe for the daily situation manifested by the patient
Basal treatment	Qualitative ordinal.	Any DMARD, glucocorticoid, NSAID or supplement.

5.6. DATA COLLECTION TECHNIQUES

Samples will be processed at the central laboratory of INCICH. Commercial reagents will be used for the determination of soluble inflammatory mediators, with the technique (enzyme-linked immunoassay or luminex platform) selected based on availability and accessibility. Mediators to be measured include leptin, TNF-α, IL-6, and CRP.

5.7. SAMPLE PROCESSING

Peripheral venous blood (4 mL) will be collected in a tube with activator gel, which will be centrifuged at 600 g for 15 minutes at 4°C. The serum obtained will be stored in aliquots of 500 µL at -70°C until analysis.

STATISTICAL ANALYSIS PLAN

Spreadsheet databases will be created using the Excel program. The Kolmogorov-Smirnov test will be used to assess variable distribution. Proportions and percentages will be used to describe dichotomous, nominal, and ordinal variables, while means with standard deviation or medians with interquartile ranges will be used to describe dimensional variables, depending on the type of distribution.

To compare differences between two independent groups, appropriate statistical tests (Fisher's exact test or chi-square test for discrete variables, Student's t-test or Mann-Whitney test for continuous variables) will be used. For dependent variables, the paired T-test or the Wilcoxon rank sum will be used. If analysis of 3 or more groups is required, the Kruskal-Wallis test or one-way ANOVA will be used.

Associations will be explored using Spearman or Pearson's rho coefficients, as appropriate. All analyses will be conducted using two-sided testing, with significance set at $P < 0.05$.

For statistical analysis, the GraphPad Prism version 6.0 (GraphPad Inc., San Diego, CA) and IBM SPSS Statistics program for macOS, version 25 (IBM, Armonk, NY) will be used.

7. SCHEDULE OF ACTIVITIES.

Schedule.

	Aug 2021	Dec 2021	Jan 2022	Aug 2022	Dec 2022	Jan 2023	Aug 2023	Dec 2023	Aug 2024
	4	5	6	7	8	9	10	11	12
Initial presentation of the protocol.	x								
Presentation of the protocol to the committee.	x								
Authorization	x	x							
Selection of study population.	x	x	x	x	x	x	x		
Start of dietary intervention	x	x	x	x	x	x	x		
Data collection.		x	x	x	x	x	x		
Results comparison.					x	x	x	x	
Analysis of results.						x	x	x	

Obtained data record.	x	x	x	x	x	x			
Realization of statistics and diagrams.							x	x	
Elaboration of the article								x	x
Publication								x	x

8. BIBLIOGRAPHIC REFERENCES.

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