**PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA**

**وزارة التعليم العالي والبحث العلمي**

**MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH**

**جامعة الإخوة منتوري قسنطينة ا**
**Frères Mentouri Constantine I University**
**Université Frères Mentouri Constantine I**


**University of Frères Mentouri Constantine**
**Faculty of Life and Natural Sciences**

**كلية علوم الطبيعة والحياة**
**قسم بيولوجيا الحيوان**


**Thesis submitted for the degree of Master II**

**Faculty: Life and Natural Sciences**

**Domain: Biological Sciences**

**Option: Immunology Molecular and Cellular**

**N° d'ordre :**
**N° de série :**

Topic :

# Interaction between hot water and high consumption of crystallize sugar in cardiovascular disease


**Presented by:** *ABDERRAHMANE Khitem*
*SERAOUI Rayene*
*NASRI Bouchra*


**Le 13/07/2022**

**Examination board:**

**Supervisor: Pr. ZERIZER S. (Prof**- Université Frères Mentouri, Constantine 1)

**Examiner1:Dr. MESSOUDIS**. (**M.C.A** - Université Frères Mentouri, Constantine1)

**Examiner2 :Dr. RAMLI I.** (**M.A.A** - Université Frères Mentouri, Constantine 1)

***Year***
***2021/2022***

# Acknowledgement

*We wish to express our deepest gratitude to our supervisor* ***Pr. ZERIZER S.****, who has patiently guided the production of this work. We own her a particular debt for her precious advices, insightful criticism and patient encouragement.*

*To all the members of the jury, we thank you for the honor you dous by agreeing to judge this work. Special thanks for* ***Dr. RAMLI Imene*** *for agreeing to chair the jury, and* ***Dr.MESSAOUDI Saber***

*For agreeing to examinate this work,* ***Mme BOUALI Kh*** *and* ***Dr. BAHRIEl-eid*** *for they helps in the animals house.*

*Finally, we extend our most sincere thanks to all our friends and families, who have always supported and encouraged us during the realisation of This Thesis.*

# Dedication

- *I thank **Allah almighty** for having given me the privilege, the chance to study, follow the path of science and who granted me the ability to accomplish this work.*
- *Thanks to My parent's **Bellala Fatiha** and **Seraoui El-hacen** for their help, who have always loved and supported me.*

❖ *To Who the Rahman said for them:*

**(وَٱخۡفِضۡ لَهُمَا جَنَاحَ ٱلذُّلِّ مِنَ ٱلرَّحۡمَةِ وَقُل رَّبِّ ٱرۡحَمۡهُمَا كَمَا رَبَّيَانِى صَغِيرًا)**

- *Special thanks to my sisters **Linda**, **Khadidja** and **Houria**, who have been a constant source of support and encouragement. I'm really grateful to have them in my life. And big thanks to my aunts **Malika**, **Habiba**, **Nadjat** and **Hayat** for their love and encouragement.*
- *Thanks to my brothers and their wifes who I love so much, **Mouhamed** and **Daoud.***
- *To my sweethearts **Baraà**, **Iyad**, **Nadjem el-din**, **Alaà**and my littles babys **Amira**,**Ghaidaà**,**Adem** and my dear **Mouad**, I wish you all the best and every success.*
- *Thanks to **Rofaida** and **Randa** for being my friends and partners, thanks for your help and for all good and bad moment we lived together.*
- *Thanks To my colleagues **Khitem** and **Bouchra**, for this work.*

***Rayene***

# Dedication

*I thank **Allah** almighty for having given me the privilege, the chance to study and follow the path of science, as I always wanted and desired..*

*I dedicate this work to my mother **ABDERRAHMANE DALILA** for her never ending love.*

*I will always be grateful to my father **ABDERRAHMANE ABDELHAMID** for his confidence and his unconditional support.*

*To my other mother **ABDERRAHMANE SALIMA** to my second father **ABDERRAHMANE YACINE** Thank you for everything.*

*To all my brothers and sisters **MOHAMED, LYES, MOHAMED, KHADIDJA, ROKIA, AMAR, SOUMIA, LILIA, BACHIR, AMINA, MARWA, YACINE, CHARAF, ADEM, YOUCEF** your unconditional help with this work and never stop believing on me.*

*To **KADI SAADEDDIN**, thank you for being in my side encouraging me to keep working, to never give up and to finish this work, I would never have done it without you.*

*To all of my nephews and niece **MALAK, IMAD, MOHAMED, HIBA, CHIHAB, NIHAL, DAYAA, AYA, ALAA, MARIEM, ABDELMOHAIMAN(MADJED)** Thank you for bringing laughter on my face and taking the stress off my shoulders by your spontaneity and nice being.*

*Thanks and appreciations to my Second family Mr. **ABDELHAMID KADI** and Mrs. **SAMIA FAHEM.***

*To all of my friends and partner **SOUAD, KENZA, YOUSRA, AYA, RANIA, CHAIMA, AMINA, BOUCHRA, RAYENE**...*

*To all my friends and family, to everyone who has been beside me during this whole journey.*

***KHITEM***

# Dedication

*THANKS TO **ALLAH** WHO HAVE LIGHTENED MY PATH AND WHO GRANTED ME THE ABILITY TO ACCOMPLISH THIS WORK AND THE FORCE TO OVERCOME ALL DIFFICULTIES.*

*This thesis work is dedicated to my parents **HALIMI FARIDA ADRAA** and **NASRI MOUSSA LAZHAR**, who have always loved me unconditionally and whose good examples have taught me to work hard for the things that I aspire to achieve.*

*Special thanks to my husband **BOUBAKHA SABER**, who has been a constant source of support and encouragement during the challenges of graduate school and life. I am truly thankful for having you in my life.*

*To my little baby my lovely daughter my dear and sweetheart **ZEINEB** who was my companion in all this journey. I know you will be proud of your mom.*

*I am very fortunate and grateful to my brothers **ABDERRAHMANE, MOUHAMED YACINE** and **KHALED** for encouraging me to continue in this work. Thank you for your support and helpful suggestions.*

*Thank you **KHITAM & RAYENE** for being my friends and partners thank you for your help and for all good and bad moment we leave together.*

***BOUCHRA***

## Table of contents



# Bibliographic part

## Chapter 1: Inflammation



## Chapter 2:Excessive sugar consumptionand inflammation

## Chapter 3: Excessive sugar consumption and cardiovascular disease



## Materials and Methods

## Results and discussion

## List of abbreviations

**AI**: Atherogenic index

**ALT:**Aspartate aminotransferase

**AST:** Aspartate aminotransferase

**BSA:**Bovine Serum Albumin

**CCT:**Cardiac computed tomography

**CDV:**Cardiovascular disease

**CMR:**Cardiac magnetic resonance

**CRP:** C-reactive protein

**DTNB:** 5, 5′-dithiobis-2 nitrobenzoicacid

**EDTA:** Ethylene diamine tetra acetic acid

**ENOS:** Endothelial nitric oxide synthase

**FcγR:**Fcγ receptors

**GSH:** Glutathione reduced

**HDL:** High density lipoproteins

**Hs-CRP:** High-sensitivity C - reactive protein

**IFNs:**Interferon-s

**IFN-γ:** Interferon-γ

**IL-1:**Interleukin 1

**IL-8:**Interleukin 8

**IL-6:**Interleukin6

**LDL:** Low density lipoprotein

**PAMPs:** Pathogen-associated molecular patterns

**PBS:** Phosphate buffered saline

**TBS:** Tris buffered saline

**TCHOL:** Total cholesterol

**TG:** Triglycerides

**TGF-β1:** Transforming growth factor-β1

**TNFα:** Tumor necrosis factor

**VEGF:**Vascular endothelial growth factor

**VLDL:** Very low density lipoprotein

## List of figure

## List of Tables

# Introduction

## Introduction

Sugar is term of all mono and disaccharides that are composed ofeither six- or five-ring sugar molecules. This is a subclassof carbohydrates that differentiates ‘sugar’ from small chains of carbohydrates (oligosaccharides) and complexcarbohydrate made up of long chains of sugar molecules **(Thornley et al., 2012)**.“Table sugar” is essentially pure sucrose, whereas fruit juice, honey and syrups contain mixtures of sucrose, glucose and fructose, and often oligosaccharides of different size. These compounds are invariably combined as “sugars” **(Polly et al., 2020)**.

Sugar is considered as one of the most elemental nutrients for humans **(Jana et al., 2015)**.That why there is debate concerning the relationship between dietary sugar intake and other health outcomes such as obesity, type 2 diabetes and cardiovascular disease **(Janette et al.,2021)**.Thereis arise in cardiovascular disease incidencedue to increased consumption of sugar **(Thornley et al., 2012)**.

There no firm conclusion that dietary sugars are directly related to diet-related diseases other than as a source of dietary energy particularly implicated as a contributor towards positive energy balance **(Janette et al .,2021)**.

Furthermore complications of atherosclerosis are the most common causes of death in Western societies. It is alreadyconsidered as a chronic inflammation resulting from interaction between modified lipoproteins, monocyte-derived macrophages T cells, and the normal cellular elements of the arterial wall. This inflammatory process can ultimately lead to the development of complex lesions, or plaques, that protrude into the arterial lumen **(Christopher, 2001)**.

Hot water helps melting the fat deposits, purifies the toxin and destroys harmful bacteria in our body. It is the most important catalyst in losing weight and can also help the gastrointestinal tract to function even better. Reduce obesity, and stroke, gastroenterisis, heart disease and cured high blood cholesterol **(Alhajri, 2010)**.

In the present research we aimed to:

• Evaluate the effect of hot water on the weight and diet of rats.

• Evalute the effect of hot water on high consumption of crystallizes sugar by measuring the levels of T-Ch, HDL-c, LDL-c, TG and liver enzymes.

• Evalute the effect of hot water on the inflammation by measuring C-reactive protein induced by high consumption of crystallize sugar

• Analyse the relationship between high consumption of crystallize sugar and cardiovascular disease.

# Chapter 1

# Inflammation

# I. Inflammation

## 1. Definition

The Word inflammation comes from the Latin "inflammo", meaning "I set alight, I ignite» **(Christian, 2015)**, it is derived from the Latin "inflammare" meaning « to burn ». Inflammation is a vital process in response to injury or infection that is followed by a sequence of events to respond to the wound healing or infection **(Nahrendorfet al., 2007).**

At the biological level, inflammation is suspected mainly by an increase in the reactive protein C or CRP and the increase in the amount of white blood cells reflects the implementation of mechanisms to fight it (**Hordece, 2014).**

Inflammation is an essential immune response that enables survival during infection or injury and maintains tissue homeostasis under a variety of noxious conditions. Inflammation comes at the cost of a transient decline in tissue function, which can in turn contribute to the pathogenesis of diseases of altered homeostasis (**Medzhitov, 2010**).

## 2. Types of inflammation

### A. Acute inflammation

Acute inflammation is an immediate, adaptive response with limited specificity caused by several noxious stimuli, such as infection and tissue damage (**Medzhitov, 2008**). It is starts after a specific injury that will cause soluble mediators like cytokines, acute phase proteins, and chemokines to promote the migration of neutrophils and macrophages to the area of inflammation (**Germolec et al., 2018**).

Acute inflammation evolves into prolonged subacute and chronic inflammation when the initial pathogenic agent persists in the tissues, or when acute inflammation recurs repeatedly in the same organ, leading to less and less repaired tissue destruction at each episode(**Figure1**) (**Zerbato, 2010**).

### B. Chronic inflammation

Chronic inflammation is also referred to as slow, long-term inflammation lasting for prolonged periods of several months to years. Generally, the extent and effects of chronic inflammation vary with the cause of the injury and the ability of the body to repair and overcome the damage (**Pahwaet al., 2021**).

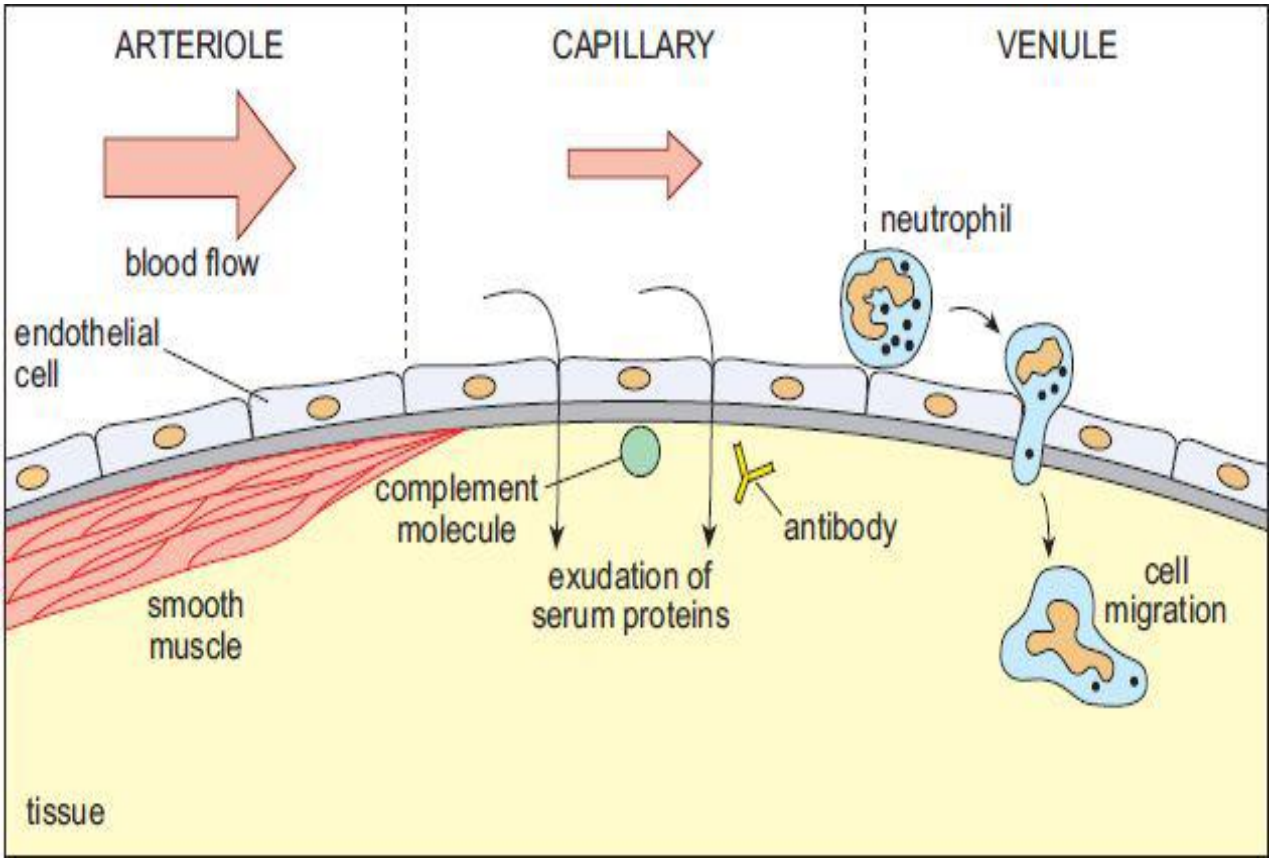


**Figure 1:** The stages of acute inflammation; vessel vasodilatation, exudate formation and neutrophil migration **(1).**

## 3. Causes

The causes or inducers of inflammation can classify into two main groups: exogenous and endogenous inducers (**Medzhitov, 2008**).

### A. Exogenous inducers

This grouping can further subdivide into two classes; microbial and non-microbial exogenous inducers.

#### i.Microbial inducers

There are two classes of microbial inducers. The first class is pathogen-associated molecular patterns (PAMPs), which are carried by all microorganisms. The second class is virulence factors restricted to pathogens. Virulence factors trigger the inflammatory response due to the effects of their activity. Examples includeenzymatic activity produced by helminths and exotoxins produced by bacteria, which will be sensed by known or unknown sensors (**Medzhitov, 2008**).

#### ii. Non-microbial

Causes include allergens, toxic compounds, irritants, and foreign bodies that are too large to be digested or cause phagosomal damage in macrophages. Exemples of foreign bodies include silica and asbestos.

### B. Endogenous inducers

These are signals released by tissues that are either dead, damaged, malfunctioned, or stressed.

The inflammatory inducers are divided to infections and non-infections factors.

#### i. Infections factors

This category includes bacteria, viruses, and other microorganisms.

#### ii. Non-infections factors

This group can be due to physical injuries such as frostbite, burn, physical injury, foreign bodies, trauma, ionizing radiation, chemical compounds such as glucose, fatty acids, toxins, alcohol, and chemical irritants such as nickel and other trace elements. Apart from that, there are also biological inducers, including signals released by damaged cells and physiological due to excitement (**Chen et al., 2018**).

## 4. Symptoms

The reaction of inflammation is typically manifested by 4 clinical signs:

- Redness.
- Pain.

- Swelling.
- Increased heat **(Figure2)** (**Hordece, 2014).**

# II. C-reactive protein

## 1. Definition

C-reactive protein (CRP) is an acute-phase serum protein and a member of the pentraxin protein family and is a phylogenetically highly conserved (**Terry, 2004**). It's have a host defense functions predate the adaptive immune system by millions of years. Our current understanding of CRP interactions with complement and with Fcγ receptors (FcγR) have led to an increased appreciation of the regulatory role of CRP in inflammation and autoimmunity (**Steven et al., 2004**).

C-reactive protein (CRP) is a pattern recognition molecule, binding to specific molecular configurations that are typically exposed during cell death or found on the surfaces of pathogens. Its rapid increase in synthesis within hours after tissue injury or infection suggests that it contributes to host defense and that it is part of the innate immune response. Recently, an association between minor CRP elevation and future major cardiovascular events has been recognized, leading to the recommendation by the centers for disease control and the American heart association that patients at intermediate risk of coronary heart disease might benefit from measurement of CRP (**Steven et al., 2004**).

C-reactive protein (CRP) is the prototypical acute phase protein in humans. Tillet and Francis discovered CRP over 70 years ago in the blood of patients with Streptococcus pneumoniae infection (**Lorraine et al., 2005**).

## 2. Structureof the C-reactive protein

The three-dimensional structure of CRP has been determined by X-ray crystallography (**Terry, 2004**).

CRP consists of five identical, non-covalently associated 23-kDa protomers arranged symmetrically around a central pore, each containing 206 amino acid residues (**Mark et al.; 2003**). The term "pentraxins" has been used to describe the family of related proteins with this structure (**Steven et al., 2004**) (**Figure3**).

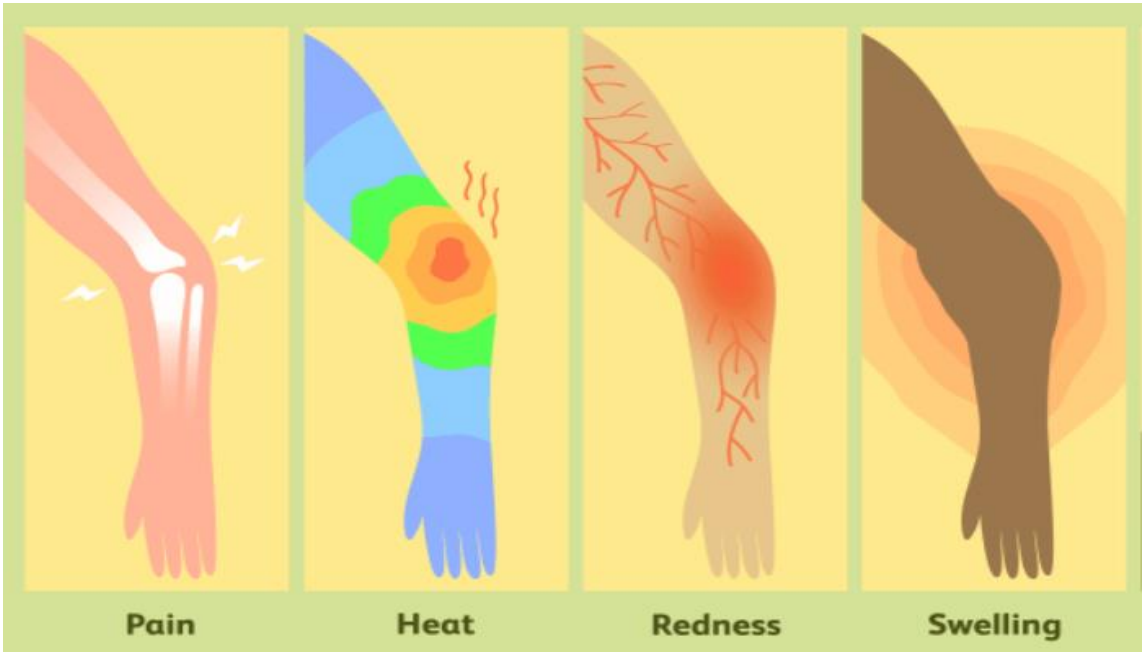


**Figure2:**The symptoms of inflammation (**Barhum, 2022**).

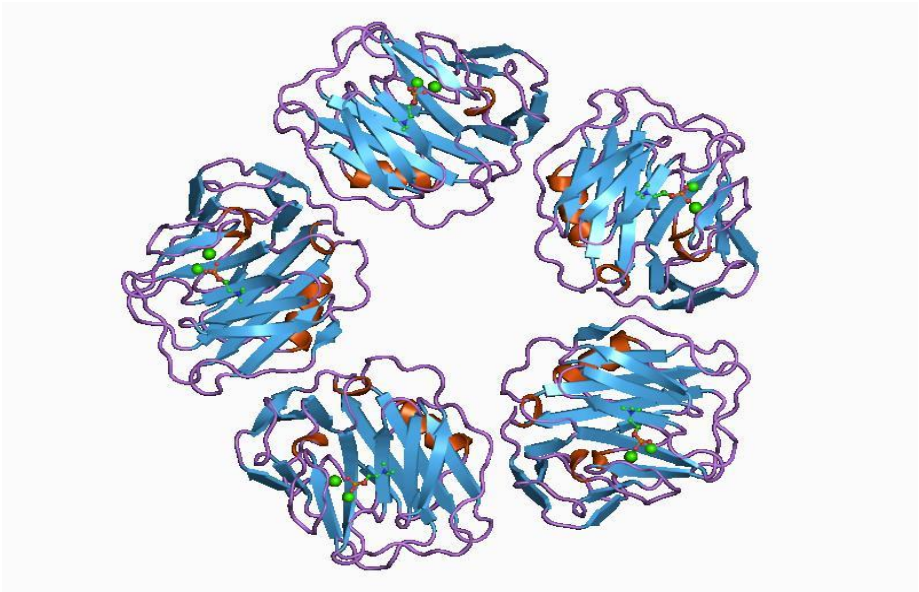

**Figure 3:** Crystal structure of C-reactive protein complexed with phosphocholine **(2).**

## 3. Dosage of the C-reactive protein

The CRP test is simple, fast (result in less than one hour) *(***Boufedeche et al., 2017***)*. It is a marker of inflammation. The standard test is used to assess an individual's inflammatory status. A version of the test called hs-CRP (high sensitivity C-reactive protein) is used only to assess the risk of cardiovascular disease**(In site3)**.

## 4. Functionof the C- reactive protein

C-reactive protein (CRP) plays an essential role in the recognition of self and foreign molecules. This interaction leads to an activation of the adaptive immune system early in the course of an inflammatory or infectious process. Through interaction with the complement system and Fc receptors on phagocytic cells, CRP plays a direct role in the clearance of these molecules (**Terry, 2000**).

C-reactive protein (CRP) is synthesized very rapidly and appears at very high levels when the inflammatory response is reaching its peak (**Terry, 2004**).

# Chapter 2
# Excessive sugar consumption and inflammation

# I. Sugar

## 1.Definition of sugar

Sugar, which refers usually to sucrose, is a natural and nontoxic, sweet testing, water soluble crystalline carbohydrates, and every 1 gram of sugar provide body 4K.calories. The main source for sugar is the beet sugar or cane sugar. At last years, the increase of consuming sugar leads to several disease especially obesity, cardiovascular disease and diabetes type 2 **(Margaret et al., 2018)**.

Sugars are simple carbohydrates and are important for everyday life biological functions such as providing energy for running vital roles of the living body. The majority of the natural sugars contain 6 or 12 carbon atoms in their molecules. Sugars are crystalline, soluble in water and generally have a sweet taste. The commercial sugar is the disaccharide sucrose white sugar **(Mohammad and Klein, 2011).**

In general, sugar is used as a preservative, a viscosity-enhancing agent, a sweetening agent, and for other reasons in foods and beverages (**John and White, 2018)**.

It is the generic name for all sweet-tasting soluble carbohydrates, many of which are used in food. Table sugar (or granulated or regular sugar) refers only to one form, sucrose that is a disaccharide (twin molecule) composed of a combination of the two common carbohydrates of glucose and fructose (**Spencer, 2021**).

## 2. Carbohydrates

### 2.1. Definition

Carbohydrates are the main source of energy that is ingested by the human body (**Jéquier, 1994**).Structurally carbohydrates are polyfunctional Compounds. They contain two types of functional groups hydroxyl and carbonyl. They may be polyhydroxy aldehydes or polyhydroxy ketones. The formula for carbohydrates is $(CH_2O)_n$ **(Mondal, 2017).**

### 2.2. Chemical structure of carbohydrate

Carbohydrates chains come in different lengths, and biologically important carbohydrates belong to three categories **(Raven et al., 2014):**

The simplest forms are monosaccharides.

The double-molecules of sugar (or disaccharides) are composed of two monosaccharides, joined by a single covalent glycosidic bond.

There are other longer chains of monosaccharides but they are not called sugars but oligsaccharides that are often used as markers or signal molecules or when many MS join together form a polysaccharide (**Spencer, 2021**).

### A.Monosaccharides

Monosaccharides consists of six carbon atoms **(Wakim and Grewal, 2021)**, are simple sugars, the most common of which is glucose. Monosaccharides have a formula of$(CH_2O)_n$.

Sugars are also named according to their number of carbons, some of the most common types are trioses (three carbons), pentoses (five carbons), and hexoses (six carbons) **(Figure 4) (Mondal, 2017).**

### B.Disaccharides

Disaccharide have 12 carbon atoms, and their chemical formula is $C_{12}H_{22}O_{11}$**(Neuman, 2013).**

The most common disaccharide is sucrose, or table sugar, which is composed of the monomers glucose and fructose. Other common disaccharides include lactose and maltose **(Figure 5) (Wakim and Grewal, 2021).**

### C.Polysaccharides

Polysaccharides are composed of chemically bonded monosaccharides, they are considered as vital bio-macromolecules for all living organisms, which are structurally comprised of homo or hetero monosaccharides and uronic acids connected with glycosidic linkages **(Zhang and Wang, 2015).**

## 2. Relationship between excessive sugar consumption and inflammation

A high sugar diet can have harmful effects on health, such as increasing the risk of chronic diseases, weight gain, and tooth decay. It can also result in chronic inflammation, where the body's immune system activates, resulting in damage to healthy cells.

Sugar stimulates the production of free fatty acids in the liver. When the body digests these free fatty acids, the resulting compounds can trigger inflammatory processes (**Marengo, 2019**).

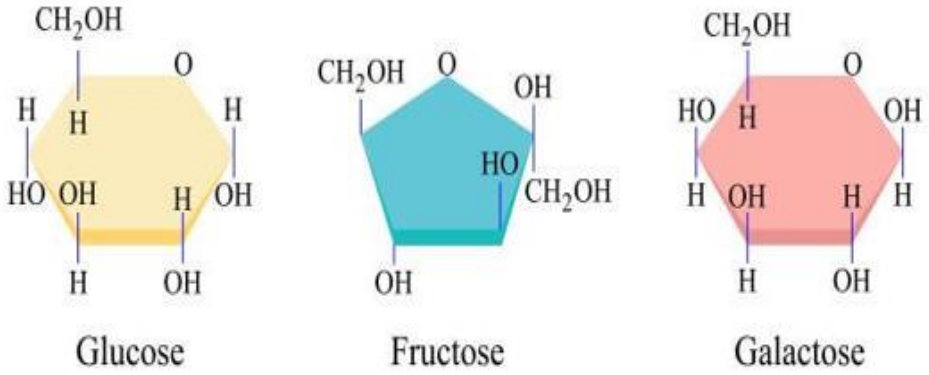


**Figure 4:** A diagram of the monosaccharide (Glucose, Fructose, and Galactose) **(4).**

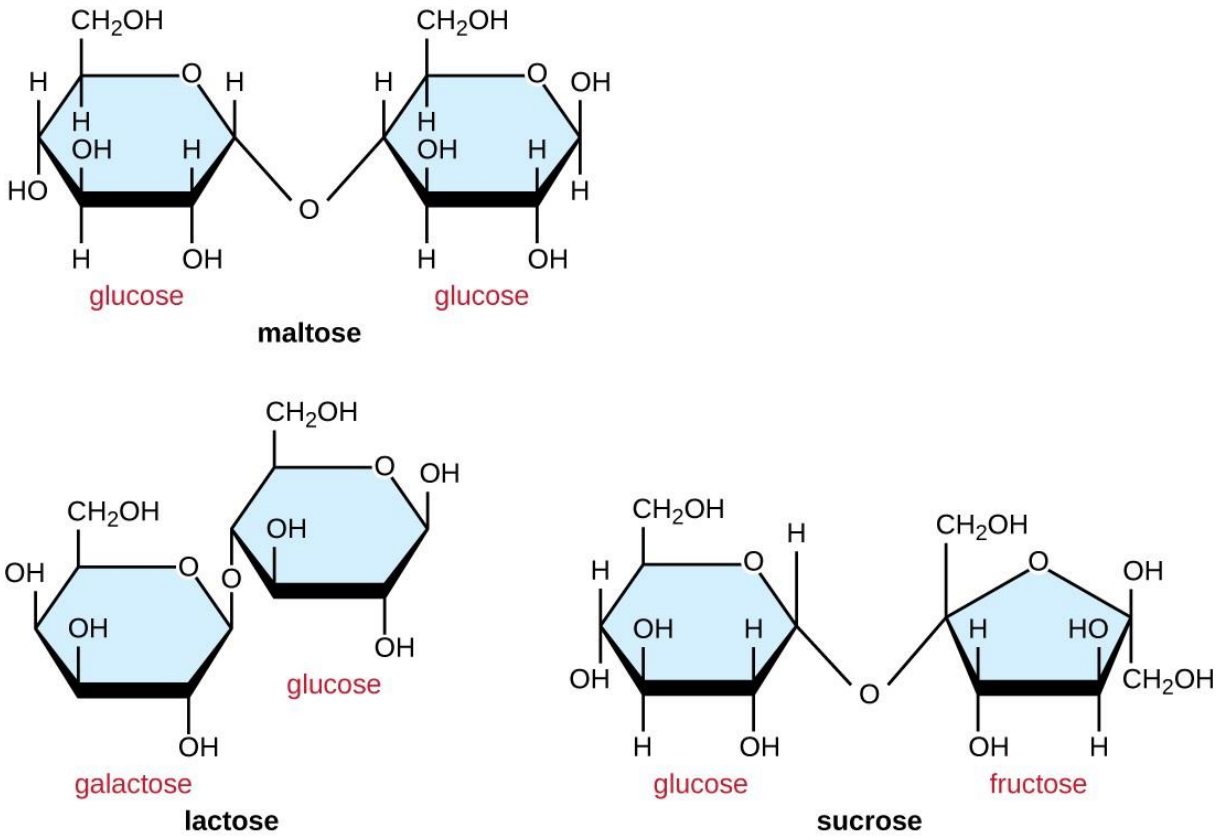


**Figure 5:** A diagram of the disaccharide (Maltose, Lactose, and sucrose) **(5).**

## 4. Inflammatory cytokines

The immune system produces cytokines and other humoral factors to protect the host when threatened by inflammatory agents, microbial invasion, or injury. In some cases this complex defense network successfully restores normal homeostasis, but at other times the overproduction of immune regulatory mediators may actually prove deleterious to the host**(Hopkins, 2003).**

There are much kind of cytokines shawn in the **(Table 1)** which are:

- **Interleukines**: That are able to promote cell growth, differentiation, and functional activation. Seven interleukins have been described; each has unique biological activities as well as some that overlap with the others **(Mizel, 1989).**
- **Chemokines:**That are essential in the recruitment of circulating leukocytes to inflammatory sites, taking part both in leukocyte trafficking and leukocyte activation. Chemokines are also involved in angiogenesis and tumor cell proliferation. They contribute to the differentiation and proliferation of hematopoietic progenitors **(Samson and Aubry, 1999).**
- **Tumour necrosis factors:**thatareimportant cytokines involved in physiological processes, systematic inflammation, tumor lysis, apoptosis and initiation of the acute phase reaction **(Chu, 2013).**
- **Interferons:** that are now recognized as central regulatory mediators of the immune response. The functions of IFNs are represented by three major biological activities: antiviral activity, antitumor activity and immune regulatory activity **(Chelbi and Wietzerbin, 2007).**
- **Growth factors :** are members of a family of polypeptides that are potent regulators of cell proliferation, differentiation, and function, These proteins play crucial roles in normal development in the maintenance of tissues, and in wound healing and, and they have also been implicated in a wide range of pathological conditions, including tumorigenesis and metastasis. They act on cells of meso-, ecto-, and endo-dermal origin, and they cause changes in migration, morphology, and function as well as proliferation(**Galzie et al., 1997**).

**Table 1** : Cytokines families**(Hopkins, 2003).**

| Family | Examples | Activities |
|---|---|---|
| Interleukins | IL-1α IL-1β, IL1ra and IL-2 – IL-26 | Regulation of innate and adaptive immunity |
| Chemokines | IL-8, monocyte chemotactic factor | Leukocyte chemotaxis and activation |
| Tumour necrosis factors | TNF-α and TNF-β | Similar to IL-1 1 tumour cytotoxicity |
| Interferons | IFN-α and β<br>FN-γ | Anti-viral<br>Immunoregulatory |
| Colony stimulating factors | Granulocyte-SF, macrophage-SF, IL-3, 5, 6 and 7 | Myelopoiesis |
| Neurotrophins and neuropoietins | Ciliaryneurotrophic factor, nerve growth factor, IL-6 | Neuronal growth and differentiation. |
| Growth factors | Fibroblast growth factor<br>Endothelial cell growth factor | Regulation of cell growth |

### a. Role of cytokines

Cytokines are a crucial component of immune response, as they aid in the intercellular communication and are involved in both health and disease, through their physiological and pathological effects. They are involved in mediating natural immunity and regulating the activation, growth, and differentiation of lymphocytes, leukocytes, as well as other cell types. Furthermore, they play a role in the regulation of immune-mediated inflammation and stimulate hematopoiesis. Their effects are mediated via cell surface receptors. Based on their physiological and pathological effects, they are ideal therapeutic agents, either by themselves or as antagonists, which also include specific monoclonal antibodies directed against them (**Khan, 2016).**

### b. Relationship between cytokines and cardiovascular disease

Complex cellular and inflammatory interactions are involved in the progress of vascular diseases. Endothelial cells, upon exposure to cytokines, undergo profound alterations of function that involve gene expression and *de novo* protein synthesis. The functional reprogramming of endothelial cells by cytokines is of importance especially in patients with chronic vascular inflammation. The intercellular network of dendritic cells, T-lymphocytes, macrophages and smooth muscle cells generates a variety of stimulatory cytokines [e.g. TNF-α (Tumor necrosis factor-α), IL-1 (interleukin), IL-6 and IFN-γ (interferon-γ)] and growth factors that promote the development of functional and structural vascular changes. High concentrations of pro-inflammatory cytokines increase oxidative stress, down-regulate ENOS (endothelial nitric oxide synthase) bioactivity and induce endothelial cell apoptosis. Chemoattractant cytokines [e.g. VEGF (vascular endothelial growth factor), TGF-β1 (transforming growth factor-β1) and IL-8] are important regulators of inflammation-induced angiogenesis and are directly modulated by nitric oxide. This review will focus on the vascular mechanisms orchestrated by cytokines and summarizes the current knowledge concerning the contribution of cytokines to cardiovascular diseases **(Kofler et al., 2005).**

**Table 2** : Inflammatory cytokines **(Hopkins, 2003).**

| | TNF-α | IL-1 | IL-6 |
|---|---|---|---|
| Eicosanoid and Nitric Oxide Induction | + | ++ | - |
| Endothelial cell activation | + | ++ | + |
| Proteolytic enzyme induction | + | ++ | - |
| Cytokine induction | + | ++ | - |
| T lymphocyte activation | | ± | + |
| B lymphocyte differentiation | - | ± | + |
| Acute phase protein induction | ± | + | ++ |
| Corticosteroid induction | ± | + | + |
| Fever | ± | + | + |
| Cachexia/anorexia | + | - | + |
| Myelopoiesis | ± | + | ++ |

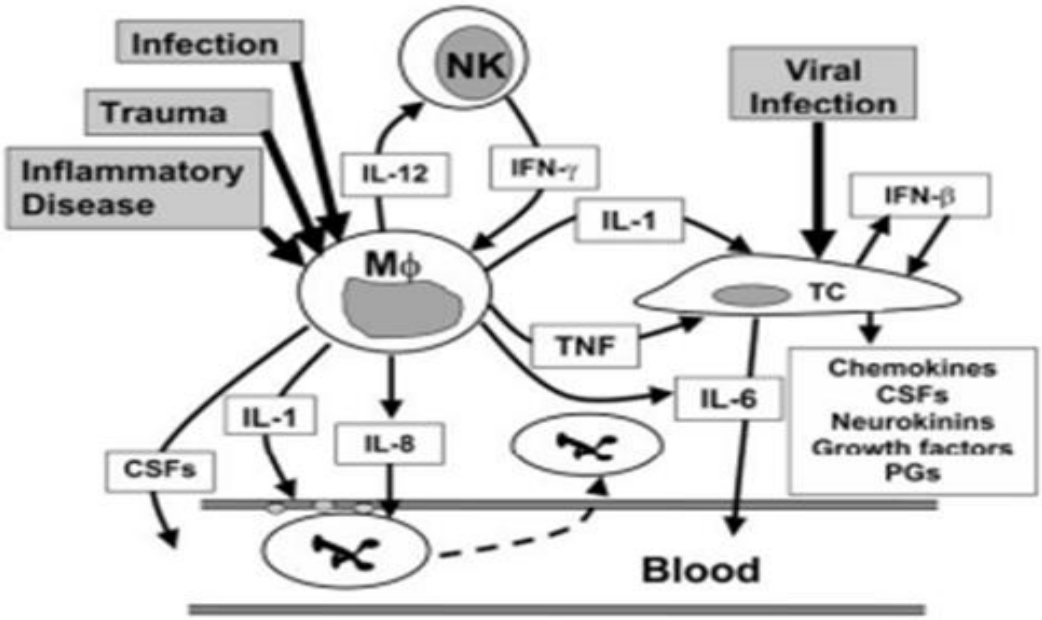


**Figure 6:** Tissue cytokine induction. This diagram represents the network of cytokine production in tissues, following typical injurious stimuli. Macrophages (Mf) and other tissue cells respond to a variety of stimuli (large arrows), by production of a variety of cytokines (small arrows). Cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-a (TNF), produced by tissue macrophages, as well as bacterial and viral products themselves may stimulate further cytokine production by other tissue cells, such as fibroblasts and endothelial cells. IL-1 and TNF also potently induce synthesis of non-cytokine inflammatory mediators such as prostaglandins (PGs). Viral infection may also lead directly to production of interferon (IFN)-a by macrophages and IFN-b by tissue cells. Colony stimulating factors (CSF) and IL-6 represent major cytokines entering the blood stream **(Hopkins, 2003).**

# Chapter 3
# Excessive sugar consumption and cardiovascular disease

# I. Cardiovascular

## 1.The heart

### 1.1. Definition

The heart is a muscular pump, which serves two functions: collect blood from the tissues of the body, pump it to the lungs, collect blood from the lungs, and pump it to all of the tissues of the body. The human heart lies in the protective thorax, posterior to the sternum and costal cartilages, and rests on the superior surface of the diaphragm (**Weinhaus, 2015**).

The heart is a fibromuscular organ in the shape of an irregular cone. Situated between the right and left pleural sacs, in the middle mediastinum **(Figure 7)** (**Vishy, 2008**).

### 1.2. Anatomy of the heart

#### a. The chambers of the heart

The heart possesses a 'fibrous skeleton' that provides anchorage for the myocardium of the cardiac chambers and for the cusps of the heart valves. The fibrous skeleton of the heart, composed of dense collagen, is a conjunction of four fibrous rings and the contiguous parts of the interatrial and interventricular septa (**Vishy, 2008**).

The heart consists of four chambers, each containing an atrium and a ventricle. The right side is responsible for collecting oxygen- poor blood and pumping it to the lungs. The left side is responsible for collecting oxygen-rich blood from the lungs and pumping it to all tissues in the body. Within each side, the atria are the sites where blood collects and passes through to the ventricles and then they contract to eject the final volumes of blood into the ventricles. The ventricle is much stronger, and it is a site for the pumping of blood out and away from the heart **(Figure 8)** (**Weinhaus, 2015**).

#### b. The heart valve anatomy

The four cardiac valves lie behind the body of the sternum along a line that is nearly vertical. The location of the valves is the pulmonary valve, aortic valve, mitral valve and tricuspid valve. Normal heart sounds are the result of abrupt apposition of valve cusps at the time of valve closure. Each of the four valves projects its closure sound with maximal intensity to a defined and distinct area over the anterior chest wall (**Vishy, 2008**).

- **Aortic valve**

The aortic valve is composed of three symmetric semilunar- shaped cusps, and each cusp acts like an upside-down parachute facing into the aortic artery .blood is pumped from the left ventricle. When the ventricle relaxes in diastole, blood is prevented from flowing back into the ventricle by the aortic semilunar valve (**Weinhaus, 2015**).

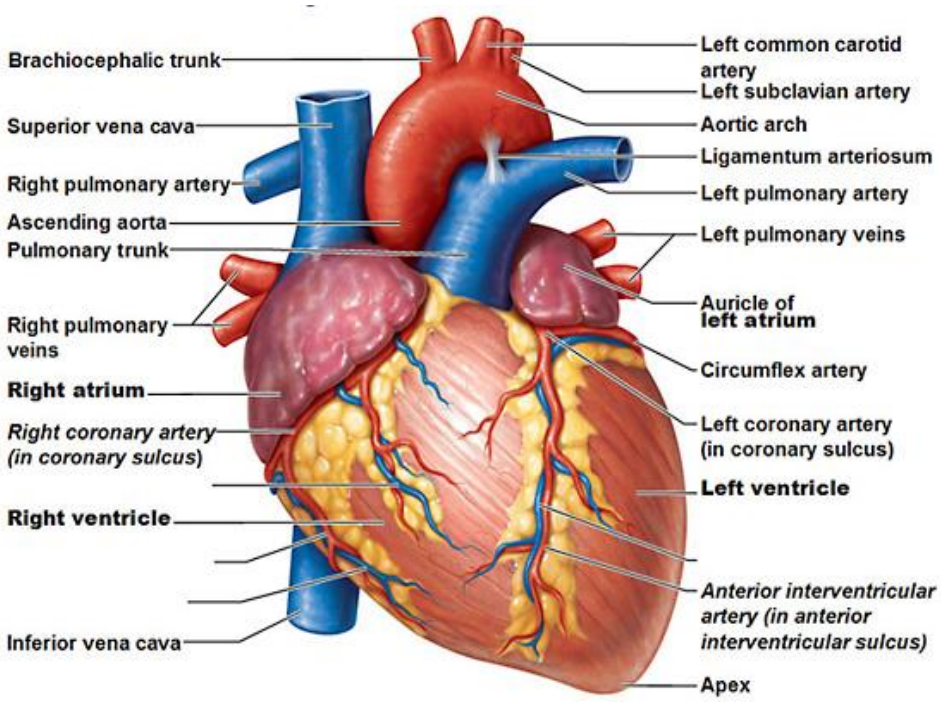


**Figure 7:** Anatomy of the heart **(6).**

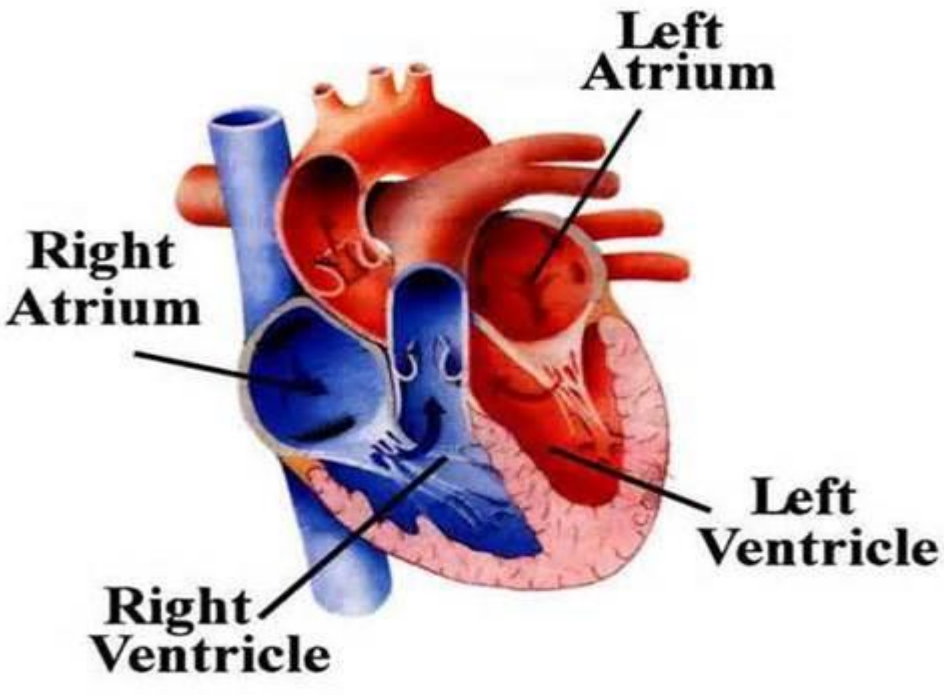


**Figure 8:** The chambers of the heart **(7).**

- **Tricuspid valve**

The valve consists of the annulus, three valvularleaflets, three papillary muscles, and three sets of chordae tendineae Blood is pumped from the right atrium. When the right ventricle contracts, blood is prevented from flowing back into the atrium by the tricuspid valve (**Weinhaus, 2015**).

- **Pulmonary valve**

The semilunar valve is composed of three symmetric semilunar-shaped cusps. Each cusp looks like a cup composed of a thin membrane. Blood is pumped from the right ventricle. When the ventricle relaxes, in diastole, blood is prevented from flowing back into the ventricle by the pulmonary semilunar valve (**Weinhaus, 2015**).

- **Mitral valve**

The valve consists of the annulus, two leaflets, two papillary muscles, and two sets of chordae tendineae. Blood is pumped from the left atrium, when the left ventricle contracts, blood is prevented from flowing back into the atrium by the bicuspid valve **(Figure 9)** (**Weinhaus, 2015**).

**c. Pericardial membrane**

The pericardium is the covering around the heart. It is a serious membrane, composed of two distinct but continuous layers. The part of the pericardium that is in contact with the heart is called the visceral pericardium or epicardium. The part of the pericardium forming the outer border is called the parietal pericardium (**Weinhaus, 2015**).

- **Anatomy of pericardium**

The pericardium is composed of visceral and parietal components. The visceral pericardium is a mesothelial cell monolayer that adheres firmly to the epicedium, reflects over the origin of the great vessels, and, fibrous tissue that envelops the heart. The pericardial space is enclosed between these 2 layers and normally, contains up to 50 mL of pericardial fluid (**Brian, 2017**).

Pericardial fluid is largely a plasma ultra-filtrate, but may include myocardial interstitial fluid and lymph drainage. Pericardial fluid volume is greatest over the atrioventricular and interventricular grooves (**Brian, 2017**).

The thickness of the pericardium varies by region (0.8–1.0 mm thick on anatomic specimens) and is slightly greater on imaging studies (0.7–1.2 mm by cardiac computed tomography [CCT] and 1.5 to 2.0 mm by cardiac magnetic resonance [CMR]) **(Figure 10)** (**Brian, 2017**).

**d. Layers of the heart walls**

The heart wall consists of three layers enclosed in the pericardium

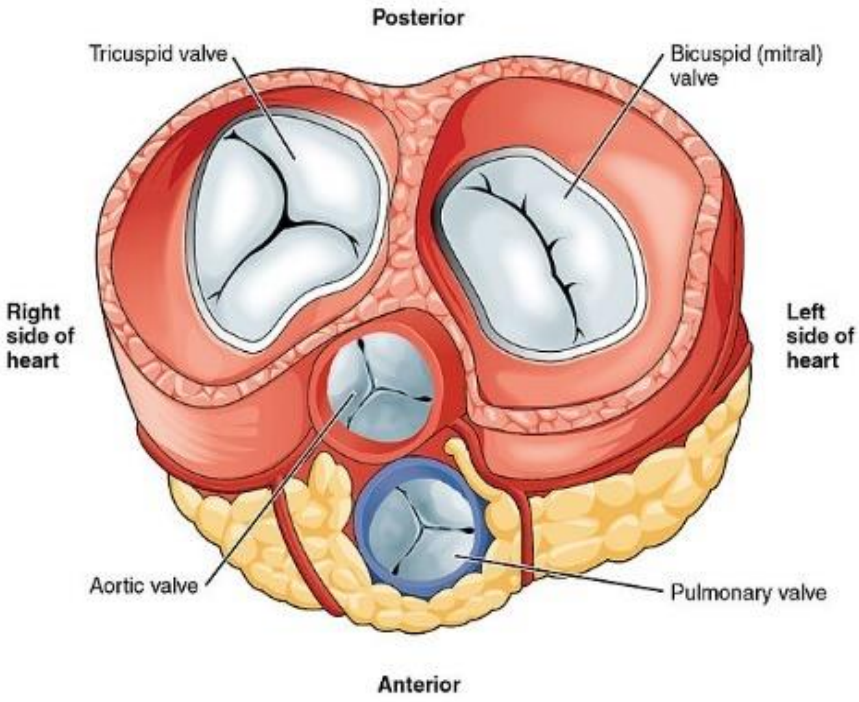


**Figure 9:** Valves of the heart **(8).**

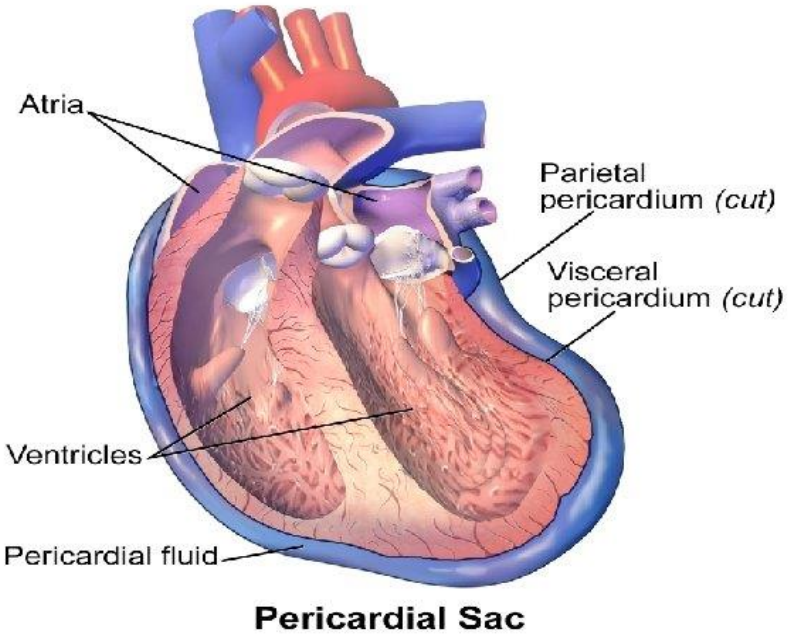


**Figure 10:** Anatomy of pericardium **(9).**

- **Myocardium**

The ventricular myocardium is represented by 3 layers of cardiomyocytes. Outside, subepicardial layer contains cardiomyocytes, forming longitudinal beams, are found in the middle layer of circularly directed beams, innesubendocardial layer containing slightly obliquely orientedbundles of cardiomyocytes. The outer and inner layers of the longitudinal direction belongs to both ventricles (**Global, 2017**).

- **Epicardium**

Epicardium is bundles of collagen and elastin fibers lie longitudinally, and have a greater packing density than the bundles of collagen and elastic fibers endocardial (**Global, 2017**).

- **Endocardium**

Endocardium isthe inner layer of heart comprises a longitudinally directed bundle of collagen fibers **(Figure 11)** (**Global, 2017**).

**e. Coronary vascular**

The arterial supply to the heart arises from the base of the aorta as the right and left coronary arteries and typically, the right coronary artery is of greater calibre than the left (**Weinhaus , 2015**).

The heart derives its arterial supply from the right and left coronary arteries, which are the earliest branches of the aorta **(Figure 12)** (**Vishy, 2008**).

## 1.3 Function

The heart has two primary functions collects oxygen-poor blood and pumps it to the lungs for the release of carbon dioxide in exchange for oxygen and collects oxygen-rich blood from the lungs and pumps it to all tissues in the body to provide oxygen in exchange for carbon dioxide (**Weinhaus, 2015**).

The heart is the main organ in the circulatory system; the structure is primarily responsible for delivering blood circulation and transportation of nutrients in all parts of the body, the cardiac cycle, ensures that blood is distributed throughout the body, and the systole-diastole relationship is the reference in measuring blood pressure.It is works with other body systems to control the heart rate and other body functions. The primary systems are nervous system and endocrine system (**Gaea, 2021**).

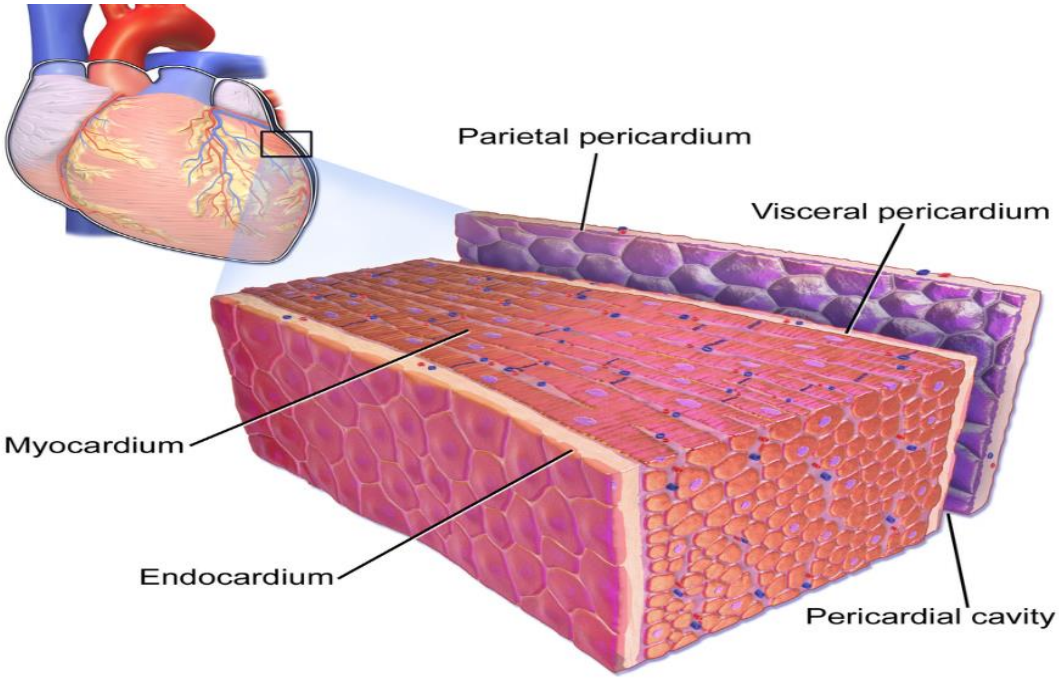


**Figure 11:** Layers of the heart walls **(10).**

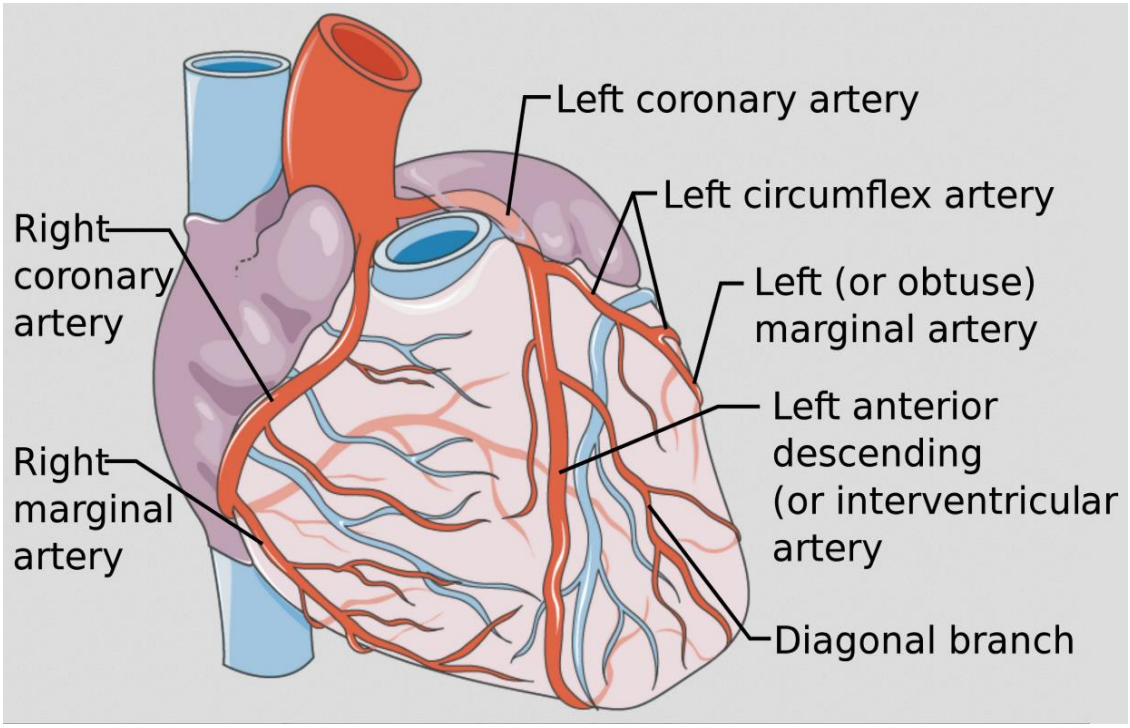


**Figure 12:** The coronary vascular of the heart **(11).**

# 2. Vessels

## 2.1. Blood vessels

The vascular system has the noble role to nourish all other tissues and organs in the human body. Composed of impressive 19 000 km of interconnecting vessels, oxygen-rich blood flows in their hollow structure from the heart through large arteries which progressively divide into smaller vessels until the capillaries of all other tissues (**Figure 13)**.

The blood then delivers nutrients and oxygen, removes metabolic by-products, and continues through veins back to the heart to be resaturated with oxygen in the pulmonary circuit

The complex structure and composition of the vascular wall impart unique mechanical features for the blood flow propagation and any disturbance on its physiology can significantly compromise its circulation. Indeed, vascular pathologies such as atherosclerosis have been the leading cause of death worldwide and the total replacement of the diseased tissue must be applied in a number of cases (**Camasão and Mantavani, 2021).**

## 2.2. The anatomy of the aorta

### a. Morphology of the aorta

The aortic arch is the segment of the aorta that helps distribute blood to the head and upper extremities via the brachiocephalic trunk, the left common carotid, and the left subclavian artery. The aortic arch also plays a role in blood pressure homeostasis via baroreceptors found within the walls of the aortic arch (**Brian, 2017**).

Arteries and veins are comprised of three distinct layers while the much smaller capillaries are composed of a single layer.

- Tunica intima

The inner layer (tunica intima) is the thinnest layer, formed from a single continuous layer of endothelial cells and supported by a subendothelial layer of connective tissue and supportive cells. In smaller arterioles or venules, this subendothelial layer consists of a single layer of cells, but can be much thicker in larger vessels such as the aorta. The tunica intima is surrounded by a thin membrane comprised of elastic fibers running parallel to the vessel. Capillaries consist only of the thin endothelial layer of cells with an associated thin layer of connective tissue **(In site 14).**

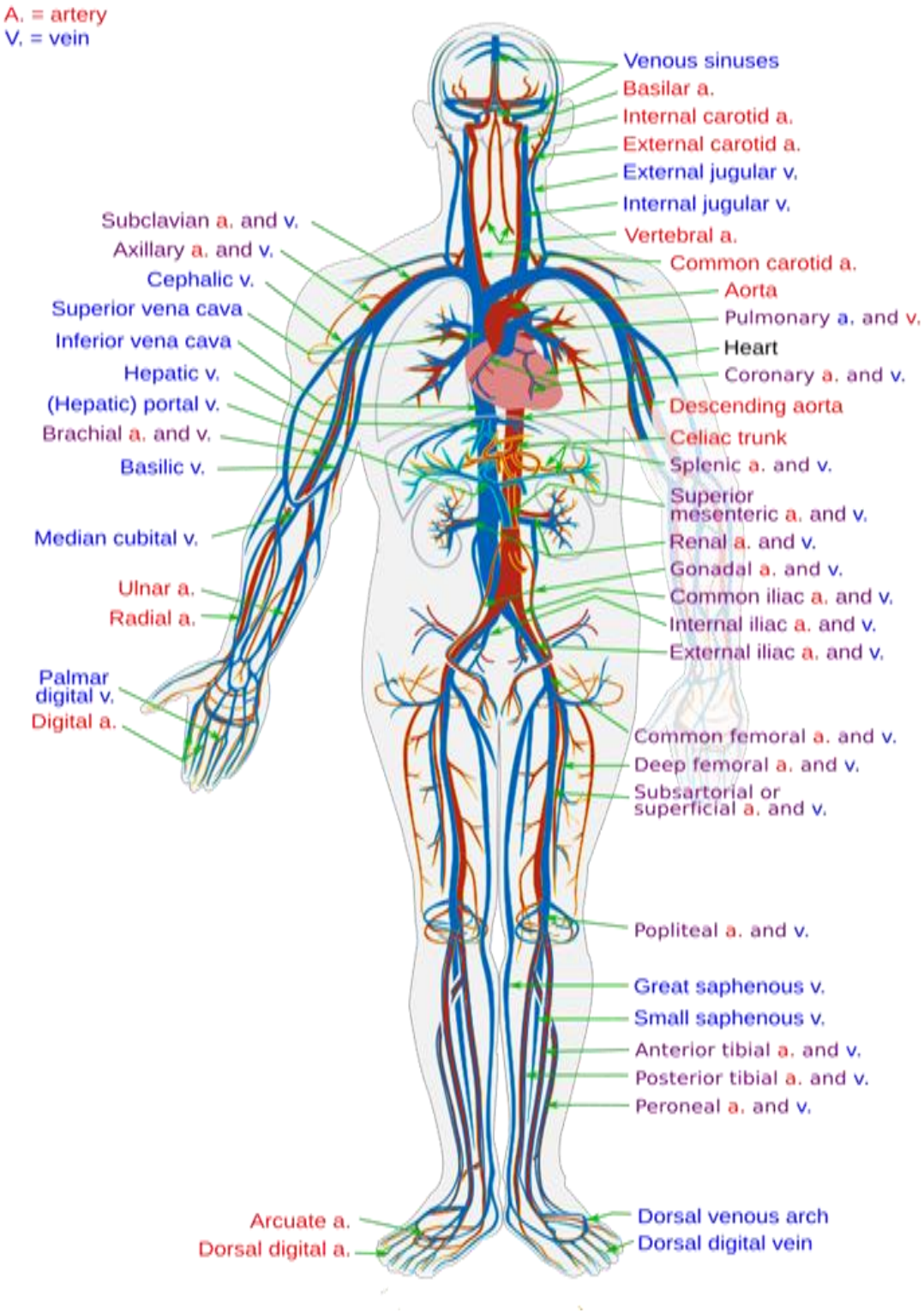


**Figure 13:** The Circulatory system: This simplified diagram of the human circulatory system (anterior view) shows arteries in red and veins in blue **(12).**

- Tunica media

Surrounding the tunica intima is the tunica media, comprised of smooth muscle cells and elastic and connective tissues arranged circularly around the vessel. This layer is much thicker in arteries than in veins. Fiber composition also differs; veins contain fewer elastic fibers and function to control caliber of the arteries, a key step in maintaining blood pressure **(In site 14).**

- Tunica externa

The outermost layer is the tunica externa or tunica adventitia, composed entirely of connective fibers and surrounded by an external elastic lamina which functions to anchor vessels with surrounding tissues. The tunica externa is often thicker in veins to prevent collapse of the blood vessel and provide protection from damage since veins may be superficially located **(Figure 14)( 14).**

### b. Function of the aorta

The function of blood vessels is to deliver blood to the organs and tissues in your body. The blood supplies them with the oxygen and nutrients they need to function. Blood vessels also carry waste products and carbon dioxide away from your organs and tissues.

Each type of blood vessel serves a different function:

- **Arteries**

  These strong, muscular blood vessels carry oxygen-rich blood from your heart to your body. They handle a large amount of force and pressure from your blood flow but don't carry a large volume of blood. At any given time, only about 10% to 15% of your body's blood is in your arteries **(15).**

- **Arterioles**

  Arteries branch into smaller vessels called arterioles. Both arteries and arterioles are very flexible. They get bigger or smaller to help maintain your body's blood pressure **(15).**

- **Venules**

  Veins begin as tiny vessels called venules and get gradually larger as they near your heart. Venules receive blood from capillaries **(15).**

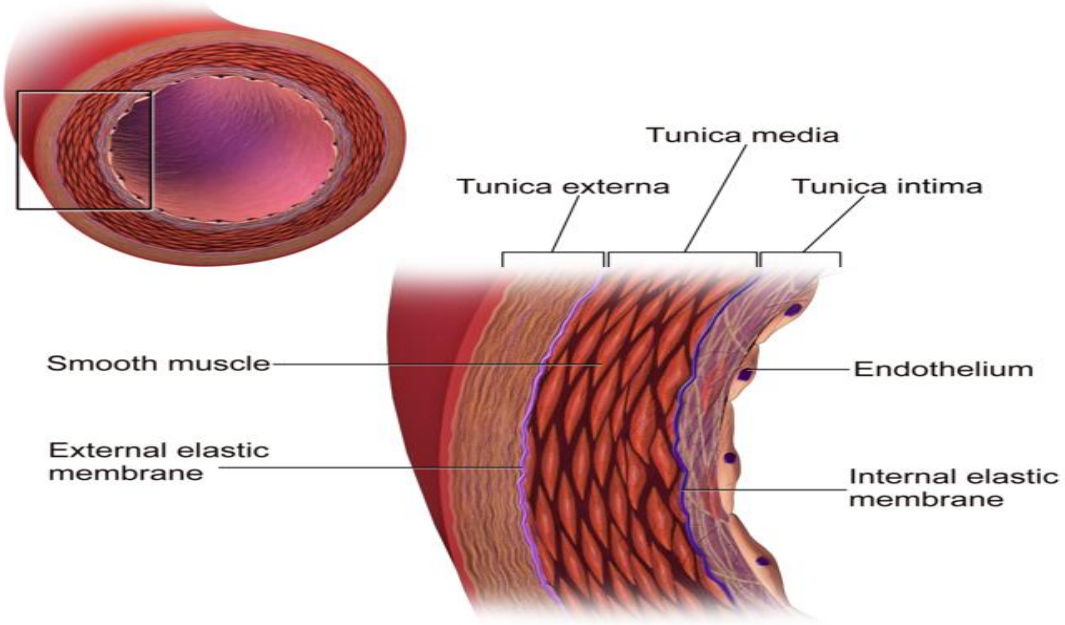


**Figure 14**: Structure of the artery wall: This diagram of the artery wall indicates the smooth muscle, external elastic membrane, endothelium, internal elastic membrane, tunica externa, tunica media, and tunica intima **(13).**

- **Capillaries**

  These tiny blood vessels have thin walls. Oxygen and nutrients from the blood can move through the walls and get into organs and tissues. The capillaries also take waste products away from your tissues. Capillaries are where oxygen and nutrients are exchanged for carbon dioxide and waste **(15).**

- **Veins**

  Unlike arteries, veins don't have to carry highly pressurized blood, but they do have to carry large volumes of deoxygenated blood back to your heart. Thin, less elastic walls help them handle high volumes and low pressure. Most veins have valves that open and close. The valves control blood flow and keep your blood flowing in one direction. About 75% of your blood is in your veins **(15).**

# II. Atherosclerosis

## 1. Definition

Atherosclerosis refers to the accumulation of fatty and/or fibrous material in the innermost layer of arteries, the intima (**Libby et al., 2019**). It is a chronic inflammatory disease process of complex etiology (**Sessa et al., 2014**)

The term atherosclerosis derives from the Greek word for 'gruel' or 'porridge', reflecting the appearance of the lipid material found in the core of the typical atherosclerotic plaque (or atheroma) (**Figure 15**) (**Libby et al., 2019**).

Atherosclerosis is a hardening and narrowing of your arteries caused by cholesterol plaques lining the artery over time. It can put blood flow at risk as your arteries become blocked (**Felson, 2021**).

Atherosclerotic cardiovascular disease (CVD) is on course to surpass infectious diseases as the leading cause of morbidity and mortality worldwide. Multiple risk factors are responsible for this trend including the increasing average life expectancy and reducing rates of communicable diseases in addition to potentially modifiable risk factors, such as tobacco use, hypertension, hyperlipidemia, and diabetes mellitus (**Beverly and Budoff, 2019**).

## 2. The mechanism of atherosclerosis

Atherosclerotic lesion development takes place in the intimal layer of the arterial wall. Adult intima is a rather thick formation with complex architecture and heterogeneous cellular composition. The intima is separated from the lumen of the vessel by a monolayer of endothelial cells. Endothelium plays a key role in the transport of cells and non-cellular components of blood from the arterial bed to the vascular wall (**Krüger-Genge et al., 2019**).

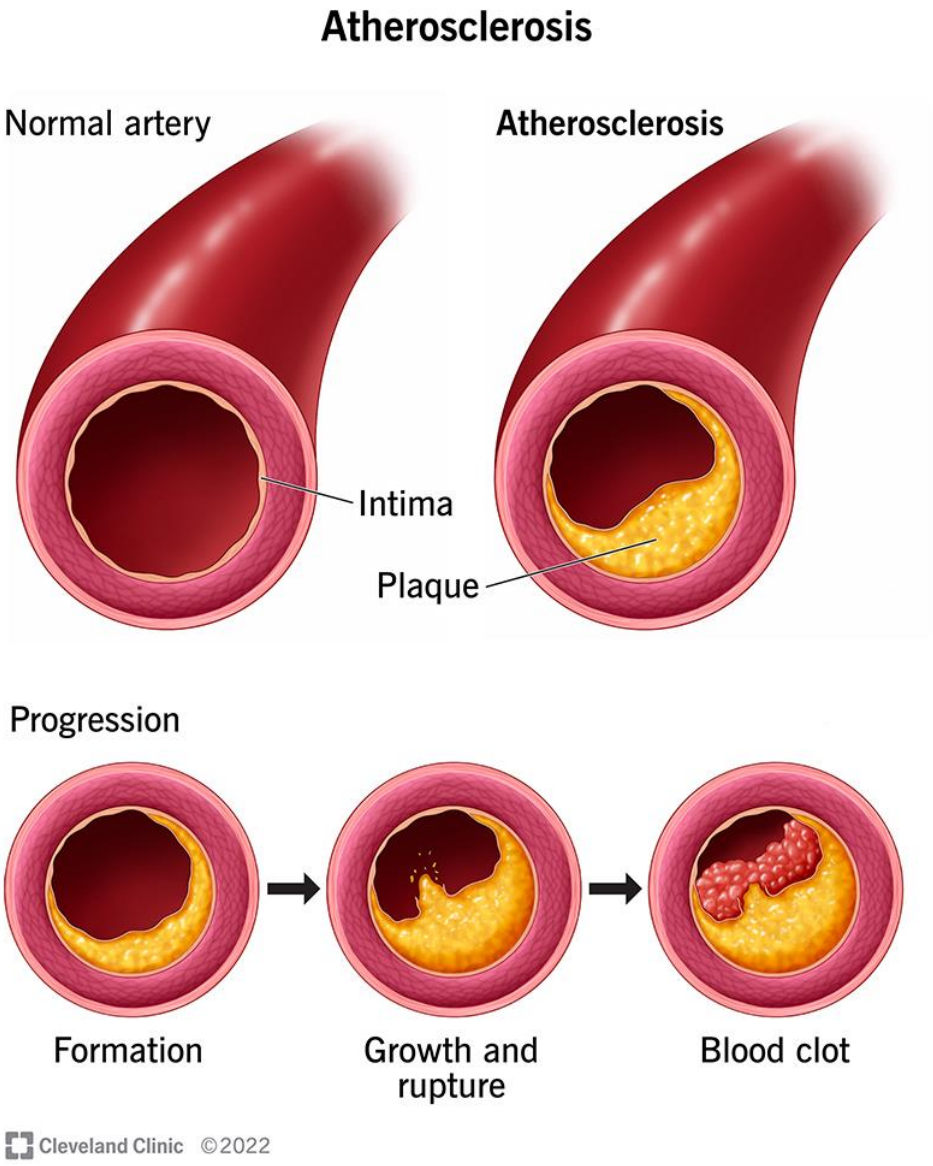


**Figure 15**: The stages of atherosclerosis **(16).**

Cellular composition of the arterial wall undergoes profound changes in atherosclerotic lesion areas (**Stary, 1990; Xu et al., 1990; Padarti and Zhang, 2018**).

The primary source of lipids that accumulate in foam cells is atherogenic modified low density lipoprotein (LDL). Particles of LDL circulate in the blood and undergo chemical modifications affecting the glycoconjugate, lipid, and protein moieties (**Ivanova et al., 2017) (Orekhov and Myasoedova, 2019**).Native (unmodified) LDL does not cause the accumulation of intracellular lipids in cultured cells. Associates of modified LDL stimulate the phagocytic activity of subendothelial macrophages and pericytes. Following phagocytosis, inflammatory cytokines are secreted, which attracts monocytes and other immune cells to the emerging site of inflammation. Inflammatory cytokines contribute to further accumulation of intracellular lipids induced by modified atherogenic LDL. Moreover, in some cases, lipid accumulation induction is observed even in absence of modified LDL.Therefore, not the accumulation of intracellular lipids caused by LDL, but the immune response to the interaction of the cell with LDL promotes or even induces the formation of foam cells. Intracellular lipid accumulation leads to the rupture of cell contacts in the three-dimensional network of pericyte-like cells (**Ivanova et al., 2015**). This is accompanied by increased proliferative activity and stimulation of extracellular matrix synthesis (**Ponticos and Smith, 2014) (Orekhov et al., 2016**). Such processes are characteristic of the reparative phase of the inflammatory reaction. Normally, only a small thickening of the intimal tissue remains at the site of inflammation (**Orekhov and Ivanova, 2016**).

This process may occur in the arteries without causing visible symptoms and lesions can accumulate over time, so that focal formations become a diffuse thickening. Diffuse intimate thickening is not an atherosclerotic lesion, but is considered normal for the arteries of an adult body (**Nakashima et al., 2008) (Subbotin, 2016**).

An increase in the concentration of pro-inflammatory cytokines leads to endoplasmic reticulum stress in the arterial wall cells, which in turn can lead to the initiation of apoptosis (**Ivanova and Orekhov, 2016**). Cytokine-induced inflammation disrupts the normal functioning of mitochondria, their synthesis and mitophagy (**Gkikas et al., 2018**), which also leads to apoptosis. These effects contribute to the prolonged circulation of pro-inflammatory agents in the vascular bed, which can further stimulate the launch of a cascade of inflammatory reactions at the site of vascular damage.

It is plausible that atherosclerotic lesion development occurs when the inflammatory process cannot be resolved in a regular way and becomes chronic (F**igure 16**) (**Orekhov and Ivanova, 2016**). Thus, the response of the innate immunity is a trigger for the formation of foam cells,

while violation of the normal immune response is the cause of inflammation chronification, which leads to the development of atherosclerotic lesions.

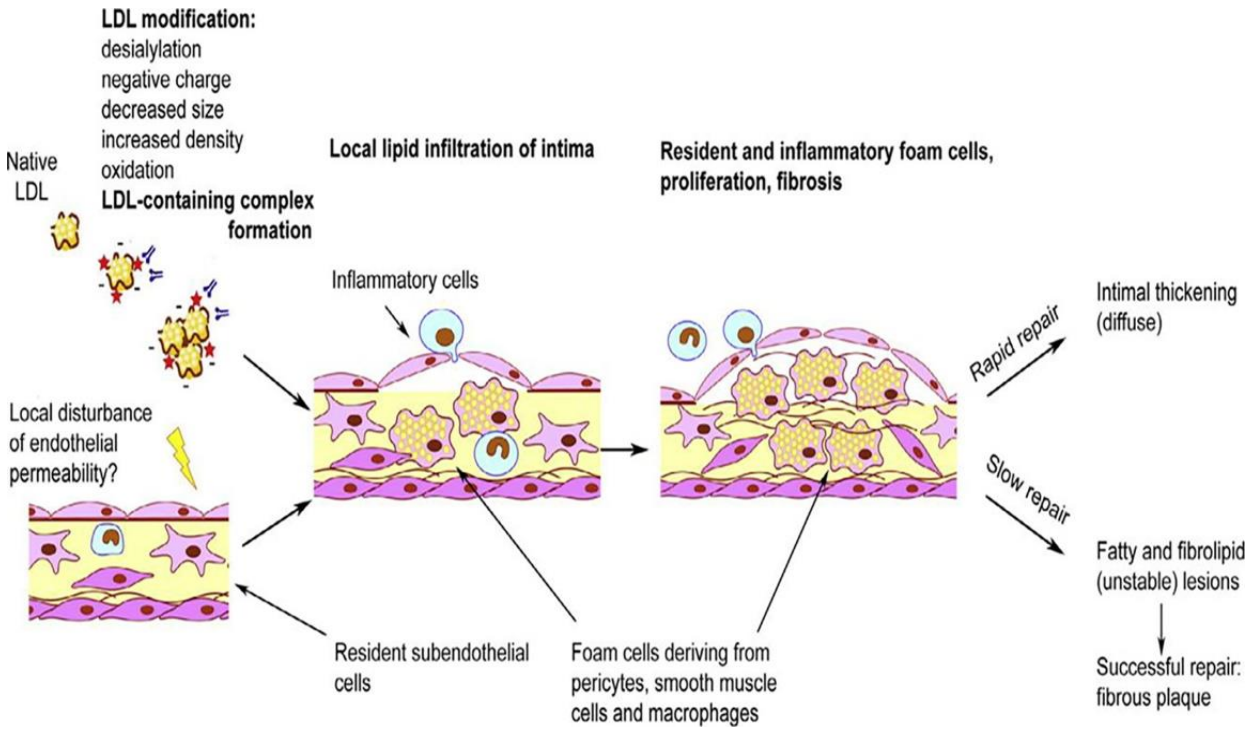


**Figure 16:** Schematic overview of initiation of atherosclerotic lesion formation **(17).**

# Materials and Methods

# Materials and Methods

## 1. Materials

### 1.1. Choice of treatment

In this experience we have used hot water at 50°C proposed by**(AlHajri, 2010; AlHajri2020)**, and crystallize sugar (50g/65kg).

### 1.2. Animals

The experiment was performed on 32 albino *Mus musculus* mice (2- 2.5-month-old).The animals were born in the Animal house of Université des frères Mentouri constantine1.

### 1.3. Chemicals products

Chemicals products used in our research are:

Chloroform, formol (10%) Nacl (0,9%) , tris buffered saline (TBS), phosphate buffered saline (PBS), H2O2, Sulfosalicylic acid, orthophosphoric acid, comassieblue, methanol, tris ethylene diamine tetra acetic acid (EDTA), bovine albumin serum (BSA), Hcl, NaOH.

### 1.4 Equipments

Precision balance (readability 0, 0001), balance (1kg), centrifuge, spectrophotometer, pH meter, heating magnetic stirrer, vortex mixer.

## 2. Methods

### 2.1. Treatment of mice

Adult male Albino *Mus musculus* mice (2- 2.5-month-old) from Université des frères Constantine1, Constantine, Algeria, weighing between (34 - 45g) were housed in polypropylene cages with soft wood and free access to water and diet every day. Were maintained under standard Animal house conditions of humidity, temperature (25°C) and light (12hday: 12h night). After the adaptive period, they were divided into four groups.

The control group ( C) was fed with normal diet ( 150g/kg/day ) the second group (S) was fed with Crystallize sugar ( 50g/65kg/day), the third group ( SHW) was fed with crystallize sugar and hot water (125ml+150g/8mice+50g/65kg /mice ) and the fourth group (HW) drunk hot water and normal diet (125ml+150g/8mice) ( Table 3). The diet and water consumed by mice throughout the experiment have been taken at the same time every day.

After Three weeks of treatment, blood samples were collected after fasting from retro orbital plexus into heparin tubes by using glass capillaries.

After taking the blood samples, the mice were scarified and the organs such as liver, aorta and heart are fixed in the formol, at the same time the rest of the liver are stored in the freezer at -20 for the dosage of antioxidants (GSH and catalase).

**Table 3**:Shows treatment of animals during 21days

| Experimental group | Treatment | Number of animals | Duration of experiment | Daily dose |
|---|---|---|---|---|
| GI(C) | Normal water +Animal Diet | 8 | 21 J | 125ml+150g/8mice |
| GII(S) | Normal water + Animal diet +Crystallize sugar | 8 | 21 J | 125ml+150g/8mice+50g/65kg /mice |
| GIII(SHW) | Hot water + +Crystallize sugar Animal diet | 7 | 21 J | 125ml+150g/8mice+50g/65kg /mice |
| GIV(HW) | Hot water +Animal diet | 9 | 21 J | 125ml+150g/8mice |

## 2.2. Biochemical analysis

Blood glucose, lipids status, CRP measurement were performed in the medical laboratory of AMINE Constantine (Algeria).

### 2.2.1 .Blood glucose determination

**Clinical significant of blood glucose**

Blood glucose determination is used in the diagnosis and treatment of disorders of carbohydrate metabolism such as diabete and hypoglycemia. Blood glucose were measured using colorimetric automatic procedures (Auto-Analyser type COBAS integraroche 400).

The reactions involved in the assay system are as follows:

$$\text{D-glucose+ATP} \xrightarrow{\text{HK}} \text{D-glucose-6-phosphate+ADP}$$

$$\text{D-glucose-6-phosphate+NAD}P^{+} \xrightarrow{\text{G6PDH}} \text{D-6-phosphogluconate+NADPH+}H^{+}$$

### 2.2.2. Lipids determinations

Total cholesterol, HDL-c, LDL-c and triglyceride concentrations were measured using colorimetric automatic procedures (Auto-Analyser type COBAS integraroche 400).

**Clinical investigation**

The objective of lipidsmeasurment is to detect the relationship between high consumption of crystallize sugar and hypercholesterolemia.

A. **Total cholesterol**

The concentration of T-cholesterol was calculated by using the following formulae.

$$\text{cholesteryl acetate} + \text{H}_2\text{O} \xrightarrow{\text{Cholesterol esterase}} \text{cholesterol} + \text{Acetic acid}$$

$$\text{cholesterol} + \text{O}_2 \xrightarrow{\text{cholesterol oxidase}} \Delta - \text{Cholestenone} + \text{H}_2\text{O}_2$$

$$2\text{H}_2\text{O} + 4 - \text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4\text{H}_2\text{O}$$

B. **Triglyceride determination**

Triglycerides are indicated for the diagnosis and treatment of disorders of lipid metabolism in sugary diabetes. It is measured using colorimetric automatic procedures (AutoAnalyser type COBAS integraroche 400).

$$\text{Triglycérides} + 3\,H_2O \xrightarrow{LPL} \text{glycerol+3RCOOH}$$

$$\text{Glycerol +ATP} \xrightarrow[\text{Mg}^{2+}]{\text{GK}} \text{glycerol-3-phosphate+ADP}$$

$$\text{Glycerol-3-phosphate+}O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone phosphate+}H_2O_2$$

$H_2O_2$+amino-4 phenazone+chloro-4 phenol$\xrightarrow{\text{POD}}$ mono-imino-p-benzoquinone-4 phénazone+2$H_2O$+HCL

C. **HDL-C**

Ester of cholesterol HDL+ $H_2O$ $\xrightarrow{\text{cholesterol-esterase modified by the PEG}}$cholesterol HDL+RCOOH

Cholesterol HDL+$O_2$ $\xrightarrow{\text{cholesterol-oxydase modofied by the PEG}}$Δ4- cholestenone+$H_2O_2$

2$H_2O_2$+aminoantipyrine+HSD$A^a$+$H^+$+$H_2O$ $\xrightarrow{\text{peroxydase}}$derived puplecolour 5$H_2O$

D. **LDL-C**

Ester of cholesterol LDL+$H_2O$ $\xrightarrow{\text{detergent cholesterol-esterase}}$ cholesterol + free fatty acid

Cholesterol LDL+ $O_2$ $\xrightarrow{\text{cholesterol oxydase}}$Δ4-cholestenone+ $H_2O_2$

2 $H_2O_2$+amino-4 phenazone+HSDAa+$H^+$+$H_2O$ $\xrightarrow{\text{peroxydase}}$derived puplecolour 5$H_2O$

### 2.2.3. Determination of aspartate aminotransferase and alanine aminotransferase vactivities

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were carry out using colorimetric automatic procedures (Auto-Analyser type COBAS integraroche 400). The derminationof AST and ALT activities in serum is used mainly to assess the liver damage **(Aklil, 2017)**.

**A. Aspartate aminotransferase (AST)**

The series of reactions involved in the assay system are as follows:

L-aspartate+2-oxoglutarate$\xrightarrow{\text{AST}}$oxaloacetate+L-glutamate

Oxaloacetate+NADH+$H^+$ $\xrightarrow{\text{MDH}}$L-malate+$NAD^+$

**B. Alanine aminotransferase (ALT)**

L-Alanine+2-oxoglutarate $\xrightarrow{\text{ALT}}$pyruvate+L-glutamate

Pyruvate+NADH+$H^+$ $\xrightarrow{\text{LDH}}$L-lactate+$NAD^+$

### 2.2.4. Determination of high sensitivity C-reactive protein

The method usedfor the determination of high sensitivity is the immune turbidimetric which was performed on the Cobas Integra 400.

**Clinical investigation**

High sensitivity C-reactive protein (CRP) is determinated to evaluate the possibility of infection or inflammatory disease.

### 2.2.5 Determination of oxidative stress parameters

**A.Tissue homogenate preparation**

From each mouse 0.5 g of liver was homogenized in 2ml of tris buffered solution (TBS). The homogenates were centrifuged at 9000g for 15min at C°4) and the supernatant was used to measure the glutathione reduced and catalase **(Aklil et al., 2017)**.

**b. Measurment of glutathione reduced**

Liver homogenate sample (0.8ml) was deproteinized with (0.2ml) of 5-sulfosalicylic acid solution (0.25%) and was allowed stand on ice for 10min. Following centrifugation at 1000 tours /5min to remove the precipitated protein. The quantity of 0.5ml of supernatant was mixed with 1ml Tris buffered solution (pH 9.6) and (0.025ml) of DTNB reagent (0.01M 5.5 dithio-bis-2-nitrobenzoic acid ), the reaction was left at room temperature for 5min, then the absorbance was measured at 412 nm using spectrophotometer (SHIMADZU UV-1280 ) against the blanc**( Weekbeker and Cory, 1988)** .

The glutathione concentration is obtained by the following formula:

$$GSH\ (nmol/mg\ protein) = DO \times 1 \times 1.525 / 13100 \times 0.8 \times 0.5 \times mg\ protein$$

**c. Measurement of catalase**

The tissu catalase was determined according to Aebi's method (1974). The principle assay based on the determination of $H_2O_2$decomposition rate at 240nm and the reaction are shown in (Table 4).

**Table 4:** Shows the reaction of catalase

| Reagents | Sample (µl) | Blank (µl) |
|---|---|---|
| Phosphate buffer (100Mm, pH 7.5) | 780 | 800 |
| $H_2O_2$ (500Mm) | 200 | 200 |
| Surnageant | 20 | 0 |

# Results and Discussion

# Results

## 1. Weight

Our results demonstrated that the weight is increased during the first week( 39.60g±0.51) and third week( 40.82g±0.40 ) in group administered with crystallized sugar, and was in the control group from the first week ( 38.63g±1.42) and third week ( 39.70g±0.64 ) and in mice treated with hot water the weight was in first and third week ( 38.62g±0.47 ) (38.66g±0.09) respectively. However the weight is decreased in group administered with crystallized sugar and treated with hot water (38.81g±0.34) (38.65g±0.16) between the first and third week **(Figure17)**.

## 2. Food

Our results demonstrated that the food consumed by mice is increased during the first and third week (104.28g±8.75) (114.00g±9.25) in the group control, in the group of mice treated with hot water in the first week was (109.75g±7.80) and in the third week was (122.79g±22.49) and in the group administered with crystallize sugar in the first and third week (80.29g±17.63)(81.43g±37.63). On the other hand, the food consumed by mice is decreased in group administered with crystallized sugar and treated with hot water (127.71g±14.56) (118.14g±23.16) **(Figure18).**

## 3. Water

Our results demonstrated that the quantity of water consumed is increased during the first and third week in the group control (24.29ml ±1.70) (26.71ml ±3.04) respectively and in the animals treated with hot water (22.00ml±1.15) (41.43ml ±12.54). However the quantity of water consumed by mice is decreased in groups administered with crystallized sugar and treated with hot water (47.20ml ±4.71) (37.43ml ±8.81) and administered with crystallized sugar in the first (30.57ml±3, 03) and third week (28.29 ml ±3.74) **(Figure19).**

## 4. Blood sugar

The figure 20 showed that there is a difference between groups ,C ( 1.76g/l±0.15), S ( 1.59g/l± 0.31) ,SHW( 1.83g/l±0.14 ) and HW ( 1.35g/l±0.21 ). The level of blood sugar is decreased in the group treated with hot water when it is compared to the other group however, the level of blood sugar is increased in the group administered with crystallized sugar and treated with hot water when it is compared to the groups (C and S).

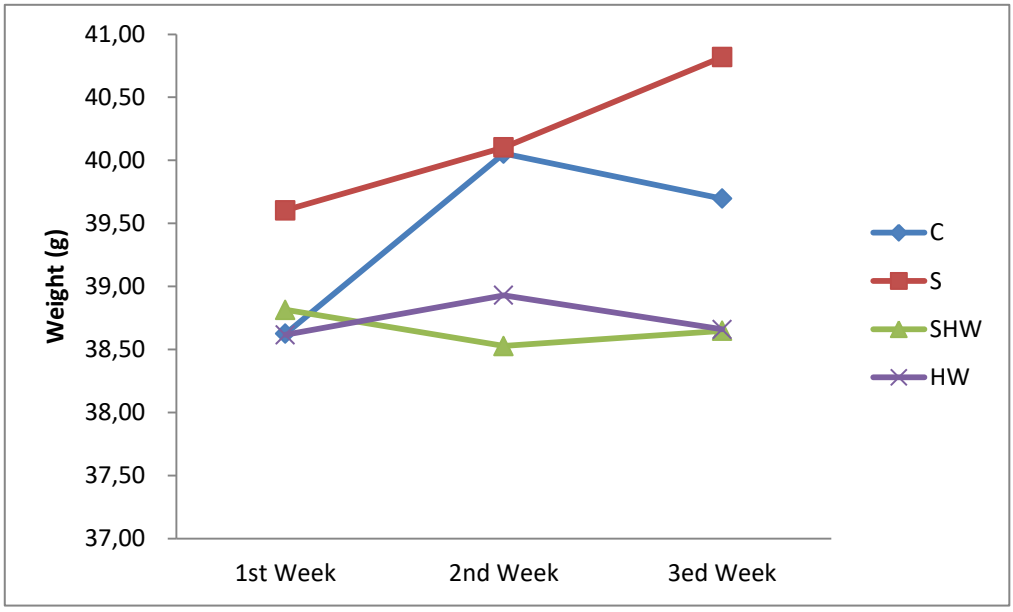


**Figure 17**: The effect of crystallize sugar and hot water on the weight in mice during 21 days

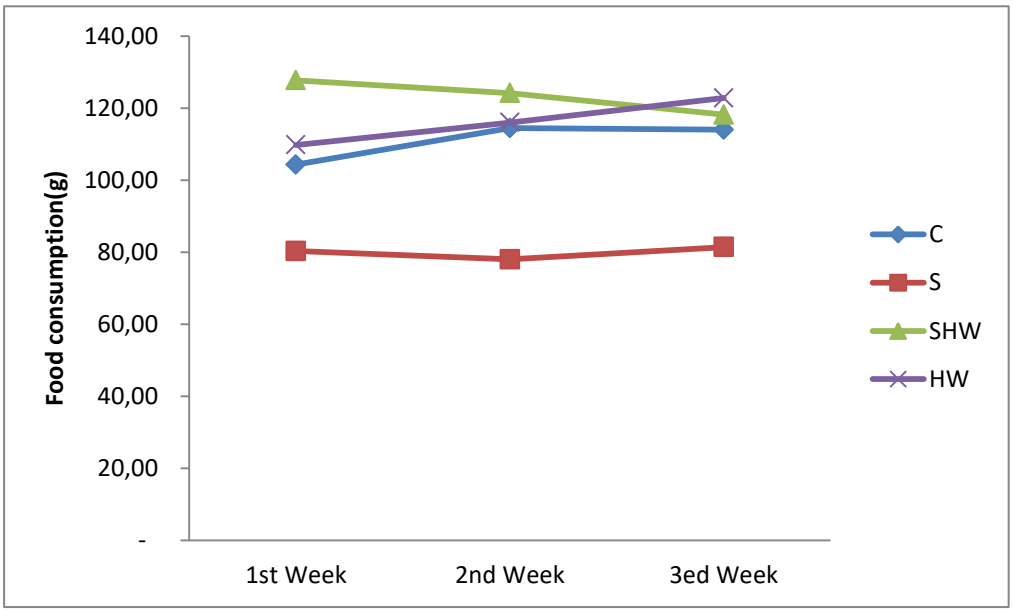


**Figure 18**: The effect of crystallize sugar and hot water on the food consumed by mice during 21 days

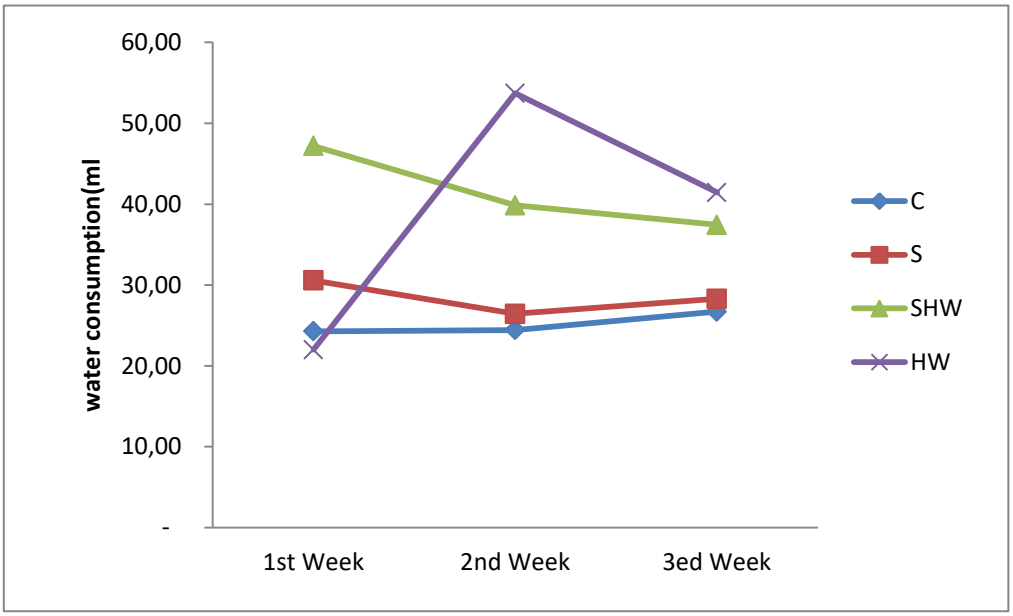


**Figure 19**: Water consumption during 21 days of treatment.

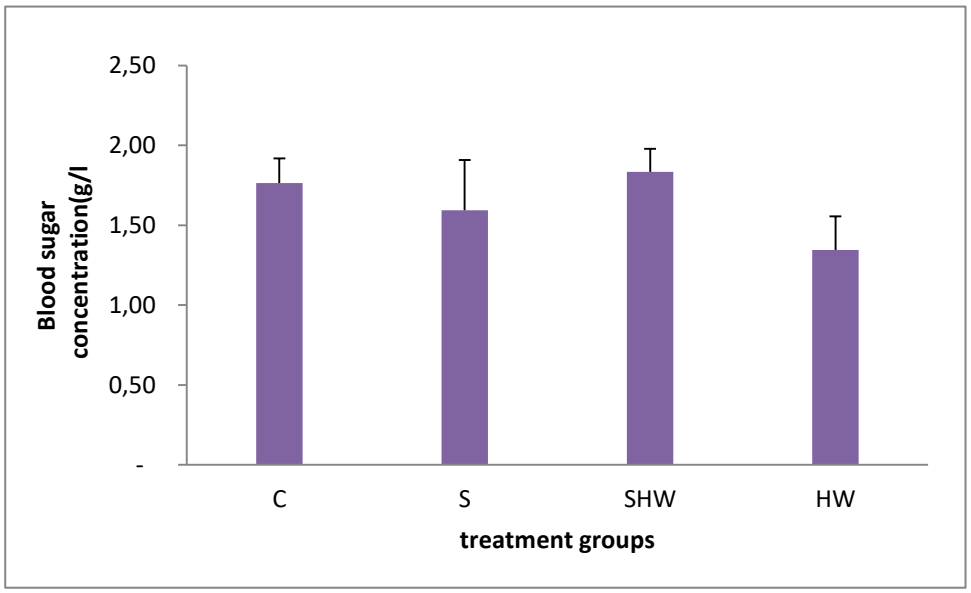


**Figure 20:** The effect of high consumption of crystallize sugar and hot water on fasting blood sugar in mice.

## 5. Lipids status

### 5.1 Total cholesterol

The figure 21 showed that the concentration of total -cholesterol is increased in the group administered with crystallized sugar (1.42g/l±0.06) and SHW (1.46g/l±0.07) when it is compared to the other group C (1.11g/l±0.17) and HW (1.28g/l±0.17).

### 5.2. Triglyceride

Our results showed that the concentration of Triglyceride is increased in the groups administered with crystallized sugar ( 1.24g/l±0.42 ) and administered with crystallized sugar and treated with hot water ( 1.72g/l±0.36 ) when compared to the groups of animals treated with hot water ( 1.00g/l±0.50 ) and control group ( 0.94g/l±0.17 ) ( **Figure 22**).

### 5.3. HDL-c

Our data demonstrated that the concentration of HDL-c is increased in the groups administered with crystallized sugar ( 1.17g/l±0.02 ) and administered with crystallized sugar and treated with hot water ( 1.18g/l±0.08 ) when compared to the groups of animals treated with hot water ( 1.04g/l±0.17 )and control group ( 0.93g/l±0.14) ( **Figure23**).

A. **LDL-c**

Our data demonstrated that the concentration of LDL-c is increased in the group administered with crystallized sugar and treated with hot water ( 0.12g/l±0.02 ) ,group of animals administered with crystallize sugar(0.11g/l±0.01)and the mice treated with hot water ( 0.12±0.01 )when compared to the control group (0.08±0.03) ( **Figure24**).

B. **VLDL-c**

Our data demonstrated that the concentration of VLDL-c is increased in the groups administered with crystallized sugar and treated with hot water ( 0.34g/l ±0.07 ) when compared to the groups of animals administered with crystallize sugar ( 0.25g/l ±0.08 ) , treated with hot water ( 0.2g/l ±0.1)and control group (0.19g/l ±003 ) ( **Figure25**).

C. **CRP**

Our data demonstrated that there is a decrease in the concentrations of CRP the groups (S= 0.50mg/l±0.06, SHW= 0.41mg/l±0.13 and HW= 0.55mg/l±0.1) when compared to the group (C= 0.76mg/l±0.20, (**Figure26**).

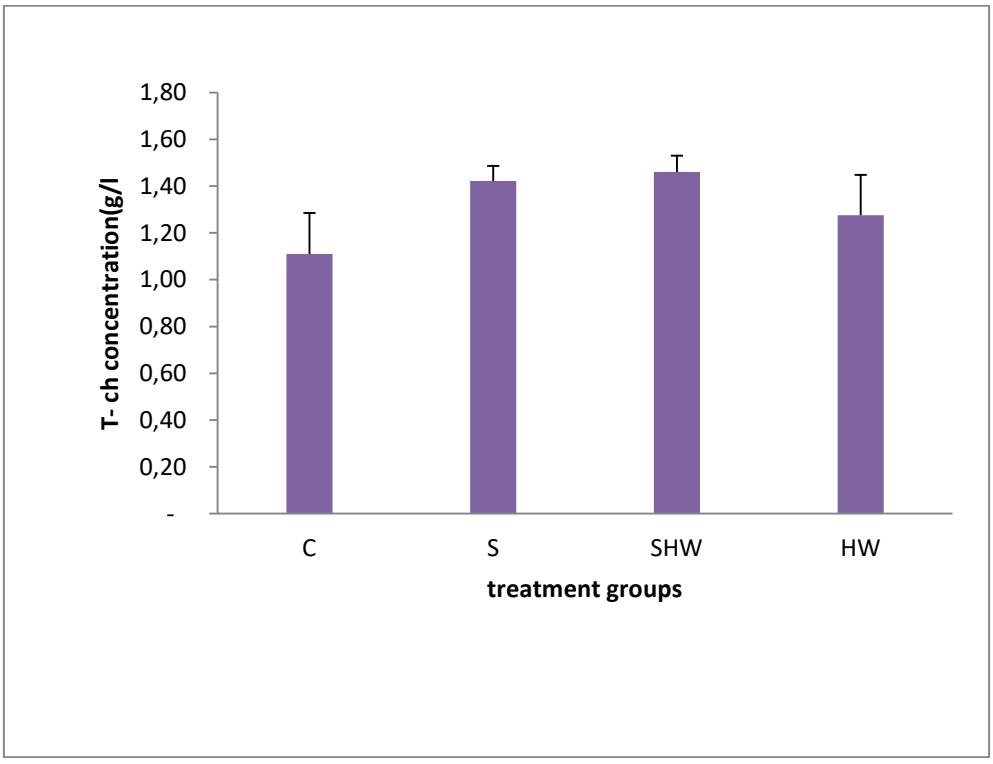


**Figure 21:** The effect of high consumption of crystallize sugar and hot water on T- cholesterol in mice.

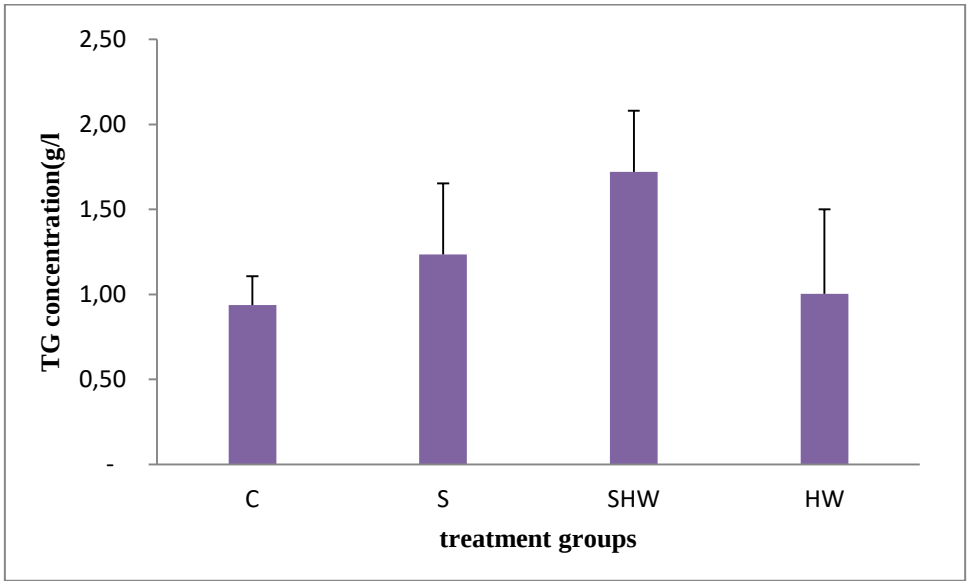


**Figure 22:** The effect of high consumption of crystallize sugar and hot water on Triglyceride in mice.

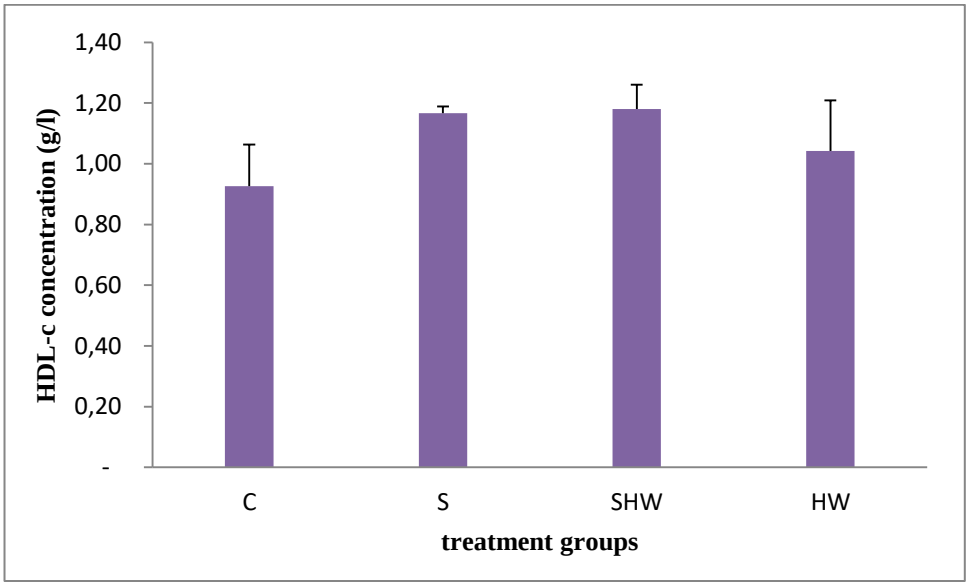


**Figure 23:** The effect of high consumption of crystallize sugar and hot water on HDL-c in mice.

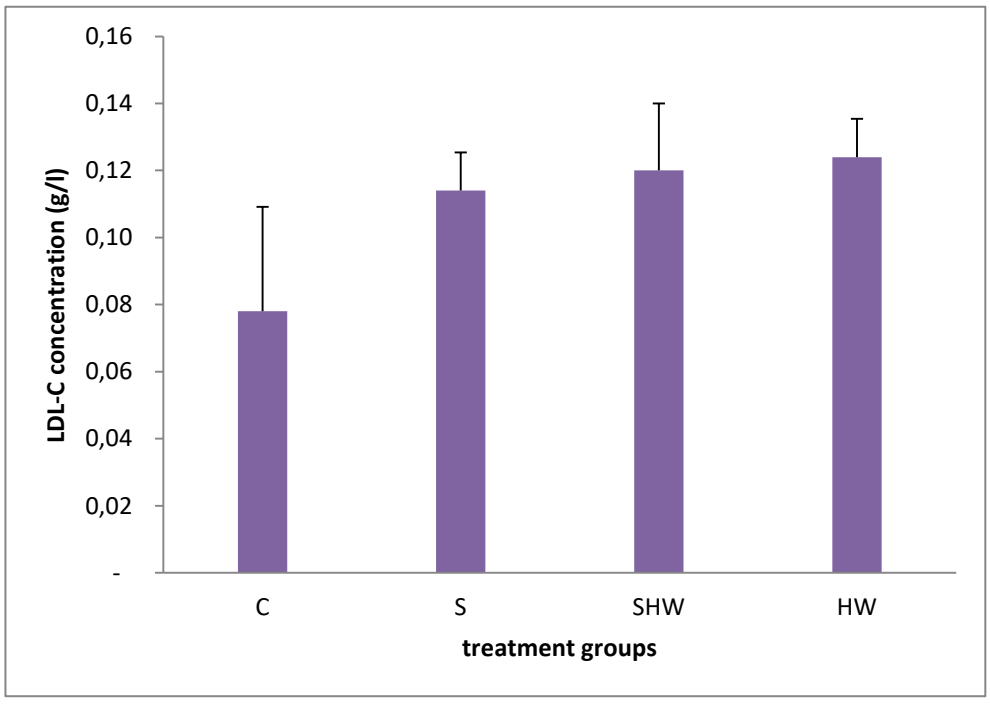


**Figure 24:** The effect of high consumption of crystallizes sugar and hot water on LDL-c in mice.

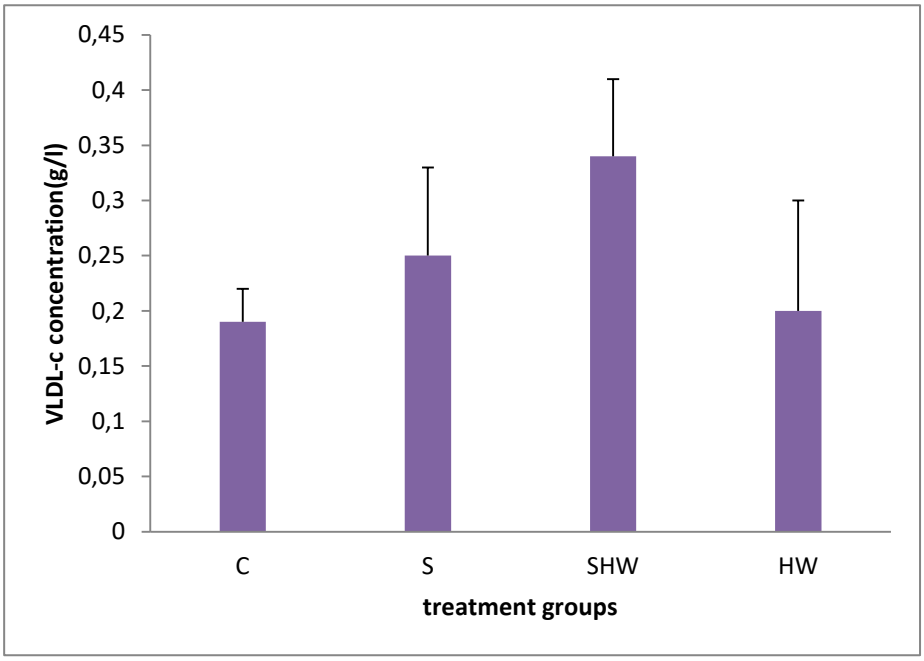


**Figure 25:** The effect of high consumption of crystallizes sugar and hot water on VLDL-c in mice.

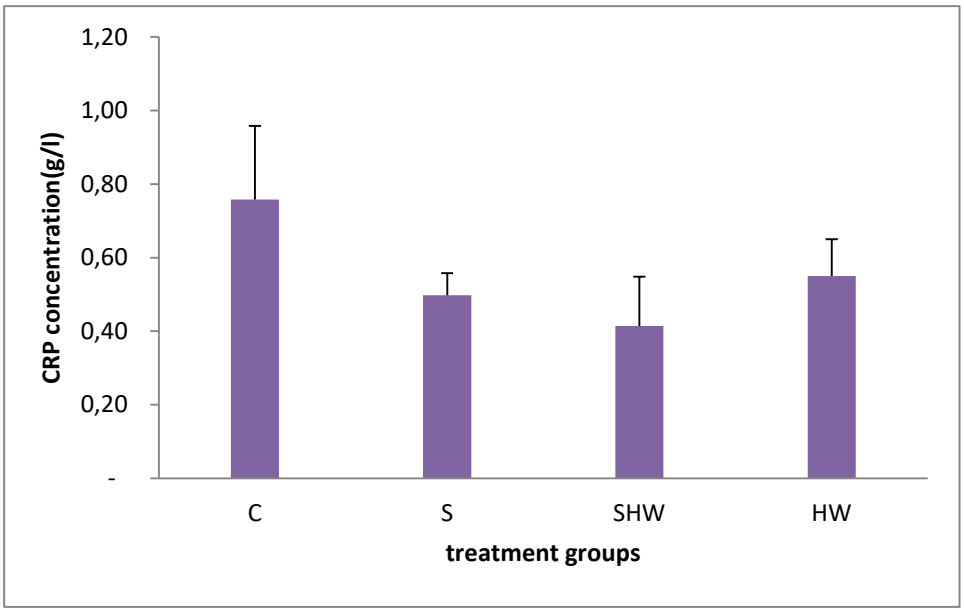


**Figure 26:** The effect of high consumption of crystallize sugar and hot water on CRP in mice.

D. **AST and ALT**

**A. AST**

Our data demonstrated that there is a difference between groups in the concentrations of AST. In addition we have detected that there is a decrease in concentration of AST in the groups S administered with crystallized sugar (78.29U/L±13.90 ) , SHW administered with sugar and treated with hot water ( 83.94U/L±26.09) and HW treated with hot water (122.50U/L±11.26) when compared to the group of control ( 125.59U/L±18.77 ) ( **Figure27**).

**A. ALT**

Our data demonstrated that there is a difference between groups in the concentrations of ALT. In addition we have detected that there is a decrease in concentration of ALT in the groups S administered with crystallized sugar ( 24.12UI/L±3.96 ) and SHW adminisered with sugar and treated with hot water (25.14UI/L±5.14) and increase in ALT level in group HW treated with hot water ( 33.17UI/L±11.77 ) when compared to the group of control ( 28.00UI/L±4.39 ) ( **Figure28**).

## 6. GSH

Our data demonstrated that there is an increase in the concentrations of GSH in the groups of mice S administered with crystallize sugar ( 74.42nmol/mg protein ±32.78 ) and SHW administered with crystallize sugar and treated with hot water ( 74.41nmol/mg protein±31.98 ) when compared to groups HW treated with hot water ( 65.01nmol/mg protein±15.43) and the control ( 65.44nmol/mg protein±40.37 ) (**Figure 29**).

## 7. Catalase

Our data demonstrated that there is a decrease in the concentrations of catalase in the groups of mice S administered with crystallize sugar ( 3.38µmole $H_2O_2$ /min/mg/protein ±5.74 ) and SHW administered with crystallize sugar and treated with hot water ( 3.26µmole $H_2O_2$ /min/mg/protein± 3.87 ) and HW treated with hot water (1.79µmole $H_2O_2$ /min/mg/protein ±1.13) when compared to C group ( 12.84µmole $H_2O_2$ /min/mg/protein ±9.02 ) (**Figure 30**).

### 8. Atherogenic index

Our data demonstrated that there is a slightly difference in the atherogenic index in the groups of mice (S) administered with crystallize sugar (0.22±0.06) and (SHW) administered with crystallize sugar and treated with hot water (0.24 ±0.03) and (HW) treated with hot water (0.23 ±0.04) when compared to group the control (0.2±0.06) (Figure 31).

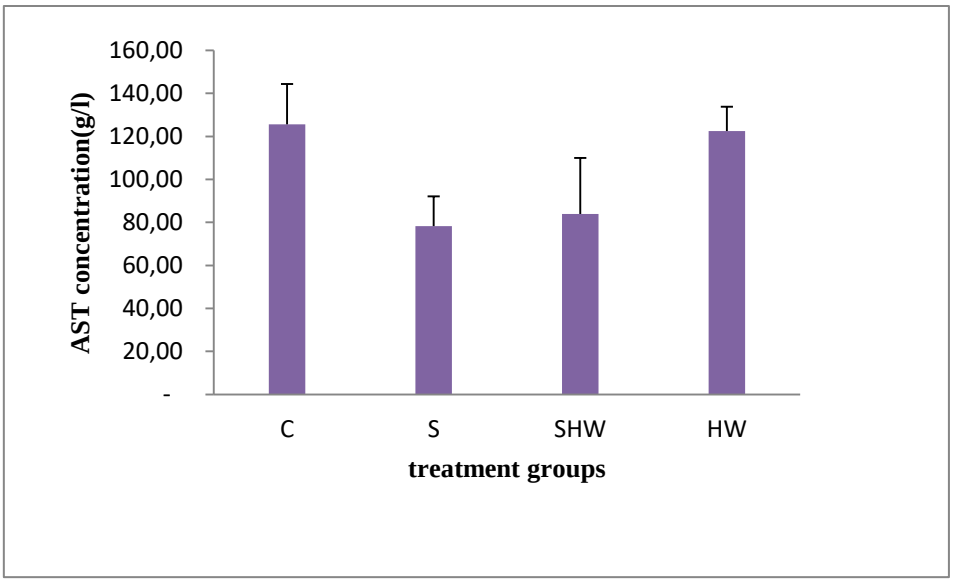


**Figure 27:** The effect of high consumption of crystallize sugar and hot water on AST in mice.

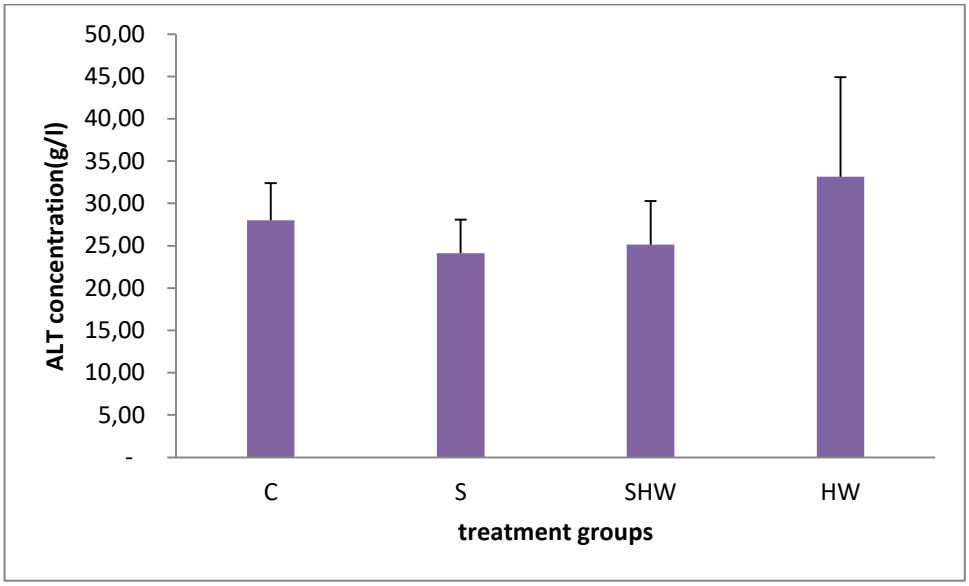


**Figure 28:** The effect of high consumption of crystallize sugar and hot water on ALT in mice.

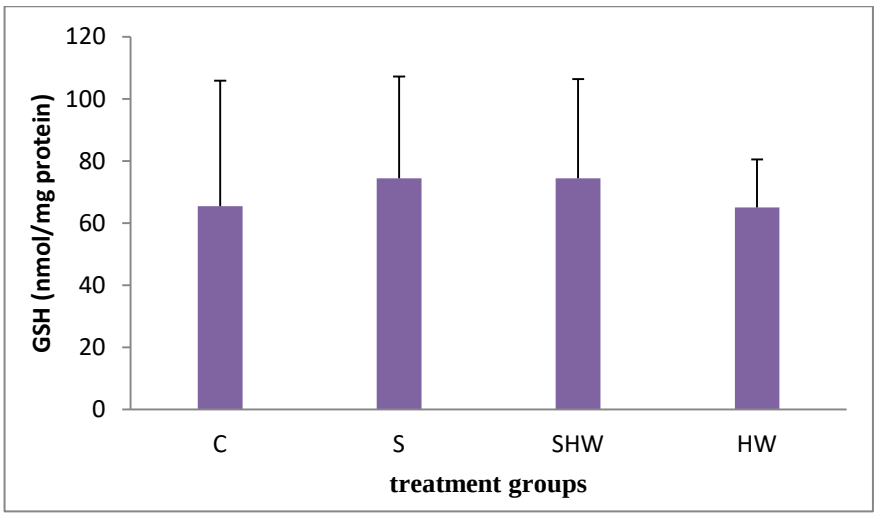


**Figure 29:** The effect of high consumption of crystallize sugar and hot water on GSH in mice.

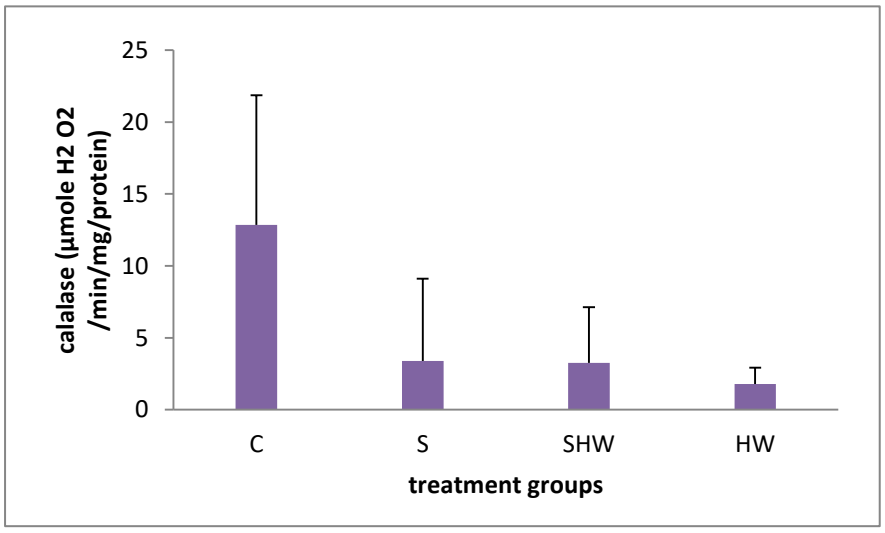


**Figure 30:**The effect of high consumption of crystallize sugar and hot water on catalase in mice.

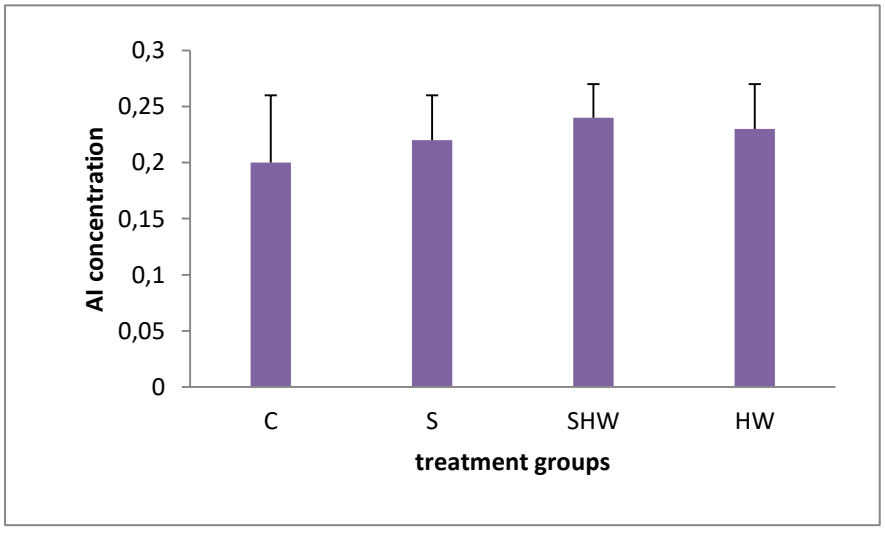


**Figure 31:** The effect of high consumption of crystallize sugar and hot water on AI index in mice.

## Discussion

Epidemiologic studies have suggested that higher intake of added sugar is associated with cardiovascular disease (CVD) risk factors. Few prospective studies have examined the association of added sugar intake with CVD mortality (Quanhe et al., 2013). In this research we aimed to investigate the interaction of high consumption of crystallize sugar on cardiovascular disease and on the other way we looking to evaluate the benefits of hot water on some biochemical parameters.

**Body weight and food**

The weight of animals are increased in group administered with crystallize sugar. This result is in agreement with the work of Zerizer (2006) who reported an increase in the weight of rats treated with 200mg/kg of L-methionine. On the other hand the weight of animal is slightly increased in the group administered with Crystallize sugar and treated with hot water. Our result is agrees with those of Guennoub and Redouane ( 2017 ), who obtained that the weight of rats is increased in group fed with diet rich in trans-fat and treated with hot water.

However, the weight in group of mice treated with hot water is decreased our result is not agrees with the work of Guennoub and Redouane (2017) who obtained that the weight is increased in rats treated with hot water during 21days.

We have detected that the quantity of diet consumed by mice is increased in the groups administered with crystallize sugar and the other administered with normal diet and treated with hot water. Our result is agrees with the work of Guennoub and Redouane ( 2017 ) who obtained that the rats consumed more food in group fed with diet rich trans-fat on the other hand is not agrees with our results because they found that the food consumed by the rats is decreased when they treated with hot water.

However, the diet consumed by mice is decreased in group control and group administered with crystallize sugar and treated with hot water our result is in accordance with Guennoub and Redouane(2017) for the control group but not in accordance with the group (SHW).

The quantity of water was high in the group of mice administered with crystallize sugar and low in groups treated with hot water, this result is agrees with the result of Guennoub and Redouane ( 2017 ) who obtained that the quantity of water consumed is decreased by rats in both groups treated with hot water.

**Biochemical results**

Our study has shown that the treatment with hot water could decrease the concentration of blood glucose when compared to the control group. There is a benefit of drinking hot water on the stimulation of insulin which converts the glucose to glycogen in the liver. Our result is not agrees

with **(Boufedeche et al.,2018)** who found that hot water increased the concentration of blood sugar in females rats treated with hot water during 21 days.

However, the group administered with crystallize sugar and treated with hot water we have detected that blood sugar is higher than the other group.

The quantity of 50g/65kg during 21 days could destroy the ß cells of pancreas langerhans islets cells which decrease the secretion of insulin and inhibited the permeability of glucose to the cells. So the quantity of hot water it is not enough to reduce the high level of glucose formed from the crystallize sugar and we cannot advise people to eat crystallize sugar and drunk hot water.

As found in this study, mice administered with crystallize sugar have shown an increase in T-ch, TG, VLDL when compared to the control group and group treated with hot water. Hot water could decrease the concentrations of TG and VLDL. Our result is agrees with the study of Guennoub and Redouane (2017) who obtained that the levels of T-ch and triglyceride are increased in group of rats fed with diet rich in trans fats .

However, our result is in accordance with that of Guennoub and Redouane (2017) who found that hot water increase T-ch but not in accordance with their results on TG.

In our research we found that hot water increase the levels of HDL-c and LDL-c with a decrease in VLDL. Same results are obtained by Guennoub and Redouane (2017) with HDL-c and LDL-c.

Our result is not agrees with Boufedeche et al.(2018) who found that hot water decrease the concentrations of LDL-c but are in agreement with that of triglycerides.

In addition we have obtained that hot water could decrease AST, CRP and increase ALT.

Boufedeche et al. (2018) confirmed that hot water could decrease the levels of C-reactive protein. Same result is obtained in our study in the three groups (S, SHW and HW), hot water at 50C° has a potential effect to reduce inflammation but the decrease of CRP in animals administered with sugar that mean that 50 g/65kg is not enough to induce the inflammation but could induce the lipid profile during 21days.

Furthermore, we reach that the concentration of GSH was increased in response to the oral crystallize sugar administration when compared to the other groups (C, HW). Our results is not agree with the results of Aklil et al. (2017) who mentioned that the concentration of GSH was decreased in mice administered with L-methionine (500mg/kg ). On the other hand we detected that the concentration of GSH was almost nearly the same in groups of animals treated with hot water and control group.

Glutathione reduced is a tripeptide, γ-L-glutamyl-L-cysteinyl-glycine.The sulfhydryl group (−SH) of the cysteine is involved in reduction and conjugation reactions that are usually

considered as the most important functions of GSH. These reactions provide the means for removal of peroxides and many xenobiotic compounds; however, GSH is also involved in regulation of the cell cycle (Meister, 1992).

A catalase is one of the crucial antioxidant enzymes that attenuates oxidative stress to a considerable extent by destroying cellular hydrogen peroxide to produce water and oxygen. Deficiency or malfunction of catalase is postulated to be related to the pathogenesis of many age-associated degenerative diseases like diabetes mellitus, hypertension, anemia, vitiligo, Alzheimer's disease, Parkinson's disease, bipolar disorder, cancer, and schizophrenia (Ankita, 2019).

Our result presented a decrease of CAT values from liver in the groups (S and SHW) compared to the control group. The liver releases CAT and GSH mainly into the system blood as part of an interorgan turnover there by maintaining a thiol redox balance in the plasma (Slimani, 2021). Tailoring of microbial fermentations has been extensively used to increase the yield and productivities of large number of bioprocesses (Khanna and Srivastava, 2005). All the tested carbon sources were found to support bacterial growth and enzyme synthesis, however sucrose was adjudged as the best source for the catalase synthesis (29,000 IU/mg of cells) from Geobacillus sp. BSS-7. The increase in sucrose concentration in medium up to 1 % (w/v) causes an increase in the catalase production (33,600 IU/mg of cells) and thereafter, the enzyme activity declined (Kauldhar and Sooch, 2005). Likewise, we detected a depletion in the catalase concentrations in the group of animals treated with hot water. This is the first study carried on the interaction of high consumption of crystallize sugar and hot water on antioxidants in cardiovascular disease which need more investigations.

.

# Conclusion and future work

## Conclusion and future work

In this research we estimated the interaction between high consumption of crystallize sugar and hot water 50C° on cardiovascular disease. There is a variation in weight during three weeks of the experiment and corrections of CRP and lipids.

We conclude that eating too much sugar more than 50g/65kg could increase the lipid profile. Additional studies are required to explore the beneficial therapeutic implication of hot water for the treatment of cardiovascular disease.

# References

## References

**Aebi H.** (1974). Methods for enzymatic analysis.Academic Press. 2: 674-84.

**Aklil B., Zerizer S., Kabouche Z.** (2017). The protective effect of Arganiaspinosa seeds against hyperhomocysteinemia induced by high methionine diet in mice. International Journal of Pharmacy and Pharmaceutical Sciences. 9 (12): 64-69.

**AlHajri F.** (2010). The miracle & wonders of treatment from hot water: hot water miracles. 1-108.

**AlHajri F**. (2020). Corona- virus (COVID) out break and the last treasure. A*uthorHouse™ UK Bloomington*, USA.

**Ankita N., Liang-Jun Y., Chandan K. J., and Nilanjana D.** (2019). Role of Catalase in Oxidative Stress- and Age-Associated Degenerative Diseases.Oxidative Medicine and Cellular Longevity. 19.

**Beverly J.K.,and Budoff M.J**. (2019).Atherosclerosis: Pathophysiology of insulin resistance, hyperglycemia, hyperlipidemia, and inflammation. Journal of diabetes.1–3.

**Boufedeche S., Grendou Y., Merikhi**.**F**. (2017). Effet de l'eau chaude sur les maladies cardiovasculaires induites par l'hypercholestérolémie chez les rats.Thèse Master II .Université des frères Mentouri Constantine1 Algeria

**Brian D.** (2017). Anatomy and physiology of the pericardium. Cardiology clinics.35:481–490.

**Camasão D. B., Mantovani D**. (2021). The mechanical characterization of blood vessels and their substitutes in the continuous quest for physiological-relevant performances.A critical review. Materials today bio.10: 100106.

**Cerami A.** (1992). Inflammatory cytokines. Clinical Immunology and Immunopathology, 62(1):3–10.

**Chen L., Deng H., Cui H., Fang J., Zuo Z., Deng J., Li Y., Wang X., Zhao L.** (2018). Inflammatoryresponses and inflammation-associated diseases in organs. Oncotarget. 9(6):7204-7218.

**Chelbi A. M. K., Wietzerbin J**. (2007). Interferon, a growing cytokine family: 50 years of interferon research. Biochimie. 89(6-7): 713–718.

**Christian N**. (2015). Inflammation: causes, symptoms and treatment. Medical news today.1-13.

**Christopher K., Glass, Joseph L., Witztum**. (2001). Atherosclerosis: The road ahead. Cell press. (104):503–516.

**Chu W.M**. (2013).Tumor necrosis factor.Cancer letters. 328(2): 222–225.

**Desai M., Seifalian A.M., Hamilton G.** (2011) .Role of prosthetic conduits in coronary artery bypass grafting.European journal of cardio-thoracic surgery. 40:394–398.

**Galzie Z., Kinsella A. R., Smith J. A.** (1997). Fibroblast grow the factors and the receptors.Biochemistry and cellbiology.75 (6): 669–685.

**Germolec D.R., Shipkowski K.A. Frawley R.P. Evans E.** (2018). Markers of inflammation. Methods in molecular biology.1803:57-79.

**Global R.S.** (2017).Structure of rat heart walls in early postnatal. International Scientific and Practical Conference "world science" .5(21): 2413-1032.

**Gkikas I., Palikaras K., Tavernarakis N.** (2018). The role of mitophagy in innate immunity. Frontiers in immunology. 9:1283.

**Guevara-Noriega K.A., Martinez-Toiran A., Alvarez-Concejo B., Pomar J.L.**(2019) Historical overview of vascular allografts transplantation. Vascular and endovascular surgery.2:19–22.

**Guennoube and Redouane**. (2017).The effect of hot water on inflammation induced by hypercholesterolemia. Thesis submitted for the degree of Master II Université des frères Mentouri Constantine1 (Algeria).

**Herman**. (2016). Physics of the human body, second ed., Springer science & business media, Switzerland.

**Hopkins S. J.** (2003).The pathophysiological role of cytokines.Legal Medicine. 5: 45–57.

**Ivanova E. A., Bobryshev Y. V., Orekhov A. N.** (2015). Intimal pericytes as the second line of immune defence in atherosclerosis.World Journal of Cardiology. 7 (10): 583–593.

**Ivanova E. A., Myasoedova V. A., Melnichenko A. A., Grechko A. V., Orekhov A. N.** (2017). Small dense low-density lipoprotein as biomarker for atherosclerotic diseases. Oxidative Medicine and Cellular Longevity. 1273042.

**Ivanova E. A., Orekhov A. N**. (2016). The role of endoplasmic reticulum stress and unfolded protein response in atherosclerosis. International journal of molecular sciences. 17 (2): 193.

**Jana O., Ladislava M., Jarmila V.A., Kobert V., Jiri M.** (2015). Fatty acids composition of vegetable oils and it contribution to dietary energy intake and dependence ofcardiovascular

mortality ondietry intake of fatty acids.International journal of molecular sciences. 16: 12871-12890.The american journal of clinical nutrition.94 (2): 479-485.

**Janette W., Haley B., Roberta R., Anne P.N.** (2021). Current perspectives on global sugar consumption: Definitions, recommendations, population intakes, challenges and future direction .Nutrition research reviews. (10): 1- 22.

**Jéquier E.** (1994). Carbohydrates as a source of energy. The American journal of clinical nutrition. 59 (3).682-685.

**John R., White, J**. (2018).Sugar. Clinical diabetes.36(1): 74–76.

**Khan M. M.** (2016). Role of cytokines.Immunopharmacology.57–92.

**Kofler S., Nickel T., Weis M**. (2005). Role of cytokines in cardiovascular diseases: a focus on endothelial responses to inflammation. Clinical Science. 108(3): 205–213.

**Kannan R.Y., Salacinski H.J., P.E. ButlerP.E., Hamilton G., Seifalian A.M**. (2005) .Current status of prosthetic bypass grafts: a review, Journal of biomedical materials research part B: Applied biomaterials. 74:570–581. DOI: 10.1002/jbm.b.30247

**Kauldhar B.S and Sooch B.S.**(2016). Tailoring nutritional and process variables for hyperproduction of catalase from a novel isolated bacterium Geobacillus sp. BSS-7. Microbial cell factory. 15-7.

**Khanna S, Srivastava AK**.( 2005) Statistical media optimization studies for growth and PHB production by Ralstoniaeutropha. Proc Biochem. 40(6):2173–82.

**Krüger-Genge A., Blocki A., Franke R. P., Jung F.** (2019). Vascular endothelial cell biology: An update. International journal of molecular sciences. 20 (18):4411.

**LevensonJ.W., SkerrettP.J., GazianoJ.M.** (2007). Reducing the global burden of cardiovascular disease: the role of risk factors. Preventative cardiology.5:188-199.

**Libby P, BuringJ.E., BadimonL**., **HanssonG.K.,DeanfieldJ., BittencourtM.S,**

**Lorraine M., Carolyn M., Tokgözoğlu L., Lewis E.F.** (2019).Atherosclerosis. Nature reviews disease primers .279(47): 48487–48490.

**Mark B. Pepys., Gideon M. Hirschfield.** (2003). C-reactive protein: a critical update. Journal of clinical investigation .111(12):1805-1812.

**Margaret Z., Maissam G., Seba H.** (2018). Sugars: Types and their functional properties in food and human health. International journal of public health research .6(4): 93-99.

**Mathers C.D., Loncar D**.(2006).Projections of global mortality and burden of disease from 2002 to 203.Plos medicine.3:442.

**MedzhitovR.** (2008). Origin and physiological roles of inflammation. Nature. 454(7203):428-35.

**Medzhitov R**. (2010).Inflammation new adventures of an old flame.Journal.10:1016.

**Melly L., Torregrossa G., Lee T., Jansens J.L., Puskas J.D**.(2018). Fifty years of coronary artery bypass grafting, Journal of thoracic disease. 10:02-43.

**Meister A** (1992).Biosynthesis and function of glutathione, an essential biofactor. Journal of Nutritional Science and Vitaminology.1-6. doi: 10.3177/jnsv.38.special_1.

**Mohammad k.A., Klein P.** (2011). Determination of sugars in honey by liquidchromatography. Saudi journal of biological sciences, 18 (1):17-21.

**Mondal S.** (2017). Chemistry of carbohydrates. Pharm vsemapplied biochemistry. 1-20.

**Mizel S. B.**(1989). The interleukins.The faseb journal, 3(12), 2379–2388.

**Neuman R.** (2013). Organic chemistry: Carbohydrates. University of california. Riverside: 20-32.

**Nahrendorf M., Swirski FK., Aikawa E., Stangenberg L., Wur-dinger T., Figueiredo JL., Libby P., Weissleder R., Pittet MJ**. (2007). the healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions.Journal of experimental medicine .204:3037–3047.

**Nakashima Y., Wight T. N., Sueishi K.** (2008). Early atherosclerosis in humans: role of diffuse intimal thickening and extracellular matrix proteoglycans. Cardiovascular research journal. 79 (1):14–23.

**Orekhov A. N., Myasoedova V. A.** (2019). Low density lipoprotein-induced lipid accumulation is a key phenomenon of atherogenesis at the arterial cell level. Vessel plus. (3):3.

**Orekhov A. N., Andreeva E. R., Bobryshev Y. V.** (2016). Cellular mechanisms of human atherosclerosis: Role of cell-to-cell communications in subendothelial cell functions. Tissue cell. 48 (1): 25–34.

**Padarti A., Zhang J.** (2018). Recent advances in cerebral cavernous malformation research. Vessel plus. 2: 29.

**Polly P., Birdem A., Toni S., Caireen R., David C.** (2020). Free and added sugar consumption and adherence to guidelines: The uk national diet and nutrition survey (2014/15–2015/16). Nutrients. 12(2):393.

**Ponticos M., Smith B. D.** (2014). Extracellular matrix synthesis in vascular disease: hypertension, and atherosclerosis. The journal of biomedical research. 28 (1): 25–39.

**Raven P. H., Johnson G. B., Mason K. A., Losos J. B., SingeS. R.** (2014). Sugar isomers have structural differences. In biology .10th ed., AP ed., New York, NY: McGraw-Hill: 38.

**Samson M., Aubry F. (1999)** - Parmentier, M, Que sont les chimiokines ?, médecine sciences paris .15 :966-73.

**SalderaKA., Ali S., Ashfaq A.** (2017). Atherosclerosis; Association of adiponecttin/leptin ratio to intima media thicknessinlocalpopulation.

**Seifu D.G.,Purnama A., Mequanint K.D**.(2013).Mantovani, Small-diameter vascular tissue engineering.Nature reviews cardiology. 10:410–421

**Sessa R., Pietro MD., Filardo S., Turriziani O.** (2014).Infectiousburden and atherosclerosis: a clinical issue.World journal of clinical cases.2:240-249.

**Slimani W.**( 2021). Biological activities of medicinal plant extracts on arthritis induced by formalin and on tumoral process. Doctoral thesis, Université des feres Mentouri-Constantine1 Algeria.

**Spadaccio C., Nappi F., Al-Attar N., Sutherland F.W., Acar C.,Nenna A., Trombetta M., Chello M., Rainer A**. (2016) . Old myths, new concerns: the long-term effects of ascending aorta replacement with dacron grafts. Not all that glitters is gold.Journal of cardiovascular medicine.9:334–342.

**Subbotin V. M.** (2016). Excessive intimal hyperplasia in human coronary arteries before intimal lipid depositions is the initiation of coronary atherosclerosis and constitutes a therapeutic target. Drug discovery today.21 (10):1578–1595.

**Stary H. C.** (1990). The sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries in the first forty years of life. European heart journal.3–19.

**Steven B., Irving K., David S.** (2002). C - reactive protein*.The journal of biological chemistry .279(47): 48487–48490.

**Terry W.** (2004). C - reactive protein an activator of innate immunity and a modulator of adaptive immunity. Immunologic research. 30(3):261–277.

**Terry W.** (2000). Function of C - reactive protein. Annals of medicine.32 (4): 274-278.

**Thornley S., Tayler R., Sikaris K.** (2012). Sugar restriction: the evidence for a drug free intervention to reduce cardiovascular disease risk.Internal medicine journal.42: 46-58.

**Vaquero F., Clara A.** (2006**).**Tratado de las enfermedadesvasculares. Madrid.Viguera. 855–864.

**Weckbercker G., Cory J.G.** (1988). Ribo nucleotide reductase activity and growth of glutathione –depended mouse leukaemia L1210 cells *in vitro*.Cancer letters. 40: 257-64.

**Xu Q. B., Oberhuber G., Gruschwitz M., Wick, G.** (1990). Immunology of atherosclerosis: cellular composition and major histocompatibility complex class II antigen expression in aortic intima, fatty streaks, and atherosclerotic plaques in young and aged human specimens. Clinical immunology and immunopathology.56 (3): 344–359.

**Yang Q.' Zhang Z., Gregg E. W. Flanders W.D., Merritt R. Hu F.B.** (2014). Added sugar intake and cardiovascular diseases mortality among US adults.JAMA internal medicine.174 (4) : 516-24.

**Zerbato M.** (2010). Intérêt du dosage par microméthode de la Protéine C Réactive au cabinet de pédiatrie.Thesis submitted for the degree of doctorat d'état University Henri Poincaré - Nancy 1 (France).

**Zerizer S.** (2006). Hyperhomocysteinemia, B vitamins and atherogenesis clinical and experimental studies. Thesis submitted for the degree of doctorat d'état University Mentouri Constantine Algeria.

**Zhang Y., Wang F**. (2015). Carbohydrate drugs: current status and development prospect. Drug discovery today.9: 79–87.

## Reference web

**Alexander M., Markin, Igor A. Sobenin., Andrey V. Grechko., Dongwei Zhang., Alexander N. Orekhov**. (2020). Cellular mechanisms of human atherogenesis: Focus on chronification of inflammation and mitochondrial mutationsL. In site: https://pubmed.ncbi.nlm.nih.gov/32528276/ .

**Barhum L.** (2022). 5 cardinal signs of inflammation. In site: https://www.verywellhealth..com/signs-of-inflammation-4580526 .

**Felson S**. (2021). Atherosclerosis. In site: https://www.webmd.com/heart-disease/what-is-atherosclerosis?fbclid=IwAR0KtZAoOtl_0S53Bk4j0UqdRCY2v3kCZdFZelGv59br1WjfNnGXplqo4mE .

**Gaea M .**(2021). Structure and function of the heart. In site: https://www.news-medical.net/health/Structure-and-Function-of-the-Heart.aspx .

**Gaziano T., Reddy K.S., Paccaud F.** (2006). Cardiovascular disease.Theworld bank. In site: https://www.ncbi.nlm.nih.gov/books/NBK11767/?report=reader .

**Hordece P.**(2014).Definition de l'inflammation cellulaire. In site :http://www.amiform.com/web/documentation-micronutrition-corse-2014/journee-2/definition-de-l27inflammation-cellulaire.pdf .

**Jonathan D., Connor C., John V.** (2021).Anatomy, Thorax, Aortic arch. In site: https://www.ncbi.nlm.nih.gov/books/NBK499911/ .

**Marengo K.**(2019).Does sugar cause inflammation in the body?.In site: https://www.medicalnewstoda.com/articles/326386 .

**Pahwa R., Goyal A., Bansal P., Jialal I.** (2021). Chronic inflammation. Stat pearls publishing. In site: https://www.ncbi.nlm.nih.gov/books/NBK493173/ .

**Spencer H. (2021).** Sugar: an essay on the impact of sugar on society. In site: https://www.researchgate.net/publication/351118014_SUGAR_an_Essay_on_THE_IMPACT_OF_SUGAR_ON_SOCIETY .

**Sally H., Dian N., Nasuruddin.**(2021).Acute inflammatory response. In site:https://pubmed.ncbi.nlm.nih.gov/32310543/.

**Vishy M**. (2008). Anatomy of the heart. In site:https://www.researchgate.net/publication/257096683_Anatomy_of_the_heart .

**WakimS.andGrewal,M**.(2021). Carbohydrates.Insite : https://bio.libretexts.org/Bookshelves/Human_Biology/Book%3A_Human_Biology_(Wakim_and_Grewal)/03%3A_Chemistry_of_Life/3.05%3A_Carbohydrates .

**Weinhaus A.J.** (2015). Anatomy of the human heart. In site: https://link.springer.com/chapter/10.1007/978-3-319-19464-6_5 .

1:https://teachmesurgery.com/skills/wounds/acute-inflammation/)

2:https://stringfixer.com/fr/C_reactive_protein

3:https://www.groupeproxim.ca/fr/article/laboratoires/dosage-de-la-proteine-c-reactive-crp

4:https://www.shutterstock.com/search/monosaccharide

5:https://www.coursehero.com/study-guides/microbiology/carbohydrates

6:https://zavaromd.com/faq/heart-anatomy

7:https://socratic.org/questions/what-are-the-4-chambers-of-the-heart

8:https://ar.m.wikipedia.org/wiki/%D9%85%D9%84%D9%81:2011_Heart_Valves.jpg

9:https://en.wikipedia.org/wiki/Pericardium

10:https://socratic.org/questions/what-are-the-names-of-three-layers-of-the-heart-wall

11:https://ccasociety.org/education/echoimage/intraoperative-imaging-coronary-arteries

12:https://study.com/learn/lesson/circulatory-system-components-parts.htm

13:https://www.researchgate.net/figure/Structure-of-the-vascular-wall-Adapted-from-Wikipedia-Disposition-of-the-three_fig1_286948064

14:https://courses.lumenlearning.com/boundless-ap/chapter/blood-vessel-structure-and-function

15:https://my.clevelandclinic.org/health/body/21640-blood-vessels

16:https://my.clevelandclinic.org/health/diseases/16753-atherosclerosis-arterial-disease

17:https://www.researchgate.net/figure/Schematic-overview-of-initiation-of-atherosclerotic-lesion-formation-From-Orekhov-and_fig1_341378253

# Summary

## Summary

Sugars occur naturally in our DNA, as well as in fruit, vegetables, honey, sugar beet and sugar cane. Animals provide us with sugars too, in dairy products for example. Sugars are part of the carbohydrate family, alongside starch and cellulose, and are central to the energy metabolism of living beings.

Researchers found that a higher intake of sugar was associated with increased cardiovascular disease (CVD).

In the present study , we evaluated *in vivo* the interaction of crystallize sugar and hot water at 50°C on cardiovascular disease during 21 days in mice , which was evaluated by using the detection of lipids ,CRP and antioxidants.

The results showed that hot water at 50°C could decrease the levels of CRP, T-ch, TG, and VLDL and increase the level of HDL-c, which reduce the deposition of lipids on artery. And an increase of GSH in groups administered with crystallize and crystallize sugar and treated with hot water. However, the level of GSH and catalase was low in the group treated with hot water.

Therefore, we considered that hot water at 50°C is a natural preventive treatment for decreasing the lipid profile and CRP in cardiovascular diseases.

Key words: Cardiovascular disease, Crystallize sugar, Lipids profile, CRP, antioxidants.

## Résumé

Les sucres sont naturellement présents dans l'ADN, ainsi que dans les fruits, les légumes, le miel, la betterave et la canne à sucre. Les animaux nous fournissent aussi des sucres, dans les produits laitiers par exemple. Les sucres font partie de la famille des glucides, dans l'amidon et de la cellulose, et sont une source du métabolisme énergétique des êtres vivants.

Les chercheurs ont constaté qu'une consommation excessive en sucre est associée à une augmentation des maladies cardiovasculaires (MCV).

Dans la présente étude, nous avons étudié *in vivo* l'interaction du sucre cristallisé et de l'eau chaude à 50°C sur l'inflammation des maladies cardiovasculaires pendant 21 jours chez la souris. Nous avons évalué les statuts lipidiques, la CRP et les antioxydants.

Les résultats ont montré que l'eau chaude à 50°C a fait baisser les niveaux de CRP, T-Ch,TG, VLDL et a augmenter le niveau de HDL-c qui réduit le dépôt de lipides sur l'artère. En outre, une élévation du GSH dans les groupes d'animaux nourris avec du sucre cristallisé et cristallisé et de l'eau chaude a été constaté. En revanche, le niveau de GSH et de catalase était faible dans le groupe traité par l'eau chaude. Par conséquent, nous avons considéré que l'eau chaude à 50°C est un traitement préventif naturel pour diminuer le profil lipidique et la CRP dans les maladies cardiovasculaires.



**Mots clés**. Maladies cardiovasculaires - Sucre cristallisé - Lipides - CRP - Antioxydants Eau chaude.

## ملخص

يوجد السكر طبيعيا في الجسم في الحمض النووي الريبي ADN الفواكه، الخضراوات، العسل، الشمندر، سكر القصب ومشتقات الحليب يعتبر من عائلة الكربوهيدرات منها النشويات، السليلوز، وهو يعتبر مصدرا للطاقة. مع ذلك فإن استهلاك كميات كبيرة من السكر قد تزيد من امراض الاوعية الدموية.

في هذه الدراسة قمنا بتقييم فوائد العلاج بالماء الساخن على الالتهاب الناجم عن استهلاك السكر المبلور خلال 21 يوم عند الفئران والتي تم تقييمها بإستخدام الكشف عن البروتين سي التفاعلي والكشف عن كمية الدهون والمركبات المضادة للأكسدة.

أظهرت النتائج ان كمية الماء الساخن في اليوم بالنسبة للفئران يمكن ان تقلل من البروتين سي التفاعلي وكمية الدهون في الدم (الكولسترول-الجلسيريدات) وزيادة البروتين الدهني عالي الكثافة.

لوحظ ارتفاع في الجلوتانيون في الفئران المتغذية على السكر والمجموعة الأخرى المتغذية على السكر مع المعالجة بالماء الساخن، بينما لوحظ انخفاض في الجلوتانيون والكاتالاز في الجموعة المعالجة بالماء الساخن فقط. ولهذا السبب اعتبرنا ان الماء الساخن عند 50 درجة مئوية هو عبارة عن علاج وقائي طبيعي لتقليل من كمية الدهون والبروتين سي التفاعلي في امراض القلب والاوعية الدموية.



الكلمات المفتاحية: امراض القلب والاوعية الدموية، السكر المتبلور، ليبيدات، بروتين سي التفاعلي، مضادات الاكسدة و الماء الساخن.

# Annex

# Annex

## 1. Preparation of solutions

### 1-NaCl

0.9 g → 100 ml

X g → 1000 ml

0.9 g ×1000 ml/100 ml=9 g

### 2-formol 10%

(10 ml formol (37%) + 27 ml of distilled water.)

### 3- TBS

((Tis (3.028g) + NaCl (4.383g)) on 500 ml distilled water) (PH=7.4)

### 4- Bradford

Coomassie blue (0.1g) in Ethanol (50ml) (agitate 2h) + Orthophosphoric acid (100ml) + Distilled Water (850ml)

### 5- BSA

BSA (0.001g) + distilled water (1ml)

### 6-TBS

( $NaH_2PO_4$ (5.995g) + $Na_2HPO_4$ (7.098 g)) in 500ml distilled water on PH= 7.5

### 7- Sulfosalic acid

Sulfosalic acid (0.25 g) →100ml distilled water

### 8- Tris-EDTA

Tris (6.06g) + EDTA (0.96g) → 12.5ml distilled water

### 9- DTNB

DTNB (0.1g) → 25ml Methanol

| year: 2021-2022 | **Présented by :** *ABDERRAHMANE Khitem*<br>SERAOUI Rayene<br>*NASRI Bouchra* |
|---|---|

# Interaction between hot water and high consumption of crystallize sugar in cardiovascular disease

**Mémoire pour l'obtention du diplôme de Master II en Molecular and Cellular Immunology**


## Summary

Sugars occur naturally in our DNA, as well as in fruit, vegetables, honey, sugar beet and sugar cane. Animals provide us with sugars too, in dairy products for example. Sugars are part of the carbohydrate family, alongside starch and cellulose, and are central to the energy metabolism of living beings. Researchers found that a higher intake of sugar was associated with increased cardiovascular disease (CVD).

In the present study , we evaluated *in vivo* the interaction of crystallize sugar and hot water at 50°C on cardiovascular disease during 21 days in mice , which was evaluated by using the detection of lipids ,CRP and antioxidants.

The results showed that hot water at 50°C could decrease the levels of CRP, T-ch, TG, and VLDL and increase the level of HDL-c, which reduce the deposition of lipids on artery. And an increase of GSH in groups administered with crystallize and crystallize sugar and treated with hot water. However, the level of GSH and catalase was low in the group treated with hot water.

Therefore, we considered that hot water at 50°C is a natural preventive treatment for decreasing the lipid profile and CRP in cardiovascular diseases.



Key words: Cardiovascular disease, Hot water, Crystallize sugar, Lipids profile, CRP, antioxidants.



**Research laboratories:**
Laboratory of Immunology (12) (Université Frères Mentouri,Constantine 1).

**Encadreur : Pr. ZERIZER S.** **(Prof**- Université Frères Mentouri, Constantine 1).

**Examinateur 1 : Dr. MESSOUDI S**. (**M.C.A** - Université Frères Mentouri, Constantine 1).

**Examinateur 2 : Dr. RAMLI I.** (**M.A.A** - Université Frères Mentouri, Constantine 1).