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The effects of intermittent fasting on spleen function in laboratory rat

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Part I: Introduction

The human body has developed physiological responses to lack of food in order to ensure the maintenance of its homeostasis. It adapts by reducing and changing the rate of metabolism, thus allowing vital functions to be maintained for as long as possible from the body's own reserves **(Mottet and Sierro, 2016).**

Intermittent fasting (IF) is an increasingly popular dietary practice, and its implementation is found throughout human civilisation in various cultural, spiritual and religious traditions **(Sumona *et al*., 2023)**. It has recently received increasing attention for its advantages on body health **(Moro *et al*., 2021).**

Emerging evidence has shown that the health benefits of IF stretch beyond calorie restriction and weight loss. These benefits include metabolic shifts in energy production, the optimisation of peripheral circadian clocks, and overall improvement in physiological markers of metabolic health. IF has been proposed to reduce systemic inflammation and have a role in the prevention and treatment of chronic diseases **(Sumona *et al*., 2023).**One area of great interest is the influence of intermittent fasting on the immune system, which responds to stressful and harmful events in the body **(Mindikoglu *et al*., 2020).**

More evidence has emerged from numerous in-depth studies that have clearly demonstrated that fasting can produce therapeutic effects in many non-communicable disorders such as obesity, diabetes, cardiovascular disease, cancers and neurodegenerative diseases. Fasting stimulates intestinal stem cell regeneration after a 24-hour fast in mice through metabolic changes from carbohydrate utilization to fat burning **(Mihaylova *et al*., 2018).**

In this study, we sought to assess the effects of Intermittent fasting, prolonged fasting, and colonic inflammation on the structure of the spleen in laboratory rats.

In order to appreciate the effect of intermittent fasting on the physiology of the spleen and the immune profile associated with colitis in laboratory rats, we carried out an experimental protocol of 30 days of fasting with an inflammation of the colon induced by 2,4,6-Tri-Nitro Benzene Sulfonic Acid (TNBS) 24 hours before dissection.

This dissertation is divided into three chapters:

- The first part which includes general information on fasting, the spleen and the pathophysiology of colitis.
- The second part, material and methods devoted to the description of the experimental equipment and the methods of analysis used.
- The third part, where we presented the results obtained with an interpretation followed by a discussion and conclusion.

Part II: Theoretical part

I- General information on fasting

I.1. Definition

According to the Robert historical dictionary of the French language, 2000, to fast comes from the Christian Latin word *jejunare* which means "to abstain, not to eat by act of penance". Figuratively, to fast means “to deprive oneself of, to keep away from, to abstain from all rejoicings”. Fasting, outside of the religious context, is used in the sense of "being deprived of food". From fasting is derived the masculine noun fasting (XIV century), with all the meanings corresponding to the verb.

Looking at other linguistic resources, the word fasting is invariably associated with food deprivation. This can be restrictive or partial deprivation of food with the exception most often of water or all food for a certain time **(Sanvictores *et al*., 2022).**

I. 2. Metabolism during fasting

I.2.1. Energy balance

Fasting involves a radical change in cellular physiology and metabolism. Blood glucose normally provides the body with sufficient energy through glycolysis. During a fast, maintenance of blood glucose levels initially relies on energy reserved (glycogen stored) in the liver and skeletal muscle. The expenditure and reserves of energy are major determinants during fasting **(Sanvictores *et al*., 2022).**

I.2.1.1- Energy expenditure

Most glycogen is stored in the liver, which has the greatest role in the maintenance of blood glucose during the first 24 hours of a fast necessary for the function of the body to satisfy:

-**Basal metabolism (BM):** It is the energy required under baseline conditions to fuel the activity of a set of cellular mechanisms essential to sustaining life.

- **Energy expended during physical activity:** any movement in addition to MB. This is the most variable part from one individual to another. It is determined by the intensity and the time devoted to physical activity.

- **The thermal effect of food or food thermogenesis:** energy spent to digest, metabolize and absorb food

After fasting for around 24 hours, glycogen stores are depleted causing the body to utilize energy stores from adipose tissue and protein stores.

I.2.1.2- Energy reserves

-Adipose catabolism (glycogenolysis): The drastic change in metabolism that follows glycogen depletion is primarily dependent on the metabolism of triglyceride stores in adipose tissue. Triglycerides are separated into free fatty acids and glycerol that the liver respectively converts into ketone bodies and glucose. Ketone bodies made from free fatty acids through the process of ketogenesis. These ketone bodies travel through the body and are reconverted back into acetyl-CoA at the tissues requiring energy **(Sanvictores *et al*., 2022).**

-Protein catabolism: Gluconeogenesis simultaneously takes place in times of fasting. Gluconeogenesis produces glucose from amino acids broken down from various tissues including muscle. After glycogen stores become depleted, the dependence of body tissues for glucose gradually declines as ketone bodies become more readily available to metabolize **(Sanvictores *et al*., 2022).**

The main form of storage in humans is by far triglycerides, much more important than proteins and even more so than proteins, carbohydrates. In a 70 kg male subject, the carbohydrate reserves total about 2500 kcal, stored in the muscle (400 g of glycogen), in the liver (100 g of glycogen) and in the extracellular fluid (20 g of glucose). Lipid reserves total approximately 112,000 kcal (12 kg of neutral fat), i.e. 80% of reserves, the rest being represented by proteins **(Beaufrère et Leverve, 2007)**.

II- Intermittent fasting

II.1- Definition

Intermittent fasting (IF) is an eating pattern in which individuals go extended periods with little or no energy intake after consuming regular food in intervening periods **(Simin *et al*., 2023)**.The most popular type of intermittent fasting is two days of restriction per week (5D:2D) **(Scholtens *et al*., 2020)** or time-restricted eating (16h restriction/24h) **(Pellegrini *et al*., 2020).**

II.2- Objectives

The short-term goal of intermittent fasting is weight loss, fat loss, and even appetite regulation. According to the adaptations of this type of diet, the other positive effects sought after on long-term health are a better life expectancy, the prevention of certain chronic and degenerative diseases, and better cognitive performance (**Pellegrini *et al*., 2020**). These suggested beneficial effects are now the subject of several clinical studies.

A special type of intermittent fasting occurs during Ramadan, when Muslims worldwide fast daily from dawn to sunset for a month **(Sedra *et al*., 2023)**.

III. Islamic fast

III.1- Definition

According to the Islamic lunar year, Ramadan is the 9th month and it is the 4th pillar of Islam. Ramadan fasting is a special form of intermittent fasting practiced by 1.9 billion Muslims worldwide during the holy month of Ramadan, the ninth month of the Islamic lunar calendar. Muslims fast for 29-30 days, each day starting with a meal at dawn (suhoor) and breaking the fast with dinner at sunset (iftar). Between dawn and dusk, Muslims refrain from eating, drinking water, smoking, and having sexual intercourse. This religious obligation is taken very seriously by the vast majority of Muslims and has been practiced for over 1,400 years, as it is ordained in the Quran **(Sedra *et al*., 2023)**.

III.2 - Difference between Islamic fasting and physiological fasting

Fasting in the month of Ramadan differs from physiological fasting by the length of time. The number of days compulsory for Ramadan fasting is 29 or 30 days (lunar month) while the number of days for physiological fasting varies. In Islamic fasting, Muslims refrain from consuming both food and water from sunrise to sunset. Therefore, the duration of daily fasting varies according to the time from sunrise to sunset in a particular location. In countries located near the equator, the duration of fasting is about 14 hours and the allowable feeding time is about 10 hours. However, in countries further away from the equator, the duration of fasting varies according to the season – longer in summer (more than 20 hours) and shorter in winter (less than 8 hours). Although Islamic fasting has some of the same elements as physiological fasting, there is no restriction on calorie intake. Ramadan meals may be calorie-dense, overeating may occur before bed and regular sleep patterns may be disturbed **(Faris *et al*., 2019; Suriani *et al*., 2019)**.

III.3- Effect of Islamic fasting on the metabolism and physiology of healthy subjects

Several studies have been carried out on the effects of fasting during the month of Ramadan on the health state of individuals. Ramadan fasting has demonstrated several health benefits including improving the gut microbiome and modifying gut hormone levels **(Sedra *et al*., 2023).** Effects of Ramadan fasting on weight vary between individuals, ranging from weight loss to weight gain, depending on whether or not energy intake in the non-fasting period

under- or over-compensates for the lack of energy intake during the fasting period. A meta-analysis that used data from 35 publications showed that Ramadan fasting caused a statistically significant decrease in weight (-1.24 kg by the end of Ramadan, -1.51 kg in men and -0.92 kg in women), while the weight loss observed at follow-up (2-6 weeks after Ramadan) was less pronounced, albeit weight was still statistically significantly lower compared to before Ramadan (-0.27 kg) **(Hamish *et al*., 2019).** Overall, the effects of Ramadan fasting differ in that the type, frequency, quantity and time of food consumption vary **(Zoughbie *et al*., 2023).**

IV. Fasting and scientific research

In experimental laboratory conditions, many mammals have been studied under imposed fasting, especially rodents. It is interesting to note that the duration of fasting tolerated by the rat is much shorter than in humans (8-10 days maximum) and that, in rodents, the main source of protein during the initial fast (known as the phase of short fast) is of hepatic origin, unlike the human species where the main reserves are muscular **(Lemar, 2011**).

Intermittent fasting from dawn to dusk for more than 14h per day for 30 days has been associated with an anticancer serum proteomic signature and upregulation of the expression of proteins involved in carbohydrate and lipid metabolism, insulin signaling, circadian clock, DNA repair Cytoskeleton remodeling, immune system and cognitive function. They have demonstrated that intermittent fasting from dawn to dusk can be a preventive therapy against cancer, metabolic syndrome and Alzheimer's disease and several neuropsychiatric diseases **(Mindikoglu *et al.*, 2020).**

IV.1. Effects of fasting on the immune system and inflammation

Studies have shown that fasting can restore the immune system. Fasting for at least 3 days allows the body to begin producing new white blood cells which rejuvenate the immune system to fight infection. Although white blood cell counts have been shown to decrease in humans and animals with long-term fasting, blood cells return when fed again. This way, Ramadan fasting mimicking diets for days (intermittent fasting during Ramadan, time-restricted feeding, and alternate day fasting) forces the body to consume glucose and fat stores, and a significant amount of white blood cells are broken down.

Therefore, changes in the body cause the regeneration of new cells in the immune system. The supportive results of studies have shown that. Inflammatory cytokines eg: IL-1, IL-6 and TNF-α and markers of oxidative stress and C-reactive protein (CRP) could be reduced by fasting **(Moghadam *et al*., 2021).**

Intermittent fasting has been shown to be protective against inflammation in multiple pathogenic processes **(Shuhui *et al*., 2022)**. It is also associated with the regulation of the hematopoietic system and induces a reduction in Insulin Growth-like Factor 1(IGF-1) and Protein Kinase A(PKA) which increases the self renewal of hematopoietic stem cells. Fasting also leads to an improved immune system **(Boudreau, 1994; Takakuwa *et al*., 2019)**.

A recent American study has just confirmed the benefits of intermittent fasting on the immune system. By strengthening the immune system, reducing inflammation, it would reduce the risk of chronic diseases. Prescribing an appropriate diet to patients, in addition to their traditional treatment, could therefore improve their chances of recovery **(Jordan *et al*., 2019)**.

This study looked at the effects of fasting on immune cells after periods of intermittent fasting. The researchers found that fasting reduced the number of monocytes, pro-inflammatory white blood cells circulating in the blood. Studies on the anti-inflammatory effects of fasting have enormous potential, given the wide range of pathologies caused by chronic inflammation, and the growing number of patients with these diseases. (**Jordan *et al.*, 2019**).

IV.2. Fasting and cancer

Epidemiology shows that in 2020, there was an estimated 19.3 million new cancer cases worldwide, and by 2040, the number of global cancer cases is expected to reach 28.4 million **(Sung *et al*., 2021).** The anti cancer effects of IF have been demonstrated through extensive animal studies, wherein IF inhibited tumor growth by impeding glucose acquisition by the tumors for a short period **(Simone *et al*., 2013).**

Fasting protects the hematopoietic system and induces a reduction in Insulin-like Growth Factor-1 (IGF-1) and Protein Kinase A (PKA) which increases the self-renewal of hematopoietic stem cells. The typical nutritional plan in Ramadan may have beneficial influences on the inflammatory state by decreasing inflammatory factors and strengthening the immune system **(Cheng *et al.*, 2014)**.

Fasting selectively protects normal cells against oxidants and certain chemotherapeutic agents and sensitizes cancer cells (**Brandhorst et Longo, 2016**; **Zhang *et al*., 2020**).

Preclinical and initial clinical data indicate that fasting or fasting-mimicking diets reduce levels of nutrients and tumor growth-promoting factors, including glucose, IGF1, and insulin. Fasting may cause an anti-Warburg effect by reducing glucose uptake via glucose transporters and aerobic glycolysis and by forcing cancer cells to increase oxidative phosphorylation; it increases the production of free radicals in the cancer cell, and consequently, oxidative DNA damage, activation of p53, cell death, especially in response to chemotherapy

In mouse models, IF improved the fix act of chemotherapy regimens used to breast, melanoma, neuroblastoma, pancreatic and colorectal cancers and reduced the harm caused by conventional therapies in humans **(Simin *et al*., 2023).**

IV.3- Effects of fasting on inflammatory bowel disease

IV.3.1- Inflammation

Inflammation is a natural response process of an organism in the face of an aggression. However, when it is too important or inappropriate, it can be the cause of pathological phenomena. Many regulatory pathways can modulate, directly or indirectly, the inflammatory response. Among them, the regulatory pathways of cellular metabolism appear more and more clearly involved in the control of the inflammatory status of many immune cells. A booming field of research, immunometabolism could reveal the pathophysiological mechanisms at the origin of the onset of many diseases (**Liang *et al*., 2020**).

IV.3.2- Colitis

IV.3.2.1- Experimental models of ulcerative colitis

Trinitrobenzene sulfonic acid (TNBS) elicits cell-mediated immune responses and induces transmural inflammation in the gut with morphological and histopathological features similar to human IBD **(Cheon *et al*., 2012; Almeida *et al*., 2013).** TNBS induces diffuse colonic inflammation, characterized by increased leukocyte infiltration, edema and ulceration **(Isik, 2011)**.

IV.3.3. Inflammation mediators

Several cytokines such as the interleukin IL-2, IL-5, IL-6 and IL-12, TNF-α and IFN-γ (interferon gamma), which are present in the microenvironment, induce local secretion of chemokines and adhesion molecules allowing the recruitment of inflammation cells, lymphocytes, macrophages and monocytes. These also secrete other chemokines inducing the recruitment and activation of resident immune cells, neutrophils and natural killer cells, then cytotoxic T cells specific for the alloantigen, in the injured sites **(Guerrot *et al*., 2012).**

Often very hydrophobic into a water-soluble molecule that is easier to remove. Phase III consists of the active excretion of the latter, using transmembrane transporters, either in the blood stream in order to be eliminated at the renal level, or in the bile to be eliminated via the faeces, after secretion of the bile in the intestine **(Modica *et al*., 2009; Sendensky and Dufour, 2011).**

IV.4. - Role in the immune system

Immunity is defined as resistance to disease, the set of cells, tissues and molecules that contribute to oppose resistance to infections and called immune system, and the coordinated reactions of it is cells and molecules against pathogenic germs is called immune response. The physiological function of the immune system is to prevent infections and eradicate reported infections **(Abbas *et al.*, 2009**).

V. Spleen

V.1- Definition

The spleen is the largest organ of the lymphatic system positioned between the fundus of the stomach and the diaphragm in the left hypochondriac region of the abdominal cavity, relatively below the left costal margin between the ninth and 11th ribs. The spleen is spongy and appears reddish purple on account of it being densely vascularized. A healthy spleen is usually not palpable in most individuals. It is encased in a weak outer connective tissue capsule which allows for protection and also the expansion of the organ and is subdivided into many smaller internal sections termed lobules **(Chaudry *et al*., 2022).**

V.2- Anatomy of the spleen

The spleen has an anterior and posterior segment and rests on the upper pole of the left kidney and tail of the pancreas. The spleen has 3 distinct borders: superior, inferior, and intermediate. The superior border of the spleen has a notch on the anterior end. The spleen has 2 surfaces, the visceral and diaphragmatic. The latter surface is convex and smooth, whereas the former

surface is concave and irregular with several imprints. The most concave imprint on the spleen is a resultant of the fundus of the stomach. The left kidney leaves an imprint on the intermediate and inferior borders. The colic imprint is from the splenic flexure of the colon. The splenic hilum is found on the inferomedial aspect of the gastric imprint. The splenic hilum contains nerves, splenic vessels, and also contains attachments for the splenorenal and gastrosplenic ligaments. It is roughly the size of an individual's fist, measuring about 10 cm to 12 cm and weighing about 150 g to 200 g.

Two types of splenic compartments are distinguished macroscopically:

Red pulp: It is composed of splenic cords (Cords of Billroth) and a large volume of venous sinuses. The splenic cords provide the organ structure through reticulin and fibrils. The cords also contain a reservoir of monocytes to aid in wound healing. Splenic cords lead to splenic sinuses where macrophages respond to antigens and filter abnormal or aging erythrocytes out of blood flow. The red pulp tissue is involved more so with the filtering aspect of the blood. The red pulp removes old, damaged, and/or useless red blood cells. Contained within the red pulp are also WBCs, particularly phagocytes (macrophages in particular) which destroy microorganisms such as viruses, bacteria, and fungi. The red pulp also acts as a storage area for WBCs and platelets, which are typically released to injury sites to aid in healing and inflammation regulation or to assist in blood loss compensation. The white and red pulp regions are separated by a border known as the marginal zone which functions as a filter, filtering pathogens out of the blood and into the white pulp **(Chaudhry *et al*., 2022).**

A thin, fibrous capsule covers the spleen from which trabeculae arise. Trabeculae are fibrous bands transporting blood vessels to and from the splenic pulp **(Vaishali *et al*., 2023).**

White pulp: It is composed of lymphatic tissue surrounding a central arteriole and contains mainly white blood cells that are involved in the initiation of the adaptive immune response. The white pulp tissue is involved with the production and maturity of WBCs, particularly lymphocytes (types B and T) and thereby the production of antibodies. The innermost area of the white pulp, the germinal center, contains B-cells while the surrounding marginal zone contains T-cells.

The marginal zone is surrounded by a periarteriolar lymphoid sheath (PALS), which also contains T-cells. White pulp throughout the spleen is surrounded by red pulp **(Vaishali *et al*., 2023).**

V.3- Function

The spleen is the only lymphoid organ that lies directly in the path of the blood circulation. Due to its heterogeneous structure, the spleen performs a variety of immunological and hematological functions. It plays a key role in both the innate and adaptive immune systems, thereby protecting the body from invading organisms **(Faryal *et al*., 2020).**

The spleen has several functions, including the filtering of blood, removing microbes and inadequate red blood cells (RBCs), producing white blood cells (WBCs), and antibody synthesis. It is important to note, that while the spleen does have a wide range of functions, it is not a vital organ. Individuals can survive without a spleen as other organs of the body, such as the liver, can adapt in its absence to serve just about the same functions.

V.4- Physiology of spleen

In the red pulp filtration of erythrocytes and platelets occurs via splenic cords. Young, flexible red blood cells pass through the epithelial cells of the splenic cords and continue through blood flow. On the other hand, older, larger, and deformed red blood cells are trapped by the splenic cords and phagocytized by macrophages waiting on the reticulum and sinus endothelium. Furthermore, splenic macrophages in red pulp are specialized to recycle iron from the breakdown of senescent and damaged red blood cells. Macrophages can either store ingested iron in the cytoplasm or export it via ferritin into the bloodstream **(Vaishali *et al*., 2023).**

Not only does the spleen play a role in the breakdown of red blood cells, but it can also play a role in hematopoiesis. While not a typical function, in pathologic conditions, such as beta-thalassemia major, extramedullary hematopoiesis may occur to help the bone marrow compensate for the hemolysis taking place. Infection prevention occurs by two major mechanisms: phagocytic filtration of the blood stream and production of opsonizing antibodies. As mentioned above, macrophages supervise the flow of red blood cells, platelets, as well as microorganisms through the splenic cords. Additionally, in the follicle of the white pulp, infectious antigens and blood-borne pathogens are presented by antigen-presenting cells. This process initiates the activation of T-cells and B-

cells, which eventually leads to the production of opsonizing antibodies. After opsonization, macrophages, dendritic cells, and neutrophils phagocytose the antigen. Opsonization is essential to clear particular microorganisms like encapsulated bacteria and intra-erythrocytic parasites **(Vaishali *et al*., 2023).**

As a reservoir for blood, the spleen weights about 100 g. The organ can respond to sympathetic stimulation by contracting its fibroelastic capsule and trabeculae to increase systemic blood supply. In particular, this vital function takes place during hemorrhage. About 25% to 30% of red blood cells (RBCs) are stored in the spleen, along with about 25% of platelets normally sequestered in the spleen **(Vaishali *et al*., 2023).**

V.5- Physiopathology

- **Splenomegaly**

Splenomegaly is the abnormal enlargement of the spleen with a length greater than 10 cm. This irregularity can result from multiple types of mechanisms: hypertrophy, infiltration, congestion, myeloproliferative, and neoplastic. RBC work hypertrophy suggests increased normal splenic function by filtering large amounts of abnormal erythrocytes from the circulation. Examples of this type include hereditary spherocytosis and sickle cell anemia.
Congestive splenomegaly is secondary to obstruction of blood flow and, therefore, engorged with blood in the red pulp. Myeloproliferative disorders include chronic myeloid metaplasia. Neoplastic origins include chronic lymphocytic leukemia. Splenomegaly may be idiopathic or secondary to an underlying disease **(Vaishali *et al*., 2023).**

- **Extravascular hemolysis**

Hemolysis of red blood cells can classify as intravascular or extravascular. As the name implies, intravascular hemolysis is the breakdown of red blood cells in vessels. Extravascular hemolysis is the breakdown of red blood cells in the reticuloendothelial system, such as the spleen and liver. In extravascular hemolysis, it is the macrophages that perform the hemolysis. Patients with extravascular hemolysis present with **splenomegaly** secondary to splenic hypertrophy and jaundice due to the increased levels of unconjugated bilirubin from broken down blood cells.Conditions that can cause extravascular hemolysis include sickle cell disease, hereditary spherocytosis, hemoglobin C, malaria, IgG immune hemolytic anemia, and beta-thalassemia major. Treatment

typically focuses on the underlying cause, with splenectomy providing a cure in some cases **(Vaishali *et al*., 2023)**.

Hyposplenia/Post-Splenectomy/Asplenia Immunization and Antibiotic prophylaxis

For any patient with splenic dysfunction, they must receive prophylactic vaccination against encapsulated organisms, such as *Streptococcus pneumoniae, Neisseria meningitides,* and *Haemophilus influenzae*. Due to the increased risk of infection patients should receive yearly influenza vaccinations as well. Because vaccinations do not cover all serotypes of the organism, many patients receive either daily prophylactic antibiotics, usually penicillin, or an emergency supply should they develop a fever **(Vaishali *et al*., 2023).**

Portal Hypertension

Portal hypertension is a result of increased pressure in the portal venous system, which drains the stomach, intestine, spleen, and pancreas. As portal hypertension worsens, the venous congestion of the splenic vein can cause splenomegaly. The spleen undergoes hyperplasia and fibrosis, which further increases the blood supply to the spleen, exacerbating the condition **(Vaishali *et al*., 2023).**

Part III :
Material and Methods

The present study aims to research the effect of intermittent fasting, prolonged fasting and inflammation in the spleen. It is carried out at the level of the Research Laboratory on Arid Zones (LRZA, USTHB and headquartered at the University of Algiers 1), and the University M'Hamed BOUGARA, Boumerdes during from March to June 2023.

I. Material

I.1.Animals

Our study is based on 36 *Wistar rats*, of which the average weight is between 180g and 190g. Before any experimentation, the animals are kept for one week in the animal facility or the temperature (24±1ºC).

I.2.Apparatus and reagents

II- Evaluation of the anti-colitis activity induced by TNBS

II.1- Constitution of groups of animals

The experiment was carried out at the animal facility of M'Hamed BOUGARA University, Boumerdes, for 30 days on 36 adult rats. The individual identification of the rats is done by marking at the level of the tail using a permanent marker. For the fasting group, the deprivation of food and water takes place every day between 3:00 p.m. and 8:00 a.m**.**

II.2- Distribution of groups

The 36 rats are initially subdivided into 3 groups :
- Control group
-Fasting group
-Prolonged fasting group

II.3 - Evaluation of weight parameters

To study the effect of fasting on the evolution of body weight and feeding behaviour in laboratory rats, we performed weekly weighing of body weight, quantity of food ingested and volume of water consumed for 30 days of experimentation.

II.4. Preparation and intra rectal administration of TNBS

The model used to study the immunopathogenesis of inflammatory bowel disease is 2, 4,6-Tri Nitro Benzene Sulfonic Acid (TNBS)**,** which induces severe colonic inflammation when administered intra rectally.

We prepared a solution composed of 2g of TNBS powder diluted in 10 ml of 50% ethanol. After 30 days of intermittent fasting, from the 12 animals from each group, 6 underwent the administration of TNBS. All 18 rats (6 control rats, 6 fasted rats and 6 prolonged fasted rats) starved overnight. 24 hours before the dissection the rats received 1 ml of TNBS (0.2 g/ml) intra rectally. The instillation is carried out by a 1ml syringe surmounted by a needle. The animals were then held in a head-down position for 20 seconds to limit the expulsion of the solution.

II.5. Histological study of the spleen

The histological study consists in studying the histology of the spleen at the microscopic level and allows understanding their normal or pathological functioning, in which the samples are passed through the following stages:

1. Fixation

The purpose of this step is to immobilize the structures, to eliminate the risk of retraction and distortion, to protect against bacterial attacks and to oppose autolysis (enzymatic action). There are several fixing agents but formalin remains the best fixative because it can fix large parts without dissection and penetrates quickly into the tissues. The parts are put in histo-cassettes by writing above the corresponding numbering (organ, animal, date), then putting them back in 10% formalin while waiting to pass them to dehydration.

2. Washing

Remove the fixed pieces and put them under tap water for at least three hours, then cut 3 to 6 1cm fragments and put them back in their cassette.

3. Inclusion in paraffin

This process has four steps:

- **Dehydration:** This step ensures the elimination of water from the fabrics in order to prepare it for inclusion in a hydrophobic medium, each piece must pass once in each alcohol bath of increasing concentration (60°, 70°, 90°, 95° and 100 °).
- **Impregnation by the intermediate liquid (lightening):** This step allows the complete elimination of traces of alcohol and the impregnation in two baths of intermediate liquid (toluene) and lasts at least two hours.
- **Waxing :** The purpose of this step is to obtain as complete an impregnation of the parts as possible with the paraffin. The part is successively passed through 2 paraffin baths lasting 15 minutes for the first bath and 12 hours for the second bath at the melting temperature (58°C).

- **Coating:** This step is performed in a device set at 55°C. Once the histo-cassette is well immersed in paraffin, the sample is placed in a mold which contains a thin layer of paraffin, then the cassette is put back and covered with paraffin**,** then it is left to cool in order to place it on a cooling plate in order to unmold it**.**

4. Microtomization and gluing of sections on slides

Once the sample is unmolded, first it goes to a coarsening of 20µm with a Leica type microtome in order to remove any excess paraffin, then thin sections of 05µm are made, the sample is deposited on slides, followed by spreading in hot water to remove paraffin wrinkles, and placing the slides on a hot plate at 41°C, to melt the paraffin , and finally they are put in the oven at a temperature of 58° C overnight.

5. Staining:

The purpose of staining is to make the different cellular and tissue constituents more evident. This is achieved with the help of the topographic staining: hematoxylin eosin. The sections will only be ready to receive the dyes after the following two steps:

- **Dewaxing :** Dewaxing removes the paraffin from the tissue so that the dyes can penetrate. The reagent used is toluene. The sections are passed through 2 baths of toluene for 7 min and 5 min gradually.

- **Hydration:** Its purpose is to remove the toluene from the tissue and replace it with water. The slides are passed through three alcohol baths, one at 100° and the other at 90° then 70° for a duration of 1 min each. The slides are finally washed in distilled water for 3 min before staining.

- **Staining**

Hematoxylin eosin staining: It is a topographic histological staining whose nuclei are colored blue-black, the acidophilic cytoplasm pink, some secretions remain colorless.

- ***Mounting*****:** Mounting is the operation which consists of preserving the staining using the Eukitt which allows adhesion between the slide and the coverslip. After mounting, the slides are dried on absorbent paper, then observed under a light microscope.

6. Taking of pictures

Pictures were taken through the use of a camera integrated into the microscope (Optika)**.**

II.6. Statistical analysis

For each group we calculated the arithmetic mean and the standard error of the mean **(**mean ± sem).

- Arithmetic mean of individual values

$$\overline{\mathbf{X}} = \frac{\Sigma x_i}{n}$$

Σx_i: Sum of individual values
n: Number of values

- **Standard Error of the Mean (SEM)**

$$\mathbf{SEM} = \frac{\sigma}{\sqrt{n}} \quad \text{with} \quad \boldsymbol{\sigma} \text{ (deviation)} = \sqrt{\frac{\Sigma (x_i - \overline{\mathbf{X}})^2}{n - 1}}$$

x_i: Individual values n : Sample number

The difference between two compared means is statistically significant if the probability *p* read as a function of the number of degrees of freedom is less than 0.05. Thus, the degree of significance is as follows:

- If $p > 0,05$: the difference is not significant (NS)
- If $p < 0,05$: the difference is significant (*)
- If $p < 0,01$: the difference is very significant (**)
- If $p < 0,001$: the difference is highly significant (***)

Differences were considered significant when $p<0.05$. Statistical tests were performed using *Graph Pad Prism software version 5* (Graph pad *software*, San Diego, CA), using the "one-factor ANOVA" test using the Bonferonni test, in order to study multiple comparisons.
The histograms were made using Excel 2016 software and word processing with Word 2016.

Part IV:Discussion

In our study, we analyzed the presence and the modifications of the effect of intermittent fasting for one month and prolonged fasting for 48 hours and thus the inflammation at the level of the parenchyma of the spleen.

This work consists of an examination of the scientific evidence relating to the beneficial effects of intermittent fasting on the prevention of several diseases, particularly colitis. The works reported on the preventive therapy of fasting will be recalled briefly and discussed according to the literature. The cellular and molecular mechanisms involved in the action of fasting, in particular those associated with the immune system, are also discussed.

Fasting is invariably associated with food deprivation. This can be restrictive or partial deprivation of food with the exception most often of water or all food for a certain time **(Sanvictores *et al*., 2022).**

The analysis of histological sections of the organ studied in fasting rats reveals more or less significant structural alterations in the splenic parenchyma which manifest themselves differently depending on the effect of intermittent fasting, prolonged fasting and also inflammation.

The morphometric analyses are made using the Cell target and Axiovision softwares. The splenic parenchyma shows a proliferation of the white pulp in rats that were put on intermittent fasting and it becomes more pronounced with prolonged fasting while the surface area of the red pulp decreases. We note that the hyperplasia of the white pulp is manifested by an increase in the number and volume of splenic nodules, the growth in diameter of the germinal center and lymphatic follicles and their number. We also note an increase in lymphocyte levels at the follicular level while there was a decrease in the red blood cell count. These signs of the effect of fasting and inflammation revealed an increase in the cell- and humoral-mediated immune response.

The spleen is a large secondary lymphoid organ located in the blood stream, primarily playing the role of a massive blood filter. It filters effete red blood cells, antigen-antibody complexes, apoptotic bodies and damaged cells**.**

The spleen’s myriad microanatomy reflects the diversity and complexity of its functions. The spleen is an important immune effector organ since it orchestrates innate and adaptive immune responses such as pathogen clearance, cytokine production, and differentiation of cells, therefore playing a modulatory role that balances pro- and anti-inflammatory responses **(Mota *et al*., 2022).** Specialized adaptative immune cells such as B cells, natural killer cells, and macrophages populate locations within the spleen. Specifically, the spleen is a crucial organ for peripheral immune tolerance and complementing central immune tolerance. As such, the spleen provides the right site to thwart autoimmunity **(Aliyu *et al*., 2021).**

Further research is needed to find which cells or pathways are linked to favorable outcomes regarding fasting and colon inflammation. Understanding the microanatomy and physiology

of the spleen will provide a framework for the treatment of diseases, especially autoimmune diseases, cancer, infections, and transplantation.

Variations of fasting have been studied for their ability to improve physiological indicators related to health. Some of these factors include insulin sensitivity, blood pressure, atherogenic lipids, body fat, and inflammation **(Terrence *et al*., 2022**). By itself, fasting can, for example, reduce the risk of suffering from chronic diseases, reduce inflammation, slow down the body's aging processes, help regain insulin sensitivity or stimulate autophagy, a process which allows the body to recycle its old cells to make new, healthier ones (**Valter, 2018**).

According to a study carried out by a group of researchers from the University of Southern California, fasting for 48 to 120 hours or 2 to 5 days would allow the immune system to regenerate completely, and this would be valid for everyone, young or old, healthy or sick. For researchers, fasting would push the body to begin a process of regeneration (**Valter, 2018)**.

Professor **Valter Longo**, who led the study, said: The good news is that the body gets rid of damaged or old and ineffective parts of the immune system during fasting. And if you are talking about a system heavily damaged by chemotherapy or aging, fasting cycles can create, quite literally, a new immune system. During fasting, the body therefore undertakes the destruction of all deficient cells, seeking them where they are, thereby including the immune system **(Valter, 2018)**.

Inflammatory bowel disease (IBD) comprises Crohn’s disease (CD) and ulcerative colitis (UC). CD and UC are chronic and relapsing inflammatory conditions of the gastrointestinal tract that have distinct pathological and clinical characteristics. **(Inés *et al*., 2022**)

Aging and chemo-therapeutic agents are known to damage hematopoietic stem cells (HSCs), leading to dysregulation of cell division and subsequent immunosuppression. For this prolonged fasting is used as a medical intervention to decrease IGF-1/PKA signaling and protect HSCs against chemo-therapeutic toxicity and promote rejuvenation **(Cheng *et al*., 2014)**.

The intermittent fasting approach has been hypothesized to positively affect inflammatory status and reduce inflammation in animal models. In fasting animals (eg: rats), there has been a tendency for the intestinal lining to regress, thus the villi are digested within a few days. During refeeding, the intestinal surface and its villi are replenished in record time **(Zhang *et al*., 2020).** Because during prolonged fasting organisms minimize energy expenditure in part by rapidly reducing the size of a wide range of tissues, organs, and cellular populations including blood cells, the reversal of this effect during re-feeding represents one of the most potent strategies to regenerate the hematopoietic and possibly other systems and organs in a coordinated manner. **(Cheng *et al*., 2014)**.

This review has shown that intermittent fasting has a significant intestinal anti-inflammatory effect in an experimental colitis model in rats, which resembles human ulcerative colitis. This activity could be associated with the immuno-modulation effect of intermittent fasting on experimental colitis to improve the body's defense system **(Zhang *et al.*, 2020)**.

These authors report that intermittent fasting had beneficial effects in improving gut function by reducing oxidative stress, gut inflammation, and altering the diversity of the gut microbiome. These modifications consist of reversing the pathological development of colitis by improving the integrity of the intestinal barrier and the length of the colon and also attenuating the behavior of anxiety and obsessive-compulsive disorders linked to colitis **(Zhang *et al.*, 2020)**.

Numerous studies demonstrated that 24 hours of caloric restriction in rats triggers the flipping of a metabolic switch, which would stimulate the regeneration of intestinal stem cells, a theory that Randle and colleagues in 1963 proposed, a theory of energy metabolism. Mihaylova's team sequenced messenger RNA from stem cells of fasting rats, and found that fasting activated transcription factors called Peroxisome Proliferator-Activated Receptor, or PPAR), which activate genes involved in fatty acid metabolism. In this case, this activation induced cells to break down fatty acids instead of glucose, while simultaneously increasing their ability to regenerate. When the researchers blocked the activation of the PPAR, they could see that the regeneration boost had ended, but that's not all. By treating rats with a molecule called GW501516, which activates the effects of PPARs, they were able to replicate some of the beneficial effects of fasting in rats. **(Mihaylova *et al.*, 2018)**

On the other hand, fasting selectively protects normal cells against oxidants and certain chemotherapeutic agents and sensitizes cancer cells. It will cause changes at the molecular level a drop in blood sugar, insulin like growth factor-1 (IGF-1) and insulin levels and an increase in sensitivity to insulin, corticosteroids and insulin-like growth factor binding proteins (IGF-BP) which will be captured by cell receptors and modulate signaling pathways. Inhibition of nutrient-dependent signaling pathways and activation of stress resistance pathways are then observed, with the aim of cell survival **(Brandhorst and Longo, 2016)**.

All of this research reveals that intermittent fasting has beneficial effects on health and mainly on the immune system and contributes to the maintenance of homeostasis by promoting the establishment of the body's protective mechanisms by increasing the production of stem cells in particular those of the intestine and bone marrow and causes an improvement in the defense of the immune system by its anti-inflammatory and anti-colitis power.

These data probably announce the emergence of new therapeutic strategies for the management of chronic metabolic, autoimmune or inflammatory diseases by intermittent fasting.

Part VI: Conclusion

Conclusion

This study shows that intermittent fasting has beneficial health effects mainly on the immune system, contributes to the maintenance of splenic homeostasis and shows a significant improvement in the defense of the immune system through its anti-inflammatory power.

This data encourages the emergence of new therapeutic strategies for the management of metabolic, autoimmune or chronic inflammatory diseases associated with splenic function by intermittent fasting.

Our results showed that intermittent fasting leads to the following changes:

- Reduction of body fat mass

- An increase in the rate of splenic B and T lymphocytes

- A proliferation of the surface of the white pulp and a reduction of the splenic red pulp

- Increase in lymphatic follicle diameter and also the germinal center of the spleen

- Decreased splenic red blood cell count

All of these observations reveal that intermittent fasting and prolonged fasting have beneficial effects on health and mainly on the immune system and contribute to the maintenance of homeostasis by promoting the establishment of the body's protective mechanisms by increasing the production of hematopoietic cells in the spleen and causing an improvement in immune system defense.

In addition, it would therefore be interesting to continue this work with a:

− Biochemical study of oxidative stress

− Studies of molecules involved in inflammation

− Study of the cellular and molecular mechanisms of the effect of fasting on the spleen.

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Abstracts

Effects of intermittent fasting on spleen structure in colite status

Abstract

This study consists of a scientific synthesis relating to the beneficial effects of intermittent fasting and prolonged fasting on the histology of the spleen during colitis. Fasting means food deprivation for a period of time. Intermittent fasting is fasting of altered periods with periods of normal eating. Fasting has a beneficial effect on the metabolism, and leads to a reduction in body weight in overweight subjects. It protects the hematopoïetic system by stimulating the self-renewal of hematopoïetic stem cells and the strengthening of immune cells. Our results have shown that intermittent fasting leads to a reduction in body fat mass, increased levels of splenic B and T lymphocytes, proliferation of the surface of the white pulp and a decrease in the splenic red pulp. Fasting improves immune defense system response and lessens the severity of inflammation in the colon.

Key words: Intermittent fasting, spleen, structure, inflammation, colon

Effet du jeûne intermittent et jeûne sur la structure splénique au cours de la colite

Résumé

Ce travail consiste en une synthèse scientifique relative aux effets bénéfiques du jeûne intermittent et jeûne prolongé sur l'histologie de la rate au cours de la colite. Le jeûne signifie la privation alimentaire pendant un certain temps. Le jeûne intermittent est un jeûne de périodes altérées avec des périodes d'alimentation normale. Le jeûne a un effet bénéfique sur le métabolisme, et entraine une réduction du poids corporel chez les sujets en surpoids. Il protège le système hématopoïétique en stimulant l'auto-renouvellement des cellules souches hématopoïétiques et le renforcement des cellules immunitaires Nos résultats ont montrés que le jeûne intermittent entraine une réduction de la masse adipeuse corporelle, augmentation du taux des lymphocytes B et T spléniques, proliferation de la surface de la pulpe blanche et une diminution de la pulpe rouge spléniques. Le jeune améliore la réponse du système de défense immunitaire et atténue la gravité de l'inflammation du côlon.

Mots clés : Jeûne intermittent, rate, structure, inflammation, côlon,.

تأثير الصيام المتقطع على فيزيولوجيا و بنية الطحال

ملخص:

يتزايد انتشار الصيام في علاج أمراض التمثيل الغذائي والالتهابات. أظهرت العديد من الدراسات والابحاث أن الفوائد الصحية للصيام المتقطع تقلل إنتاج الجذور الحرة أو فقدان الوزن، وتحسين تنظيم الجلوكوز، وزيادة مقاومة الإجهاد، وقمع الالتهاب. تم التوصل الى أن الصيام المتقطع له آثار مفيدة في تحسين وظيفة الطحال واستجابته المناعيه . أظهرت نتائج البحث أن الصيام المتقطع يؤدي إلى انخفاض كتلة الدهون في الجسم ، وزيادة مستوى الخلايا الليمفاوية B و T في الطحال ، زياده مساحه اللب الأبيض وانخفاض في اللب الأحمر في الطحال. الصيام المتقطع هو الصيام الذي يمنع الحالة الصحية وبشكل رئيسي على الجهاز المناعي ويساهم في الحفاظ على التوازن من خلال قوته المضادة للالتهابات وتأثيره المفيد على الفيزيولوجيا النسيجية للطحال.ان النظام الغذائي له تأثير عميق على تجديد الأنسجة في الكائنات الحية المتنوعة ، وحالات السعرات الحرارية المنخفضة لها آثار مفيدة على صحة الكائنات الحية ووظائف الطحال.

الكلمات المفتاحية : الصيام المتقطع، الطحال، البنيه ، التهاب، القولون