



UNIONE EUROPEA
Fondo Sociale Europeo



Ministero dell'Università
e della Ricerca



REACT EU



UNIVERSITÀ
DEGLI STUDI DI BARI
ALDO MORO



UNIVERSITÀ
DEGLI STUDI DI BARI
ALDO MORO

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

Dip. di MEDICINA di PRECISIONE e RIGENERATIVA e AREA JONICA

DOTTORATO DI RICERCA IN

SANITÀ PUBBLICA, MEDICINA CLINICA E ONCOLOGIA

CICLO 37

“Epidemiological and clinical studies: Lipo-metabolic ultrasonography and prevention”

Coordinatore:

Prof. Jean-François Desaphy

Tutor: Prof. Piero Portincasa

Co-Tutor: Prof. Agostino Di Ciaula

Dottoranda: Hala ABDALLAH

Anno accademico 2023/2024

30/01/2025

Contents

Abstract	3
Abstract	3
Introduction.....	5
1. Obesity	5
2. Visceral and subcutaneous fat.....	5
3. Liver steatosis	6
3.1. Steatotic liver disease (SLD)	7
3.2. METABOLIC-ASSOCIATED STEATOTIC LIVER DISEASE (MASLD):	8
3.3. Diagnostic options	8
3.4. Pathogenesis of MASLD	9
4. CARDIOVASCULAR DISEASE (CVD)	9
4.1. Types of CVD	9
4.2. Pathogenesis of CVD	9
4.3. Atherosclerotic Cardiovascular Disease (ASCVD)	10
4.4. Risk factors for ASCVD	10
4.5. From obesity to MASLD to ASCVD	11
5. Strategies for losing weight	12
5.1. Diets	12
5.2. Fasting.....	15
5.3. Anti-obesity medications	17
5.4. Surgical interventions for weight loss.....	18
6. Experimental studies.....	22
7. References.....	23
8. Ramadan and gastrointestinal motility.....	32
9. Ramadan or traditional intermittent fasting?	60
10. Liver steatosis and metabolic dysfunction.....	85
11. Conclusion	102
12. Paper	102

Abstract

Over the last few decades, obesity has become a severe medical condition worldwide, and a risk factor for many cardio-metabolic diseases. It is described as an excessive accumulation of fat in the body leading to other complications such as type 2 diabetes mellitus (T2DM), hypertension, dyslipidaemia, liver steatosis, and cardiovascular diseases [1]. In other cases, obesity may lead to many types of cancer (i.e. breast, ovary, liver, gallbladder, colon and kidney cancer) [2].

The journey from obesity to metabolic dysfunction-associated steatotic liver disease (MASLD) and ultimately to cardiovascular disease (CVD) highlights a critical and interconnected health pathway. Obesity, a central feature of metabolic syndrome, significantly increases the risk of developing MASLD, a condition characterized by excessive fat accumulation in the liver. The presence of MASLD is strongly associated with an elevated risk of cardiovascular disease, which remains the leading cause of mortality among individuals with this liver condition. Shared pathophysiological mechanisms, including insulin resistance, chronic inflammation, and oxidative stress, underpin the link between MASLD and CVD. Addressing obesity is therefore crucial not only for preventing liver-related complications but also for mitigating the risk of cardiovascular events.

Many approaches were studied to find a healthy way to lose weight and prevent and/or cure obesity-related metabolic diseases. Since the timing of meals during the day plays a critical role in losing weight besides the calorie deficit, physical activity, medical interventions, and surgeries, intermittent fasting, especially Ramadan intermittent fasting has been gaining popularity as a healthy strategy to improve weight and metabolic diseases.

Abstract

Negli ultimi decenni, l'obesità è diventata una grave condizione medica in tutto il mondo e un fattore di rischio per molte malattie cardio-metaboliche. È descritta come un accumulo eccessivo di grasso che genera complicanze come diabete mellito tipo 2 (T2DM), ipertensione arteriosa, dislipidemia, steatosi epatica, malattie cardiovascolari [1]. In altri casi, l'obesità può aumentare il rischio per diverse neoplasie (ad es. cancro al seno, alle ovaie, al fegato, alla cistifellea, al colon e ai reni) [2].

Il percorso dall'obesità alla malattia epatica steatosica associata a disfunzione metabolica (MASLD) e, infine, alle malattie cardiovascolari (CVD) è critico e composto da fattori patogenetici interconnessi. L'obesità, una caratteristica centrale della sindrome metabolica, aumenta significativamente il rischio di sviluppare MASLD, una condizione caratterizzata da un accumulo eccessivo di grasso nel fegato, associata a un rischio elevato di malattie cardiovascolari. Queste rappresentano di fatto la principale causa di mortalità nei pazienti con steatosi. Meccanismi patofisiologici comuni (ad es. insulino-resistenza, infiammazione cronica di basso grado, stress ossidativo), sostengono il legame tra MASLD e CVD. Affrontare l'obesità è quindi una sfida fondamentale non solo per prevenire complicazioni correlate al fegato, ma anche per mitigare il rischio di eventi cardiovascolari. Sono stati studiati molti approcci finalizzati al calo ponderale e alla prevenzione e/o alla terapia di malattie metaboliche correlate all'obesità. Poiché la tempistica dei pasti durante il giorno gioca un ruolo fondamentale nella perdita di peso oltre al deficit calorico, all'attività fisica, agli interventi medici e agli interventi chirurgici, il digiuno

intermittente, in particolare il digiuno intermittente del Ramadan, sta guadagnando popolarità come strategia sana per il controllo del peso corporeo e per la gestione di malattie metaboliche.

Introduction

1. Obesity

Worldwide, obesity has become a serious and highly prevalent medical condition, that requires new approaches to prevent and treat obesity-related comorbidities. It consists of an excessive accumulation or an abnormal distribution of body fat [3]. The prevalence of obesity and obesity-related metabolic diseases is currently increasing worldwide and in all age classes.

To firstly identify obesity, the body mass index (BMI) is used by dividing the weight (Kg) to the height (m²) [4]. For adults, a BMI of 25.0 to 29.9 kg/m² is defined as overweight and a BMI of 30 kg/m² or higher is defined as obese [5]. An increase of 5-unit of BMI above 25 kg/m², leads to increase the prevalence of the overall mortality by about 29%, the vascular mortality by 41%, and the diabetes-related mortality by 210% [6]. Different subtypes of obesity exist depending on its metabolic effect as depicted in **Table 1**.

Table 1. Different types and characteristics of obesity.

Obesity types	Characteristics	References
Metabolically healthy obese (MHO)	-Obesity with a lack of metabolic diseases. -Less visceral adipose tissue, smaller adipocytes, and lower inflammatory profile. -High risk of conversion from MHO to MAO over time.	[7]; [8]; [9]; [10]; [11].
Metabolically abnormal obese (MAO)	-Subjects are overweight or obese with metabolic diseases. - Significant difference between MAO and MHO in levels of postprandial blood glucose, high-density lipoprotein cholesterol, insulin and triglycerides.	[12].
Sarcopenic obesity	- Characterized by a reduction of lean mass. -Associated with predicting factors: age, low physical activity, smoking, low socio-economic status, pulmonary disease, and atherosclerosis. - Accumulation of body fat and a decrease of the skeletal muscle mass and muscle strength.	[13]; [14].

2. Visceral and subcutaneous fat

Subcutaneous fat (SAT) is the layer of fat located just under the skin. It serves many important functions, i.e., energy storage, maintaining of the body temperature by providing insulation, and protection of underlying muscles and bones. Unlike visceral fat, which surrounds internal organs, subcutaneous fat is generally considered less harmful to metabolic health. However, excessive accumulation can still contribute to obesity-related complications. Recent studies have shown that subcutaneous fat can secrete various adipokines, which play critical roles in chronic inflammation and insulin resistance [15].

The SAT represents the normal storage of excessive energy intake due to a high fat diet or/and low physical activity, where the excess glycerol and free fatty acids are stored in the adipocytes as triglyceride. Once the storage capacity of SAT or its ability to generate new adipocytes is full because of genetic predisposition or physiological and mental stress, the level of cortisol increases and leads to the accumulation of visceral adipose tissue (VAT) [16]. VAT, also known as intra-abdominal fat, is stored intra-abdominally surrounding vital organs and is associated with increased risk of cardiovascular and metabolic diseases [17]. VAT represents the fat stored within the abdominal cavity and surrounds vital organs such as the liver, pancreas, and intestines. This type of fat is metabolically active and has been linked to various health risks. Unlike subcutaneous fat, visceral fat is associated with a higher risk of metabolic syndrome, type 2 diabetes, cardiovascular diseases, and certain cancers [18]. Visceral fat secretes pro-inflammatory cytokines and adipokines, such as tumour necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6), which contribute to chronic systemic inflammation and insulin resistance. Additionally, it affects lipid metabolism and increases the production of free fatty acids, which can lead to dyslipidaemia and fatty liver disease. Recent studies have highlighted the role of visceral fat in the development of insulin resistance and its impact on glucose metabolism. The accumulation of visceral fat is influenced by factors such as genetics, diet, physical activity, environmental factors and hormonal changes, particularly in postmenopausal women. Effective strategies to reduce visceral fat include regular physical activity, a balanced diet low in refined sugars and saturated fats, and lifestyle modifications aimed at reducing stress and improving sleep quality. Understanding the mechanisms and health implications of visceral fat is crucial for developing targeted interventions to mitigate its adverse effects.

3. Liver steatosis

Liver steatosis is defined as a chronic, progressive condition that affects the liver, characterized by excessive lipid accumulation in the form of intracellular triglycerides within hepatocytes. This accumulation manifests as visible lipid droplets when examined microscopically [19]. Liver steatosis is confirmed if fat content exceeds 5% of the hepatocytes observed on histological evaluation, [20]. The nomenclature of liver steatosis has evolved over time. The origins of liver steatosis date back to 1836, when T. Addison, first documented fatty degeneration of the liver among individuals who consumed excessive amounts of alcohol [21]. In 1986, the nomenclature of liver steatosis became “Non-alcoholic fatty liver disease (NAFLD)”, which was considered as a spectrum of diseases involving the liver, rather than a single entity, ranging from simple liver steatosis to steatohepatitis, having the potential to progress into liver cirrhosis and hepatocellular carcinoma [22]. While focusing specifically on liver steatosis, a distinction was made between two main conditions, ALD and NAFLD [23] (a condition which was typically considered a diagnosis of exclusion, confirmed only after ruling out all other potential secondary causes of liver disease and ensuring minimal or no alcohol consumption [24]). Both ALD and NAFLD clinically presented with similar manifestations, however, they showed differences in prognosis and management, making an early and precise diagnosis challenging yet essential [23]. Over time, the strong association between NAFLD and metabolic dysfunction prompted the need for new terminology, to better describe affected individuals [21]. Therefore, in 2020, the new terminology “MAFLD - Metabolic-associated fatty liver disease” was introduced by Eslam et al [25], to emphasize the metabolic background of liver steatosis and therefore substitute the term NAFLD [26].

However, none of the previously mentioned terms were ideal. The term NAFLD did not emphasize the metabolic pathological processes driving the disease but rather focused on its “non-alcoholic” nature. On the other hand, MAFLD successfully highlighted the underlying metabolic pathological process, however, did not consider the impact of alcohol consumption on steatosis pathogenesis, which is commonly known to exacerbate the hepatic and mortality outcomes [27].

Recognition of these limitations prompted the further need for modifications in nomenclature, therefore in 2023, a new liver steatosis terminology has been introduced “Metabolic-associated steatotic liver disease (MASLD)” [28], replacing the previous terms NAFLD and MAFLD (Figure 1).

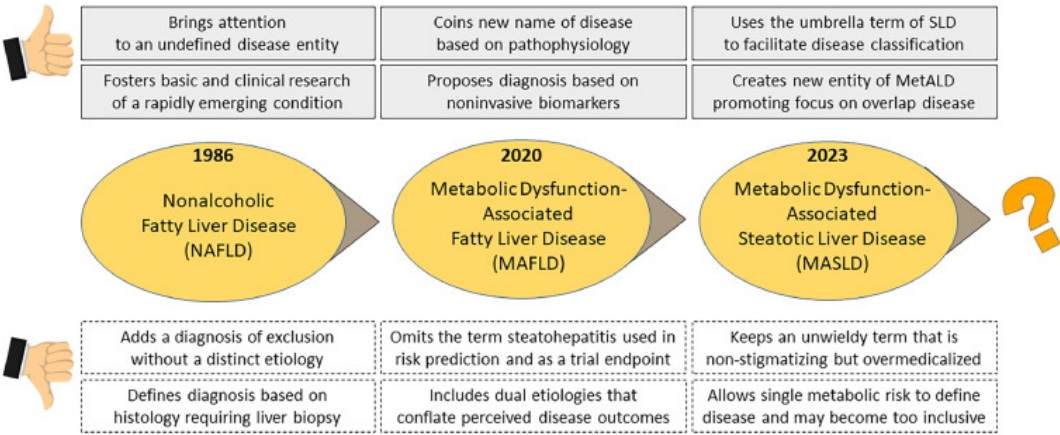


Figure 1: Change of Liver Steatosis terminology [22].

A new term “Steatotic liver disease (SLD)” was introduced to replace the term “fatty liver disease”.

3.1. Steatotic liver disease (SLD)

Based on the presence of metabolic risk factors and alcohol consumption, patients with SLD are classified as follows [21]:

- MASLD: Patients with liver steatosis and at least one of the following metabolic risk factor [29]:
 - Body Mass Index ≥ 25 kg/m² [23 Asia] or waist circumference > 94 (males), 80 cm (females) or ethnicity adjusted
 - Fasting serum glucose ≥ 5.6 mmol/L [100 mg/ dL] or 2-hour post-load glucose levels ≥ 7.8 mmol/L [140 mg/dL] or HbA1c $\geq 5.7\%$ [39 mmol/L] or type 2 diabetes or treatment for type 2 diabetes
 - Blood pressure $\geq 130/85$ mmHg or specific antihypertensive drug treatment
 - Plasma triglycerides ≥ 1.70 mmol/L [150 mg/dL] or lipid-lowering treatment
 - Plasma HDL-cholesterol ≤ 1.0 mmol/L [40 mg/dL] (males) and ≤ 1.3 mmol/L [50 mg/dL] (female) or lipid-lowering treatment [22]
- MetALD: MASLD with higher alcohol consumption [29], average daily alcohol intake exceeds 50 g/day for females and 60 g/day for males, resulting in related liver damage [23].
- Cryptogenic SLD: Patients with liver steatosis without underlying associated metabolic risk factors and no significant alcohol consumption [30].

3.2. METABOLIC-ASSOCIATED STEATOTIC LIVER DISEASE (MASLD):

MASLD has rapidly become a global health issue. It currently stands as the most prevalent chronic liver condition, being the leading cause of primary liver-related illness and death. The prevalence of MASLD has been continuously increasing over the past 30 years and is currently estimated to affect between 25-40% of the general adult population worldwide [26]. The growing prevalence is directly associated with increasing rates of metabolic dysfunctions such as obesity, insulin resistance, hypertension, and dyslipidaemia [31].

Nowadays, MASLD is known to affect around 13% of children and adolescents, given the increasing global prevalence of diabetes and obesity as well as a sedentary lifestyle. The prevalence is the highest in Latin America (44%), followed by Middle East/North Africa (37%), South Asia (34%), North America (31%) and Western Europe (25%) [8] and is relatively more prevalent in males (33%) compared to females (22%) [7].

3.3. Diagnostic options

MASLD is frequently asymptomatic in most patients [32]. Early diagnosis and appropriate management strategies are essential for preventing progression to hepatic complication [32], as well as minimizing extra-hepatic outcomes, particularly CVD, which is the leading cause of death in MASLD patients [33]. To identify the risk factor, patients should undergo an 1) anthropometric screening of BMI and waist circumference; 2) a blood analyses including glycaemic profile, lipid profile (HDL-cholesterol, triglycerides (TG), LDL-cholesterol, and total cholesterol (TC); liver enzymes (ALT, AST, GGT, ALP), 3) assessment of medical history (comorbidities and drugs), 4) alcohol consumption, and 4) physical activity [34]. Moreover, the exclusion of other possible causes of liver steatosis should be performed such as screening for Hepatitis B or C infection. The diagnosis tools are:

Ultrasound is widely available and commonly used for diagnosing and screening liver steatosis. However, it is operator-dependent and may not be effective in obese patients [34]. The severity of liver steatosis is typically graded on a four-point scale: grade 0 (normal, healthy liver without steatosis), grade 1 (mild), grade 2 (moderate), and grade 3 (severe) [35]. Ultrasound shows a sensitivity of 85% and specificity of 94% [34].

Liver biopsy is considered the gold standard for diagnosing liver steatosis. It involves extracting a small tissue sample from the liver, which is then examined under a microscope to assess the extent of fat accumulation. Despite being invasive, liver biopsy provides detailed information about the degree of steatosis, inflammation, and fibrosis, which are crucial for accurate diagnosis and management of the condition [36]. Studies have shown that liver biopsy remains a reliable method for detecting liver steatosis, offering high sensitivity and specificity compared to non-invasive techniques [36].

Magnetic Resonance Imaging (MRI) is a highly effective non-invasive tool for detecting liver steatosis. MRI techniques, such as proton density fat fraction (PDFF), provide precise and reproducible measurements of liver fat content, making it the gold standard for non-invasive quantification [37]. MRI can accurately assess the degree of steatosis and monitor changes over time, which is crucial for diagnosing and managing conditions like non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) [38]. Additionally,

MRI offers the advantage of detecting even mild steatosis, which might be missed by other imaging modalities [39].

Liver indices (biomarkers). Currently, several algorithms are employed in clinical practice for the successful assessment of liver steatosis and fibrosis. Commonly utilized predictive indices include hepatic steatosis index (HIS), fatty liver index (FLI), FIB-4, NAFLD fibrosis score (NFS), Enhanced liver fibrosis (ELF) [40], and AST platelet ratio index (APRI) [34].

3.4. Pathogenesis of MASLD

Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) is a complex condition linked to metabolic dysfunction, involving genetic, epigenetic, environmental, and lifestyle factors. Obesity, insulin resistance, and unhealthy lifestyles are primary risk factors. Dysfunctional adipose tissue releases molecules that impair insulin signaling, leading to insulin resistance and metabolic disturbances [41]. Adipose tissue expansion, accompanying obesity, leads to its dysfunction. Dysfunctional adipose tissue releases molecules such as free fatty acids (FFA), reactive oxygen species (ROS), and pro-inflammatory cytokines (leptin, IL-6, TNF-Alpha, etc) leading to impairment in insulin-signalling pathways and subsequent insulin-resistance [30]. Insulin resistance disturbs the body's balance leading to a hyperinsulinemia state. This imbalance affects glucose and lipid metabolism, thereby contributing to the development of T2DM and de novo lipogenesis, which in turn promotes the development of dyslipidemia[31]. Moreover, the release of pro-inflammatory molecules from adipose tissue contributes to the development of a state of low-grade systemic inflammation, which further impairs the insulin signalling pathway [30]. Additionally, factors such as alcohol, smoking, air pollutants, gut dysbiosis, and food contaminants may contribute to liver steatosis development and progression [7]. Management focuses on lifestyle modifications, including weight loss, dietary changes, and physical activity, as no specific medications are approved for MASLD treatment [42]. Routine monitoring with non-invasive tools is recommended to track disease progression [42].

4. CARDIOVASCULAR DISEASE (CVD)

4.1. Types of CVD

Cardiovascular disease is a term used to describe diseases affecting the cardiovascular system. CVD continues to be the primary cause of disease burden globally and is estimated to be the leading cause of death worldwide. Over the past 30 years, the global incidence of CVD has markedly increased, with the prevalence nearly doubling and CVD-related deaths rising significantly. Currently, Coronary Artery Disease (CAD), such as myocardial infarction, and Cerebrovascular Disease, including stroke, account for the most deaths and disabilities from CVD, highlighting their substantial impact on global health [43].

Cardiovascular disease encompasses various conditions; however, the primary four types include: coronary artery disease (CAD), cerebrovascular disease, peripheral artery disease (PAD), and aortic atherosclerosis [44].

4.2. Pathogenesis of CVD

CVD refers to a group of diseases primarily affecting the heart and vessels, that occur as the result of the narrowing of the vessel's lumen, possibly due to clot formation (thrombosis), artery blockage (embolism), or blood vessel

constriction (stenosis). However, the most common cause of CVD development is the narrowing of the arterial lumen due to the formation of atherosclerotic lipid plaques within the walls of arteries, a condition known as atherosclerosis (ASCVD) [45].

4.3. Atherosclerotic Cardiovascular Disease (ASCVD)

ASCVD is the most common pathological mechanism leading to the development of CVD [45], [46]. It is a chronic inflammatory condition affecting large and medium-sized arteries [47] by the deposition of lipids and fibrous material within the inner walls of vessels with possible further calcification [48], leading to the development of CVD, such as coronary artery disease, strokes, and peripheral vascular disease [49]. The pathogenesis of ASCVD may be due to several risk factors such as hypertension, smoking, diabetes, and dyslipidemia [50].

4.4. Risk factors for ASCVD

Development of ASCVD is associated with several conditions that are recognized to increase the ASCVD risk [51]. Among these factors, a distinction can be drawn between modifiable factors, which are amenable to intervention and modification, and non-modifiable factors, which are inherent and immutable [52]. Given the fact that ASCVD is the most common disease prevalent worldwide [53], all possible preventive measures should be undertaken to potentially assess and prevent the ASCVD [52].

ASCVD risk factors include increasing age, male gender, ethnicity, family history of ASCVD, genetic predisposition, unhealthy dietary habits (western diet, high caloric intake), low physical activity/inactivity, sedentary lifestyle, alcohol consumption, tobacco smoking, primary cholesterolemia, chronic inflammatory conditions, chronic kidney disease, among the female population (polycystic ovarian syndrome (PCOS), use of oral contraceptives, and post-menopausal status), metabolic risk factors (central obesity, insulin resistance, elevated blood pressure, dyslipidemia (particularly high triglycerides and low HDL cholesterol blood levels)) [49,51,54], and metabolic-dysfunction associated pathologies, such as MASLD [55].

Among all the risk factors, the major one for CVD development is metabolic dysfunction. Metabolic Syndrome (MetS) is a term that was introduced to describe individuals at higher risk of CVD due to the presence of underlying cardiometabolic risk factors. MetS is characterized by the presence of 3/5 of the following cardiometabolic risk factors: central obesity, insulin resistance, high blood pressure, abnormal glucose metabolism, and dyslipidemia, including elevated triglycerides and low HDL blood levels [54]. These individuals were established to have higher CVD [56] and therefore the following metabolic alterations are recognized as the major CVD risk factors [51].

Nowadays, with the increasing prevalence of cardiometabolic risk factors in the population, the assessment of atherosclerotic cardiovascular disease (ASCVD) risk holds significant importance [57]. Assessing is fundamental for primary prevention strategies aimed at minimizing morbidity and mortality from ASCVD [52,58]. ASCVD risk can be calculated by a mathematical tool, designed to assess the CVD risk in individuals aged 40-79 years old [59]. These tools utilize parameters such as age, gender, race, smoking status, systolic blood pressure, and the presence of metabolic dysfunction (including T2DM, hypertension, and dyslipidemia) based on plasma lipid profile levels, together with treatments of the following conditions.

4.5. From obesity to MASLD to ASCVD

Obesity contributes to the development of MASLD and CVD through several interconnected mechanisms. Excessive fat accumulation leads to insulin resistance, which disrupts normal metabolic processes and promotes hepatic lipid accumulation. Additionally, obesity-induced systemic inflammation and oxidative stress further exacerbate liver damage and contribute to the progression from simple steatosis to more severe forms like steatohepatitis and fibrosis. These metabolic disturbances also affect cardiovascular health by promoting endothelial dysfunction, atherosclerosis, and hypertension, thereby increasing the risk of CVD [60], [41]. **(Figure 2).**

Initially, liver steatosis was not considered one of the risk factors for cardiovascular disease (CVD) development. However, with the introduction of novel terminology MASLD, there has been a greater understanding of the condition. This terminology sheds light on the association between liver steatosis and metabolic dysfunction, subsequently linking it to an increased risk of CVD [55]. Indeed, contemporary understanding confirms that MASLD has an impact on cardiovascular health and currently it is recognized as a risk factor for major adverse cardiovascular events [61].

Patients with MASLD are at increased risk of developing ASCVD and related CVD mortality, driven by several MASLD-associated mechanisms [62]. MASLD is strongly associated with metabolic dysfunctions including overweight and obesity, insulin resistance, T2DM, dyslipidemia, and hypertension[63]. These factors, together with low-grade systemic chronic inflammation, endothelial dysfunction, and increased oxidative stress, represent the predominant pathological mechanisms contributing to the elevated CVD risk in MASLD patients [64]. Additionally, the pathological process inherent in MASLD further increases the CVD risk, through impaired hepatic lipid metabolism owing to hepatic dysfunction from the lipotoxic effect of accumulated lipids within hepatocytes, overall resulting in exacerbating dyslipidaemia [63].

The precise mechanisms linking MASLD to elevated CVD risk are intricate and multifaceted, involving interactions among metabolic, vascular, and inflammatory processes [55].

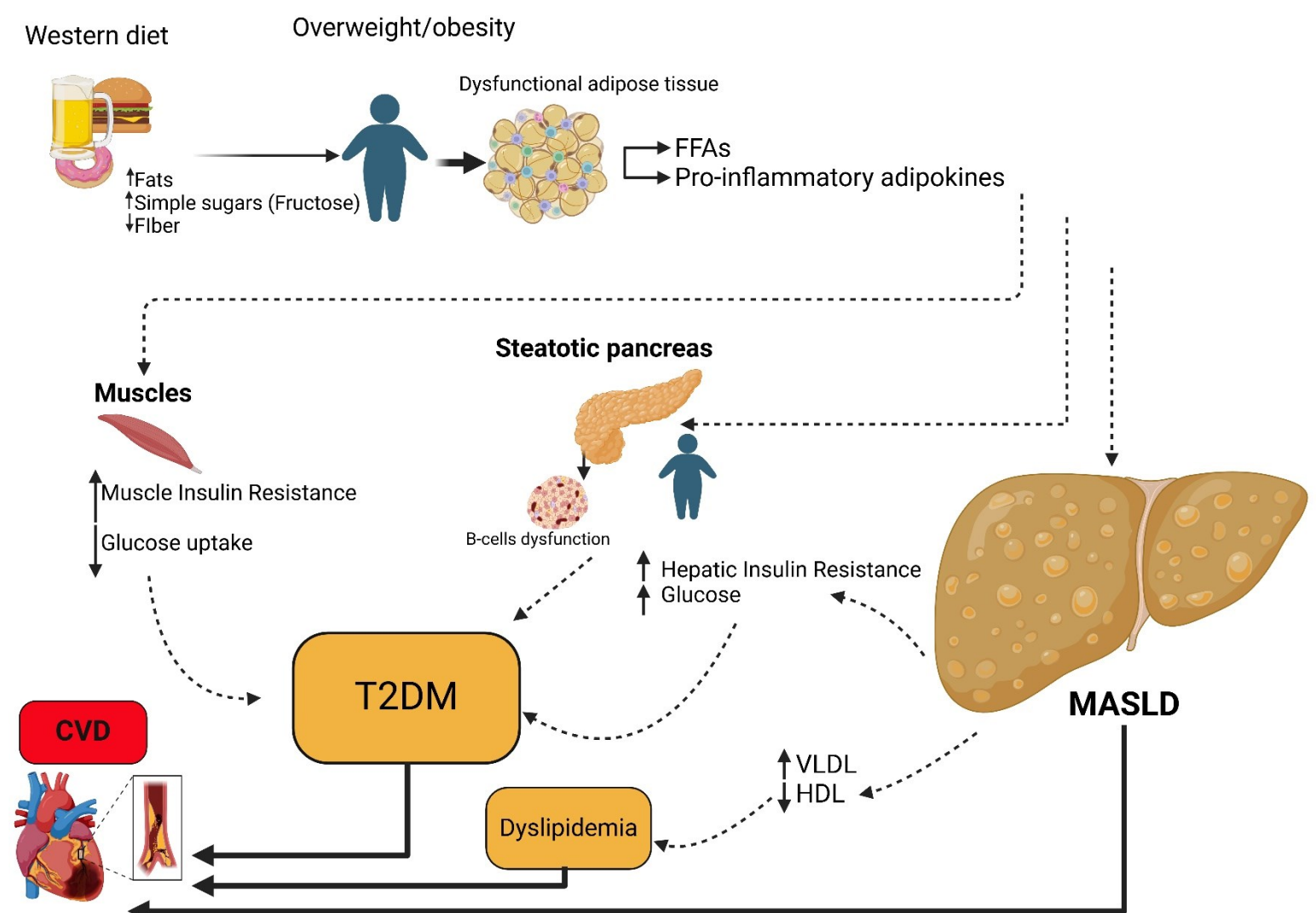


Figure 2. From obesity to MASLD to CVD

5. Strategies for losing weight

Weight loss strategies can be categorized into dietary changes, physical activity, behavioural modifications, and medical interventions. Dietary changes often involve reducing calorie intake, prioritizing whole, nutrient-dense foods, and limiting processed foods and added sugars. Physical activity is crucial, with a combination of aerobic exercises (like walking, running, or cycling) and strength training to build muscle and boost metabolism [66]. Behavioural modifications include setting realistic goals, tracking food intake, and developing healthy eating habits. In some cases, medical interventions such as prescription medications or bariatric surgery may be recommended for individuals with significant weight to lose or underlying health conditions. Combining these strategies can lead to sustainable weight loss and improved overall health. However, due to the high cost of physical activity or the psychological and behavioural interventions, many types and subtypes of diet exists, and the majority targets the caloric intake and the macronutrients range [67].

5.1. Diets

5.1.1. *Ketogenic diet*

Ketogenic diet is one of the most popular diets in the last few years, is consist of a very low carbohydrates consumption with varying levels of fat and protein [68]. The low carbohydrates intake induces Ketosis, that forces the body to burn fats rather than carbohydrates [69]. Previous studies showed its effect in reducing fat mass, and visceral fat mass, while preserving the lean mass in subjects [70].

5.1.2. *Very low-calorie diet*

The very low caloric intake diet is considered as a strategy for rapid weight loss. It consists of the consumption of a very low caloric amount, about 400 to 800 Kcal per day [71], and a replacement of meals by soups, shakes, bars, ready-made meals, powders or ready to drink options [72]. Subjects practicing this type of diet lost a high percent of their weight, divided into fat mass and lean mass, which make it not the best way to improve body composition and maintaining the weight loss [73].

5.1.3. *High protein diet*

The high protein diet consists of the consumption of more than 0.8 g of protein per Kg per day, or 10 to 35% of total calories from protein [74]. This type of diet maintains the lean body mass, since a high consumption of protein enhance the muscle protein synthesis and increase the satiety [75]. Another study shows that a consumption of more than 2g of protein per gram per day is required for maintaining the lean body mass within a caloric deficit diet [76].

5.1.4. *Low fat diet*

Since a gram of fat contains more calories than a gram of protein or a gram of carbohydrates, a low-fat diet may be a strategy for weight loss. It may be a very low fat-diet with <10% of calories from fat, a moderate fat-diet with $\leq 30\%$ of calories from fat and <7%–10% from saturated fatty acids [77]. Although several studies have shown the effect of high fat diets and the consumption of junk food high in saturated fatty acids in the alteration of the gut microbiota and its association with obesity-related metabolic diseases, a low-fat diet seems to be a healthy option for weight loss and prevention of its consequences [78].

5.1.5. *Mediterranean diet (MD)*

MD is a proper diet to maintain a healthy lifestyle promote good health, since poor dietary habits together with low physical activity and a sedentary lifestyle contribute to the development of numerous cardio-metabolic diseases[79].

Mediterranean Diet is a diet emphasizing plant-based foods and healthy fats, where the main components are vegetables, fruits, whole grains, fish, and extra virgin olive oil is the primary source of additional fat, with low-alcohol intake allowed [80].

A famous graphical presentation of the MD dietary pyramid (**Figure 3**) represents the main components of MD, together with their relatively recommended quantity of consumption related to daily/weekly intake[81].

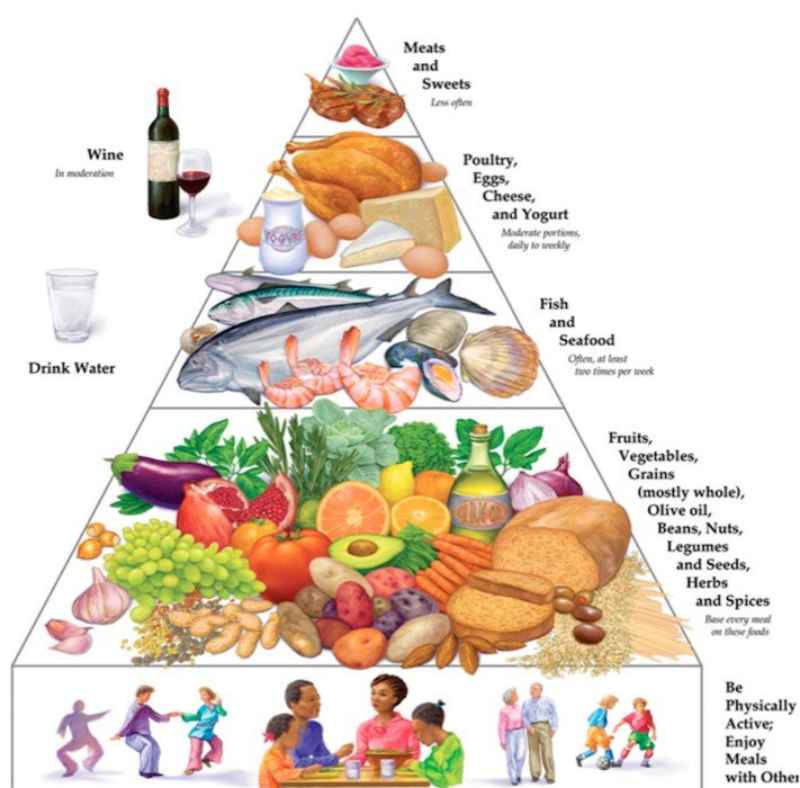


Figure 3. Mediterranean diet pyramid [82]

Beneficial effects of Mediterranean diet on MASLD and CVD:

The MD exerts a variety of beneficial effects on health [83]. Its components are abundant sources of antioxidants such as polyphenols and carotenoids. MD also contains vitamins, minerals, fibers, and healthy fat including monounsaturated fat mainly from olive oil, all recognized for their potential health-promoting effect [84]. It also features complex carbohydrates sourced from legumes and is rich in fibre, predominantly from vegetables and fruits.

Studies suggest that high adherence to a Mediterranean diet may help to reduce liver fat accumulation, increase insulin sensitivity, and improve liver function overall, subsequently leading to improvement of liver steatosis [85] and reduction of development and progression of ASCVD [86]. These are the mechanisms by which MD is believed to lower the rate of liver steatosis [87] and CVD risk [88] (**Figure 4**). Therefore, MD is recommended by the European Society for Clinical Nutrition and Metabolism [26] to improve liver steatosis [89] and lowering of CVD risk[90].

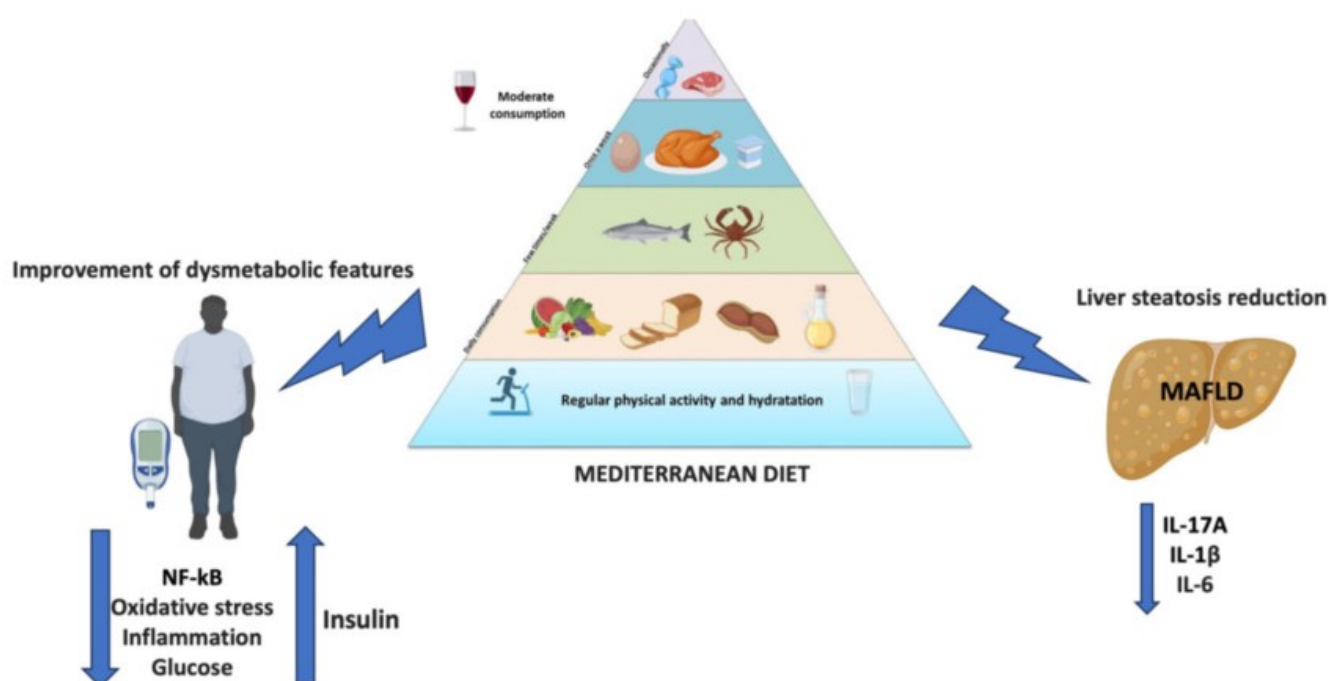


Figure 4. Beneficial effect of the MD in liver steatosis [91].

5.2. Fasting

5.2.1. Intermittent fasting

Intermittent fasting (IF) has been gaining popularity in the past few years as a healthy strategy for weight loss. It is described as an alternation between eating times and fasting times. [92]. Research indicates that intermittent fasting can offer numerous health benefits, including weight loss, improved insulin sensitivity, and reduced risk of type 2 diabetes [93]. Additionally, intermittent fasting may enhance heart health by lowering blood pressure and cholesterol levels [94]. It also promotes cellular repair processes and may reduce inflammation and oxidative stress [95].

Several types of intermittent fasting have been studied, that alternate between periods of fasting and eating. The most common types include:

5.2.2. Alternate-Day Fasting (ADF)

This method involves alternating between days of no caloric intake and days of unrestricted eating. Modified versions allow for a small caloric intake (up to 600 kcal) on fasting days [96]. A systematic review and meta-analysis of randomized controlled trials found that ADF significantly reduces body weight, body mass index (BMI), total cholesterol, low-density lipoprotein (LDL), triglycerides, and blood pressure [97].

5.2.3. Time-Restricted Eating (TRE)

This approach limits the daily eating window to a specific number of hours, typically ranging from 4 to 12 hours, with the remaining hours spent fasting. Previous study found that early TRE (eating within a 6-hour window starting in the morning) was more effective for weight loss and fat loss compared to a control eating schedule [98]. Additionally, TRE has been shown to improve cardiovascular health markers, such as lowering blood pressure and cholesterol levels [99]. However, the long-term sustainability and potential risks of TRE, particularly in different populations, require further investigation [100].

5.2.4. *The 16:8 method*

The 16:8 intermittent fasting regimen, where individuals fast for 16 hours and eat during an 8-hour window, has garnered significant attention for its potential health benefits. Research indicates that this eating pattern can lead to weight loss, improved metabolic health, and enhanced insulin sensitivity. A study published in the journal *Obesity* found that participants following the 16:8 regimen experienced a reduction in body weight and blood pressure. Additionally, intermittent fasting has been shown to decrease levels of insulin and improve insulin sensitivity, which can be beneficial for individuals with or at risk of type 2 diabetes. Another study highlighted in *Cell Metabolism* reported that intermittent fasting could improve circadian rhythms and reduce the risk of chronic diseases such as cardiovascular disease. These findings suggest that the 16:8 intermittent fasting method may offer a sustainable and effective approach to improving overall health and preventing metabolic disorders.

5.2.5. *Ramadan intermittent fasting*

In the Islamic population, the intermittent fasting is one of the religious practices, under the name “Ramadan intermittent fasting” (RIF), where Islamic healthy adult people practice fasting from food and drinks including water from dawn to sunset for 30 days “The Islamic lunar calendar’s ninth month”, per year [101]. The fasting time per day varies between 12 and 22 h (mean 12–14 h), depending on the geographical location and the season of Ramadan’s month [102]. Fasting is not required for premature children, the elderly, the sick, and pregnant and lactating women [103]. The synodic nature of the Islamic calendar means that Ramadan migrates across all four seasons over an approximately 33-year cycle.

Ramadan intermittent fasting has been associated with potential beneficial effects on metabolic syndrome parameters, including reductions in waist circumference, systolic blood pressure, fasting serum glucose, serum lipid profile, and pro-inflammatory cytokines, as well as improvements in insulin sensitivity [104], [105]. Research indicates that RIF can result in significant decreases in body weight and fat mass, although these outcomes are often accompanied by dehydration [106], [107]. Furthermore, RIF has been observed to reduce habitual physical activity due to the inability to refuel and rehydrate during fasting periods [108]. Hormonal changes, such as variations in leptin and adiponectin levels, which regulate appetite and satiety, have also been documented [109], [110]. Overall, while RIF can aid in weight management and metabolic health, it necessitates careful attention to hydration and physical activity levels to optimize its benefits [108]. The lunar nature of the Islamic calendar causes Ramadan to shift through different seasons over a 33-year cycle, influencing the duration and conditions of fasting. This variability can pose challenges for individuals with health conditions such as diabetes [111]. Despite valid exemptions, many individuals choose to fast, reflecting their strong commitment and faith.

Ramadan and gastrointestinal tract

During Ramadan, the practice of fasting from dawn until sunset can significantly impact gastrointestinal motility, affecting the stomach, intestines, and gallbladder. Fasting leads to changes in gastric emptying, with some studies indicating a delay in the process due to prolonged periods without food intake [112]. This can result in a sensation of fullness and discomfort after breaking the fast. In the intestines, fasting may alter the normal rhythmic contractions, potentially slowing down transit time and affecting bowel movements [113]. The gallbladder, which stores bile produced by the liver, may also experience changes in motility. Fasting can lead to reduced gallbladder contractions, which might increase the risk of gallstone formation due to bile stasis [114]. These physiological changes highlight

the importance of mindful eating and hydration during non-fasting hours to maintain gastrointestinal health during Ramadan.

Ramadan and gut microbiota

During Ramadan, the practice of fasting from dawn until sunset can influence gut microbiota and intestinal permeability. Fasting has been shown to alter the composition of gut microbiota, potentially increasing the abundance of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* [115]. These changes can enhance gut health by promoting the production of short-chain fatty acids (SCFAs), which have anti-inflammatory properties [115]. Additionally, fasting may impact intestinal permeability, often referred to as "leaky gut." Some studies suggest that fasting can strengthen the intestinal barrier by increasing the expression of tight junction proteins, thereby reducing intestinal permeability [116]. This can help prevent the translocation of harmful substances from the gut into the bloodstream, which is crucial for maintaining overall health [116].

5.3. Anti-obesity medications

The use of pharmacological therapy for weight loss are usually recommended for people with obesity (BMI higher than 30 Kg/m²), or for overweight people (BMI higher than 27 Kg/m²) with at least one metabolic dysfunction [5]. Following we depicts some of the anti-obesity medication used recently.

5.3.1. Benaglutide

Benaglutide, is a recombinant human GLP1 with 100% similarity to human GLP 1 [117]. It is used as a treatment for the type 2 diabetes [118]. Beinaglutide, like other GLP1RAs, was efficacious in decreasing HbA1c, fasting plasma glucose (FPG), and postprandial plasma glucose (PPG) in T2DM patients [118], [117]. Furthermore, benaglutide effectively lowers the body weight and BMI of overweight or obese diabetes patients [118].

5.3.2. Retatrutide

Retatrutide (LY-3437943) has a triple mechanism, with a combined action on GLP1/GIP/GR. it is being developed for patients with obesity and diabetes. It was administered once daily subcutaneously. This drug candidate targets glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), and glucagon receptor (GR) [119].

5.3.3. Beta 3 adrenergic agonist (3 AR)

Previous study showed improved glucose control in mice's beige adipose tissue [120]. It has been anticipated that 3-AR agonist, mirabegron treatment of obese, insulin-resistant individuals, can enhance beige adipose development in subcutaneous white adipose tissue (SC WAT). Mirabegron therapy significantly enhances glucose homeostasis. Inhibiting SC WAT with mirabegron improves muscle oxidative capacity and cell function [120].

5.3.4. *Tesofensine*

Tesofensine, a drug that blocks the presynaptic neurotransmitters uptake such as noradrenaline, dopamine, and serotonin. Tesofensine 0.5 mg is shown to be a more potent weight loss medication than other drugs. However, the findings need to be ascertained in phase 3 trials [121]. Both doses of Tesofensine did not significantly increase blood pressure than the placebo.

5.4. Surgical interventions for weight loss

Since obesity became a disease that leads to many other obesity-related disorders, a surgical therapy is recommended for those who was not able to lose weight with normal diets and/or medications, and those unable to sustain weight loss. National Institutes of Health (NIH) indicated that the BMI of patients with obesity required to do a bariatric surgery should be higher than 40 Kg/m², or for overweight patients with a BMI between 35 and 40 Kg/m² with at least one obesity-related disease [122]. Bariatric surgery consist of the reduction of the size of gastric pouch, which affects eating behaviours through endocrine and neural signalling, appetite reduction, increase the satiety. These effects in addition to an increase in physical activity increase and maintain the weight loss [123]. Following are the most popular surgical interventions for weight loss:

5.4.1. *Sleeve Gastrectomy*

Sleeve gastrectomy (SG) is a popular bariatric surgery that consists of the removal of approximately 75-80% of the stomach, resulting in a tubular gastric sleeve. This surgery is effective for significant weight loss and improvement in obesity-related comorbidities. SG results in the loss of approximately 50% to 60% of the weight with 2 years after surgery. Studies showed that SG improves the type 2 diabetes, dyslipidaemia and hypertension in studied patients [124]. However, this type of surgeries may lead to some complications such as nutritional deficiencies and gastroesophageal reflux disease (GERD) due to the small size of stomach [125].

5.4.2. *Roux-en-Y Gastric Bypass*

The Roux-en-Y Gastric bypass (RYGB) is also known to significantly decrease the body weight, and to improve obesity-related diseases such as diabetes type 2, hypertension and dyslipidaemia [126]. It consists of the creation of a small pouch and rerouting the small intestine into this pouch. Patients are expected to lose about 70% of their excess body weight within 2 years post-surgery [127] and may maintain a substantial portion of this weight loss even 10 to 15 years post-surgery. Previous study indicates that RYGB is more effective than the SG in the long-term remission of T2DM and other metabolic disorders [126].

5.4.3. *Adjustable Gastric Banding*

Adjustable Gastric Banding (AGB) is a bariatric surgical procedure designed to treat obesity by limiting food intake. The procedure involves placing an adjustable silicone band around the upper part of the stomach to create a small gastric pouch, which restricts the amount of food that can be consumed at one time and promotes

a feeling of fullness. Studies have shown that AGB can lead to significant weight loss and improvements in obesity-related comorbidities, however, the long-term success of AGB can be variable, with some patients experiencing complications such as band slippage, erosion, or the need for reoperation [128]. Despite these challenges, AGB remains a viable option for weight loss in selected patients, particularly those who prefer a less invasive procedure compared to other bariatric surgeries [129].

5.4.4. *Elipse Gastric balloon(s)*

Intragastric balloons (IGBs) are a minimally invasive and reversible therapy for weight loss. the Elipse™ balloon is swallowed in the form of a capsule and then filled with liquid once it reaches the stomach. The balloon remains in the stomach for about 16 weeks, after which it naturally deflates and is excreted from the body. By occupying space in the stomach, the balloon helps patients feel fuller faster and reduces their overall food intake [130]. Clinical studies have shown that the Elipse™ balloon can lead to significant weight loss and improvements in metabolic parameters such as blood sugar levels and cholesterol [131]. The procedure is generally well-tolerated, with common side effects including nausea and abdominal discomfort, which typically resolve within a few days [132].

Table 2. Characteristic, advantages and disadvantages of different strategies of losing weight

	Characteristics	Advantages	Disadvantages	Ref.
Diets				
Ketogenic diet	-Very low carbohydrates -Varing levels of fat and protein	-Burn fats rather than carbohydrates. -Reduce visceral fat mass -Preserve the lean mass	-Headaches, fatigue, nausea, and dizziness - Kidney Stress. - Constipation	[56]; [57]; [133]; [134]
Very low calorie diet	-Very low caloric intake (400 to 800 Kcal/day)	-Rapid weight loss -Loss of fat mass	- Loss of lean mass -Hard maintain of weight loss	[71]; [72]
High protein diet	- About 10 to 35% of total calories from protein [75].	-Maintains the lean mass -Enhance the muscle protein synthesis -Increase the satiety	-Pressure on the kidneys - Constipation, nausea - Dehydration (protein intake lead to frequent urination)	[74]; [76]
Low fat diet	-Very low fat-diet (<10% of calories from fat) -Moderate fat-diet (≤30% of calories from fat)	- Lower cholesterol and lower risk of heart disease -Maintain weigh loss	-Hormonal imbalance -Satiety issues	[64]; [65].
Mediterranean diet	-Plant-based food -Healthy fats -Vegetables, fruits, whole grains, fish, and extra virgin olive oil is the primary source of additional fat	-Low cholesterol, low risk of heart attacks and strokes -Manage blood sugar levels	-	[135]

	-Low alcohol intake	- Lower risk of type 2 diabetes		
Fasting				
Intermittent fasting	-Alternation between eating times and fasting times	-Improve insulin sensitivity, type 2 diabetes, and blood pressure -Lower cholesterol levels - Reduce inflammation and oxidative stress	-Hunger and cravings -Fatigue and low energy -Constipation, bloating, nausea	[92]; [93]; [94]; [95]
Alternate day fasting	-Alternating between days of no caloric intake and days of unrestricted eating -Modified versions: small caloric intake (≤ 600 kcal) on fasting days.	-Reduces body weight, BMI, total cholesterol, LDL, triglycerides, and blood pressure	-Hunger and cravings -Fatigue and low energy -Constipation, bloating, nausea	[96]; [136];
Time restricted eating	- Specific number of eating hours (from 4 to 12), with the remaining hours spent fasting.	- Fat loss -Improve cardiovascular health markers (lowering blood pressure and cholesterol levels)	-Constipation and bloating -Fatigue and low energy	[98]; [99]; [100].
16:8 fasting	-Fasting for 16 hours and eating during an 8-hour window.	-Improve metabolic health -Enhance insulin sensitivity -Reduce blood pressure - reduce the risk of cardiovascular disease.	-Hunger and cravings -Fatigue and low energy -Digestive issues (constipation, bloating, nausea)	[137]
Ramadan fasting	-Fasting from dawn until sunset	-Reduce visceral and subcutaneous fat -Improve gastrointestinal motility (intestine, stomach, gallbladder) -Reduce gastrointestinal symptoms -Improve the gut microbiota	-Sensation of fullness and discomfort after breaking the fast. -May slowing down the transit time and affecting bowel movements	[112]; [113]
Medications				
Benaglutide	- 100% similarity to human GLP 1. - It is used as a treatment for the type 2 diabetes.	-Decrease HbA1c, fasting plasma glucose (FPG), and postprandial plasma	- Gastrointestinal issues (nausea, vomiting, diarrhea, constipation) - Hypoglycemia	[117]; [118]; [138]; [139]

		glucose (PPG) in T2DM patients	- Pancreatitis	
Retatrutide	-Triple mechanism (GLP1/GIP/GR) -A new emerging molecular entity, retatrutide (LY-3437943), is being developed for diabetes patients with obesity -Administered once daily subcutaneously.	- Weight loss - Improves blood sugar levels - Improves lipid profiles	-Gastrointestinal issues (nausea, vomiting, diarrhea) -Hypoglycemia -Injection site reactions	[119]; [139]
Beta 3 adrenergic agonist	-3-AR agonist - primarily used to treat overactive bladder - Enhance beige adipose development in subcutaneous white adipose tissue (SC WAT).	-Improves muscle oxidative capacity and cell function - Improved bladder functions. -Cardiovascular safety	- Increase blood pressure -Gastrointestinal issues (nausea, diarrhea, constipation). -Headache	[120]; [140]; [141]
Tesofensine	-A drug that blocks the presynaptic neurotransmitters uptake such as noradrenaline, dopamine, and serotonin.	- Do not increase blood pressure - Improve metabolic health	- Gastrointestinal issues (nausea, diarrhea, constipation)	[121]
Surgeries				
Sleeve gastrectomy	-Bariatric surgery that consists of the removal of approximately 75-80% of the stomach, resulting in a tubular gastric sleeve	-Significant weight loss (50% to 60% of the weight with 2 years after surgery) -Improve type 2 diabetes, dyslipidaemia and hypertension	- Nutritional deficiencies Gastroesophageal reflux disease (GERD) due to the small size of stomach	[124]; [125]
Roux-en-Y gastric bypass	-Creation of a small pouch and rerouting the small intestine into this pouch	-Significantly decrease the body weight (about 70% of their excess body weight within 2 years post-surgery) - Maintain a substantial portion of this weight loss even 10 to 15 years post-surgery -Improve diabetes type 2, hypertension and dyslipidaemia	- Deficiencies in vitamins and minerals such as vitamin B12, iron, calcium, and vitamin D due to reduced absorption - Bowel Obstruction - Gallstones	[126]; [127]; [142]

Adjustable gastric banding	-Placing an adjustable silicone band around the upper part of the stomach to create a small gastric pouch	-Promotes a feeling of fullness - Significant weight loss and improvements in obesity-related comorbidities -Less invasive procedure	-Complications such as band slippage, erosion, or the need for reoperation	[128]; [129]
Elipse gastric balloon(s)	-The Elipse™ balloon is swallowed in the form of a capsule and then filled with liquid once it reaches the stomach -The balloon remains in the stomach for about 16 weeks, after which it naturally deflates and is excreted from the body	-Minimally invasive procedure -Reversible therapy -Faster feeling of fullness -Reduces their overall food intake - Improve blood sugar levels and cholesterol	-Nausea, abdominal discomfort	[130]; [131]; [132]

6. Experimental studies

During my 3 years oh PhD, we focused on collectively highlighting the beneficial effects of Ramadan intermittent fasting (RIF) on various health parameters: caloric intake, anthropometric measurements, subcutaneous and visceral fat, insulin resistance, the gastrointestinal motility on the level of stomach, gallbladder, and intestine, and the gastrointestinal symptoms. In our first work we studied the effect of Ramadan intermittent fasting on gastrointestinal motility and visceral fat, where we found that RIF led to a decrease in caloric intake, body weight, body mass index, and waist circumference, along with improvements in subcutaneous and visceral fat thickness, insulin resistance, and gastrointestinal motility. On the level of stomach, it showed a smaller fasting and maximal postprandial antral area, which means a less stressed stomach, in addition to a significantly faster postprandial emptying speed (i.e., T1/2) after- than before RIF. On the level of level of gallbladder, a 6% smaller fasting volume and a greater postprandial contraction, meaning more bile flowing in intestine, and faster postprandial emptying time were detected after- than before RIF. On the level of intestine, RIF was associated with a greater microbiota fermentation detected by an earlier H2 peaks and a higher H2 levels, in addition to a shorter Orocecal transit time (OCTT) than before Ramadan. After Ramadan, a signification improvement of gastrointestinal symptoms including gastric fullness, epigastric pain, nausea and heartburn was detected.

Then, the second study focused on studying the effect of intermittent fasting on body composition: anthropometric measurements, serum insulin, glucose, cortisol, non-esterified fatty acid (NEFA), body fat composition (bioelectrical impedance analysis, BIA) and the ultrasonographic measurements of liver steatosis (Hepatorenal

index, HRI) and thickness of subcutaneous (SAT) and visceral (VAT) fats, and the comparison between two types of intermittent fasting: RIF and 16hours IF. Our study showed that both RIF and 16-hour intermittent fasting (16IF) reduced body weight, but RIF had additional benefits in reducing body circumferences, subcutaneous fat, total body fat, and liver steatosis.

And a third study on RIF is ongoing to study the effect of RIF on gut microbiota and intestinal permeability.

Moreover, we studied the correlation between MASLD and CVD and the effect of MD, where we confirmed the association between metabolic-associated steatotic liver disease (MASLD) and cardiovascular disease (CVD) risk, emphasizing the preventive role of the Mediterranean diet (MD) in liver steatosis and the importance of screening for CVD risk factors in MASLD patients. Our results showed that patients with liver steatosis have an increased prevalence of CVD history, cardiometabolic risk factors and an increasing trend of ASCVD compared to non steatotic subjects, and that the highest CVD risk observed in Met-ALD subjects indicates the additional harmful impact of alcohol consumption on CVD

These findings suggest that RIF can generate multiple systemic beneficial effects, and further studies are needed to explore its potential in managing obesity, obesity-related metabolic conditions, liver steatosis, and cardiovascular diseases.

7. References

1. Mayoral, L.P.; Andrade, G.M.; Mayoral, E.P.; Huerta, T.H.; Canseco, S.P.; Rodal Canales, F.J.; Cabrera-Fuentes, H.A.; Cruz, M.M.; Perez Santiago, A.D.; Alpuche, J.J.; et al. Obesity subtypes, related biomarkers & heterogeneity. *Indian J Med Res* **2020**, *151*, 11-21, doi:10.4103/ijmr.IJMR_1768_17.
2. Avgerinos, K.I.; Spyrou, N.; Mantzoros, C.S.; Dalamaga, M. Obesity and cancer risk: Emerging biological mechanisms and perspectives. *Metabolism* **2019**, *92*, 121-135, doi:10.1016/j.metabol.2018.11.001.
3. Bray, G.A. Evaluation of obesity. Who are the obese? *Postgrad Med* **2003**, *114*, 19-27, 38, doi:10.3810/pgm.2003.12.1544.
4. Gallagher, D.; Visser, M.; Sepulveda, D.; Pierson, R.N.; Harris, T.; Heymsfield, S.B. How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? *Am J Epidemiol* **1996**, *143*, 228-239, doi:10.1093/oxfordjournals.aje.a008733.
5. Jensen, M.D.; Ryan, D.H.; Apovian, C.M.; Ard, J.D.; Comuzzie, A.G.; Donato, K.A.; Hu, F.B.; Hubbard, V.S.; Jakicic, J.M.; Kushner, R.F.; et al. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. *J Am Coll Cardiol* **2014**, *63*, 2985-3023, doi:10.1016/j.jacc.2013.11.004.
6. Prospective Studies, C.; Whitlock, G.; Lewington, S.; Sherliker, P.; Clarke, R.; Emberson, J.; Halsey, J.; Qizilbash, N.; Collins, R.; Peto, R. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *Lancet* **2009**, *373*, 1083-1096, doi:10.1016/S0140-6736(09)60318-4.
7. Munoz-Garach, A.; Cornejo-Pareja, I.; Tinahones, F.J. Does Metabolically Healthy Obesity Exist? *Nutrients* **2016**, *8*, doi:10.3390/nu8060320.
8. Puri, R. Is it finally time to dispel the concept of metabolically-healthy obesity? *J Am Coll Cardiol* **2014**, *63*, 2687-2688, doi:10.1016/j.jacc.2014.03.043.

9. Maki, Y.; Nunoi, N.; Kikuchi, M.; Fujishima, M. A profile of plasma branched chain amino acids in a totally pancreatectomized patient: effects of glucagon replacement under a steady feeding state. *Horm Metab Res* **1987**, *19*, 226-227, doi:10.1055/s-2007-1011784.
10. Navarro, E.; Funtikova, A.N.; Fito, M.; Schroder, H. Can metabolically healthy obesity be explained by diet, genetics, and inflammation? *Mol Nutr Food Res* **2015**, *59*, 75-93, doi:10.1002/mnfr.201400521.
11. Wang, Y.C.; Liang, C.S.; Gopal, D.M.; Ayalon, N.; Donohue, C.; Santhanakrishnan, R.; Sandhu, H.; Perez, A.J.; Downing, J.; Gokce, N.; et al. Preclinical Systolic and Diastolic Dysfunctions in Metabolically Healthy and Unhealthy Obese Individuals. *Circ Heart Fail* **2015**, *8*, 897-904, doi:10.1161/CIRCHEARTFAILURE.114.002026.
12. Salgado, A.L.; Carvalho, L.; Oliveira, A.C.; Santos, V.N.; Vieira, J.G.; Parise, E.R. Insulin resistance index (HOMA-IR) in the differentiation of patients with non-alcoholic fatty liver disease and healthy individuals. *Arg Gastroenterol* **2010**, *47*, 165-169, doi:10.1590/s0004-28032010000200009.
13. Tyrovolas, S.; Koyanagi, A.; Olaya, B.; Ayuso-Mateos, J.L.; Miret, M.; Chatterji, S.; Tobiasz-Adameczyk, B.; Koskinen, S.; Leonardi, M.; Haro, J.M. Factors associated with skeletal muscle mass, sarcopenia, and sarcopenic obesity in older adults: a multi-continent study. *J Cachexia Sarcopenia Muscle* **2016**, *7*, 312-321, doi:10.1002/jcsm.12076.
14. Sakuma, K.; Yamaguchi, A. Sarcopenic obesity and endocrinal adaptation with age. *Int J Endocrinol* **2013**, *2013*, 204164, doi:10.1155/2013/204164.
15. Ministerial Meeting on Population of the Non-Aligned, M. Denpasar Declaration on Population and Development. *Integration* **1994**, 27-29, doi:10.1234/2013/999990.
16. Ibrahim, M.M. Subcutaneous and visceral adipose tissue: structural and functional differences. *Obes Rev* **2010**, *11*, 11-18, doi:10.1111/j.1467-789X.2009.00623.x.
17. Staiano, A.E.; Katzmarzyk, P.T. Ethnic and sex differences in body fat and visceral and subcutaneous adiposity in children and adolescents. *Int J Obes (Lond)* **2012**, *36*, 1261-1269, doi:10.1038/ijo.2012.95.
18. Batista, M.V.; Ulrich, J.; Costa, L.; Ribeiro, L.A. Multiple Primary Malignancies in Head and Neck Cancer: A University Hospital Experience Over a Five-Year Period. *Cureus* **2021**, *13*, e17349, doi:10.7759/cureus.17349.
19. Wang, D.Q.; Portincasa, P.; Neuschwander-Tetri, B.A. Steatosis in the liver. *Compr Physiol* **2013**, *3*, 1493-1532, doi:10.1002/cphy.c130001.
20. Santoro, S.; Khalil, M.; Abdallah, H.; Farella, I.; Noto, A.; Dipalo, G.M.; Villani, P.; Bonfrate, L.; Di Ciaula, A.; Portincasa, P. Early and accurate diagnosis of steatotic liver by artificial intelligence (AI)-supported ultrasonography. *Eur J Intern Med* **2024**, *125*, 57-66, doi:10.1016/j.ejim.2024.03.004.
21. Loomba, R.; Wong, V.W. Implications of the new nomenclature of steatotic liver disease and definition of metabolic dysfunction-associated steatotic liver disease. *Aliment Pharmacol Ther* **2024**, *59*, 150-156, doi:10.1111/apt.17846.
22. Portincasa, P.; Baffy, G. Metabolic dysfunction-associated steatotic liver disease: Evolution of the final terminology. *Eur J Intern Med* **2024**, *124*, 35-39, doi:10.1016/j.ejim.2024.04.013.
23. Staufer, K.; Stauber, R.E. Steatotic Liver Disease: Metabolic Dysfunction, Alcohol, or Both? *Biomedicines* **2023**, *11*, doi:10.3390/biomedicines11082108.
24. Portincasa, P. NAFLD, MAFLD, and beyond: one or several acronyms for better comprehension and patient care. *Intern Emerg Med* **2023**, *18*, 993-1006, doi:10.1007/s11739-023-03203-0.
25. Eslam, M.; Sanyal, A.J.; George, J.; International Consensus, P. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* **2020**, *158*, 1999-2014 e1991, doi:10.1053/j.gastro.2019.11.312.

26. Chan, W.K.; Chuah, K.H.; Rajaram, R.B.; Lim, L.L.; Ratnasingam, J.; Vethakkan, S.R. Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD): A State-of-the-Art Review. *J Obes Metab Syndr* **2023**, *32*, 197-213, doi:10.7570/jomes23052.
27. Osna, N.A.; Donohue, T.M., Jr.; Kharbanda, K.K. Alcoholic Liver Disease: Pathogenesis and Current Management. *Alcohol Res* **2017**, *38*, 147-161.
28. Rinella, M.E.; Lazarus, J.V.; Ratziu, V.; Francque, S.M.; Sanyal, A.J.; Kanwal, F.; Romero, D.; Abdelmalek, M.F.; Anstee, Q.M.; Arab, J.P.; et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *Hepatology* **2023**, *78*, 1966-1986, doi:10.1097/HEP.0000000000000520.
29. Yoon, E.L.; Jun, D.W. Correspondence on Letter regarding "Waiting for the changes after the adoption of steatotic liver disease". *Clin Mol Hepatol* **2024**, *30*, 126-128, doi:10.3350/cmh.2023.0500.
30. Moon, J.H.; Jeong, S.; Jang, H.; Koo, B.K.; Kim, W. Metabolic dysfunction-associated steatotic liver disease increases the risk of incident cardiovascular disease: a nationwide cohort study. *EClinicalMedicine* **2023**, *65*, 102292, doi:10.1016/j.eclinm.2023.102292.
31. Bader, H.; Yamin, S.; Alshahwan, H.; Farraj, H.; Maghnam, J.; Abu Omar, Y. Association between Metabolic-Dysfunction-Associated Steatotic Liver Disease and Hepatic Cancer: Current Concepts and Future Challenges. *Journal of clinical medicine* **2024**, *13*, doi:10.3390/jcm13113132.
32. Raggi, P.; Milic, J.; Manicardi, M.; Cinque, F.; Swain, M.G.; Sebastiani, G.; Guaraldi, G. Metabolic dysfunction-associated steatotic liver disease: An opportunity for collaboration between cardiology and hepatology. *Atherosclerosis* **2024**, *392*, 117523, doi:10.1016/j.atherosclerosis.2024.117523.
33. Mellekjaer, A.; Kjaer, M.B.; Haldrup, D.; Gronbaek, H.; Thomsen, K.L. Management of cardiovascular risk in patients with metabolic dysfunction-associated steatotic liver disease. *Eur J Intern Med* **2024**, *122*, 28-34, doi:10.1016/j.ejim.2023.11.012.
34. Mantovani, G.; Bastepe, M.; Monk, D.; de Sanctis, L.; Thiele, S.; Usardi, A.; Ahmed, S.F.; Bufo, R.; Choplin, T.; De Filippo, G.; et al. Diagnosis and management of pseudohypoparathyroidism and related disorders: first international Consensus Statement. *Nat Rev Endocrinol* **2018**, *14*, 476-500, doi:10.1038/s41574-018-0042-0.
35. Lee, D.H. Imaging evaluation of non-alcoholic fatty liver disease: focused on quantification. *Clin Mol Hepatol* **2017**, *23*, 290-301, doi:10.3350/cmh.2017.0042.
36. Shabangu, C.S.; Huang, J.F.; Hsiao, H.H.; Yu, M.L.; Chuang, W.L.; Wang, S.C. Liquid Biopsy for the Diagnosis of Viral Hepatitis, Fatty Liver Steatosis, and Alcoholic Liver Diseases. *Int J Mol Sci* **2020**, *21*, doi:10.3390/ijms21103732.
37. Kim, Y.J.; Choi, J.Y.; Ryu, R.; Lee, J.; Cho, S.J.; Kwon, E.Y.; Lee, M.K.; Liu, K.H.; Rina, Y.; Sung, M.K.; et al. Platycodon grandiflorus Root Extract Attenuates Body Fat Mass, Hepatic Steatosis and Insulin Resistance through the Interplay between the Liver and Adipose Tissue. *Nutrients* **2016**, *8*, doi:10.3390/nu8090532.
38. Choi, E.S.; Kim, J.S.; Nickel, M.D.; Sung, J.K.; Lee, J.K. Free-breathing contrast-enhanced multiphase MRI of the liver in patients with a high risk of breath-holding failure: comparison of compressed sensing-accelerated radial and Cartesian acquisition techniques. *Acta Radiol* **2022**, *63*, 1453-1462, doi:10.1177/02841851211052988.
39. Kang, K.A.; Lee, S.R.; Jun, D.W.; Do, I.G.; Kim, M.S. Diagnostic performance of a novel ultrasound-based quantitative method to assess liver steatosis in histologically identified nonalcoholic fatty liver disease. *Med Ultrason* **2023**, *25*, 7-13, doi:10.11152/mu-3815.
40. Kjaergaard, M.; Lindvig, K.P.; Thorhauge, K.H.; Andersen, P.; Hansen, J.K.; Kastrup, N.; Jensen, J.M.; Hansen, C.D.; Johansen, S.; Israelsen, M.; et al. Using the ELF test, FIB-4 and NAFLD fibrosis score to screen the population for liver disease. *Journal of hepatology* **2023**, *79*, 277-286, doi:10.1016/j.jhep.2023.04.002.

41. Li, Y.; Yang, P.; Ye, J.; Xu, Q.; Wu, J.; Wang, Y. Updated mechanisms of MASLD pathogenesis. *Lipids in Health and Disease* **2024**, *23*, 117, doi:10.1186/s12944-024-02108-x.
42. Portincasa, P.; Khalil, M.; Mahdi, L.; Perniola, V.; Idone, V.; Graziani, A.; Baffy, G.; Di Ciaula, A. Metabolic Dysfunction-Associated Steatotic Liver Disease: From Pathogenesis to Current Therapeutic Options. *Int J Mol Sci* **2024**, *25*, doi:10.3390/ijms25115640.
43. Roth, G.A.; Mensah, G.A.; Johnson, C.O.; Addolorato, G.; Ammirati, E.; Baddour, L.M.; Barengo, N.C.; Beaton, A.Z.; Benjamin, E.J.; Benziger, C.P.; et al. Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study. *J Am Coll Cardiol* **2020**, *76*, 2982-3021, doi:10.1016/j.jacc.2020.11.010.
44. Olvera Lopez, E.; Ballard, B.D.; Jan, A. Cardiovascular Disease. In *StatPearls*; Treasure Island (FL), 2024.
45. Frak, W.; Wojtasinska, A.; Lisinska, W.; Mlynarska, E.; Franczyk, B.; Rysz, J. Pathophysiology of Cardiovascular Diseases: New Insights into Molecular Mechanisms of Atherosclerosis, Arterial Hypertension, and Coronary Artery Disease. *Biomedicines* **2022**, *10*, doi:10.3390/biomedicines10081938.
46. Frostegard, J. Immunity, atherosclerosis and cardiovascular disease. *BMC Med* **2013**, *11*, 117, doi:10.1186/1741-7015-11-117.
47. Kobiyama, K.; Ley, K. Atherosclerosis. *Circ Res* **2018**, *123*, 1118-1120, doi:10.1161/CIRCRESAHA.118.313816.
48. Jebari-Benslaiman, S.; Galicia-Garcia, U.; Larrea-Sebal, A.; Olaetxea, J.R.; Alloza, I.; Vandenbroeck, K.; Benito-Vicente, A.; Martin, C. Pathophysiology of Atherosclerosis. *Int J Mol Sci* **2022**, *23*, doi:10.3390/ijms23063346.
49. Lusis, A.J. Atherosclerosis. *Nature* **2000**, *407*, 233-241, doi:10.1038/35025203.
50. Falk, E. Pathogenesis of atherosclerosis. *J Am Coll Cardiol* **2006**, *47*, C7-12, doi:10.1016/j.jacc.2005.09.068.
51. Wong, N.D.; Budoff, M.J.; Ferdinand, K.; Graham, I.M.; Michos, E.D.; Reddy, T.; Shapiro, M.D.; Toth, P.P. Atherosclerotic cardiovascular disease risk assessment: An American Society for Preventive Cardiology clinical practice statement. *Am J Prev Cardiol* **2022**, *10*, 100335, doi:10.1016/j.ajpc.2022.100335.
52. Francula-Zaninovic, S.; Nola, I.A. Management of Measurable Variable Cardiovascular Disease' Risk Factors. *Curr Cardiol Rev* **2018**, *14*, 153-163, doi:10.2174/1573403X14666180222102312.
53. Mahmood, T.; Shapiro, M.D. The Questions on Everyone's Mind: What is and Why Do We Need Preventive Cardiology? *Methodist Debaquey Cardiovasc J* **2021**, *17*, 8-14, doi:10.14797/mdcvj.698.
54. Huang, P.L. A comprehensive definition for metabolic syndrome. *Dis Model Mech* **2009**, *2*, 231-237, doi:10.1242/dmm.001180.
55. Platek, A.E.; Szymanska, A. Metabolic dysfunction-associated steatotic liver disease as a cardiovascular risk factor. *Clin Exp Hepatol* **2023**, *9*, 187-192, doi:10.5114/ceh.2023.130744.
56. Mottillo, S.; Filion, K.B.; Genest, J.; Joseph, L.; Pilote, L.; Poirier, P.; Rinfret, S.; Schiffrin, E.L.; Eisenberg, M.J. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. *J Am Coll Cardiol* **2010**, *56*, 1113-1132, doi:10.1016/j.jacc.2010.05.034.
57. Huang, H.; Liu, J.; Liang, X.; Fang, L.; Yang, C.; Ke, K.; Bai, H.; Xu, W.; Li, W.; Meng, F.; et al. Trends in the prevalence of elevated cardiovascular risk and the control of its risk factors Among US adults, 2001-2020. *Front Cardiovasc Med* **2023**, *10*, 1153926, doi:10.3389/fcvm.2023.1153926.
58. Goldsborough, E., 3rd; Osuji, N.; Blaha, M.J. Assessment of Cardiovascular Disease Risk: A 2022 Update. *Endocrinol Metab Clin North Am* **2022**, *51*, 483-509, doi:10.1016/j.ecl.2022.02.005.
59. Rosu, M.M.; Popa, S.G.; Mota, E.; Popa, A.; Manolache, M.; Guja, C.; Bala, C.; Mota, C.; Mota, M. Cardiovascular Risk Assessment in the Adult (Aged 40-79 Years) Romanian Population. *Acta Endocrinol (Buchar)* **2018**, *14*, 227-234, doi:10.4183/aeb.2018.227.

60. Zheng, H.; Sechi, L.A.; Navarese, E.P.; Casu, G.; Vidili, G. Metabolic dysfunction-associated steatotic liver disease and cardiovascular risk: a comprehensive review. *Cardiovascular Diabetology* **2024**, *23*, 346, doi:10.1186/s12933-024-02434-5.
61. Targher, G.; Byrne, C.D.; Tilg, H. MASLD: a systemic metabolic disorder with cardiovascular and malignant complications. *Gut* **2024**, *73*, 691-702, doi:10.1136/gutjnl-2023-330595.
62. Yanai, H.; Adachi, H.; Hakoshima, M.; Iida, S.; Katsuyama, H. Metabolic-Dysfunction-Associated Steatotic Liver Disease-Its Pathophysiology, Association with Atherosclerosis and Cardiovascular Disease, and Treatments. *Int J Mol Sci* **2023**, *24*, doi:10.3390/ijms242015473.
63. Rao, G.; Peng, X.; Li, X.; An, K.; He, H.; Fu, X.; Li, S.; An, Z. Unmasking the enigma of lipid metabolism in metabolic dysfunction-associated steatotic liver disease: from mechanism to the clinic. *Front Med (Lausanne)* **2023**, *10*, 1294267, doi:10.3389/fmed.2023.1294267.
64. Pacinella, G.; Ciaccio, A.M.; Tuttolomondo, A. Endothelial Dysfunction and Chronic Inflammation: The Cornerstones of Vascular Alterations in Age-Related Diseases. *Int J Mol Sci* **2022**, *23*, doi:10.3390/ijms232415722.
65. Galatou, E.; Mourelatou, E.; Hatziantoniou, S.; Vizirianakis, I.S. Nonalcoholic Steatohepatitis (NASH) and Atherosclerosis: Explaining Their Pathophysiology, Association and the Role of Incretin-Based Drugs. *Antioxidants (Basel)* **2022**, *11*, doi:10.3390/antiox11061060.
66. Cowan, S.; Lim, S.; Alycia, C.; Pirotta, S.; Thomson, R.; Gibson-Helm, M.; Blackmore, R.; Naderpoor, N.; Bennett, C.; Ee, C.; et al. Lifestyle management in polycystic ovary syndrome - beyond diet and physical activity. *BMC Endocr Disord* **2023**, *23*, 14, doi:10.1186/s12902-022-01208-y.
67. Willoughby, D.; Hewlings, S.; Kalman, D. Body Composition Changes in Weight Loss: Strategies and Supplementation for Maintaining Lean Body Mass, a Brief Review. *Nutrients* **2018**, *10*, doi:10.3390/nu10121876.
68. McGaugh, E.; Barthel, B. A Review of Ketogenic Diet and Lifestyle. *Mo Med* **2022**, *119*, 84-88.
69. Frisch, S.; Zittermann, A.; Berthold, H.K.; Gotting, C.; Kuhn, J.; Kleesiek, K.; Stehle, P.; Kortke, H. A randomized controlled trial on the efficacy of carbohydrate-reduced or fat-reduced diets in patients attending a telemedically guided weight loss program. *Cardiovasc Diabetol* **2009**, *8*, 36, doi:10.1186/1475-2840-8-36.
70. Gomez-Arbelaez, D.; Bellido, D.; Castro, A.I.; Ordonez-Mayan, L.; Carreira, J.; Galban, C.; Martinez-Olmos, M.A.; Crujeiras, A.B.; Sajoux, I.; Casanueva, F.F. Body Composition Changes After Very-Low-Calorie Ketogenic Diet in Obesity Evaluated by 3 Standardized Methods. *J Clin Endocrinol Metab* **2017**, *102*, 488-498, doi:10.1210/jc.2016-2385.
71. Aragon, A.A.; Schoenfeld, B.J.; Wildman, R.; Kleiner, S.; VanDusseldorp, T.; Taylor, L.; Earnest, C.P.; Arciero, P.J.; Wilborn, C.; Kalman, D.S.; et al. International society of sports nutrition position stand: diets and body composition. *J Int Soc Sports Nutr* **2017**, *14*, 16, doi:10.1186/s12970-017-0174-y.
72. Brown, R.E.; Canning, K.L.; Fung, M.; Jiandani, D.; Riddell, M.C.; Macpherson, A.K.; Kuk, J.L. Calorie Estimation in Adults Differing in Body Weight Class and Weight Loss Status. *Med Sci Sports Exerc* **2016**, *48*, 521-526, doi:10.1249/MSS.0000000000000796.
73. Hoie, L.H.; Bruusgaard, D.; Thom, E. Reduction of body mass and change in body composition on a very low calorie diet. *Int J Obes Relat Metab Disord* **1993**, *17*, 17-20.
74. Trumbo, P.; Schlicker, S.; Yates, A.A.; Poos, M.; Food; Nutrition Board of the Institute of Medicine, T.N.A. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *J Am Diet Assoc* **2002**, *102*, 1621-1630, doi:10.1016/s0002-8223(02)90346-9.
75. Hector, A.J.; Phillips, S.M. Protein Recommendations for Weight Loss in Elite Athletes: A Focus on Body Composition and Performance. *Int J Sport Nutr Exerc Metab* **2018**, *28*, 170-177, doi:10.1123/ijsnem.2017-0273.

76. Mettler, S.; Mitchell, N.; Tipton, K.D. Increased protein intake reduces lean body mass loss during weight loss in athletes. *Med Sci Sports Exerc* **2010**, *42*, 326-337, doi:10.1249/MSS.0b013e3181b2ef8e.
77. Liu, A.G.; Ford, N.A.; Hu, F.B.; Zelman, K.M.; Mozaffarian, D.; Kris-Etherton, P.M. A healthy approach to dietary fats: understanding the science and taking action to reduce consumer confusion. *Nutr J* **2017**, *16*, 53, doi:10.1186/s12937-017-0271-4.
78. Kim, J.Y. Optimal Diet Strategies for Weight Loss and Weight Loss Maintenance. *J Obes Metab Syndr* **2021**, *30*, 20-31, doi:10.7570/jomes20065.
79. Sofi, F.; Casini, A. Mediterranean diet and non-alcoholic fatty liver disease: new therapeutic option around the corner? *World J Gastroenterol* **2014**, *20*, 7339-7346, doi:10.3748/wjg.v20.i23.7339.
80. Lacatusu, C.M.; Grigorescu, E.D.; Floria, M.; Onofriescu, A.; Mihai, B.M. The Mediterranean Diet: From an Environment-Driven Food Culture to an Emerging Medical Prescription. *Int J Environ Res Public Health* **2019**, *16*, doi:10.3390/ijerph16060942.
81. Bach-Faig, A.; Berry, E.M.; Lairon, D.; Reguant, J.; Trichopoulou, A.; Dernini, S.; Medina, F.X.; Battino, M.; Belahsen, R.; Miranda, G.; et al. Mediterranean diet pyramid today. Science and cultural updates. *Public Health Nutr* **2011**, *14*, 2274-2284, doi:10.1017/S1368980011002515.
82. Tuttolomondo, A.; Simonetta, I.; Daidone, M.; Mogavero, A.; Ortello, A.; Pinto, A. Metabolic and Vascular Effect of the Mediterranean Diet. *Int J Mol Sci* **2019**, *20*, doi:10.3390/ijms20194716.
83. Franco, G.A.; Interdonato, L.; Cordaro, M.; Cuzzocrea, S.; Di Paola, R. Bioactive Compounds of the Mediterranean Diet as Nutritional Support to Fight Neurodegenerative Disease. *Int J Mol Sci* **2023**, *24*, doi:10.3390/ijms24087318.
84. Santos-Buelga, C.; Gonzalez-Manzano, S.; Gonzalez-Paramas, A.M. Wine, Polyphenols, and Mediterranean Diets. What Else Is There to Say? *Molecules* **2021**, *26*, doi:10.3390/molecules26185537.
85. Leone, A.; Bertoli, S.; Bedogni, G.; Vignati, L.; Pellizzari, M.; Battezzati, A. Association between Mediterranean Diet and Fatty Liver in Women with Overweight and Obesity. *Nutrients* **2022**, *14*, doi:10.3390/nu14183771.
86. Jimenez-Torres, J.; Alcalá-Díaz, J.F.; Torres-Pena, J.D.; Gutierrez-Mariscal, F.M.; Leon-Acuna, A.; Gomez-Luna, P.; Fernandez-Gandara, C.; Quintana-Navarro, G.M.; Fernandez-Garcia, J.C.; Perez-Martinez, P.; et al. Mediterranean Diet Reduces Atherosclerosis Progression in Coronary Heart Disease: An Analysis of the CORDIOPREV Randomized Controlled Trial. *Stroke* **2021**, *52*, 3440-3449, doi:10.1161/STROKEAHA.120.033214.
87. Anania, C.; Perla, F.M.; Olivero, F.; Pacifico, L.; Chiesa, C. Mediterranean diet and nonalcoholic fatty liver disease. *World J Gastroenterol* **2018**, *24*, 2083-2094, doi:10.3748/wjg.v24.i19.2083.
88. Dontas, A.S.; Zerefos, N.S.; Panagiotakos, D.B.; Vlachou, C.; Valis, D.A. Mediterranean diet and prevention of coronary heart disease in the elderly. *Clin Interv Aging* **2007**, *2*, 109-115, doi:10.2147/cia.2007.2.1.109.
89. Montemayor, S.; Mascaro, C.M.; Ugarriza, L.; Casares, M.; Llompert, I.; Abete, I.; Zulet, M.A.; Martinez, J.A.; Tur, J.A.; Bouzas, C. Adherence to Mediterranean Diet and NAFLD in Patients with Metabolic Syndrome: The FLIPAN Study. *Nutrients* **2022**, *14*, doi:10.3390/nu14153186.
90. Bonaccio, M.; Di Castelnuovo, A.; Pounis, G.; Costanzo, S.; Persichillo, M.; Cerletti, C.; Donati, M.B.; de Gaetano, G.; Iacoviello, L.; Moli-sani Study, I. High adherence to the Mediterranean diet is associated with cardiovascular protection in higher but not in lower socioeconomic groups: prospective findings from the Moli-sani study. *Int J Epidemiol* **2017**, *46*, 1478-1487, doi:10.1093/ije/dyx145.
91. Abenavoli, L.; Gambardella, M.L.; Scarlata, G.G.M.; Lenci, I.; Baiocchi, L.; Luzza, F. The Many Faces of Metabolic Dysfunction-Associated Fatty Liver Disease Treatment: From the Mediterranean Diet to Fecal Microbiota Transplantation. *Medicina (Kaunas)* **2024**, *60*, doi:10.3390/medicina60040563.

92. Varady, K.A.; Cienfuegos, S.; Ezpeleta, M.; Gabel, K. Clinical application of intermittent fasting for weight loss: progress and future directions. *Nat Rev Endocrinol* **2022**, *18*, 309-321, doi:10.1038/s41574-022-00638-x.
93. Nowosad, K.; Sujka, M. Effect of Various Types of Intermittent Fasting (IF) on Weight Loss and Improvement of Diabetic Parameters in Human. *Curr Nutr Rep* **2021**, *10*, 146-154, doi:10.1007/s13668-021-00353-5.
94. Carvajal, V.; Marin, A.; Gihardo, D.; Maluenda, F.; Carrasco, F.; Chamorro, R. [Intermittent fasting and human metabolic health]. *Rev Med Chil* **2023**, *151*, 81-100, doi:10.4067/s0034-98872023000100081.
95. Malinowski, B.; Zalewska, K.; Wesierska, A.; Sokolowska, M.M.; Socha, M.; Liczner, G.; Pawlak-Osinska, K.; Wicinski, M. Intermittent Fasting in Cardiovascular Disorders-An Overview. *Nutrients* **2019**, *11*, doi:10.3390/nu11030673.
96. Patikorn, C.; Roubal, K.; Veettil, S.K.; Chandran, V.; Pham, T.; Lee, Y.Y.; Giovannucci, E.L.; Varady, K.A.; Chaiyakunapruk, N. Intermittent Fasting and Obesity-Related Health Outcomes: An Umbrella Review of Meta-analyses of Randomized Clinical Trials. *JAMA Network Open* **2021**, *4*, e2139558-e2139558, doi:10.1001/jamanetworkopen.2021.39558 %J JAMA Network Open.
97. Cui, Y.; Cai, T.; Zhou, Z.; Mu, Y.; Lu, Y.; Gao, Z.; Wu, J.; Zhang, Y. Health Effects of Alternate-Day Fasting in Adults: A Systematic Review and Meta-Analysis. *Front Nutr* **2020**, *7*, 586036, doi:10.3389/fnut.2020.586036.
98. Zheng, Y.; Wang, J.; Liu, M.; Zhou, X.; Lin, X.; Liang, Q.; Yang, J.; Zhang, M.; Chen, Z.; Li, M.; et al. Time-restricted eating with or without a low-carbohydrate diet improved myocardial status and thyroid function in individuals with metabolic syndrome: secondary analysis of a randomized clinical trial. *BMC Medicine* **2024**, *22*, 362, doi:10.1186/s12916-024-03595-6.
99. Gabel, K.; Cienfuegos, S.; Kalam, F.; Ezpeleta, M.; Varady, K.A. Time-Restricted Eating to Improve Cardiovascular Health. *Current Atherosclerosis Reports* **2021**, *23*, 22, doi:10.1007/s11883-021-00922-7.
100. Fanti, M.; Mishra, A.; Longo, V.D.; Brandhorst, S. Time-Restricted Eating, Intermittent Fasting, and Fasting-Mimicking Diets in Weight Loss. *Current Obesity Reports* **2021**, *10*, 70-80, doi:10.1007/s13679-021-00424-2.
101. Abdallah, H.; Khalil, M.; Farella, I.; JohnBritto, J.S.; Lanza, E.; Santoro, S.; Garruti, G.; Portincasa, P.; Di Ciaula, A.; Bonfrate, L. Ramadan intermittent fasting reduces visceral fat and improves gastrointestinal motility. *Eur J Clin Invest* **2023**, *53*, e14029, doi:10.1111/eci.14029.
102. Rashed, A.H. The fast of Ramadan. *BMJ* **1992**, *304*, 521-522, doi:10.1136/bmj.304.6826.521.
103. Bazzano, A.N.; Potts, K.S.; Mulugeta, A. How do pregnant and lactating women, and young children, experience religious food restriction at the community level? A qualitative study of fasting traditions and feeding behaviors in four regions of Ethiopia. *PLoS One* **2018**, *13*, e0208408, doi:10.1371/journal.pone.0208408.
104. Faris, M.e.A.-I.E.; Jahrami, H.A.; Alsibai, J.; Obaideen, A.A. Impact of Ramadan diurnal intermittent fasting on the metabolic syndrome components in healthy, non-athletic Muslim people aged over 15 years: a systematic review and meta-analysis. *British Journal of Nutrition* **2020**, *123*, 1-22, doi:10.1017/S000711451900254X.
105. Jahrami, H.; Ammar, A.; Glenn, J.M.; Saif, Z.; Chtourou, H.; Trabelsi, K. An umbrella review and meta-analysis of meta-analyses of the impact of Ramadan fasting on the metabolic syndrome components. *Nutrition Reviews* **2025**, *83*, e711-e721, doi:10.1093/nutrit/nuae001.
106. Correia, J.M.; Santos, I.; Pazarat-Correia, P.; Silva, A.M.; Mendonca, G.V. Effects of Ramadan and Non-ramadan Intermittent Fasting on Body Composition: A Systematic Review and Meta-Analysis. *Front Nutr* **2020**, *7*, 625240, doi:10.3389/fnut.2020.625240.
107. Al-Jafar, R.; Wahyuni, N.S.; Belhaj, K.; Ersi, M.H.; Boroghani, Z.; Alreshidi, A.; Alkhalaf, Z.; Elliott, P.; Tsilidis, K.K.; Dehghan, A. The impact of Ramadan intermittent fasting on anthropometric measurements and body composition: Evidence from LORANS study and a meta-analysis. *Front Nutr* **2023**, *10*, 1082217, doi:10.3389/fnut.2023.1082217.

108. Farooq, A.; Chamari, K.; Sayegh, S.; El Akoum, M.; Al-Mohannadi, A.S. Ramadan daily intermittent fasting reduces objectively assessed habitual physical activity among adults. *BMC Public Health* **2021**, *21*, 1912, doi:10.1186/s12889-021-11961-9.
109. Gaeini, Z.; Mirmiran, P.; Bahadoran, Z. Effects of Ramadan intermittent fasting on leptin and adiponectin: a systematic review and meta-analysis. *Hormones* **2021**, *20*, 237-246, doi:10.1007/s42000-021-00285-3.
110. Kazeminasab, F.; Behzadnejad, N.; Cerqueira, H.S.; Santos, H.O.; Rosenkranz, S.K. Effects of intermittent fasting combined with exercise on serum leptin and adiponectin in adults with or without obesity: a systematic review and meta-analysis of randomized clinical trials. *Front Nutr* **2024**, *11*, 1362731, doi:10.3389/fnut.2024.1362731.
111. Al-Sofiani, M.E.; Alharthi, S.; Albunyan, S.; Alzaman, N.; Klonoff, D.C.; Alguwaihes, A. A Real-World Prospective Study of the Effectiveness and Safety of Automated Insulin Delivery Compared With Other Modalities of Type 1 Diabetes Treatment During Ramadan Intermittent Fasting. *Diabetes Care* **2024**, *47*, 683-691, doi:10.2337/dc23-1968.
112. Patricia, J.J.; Dhamoon, A.S. Physiology, Digestion. In *StatPearls*; Treasure Island (FL) ineligible companies. Disclosure: Amit Dhamoon declares no relevant financial relationships with ineligible companies., 2025.
113. Nam, Y.; Lee, J.M.; Wang, Y.; Ha, H.S.; Sohn, U.D. The effect of Flos Lonicerae Japonicae extract on gastrointestinal motility function. *J Ethnopharmacol* **2016**, *179*, 280-290, doi:10.1016/j.jep.2015.12.056.
114. Kojima, K.; Chambers, J.K.; Nakashima, K.; Goto-Koshino, Y.; Uchida, K. Immunophenotyping of intraepithelial lymphocytes in canine chronic enteropathy and intestinal T-cell lymphoma using endoscopic samples. *Vet Pathol* **2022**, *59*, 227-235, doi:10.1177/03009858211057220.
115. Wang, Y.; Wu, Y.; Sailike, J.; Sun, X.; Abuduwaili, N.; Tuoliuhan, H.; Yusufu, M.; Nabi, X.H. Fourteen composite probiotics alleviate type 2 diabetes through modulating gut microbiota and modifying M1/M2 phenotype macrophage in db/db mice. *Pharmacol Res* **2020**, *161*, 105150, doi:10.1016/j.phrs.2020.105150.
116. Al-Obaide, M.A.I.; Singh, R.; Datta, P.; Rewers-Felkins, K.A.; Salguero, M.V.; Al-Obaidi, I.; Kottapalli, K.R.; Vasylyeva, T.L. Gut Microbiota-Dependent Trimethylamine-N-oxide and Serum Biomarkers in Patients with T2DM and Advanced CKD. *Journal of clinical medicine* **2017**, *6*, doi:10.3390/jcm6090086.
117. Zhang, Y.L.; Zhou, C.; Li, X.F.; Yang, M.N.; Tao, L.; Zheng, X.Y.; Jia, Y.S. Beinaglutide showed significant weight-loss benefit and effective glycaemic control for the treatment of type 2 diabetes in a real-world setting: a 3-month, multicentre, observational, retrospective, open-label study. *Obes Sci Pract* **2019**, *5*, 366-375, doi:10.1002/osp4.342.
118. Wang, G.; Wu, P.; Qiu, Y.; Dong, X.; Wang, Y.; Chi, Y.; Zhang, F.; Li, Y.; Zhang, J.; Huang, Z.; et al. Effect of beinaglutide treatment on weight loss in Chinese patients with type 2 diabetes mellitus and overweight/obesity. *Arch Endocrinol Metab* **2021**, *65*, 421-427, doi:10.20945/2359-3997000000388.
119. Coskun, T.; Urva, S.; Roell, W.C.; Qu, H.; Loghin, C.; Moyers, J.S.; O'Farrell, L.S.; Briere, D.A.; Sloop, K.W.; Thomas, M.K.; et al. LY3437943, a novel triple glucagon, GIP, and GLP-1 receptor agonist for glycemic control and weight loss: From discovery to clinical proof of concept. *Cell Metab* **2022**, *34*, 1234-1247 e1239, doi:10.1016/j.cmet.2022.07.013.
120. Finlin, B.S.; Memetimin, H.; Zhu, B.; Confides, A.L.; Vekaria, H.J.; El Khouli, R.H.; Johnson, Z.R.; Westgate, P.M.; Chen, J.; Morris, A.J.; et al. The beta3-adrenergic receptor agonist mirabegron improves glucose homeostasis in obese humans. *J Clin Invest* **2020**, *130*, 2319-2331, doi:10.1172/JCI134892.
121. Astrup, A.; Madsbad, S.; Breum, L.; Jensen, T.J.; Kroustrup, J.P.; Larsen, T.M. Effect of tesofensine on bodyweight loss, body composition, and quality of life in obese patients: a randomised, double-blind, placebo-controlled trial. *Lancet* **2008**, *372*, 1906-1913, doi:10.1016/S0140-6736(08)61525-1.
122. Fruhbeck, G. Bariatric and metabolic surgery: a shift in eligibility and success criteria. *Nat Rev Endocrinol* **2015**, *11*, 465-477, doi:10.1038/nrendo.2015.84.

123. Wolfe, B.M.; Kvach, E.; Eckel, R.H. Treatment of Obesity: Weight Loss and Bariatric Surgery. *Circ Res* **2016**, *118*, 1844-1855, doi:10.1161/CIRCRESAHA.116.307591.
124. Howard, R.; Chao, G.F.; Yang, J.; Thumma, J.; Chhabra, K.; Arterburn, D.E.; Ryan, A.; Telem, D.A.; Dimick, J.B. Comparative Safety of Sleeve Gastrectomy and Gastric Bypass Up to 5 Years After Surgery in Patients With Severe Obesity. *JAMA Surg* **2021**, *156*, 1160-1169, doi:10.1001/jamasurg.2021.4981.
125. Salminen, P.; Helmio, M.; Ovaska, J.; Juuti, A.; Leivonen, M.; Peromaa-Haavisto, P.; Hurme, S.; Soinio, M.; Nuutila, P.; Victorzon, M. Effect of Laparoscopic Sleeve Gastrectomy vs Laparoscopic Roux-en-Y Gastric Bypass on Weight Loss at 5 Years Among Patients With Morbid Obesity: The SLEEVEPASS Randomized Clinical Trial. *JAMA* **2018**, *319*, 241-254, doi:10.1001/jama.2017.20313.
126. Gu, L.; Huang, X.; Li, S.; Mao, D.; Shen, Z.; Khadaroo, P.A.; Ng, D.M.; Chen, P. A meta-analysis of the medium- and long-term effects of laparoscopic sleeve gastrectomy and laparoscopic Roux-en-Y gastric bypass. *BMC Surgery* **2020**, *20*, 30, doi:10.1186/s12893-020-00695-x.
127. Eghbali, F.; Bahardoust, M.; Pazouki, A.; Barahman, G.; Tizmaghz, A.; Hajmohammadi, A.; Karami, R.; Hosseini-Baharanchi, F.S. Predictors for weight loss after Roux-en-Y gastric bypass: the trend and associated factors for weight loss. *BMC Surgery* **2022**, *22*, 310, doi:10.1186/s12893-022-01760-3.
128. Giet, L.; Baker, J.; Favretti, F.; Segato, G.; Super, P.; Singhal, R.; Ashton, D. Medium and long-term results of gastric banding: outcomes from a large private clinic in UK. *BMC Obesity* **2018**, *5*, 12, doi:10.1186/s40608-018-0189-1.
129. Himpens, J.; Cadiere, G.B.; Bazi, M.; Vouche, M.; Cadiere, B.; Dapri, G. Long-term outcomes of laparoscopic adjustable gastric banding. *Arch Surg* **2011**, *146*, 802-807, doi:10.1001/archsurg.2011.45.
130. Ramai, D.; Singh, J.; Mohan, B.P.; Madedor, O.; Brooks, O.W.; Barakat, M.; Ofosu, A.; Khan, S.R.; Chandan, S.; Dhindsa, B.; et al. Influence of the Elipse Intra-gastric Balloon on Obesity and Metabolic Profile: A Systematic Review and Meta-Analysis. *J Clin Gastroenterol* **2021**, *55*, 836-841, doi:10.1097/MCG.0000000000001484.
131. Ienca, R.; Al Jarallah, M.; Caballero, A.; Giardiello, C.; Rosa, M.; Kolmer, S.; Sebbag, H.; Hansoulle, J.; Quartararo, G.; Zouaghi, S.A.S.; et al. The Procedureless Elipse Gastric Balloon Program: Multicenter Experience in 1770 Consecutive Patients. *Obesity Surgery* **2020**, *30*, 3354-3362, doi:10.1007/s11695-020-04539-8.
132. Vantanasiri, K.; Matar, R.; Beran, A.; Jaruvongvanich, V. The Efficacy and Safety of a Procedureless Gastric Balloon for Weight Loss: a Systematic Review and Meta-Analysis. *Obesity Surgery* **2020**, *30*, 3341-3346, doi:10.1007/s11695-020-04522-3.
133. Hayashi, A.; Kumada, T.; Nozaki, F.; Hiejima, I.; Miyajima, T.; Fujii, T. [Changes in serum levels of selenium, zinc and copper in patients on a ketogenic diet using Ketonformula]. *No To Hattatsu* **2013**, *45*, 288-293.
134. Lorenzo, P.M.; Sajoux, I.; Izquierdo, A.G.; Gomez-Arbelaes, D.; Zulet, M.A.; Abete, I.; Castro, A.I.; Baltar, J.; Portillo, M.P.; Tinahones, F.J.; et al. Immunomodulatory effect of a very-low-calorie ketogenic diet compared with bariatric surgery and a low-calorie diet in patients with excessive body weight. *Clin Nutr* **2022**, *41*, 1566-1577, doi:10.1016/j.clnu.2022.05.007.
135. Real, H.; Queiroz, J.; Graca, P. Mediterranean food pattern vs. Mediterranean diet: a necessary approach? *Int J Food Sci Nutr* **2020**, *71*, 1-12, doi:10.1080/09637486.2019.1617838.
136. Cherif, A.; Roelands, B.; Meeusen, R.; Chamari, K. Effects of Intermittent Fasting, Caloric Restriction, and Ramadan Intermittent Fasting on Cognitive Performance at Rest and During Exercise in Adults. *Sports Med* **2016**, *46*, 35-47, doi:10.1007/s40279-015-0408-6.
137. Baekdal, T.A.; Breitschaft, A.; Donsmark, M.; Maarbjerg, S.J.; Sondergaard, F.L.; Borregaard, J. Effect of Various Dosing Conditions on the Pharmacokinetics of Oral Semaglutide, a Human Glucagon-Like Peptide-1 Analogue in a Tablet Formulation. *Diabetes Ther* **2021**, *12*, 1915-1927, doi:10.1007/s13300-021-01078-y.

138. Eendebak, R.; Ahern, T.; Swiecicka, A.; Pye, S.R.; O'Neill, T.W.; Bartfai, G.; Casanueva, F.F.; Maggi, M.; Forti, G.; Giwercman, A.; et al. Elevated luteinizing hormone despite normal testosterone levels in older men-natural history, risk factors and clinical features. *Clin Endocrinol (Oxf)* **2018**, *88*, 479-490, doi:10.1111/cen.13524.
139. Zhou, Z.; Ma, T.; Zhang, H.; Chheda, S.; Li, H.; Wang, K.; Ehrling, S.; Giovine, R.; Li, C.; Alawadhi, A.H.; et al. Carbon dioxide capture from open air using covalent organic frameworks. *Nature* **2024**, *635*, 96-101, doi:10.1038/s41586-024-08080-x.
140. Chatterjee, P.; Schweizer, M.T.; Lucas, J.M.; Coleman, I.; Nyquist, M.D.; Frank, S.B.; Tharakan, R.; Mostaghel, E.; Luo, J.; Pritchard, C.C.; et al. Supraphysiological androgens suppress prostate cancer growth through androgen receptor-mediated DNA damage. *J Clin Invest* **2019**, *129*, 4245-4260, doi:10.1172/JCI127613.
141. Fukuda, N.; Horita, N.; Kaneko, A.; Goto, A.; Kaneko, T.; Ota, E.; Kew, K.M. Long-acting muscarinic antagonist (LAMA) plus long-acting beta-agonist (LABA) versus LABA plus inhaled corticosteroid (ICS) for stable chronic obstructive pulmonary disease. *Cochrane Database Syst Rev* **2023**, *6*, CD012066, doi:10.1002/14651858.CD012066.pub3.
142. James, H.; Lorentz, P.; Collazo-Clavell, M.L. Patient-Reported Adherence to Empiric Vitamin/Mineral Supplementation and Related Nutrient Deficiencies After Roux-en-Y Gastric Bypass. *Obes Surg* **2016**, *26*, 2661-2666, doi:10.1007/s11695-016-2155-7.

8. Ramadan and gastrointestinal motility

Effect of Ramadan intermittent fasting on metabolic pattern and gastrointestinal kinetics and symptoms in healthy subjects.

Hala Abdallah,^{1,*} Mohamad Khalil^{1,*} Ilaria Farella,¹ Stephy JohnBritto,¹ Elisa Lanza,¹ Sergio Santoro,² Gabriella Garruti,¹ Piero Portincasa,¹ Agostino Di Ciaula,¹ Leonilde Bonfrate,^{1,*}

¹Clinica Medica “A. Murri”, Department of Precision and Regenerative Medicine and Ionian Area (DiMePre-J), University of Bari “Aldo Moro” Medical School, Bari, Italy, ²Section of Internal Medicine, Endocrinology, Andrology and Metabolic Diseases, Department of Precision and Regenerative Medicine and Ionian Area (DiMePre-J), University of Bari “Aldo Moro” Medical School, Bari, Italy

*Equal contributors

Corresponding author:

Dr. Agostino Di Ciaula, MD

Department of Precision and Regenerative Medicine and Ionian Area (DiMePre-J)

University of Bari Aldo Moro

Policlinico Hospital, Piazza G. Cesare 11 - 70124 Bari – Italy

Tel.+39-080-5592731

Email: agostinodiciaula@tiscali.it

Keywords: digestion, intermittent fasting, gallbladder motility, gastric emptying, gastrointestinal symptoms, metabolic syndrome, microbiota, orocecal transit time, subcutaneous fat, visceral fat

Abstract

Few studies have shown the positive effect of modified fasting formula on human health, aging, and metabolic diseases, namely obesity and diabetes. The beneficial effects of intermittent fasting have recently gained more attention among clinical researchers. Intermittent fasting is defined as the voluntary abstinence of individuals from food, i.e., partial nutrient deprivation, or complete food deprivation with or without drink restriction, for a period longer than a typical overnight 12 h fast. The digestibility of food is a critical trait, associated with the concepts of health and well-being. Digestive motility disorder leads to various digestive problems like difficulty in swallowing, acid reflux disease, severe constipation, diarrhea, abdominal pain, vomiting, and bloating. Hence, we aimed to assess the impact of intermittent fasting (religious “Ramadan” fasting) on digestibility (gastrointestinal motility) on the level of stomach, gallbladder, and intestine, weight, blood glucose level, gastrointestinal symptoms, and gut microbiota-associated carbohydrate fermentation. In a cohort of 21 subjects homogeneously distributed by sex, age, and BMI and prospectively enrolled, the changes in digestibility, plasma glycaemia and abdominal fat distribution were analyzed before and after one-month intermittent fasting.

1. Introduction

Intermittent fasting implies food deprivation for several hours. Fasting can occur during the day or for one or more days and may involve religious reasons or medical prescriptions to promote weight loss. Ramadan represents the ninth month of the Islamic lunar calendar. During Ramadan, healthy Muslim adults have the obligation to abstain from assuming food and drinks, including water from dawn to sunset. Levels of activity are often reduced during the day but people become more active after sunset and at night [1].

As a model of intermittent fasting, Ramadan has been linked with possible positive effects on components of the metabolic syndrome such as reduced waist circumference, systolic blood pressure, fasting serum glucose, serum lipid profile, and insulin sensitivity [2,3]. One additional effect might involve a decrease of proinflammatory cytokines [4]. Scarce information, however, are available on the effects of Ramadan intermittent fasting (RIF) on physiologic gastrointestinal motility and symptoms.

This study included a discrete Muslim community living in the city of Bari, Apulia region, Italy during the year 2022, and analyses the effects of RIF in adult Muslims of both genders on anthropometric indices, caloric intake, metabolic pattern, fat distribution, extensive gastrointestinal motility, and symptoms.

2. SUBJECTS AND METHODS

2.1 Subjects

A total of 21 Muslim individuals (10 Males, 11 Females) aged between 20 and 45 joined the study. Participants were mainly students at the University of Bari.

During the enrolment, subjects underwent a full clinical assessment including detailed medical history and physical examination to rule out any known organic or functional disease potentially interfering with the study. Subjects diagnosed with cancer, inflammatory diseases, gallstone disease, liver diseases, diabetes, cardiovascular diseases, or taking medications able to affect gastrointestinal motility and/or secretions were excluded. Excluded were also subjects reporting signs and/or symptoms of functional dyspepsia, irritable bowel syndrome, functional constipation and/or diarrhoea. Pregnant women were not enrolled due to potential negative effects of intermittent fasting on fetal growth [1]. At enrolment, inclusion criteria included a normal physical examination and abdominal ultrasonography. Subjects were asked to provide a list of recent routine blood analyses (not older than 3 months) showing a normal blood count, normal liver transaminases, and renal function.

In 2022, Ramadan started on April 3rd and ended on May 2nd (30 days). The period of fasting is traditionally set between sunrise and sunset. Notably, daylight times and fasting times can vary according to the geographical location and the Gregorian month in which Ramadan falls. By contrast on the equator, day light times are about the same each year, i.e., about 13.5 hours [1]. In Apulia at the longitude of 41.1171°N, the 2022 Ramadan started on April 3 with sunrise at 6:31AM and sunset at 19:20PM and a fasting day time of 12h:49min. The Ramadan ended on May 2 with sunrise at 5:47AM and sunset at 19:51PM and a daytime fasting of 14h:04min. The mean daily temperature was approximately 24 °C.

During fasting (Ramadan), subjects are not allowed to eat, drink (including water, coffee, or any other drinks), or smoke. Refeeding starts immediately after sunset and can last until sunrise, although most people concentrate refeeding within 2-3 hours after sunset. Breakfast is not allowed after sunrise and for this, some individuals can decide to eat a limited amount of food before sunrise. Notably, women are exempted from Ramadan during menstruation, i.e., 3-6 days. Ramadan does not apply to women before menarche and to men before puberty.

All enrolled subjects provided a full informed written consent. The study was approved by the Ethical Board at the University of Bari (Study number 7275, protocol number 0063719). The study was registered in *clinicaltrials.gov* (NCT05571891).

2.1 Study design

The study design is provided in **Figure 1**. Following the enrolment, subjects were interviewed by a questionnaire investigating dietary habits, physical activity, and gastrointestinal symptoms during few days before Ramadan, on days 14-16 during Ramadan, and 3 days after the end of Ramadan. On day 0 and at the end of Ramadan subjects underwent anthropometric measurements, the ultrasonographic study of body fat distribution, the assessment of gastrointestinal motility, and blood analyses.

2.2 Assessment of physical activity Level

The validated physical activity-designed questionnaire (International Physical Activity Questionnaire; long-format version) provides a simple and rapid evaluation of physical activity during leisure, occupational, and sedentary time on a weekly basis. Physical activity levels were calculated based on metabolic equivalent tasks (METs), with 1 MET equal to 3.5 ml of O₂/kg/min. MET thresholds constitute valid indicators of physical activity intensity with relatively high accuracy [5].

2.3 Assessment of dietary habits

All participants recorded their standard dietary habits using a 3-day dietary recall (i.e., type, amount, portion size) two on a weekday and one on a weekend day. A trained clinical dietitian assessed each recorded food. The WinFood software (Medimatica, Martinsicuro, Teramo, Italy), based on the Italian food composition tables, was then used for the estimation of nutrient and caloric intake, using the mean of the 3 days before and 3 days after fasting to estimate the dietary life style of the subject [5-7].

2.4 Assessment of gastrointestinal symptoms

A questionnaire served to assess the severity of four main symptoms i.e., gastric fullness, epigastric pain, nausea and/or vomiting, and heartburn before and after Ramadan. Those symptoms are related to the change of the dietary habits and may be affected during the Ramadan fasting. Severity was scored on a 0-100 mm visual analogue scale (VAS) with 0 equal to “no symptom” and 100 corresponding to “maximal intensity”. Frequency and duration of symptoms were summarized on a semiquantitative scale reporting: frequency 0=absent, 1=at least one episode every two months, 2=at least one episode in a month, and 3=at least one episode in a week. The duration was measured as follows: 0=absent, 1=less than 5 min, 2=from 5 to 10 min, and 3=more than 10 min [8].

2.5 Anthropometric and ultrasonographic measurement of fat distribution

Physical anthropometrics included height (to the nearest 0.5 cm), weight (to the nearest 0.1 kg), and body mass index (BMI) which is reliable, easy to calculate, and correlates with percentage of body fat and body fat mass. BMI is calculated using the following formula: $BMI = \text{Weight (kg)} / \text{Height (m}^2\text{)}$ [9]. Waist circumference (WC) was also measured to detect abdominal obesity. WC provides independent risk information not provided by BMI [10] and was measured the nearest 0.5 cm using a flexible tape placed on a horizontal plane at the level of the iliac crest. A waist circumference of ≥ 102 cm for males and ≥ 88 cm for females is indicative for increased cardiometabolic risk [11].

For better quantification of body fat, we used abdominal ultrasonography (Noblus-E; Hitachi Medical, Tokyo, Japan) equipped with a 3.5 MHz probe to accurately measured subcutaneous (SAT) and visceral (VAT) abdominal fat thickness. Measurements of fat thickness were performed at 5 different locations, i.e., pre-hepatic, pre-pancreatic, pre-aortic, hepato-

renal, and splenorenal. Abdominal ultrasonography was also used to assess the presence of a bright liver as a marker of liver steatosis.

2.6 Blood analyses

Peripheral venous blood (2.5 ml) was drawn into serum-separating test tubes. Within 30 min, samples were centrifuged at 3,000 rpm for 10 min at room temperature and about 1 mL of serum was separated. Measurements included serum concentrations of insulin ($\mu\text{U/mL}$), plasma glucose (mg/dL), alanine aminotransferase (ALT, U/L), aspartate aminotransferase (AST, U/L); gamma-glutamyltransferase (GGT, U/L), creatinine (mg/dL), total cholesterol (TC, mg/dL), low-density lipoprotein cholesterol (LDL, mg/dL), high-density lipoprotein cholesterol (HDL, mg/dL), and triglycerides (TG, mg/dL). The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) measures the severity of insulin resistance and was calculated by the following formula: $(\text{plasma glucose [mg/dL]} \times \text{plasma insulin [\mu U/mL]})/405$ [12].

2.7 Assessment of gastrointestinal motility

Gastric and gallbladder motility, and oro-cecal transit time (OCTT) were determined dynamically for two hours following meal ingestion to have an overall illustration of the gastrointestinal digestion after food intake [13,14]. The test meal consisted of a liquid solution of 215 mL made of 200 mL Nutridrink® (Nutricia, Milano, Italy) with 13 g (39%) fat, 10 g (13%) protein and 35 g (48%) carbohydrates for a total of 300 kcal, 1270 kJ, 365 mOsm/l, and 15 mL Duphalac Dry® (Solvay Pharma, Torino, Italy) with 10 g of lactulose.

The drink was provided at room temperature and drunk in one minute in the presence of the examiner. During the 2 weeks before the test, subjects were not allowed to take antibiotics, probiotics, prokinetics or other drugs known to affect gastrointestinal motility. To avoid prolonged intestinal H_2 -production secondary to the presence of non-absorbable or slowly fermentable material in the colonic lumen, a special diet was given starting the day before the test and consisting of proteins and fat (meat, fish, eggs, olive oil). The meal was followed by a 12-hour fasting period. Between 08:00 and 09:00 AM the test was started. Smoking and physical exercise were prohibited one hour before and during the test.

Gastric motility

Time-dependent changes of fasting postprandial antral areas (cm^2) and gallbladder volumes (mL) were measured simultaneously by ultrasound scans using a Noblus ultrasound system (Hitachi Medical, Tokyo, Japan) equipped with the 3.5 MHz convex transducer. Gastric emptying was expressed as time-dependent changes of absolute (cm^2) and percent (%) antral areas. Measurements were taken at baseline (duplicates at -5 min -5 min), after maximal antral dilatation at 0 min, and every 15 min during the 120 min emptying process. Markers of gastric emptying kinetics were basal antral area (cm^2), maximal antral area after test meal ingestion, minimal antral area (cm^2 and % of maximal antral area). The half-emptying time ($T_{1/2}$, min) was calculated from the linear regression analysis of the descending part of the emptying curves and expressed as “true” $T_{1/2}$ ($tT_{1/2}$) from the absolute emptying curve (i.e., antrum area in cm^2) or estimated $T_{1/2}$ ($eT_{1/2}$) looking at the intercept between the regression line and a horizontal line corresponding to the 50% decrease of antral area expressed as percent of maximal area. The area under the emptying curve (AUC) was calculated during 120 min as absolute value ($\text{cm}^2 \times 120 \text{ min}$) and percent ($\% \times 120 \text{ min}$) [15].

Gallbladder motility

Gallbladder emptying indices were obtained from gallbladder volumes expressed as mL and % at baseline and at regular 15 min intervals during 120 minutes after meal ingestion [16]. Fasting volume was the volume of the gallbladder measured at 0 min. The minimum postprandial volume (mL and %) after meal ingestion was the residual volume. Further indices included the area under the emptying and the filling curve (AUC), the rate of gallbladder emptying and refilling (mL/min),

the volume of ejected bile (expressed as mL and % of basal) and the half-emptying time ($T_{1/2}$) were calculated. $T_{1/2}$ was calculated by linear regression analysis from the descending part of the emptying curves and expressed as “true” $T_{1/2}$ ($tT_{1/2}$) from the absolute emptying curve (volume as mL) or estimated $T_{1/2}$ ($eT_{1/2}$), looking at the intercept between the regression line and the horizontal line corresponding to the 50% decrease of percent gallbladder volume. Gallbladder time to residual volume was the time requested to reach the postprandial residual volume.

Oro-cecal transit time (OCTT) and microbiota-associated

OCTT was measured by lactulose hydrogen (H_2) breath test [8]. Two breath samples were taken in the fasting state and afterwards the test meal containing lactulose. Respiratory breath samples were analysed every 10 minutes for 2 hours after lactulose ingestion, using a pre-calibrated, portable hydrogen sensitive electrochemical devices (EC60-Gastrolyzer, Bedfont, USA and Lactofan H_2 Analyzer, Fisher Analysen Instr. Leipzig, Germany, kindly donated by Italchimici SpA, Pomezia, Rome, Italy). The concentration of hydrogen was measured in parts per million (ppm), and the accuracy of the detector was ± 2 ppm. A rise of 10 ppm above baseline on two consecutive measurements was considered as OCTT and expressed in minute [16].

3. Statistical analysis

We first examined the normality for continuous variables by the Shapiro-Wilk test. Variables with normal distribution are expressed as means $\pm$ SE; otherwise, they were presented as medians (Max-Min). Differences between Before and After were analysed using a paired t-test, for the same group at 2 timelines. Differences between males and females were analysed by unpaired t-test, for two different groups. Differences between proportions were analysed by chi-square test. Inter-group differences were tested by Kruskal- Wallis test, a non-parametric test to compare three or more independent groups to determine if there are statistically significant differences between them. All statistical analyses were performed using NCSS21 (NCSS LLC, Kaysville, UT, USA) [17].

4. Results

Baseline assessment

The 21 participants included 10 males (mean age 35.4 ± 1.9 yrs., range 16 years (28-44), BMI 24.8 ± 1.1 kg.m⁻²) and 11 females (26.3 ± 1.1 yrs.; range 13 years (24-37), BMI 22.0 ± 0.9 kg.m⁻²). At baseline the study group consisted of 18 normal weight subjects (8 Males, 10 Females), 2 overweight subjects (1 Male, 1 Female), and 1 obese male subject (**Table 1**). Males were slightly older and had slightly higher BMI than females.

Assessment of dietary behaviour and physical activity status

Periodic changes of lifestyle can greatly influence body composition and metabolic profile. Thus, we assessed changes of caloric, fiber intake and level of physical activity in the study group by comparing three periods, i.e., before, during and after Ramadan (**Table 2**). Mean total caloric intake decreased significantly from 2069 Kcal before Ramadan to 1798 Kcal during Ramadan and increased to 2000 after Ramadan ($P=0.04$). This change depended mainly on the combined decreased caloric intake of fat and proteins during Ramadan, with a trend for carbohydrates. After Ramadan, caloric intake of proteins and fat increased significantly and tended to increase, respectively, as compared to the Ramadan period.

The amount of fiber (in grams) tended to decrease during Ramadan and significantly increased after Ramadan.

Individual changes of total caloric intake according to gender across Ramadan are depicted in **Figure 2A**. Only 2 males and 2 females had increased caloric intake during Ramadan. By contrast, the general trend was that of decreasing Kcal intake during Ramadan in both males and females. After Ramadan all but three subjects increased again their caloric intake. The change of caloric intake as percent during Ramadan is shown in **Figure 2B**. The percent decrease ranged -5-30% in

both genders, except for the 2 males and 2 females reporting increased percent caloric intake of 5-55% on Ramadan, compared to baseline.

The total physical activity, expressed as metabolic equivalent tasks (METs) remained stable before, during, and after Ramadan (**Table 2**).

Anthropometric data and fat distribution

Ultrasonographic evidence of fatty liver (hyperechoic “bright” liver) was absent in all subjects before and after Ramadan. Anthropometric data and fat distribution by ultrasonography were assessed before, during, and after Ramadan, and results are shown in **Table 3**, while individual changes of weight and BMI according to gender are given in **Figure 3A-D**.

Body weight, BMI, and waist circumference decreased significantly in all subjects and in both genders. Such anthropometric decrease was associated with an overall and gender significant decrease of subcutaneous fat (pre-hepatic in both genders, pre-pancreatic and pre-aortic in females) and visceral fats (pre-aortic and spleno-renal in females) (**Table 3**). More in detail and studying the individual changes of weight, we observed that during Ramadan body weight decreased in 9/11 females, and 8/10 males with up to -8Kg (-13%) change (**Figure 3A-C**). With respect to BMI, such changes shifted body size in 2 females from normal weight to underweight and from overweight to normal weight (**Figure 3D**).

Metabolic biomarkers

After Ramadan fasting serum glucose decreased significant in males from 92.7 ± 3.0 at baseline to 86.0 ± 3.7 mg/L ($P=0.02$) after Ramadan and was associated with a significant decrease of serum insulin and HOMA although a trend was also evident in females (**Table 4**). No other change as documented for lipid profiles, liver enzymes, and creatinine levels (**Table 4**).

Gastric motility

Data concerning the kinetic of gastric emptying are depicted in **Table 5**.

Fasting antral area, the absolute (but not percentage) minimal postprandial area and the area under the emptying curve were significantly lower after- than before RIF. The postprandial emptying speed (i.e., $T_{1/2}$) was significantly faster after- than before RIF.

Gallbladder motility

Data on gallbladder kinetics are depicted in **Table 5**. After RIF, the fasting GB volume was about 6% smaller than that measured before Ramadan. After Ramadan, moreover, the test meal induced a stronger and faster contraction, as confirmed by all dynamic indices (i.e., residual volume, time to residual volume, emptying rate, and $T_{1/2}$). We noticed a fasting refilling rate after Ramadan, apparently responsible for a greater final volume (in %). Overall, the AUC remained comparable before and after Ramadan (**Figure 5**).

Small intestinal transit

Fasting levels of H_2 were comparable before and after Ramadan. After the RIF, the postprandial peak of H_2 increased, and the OCTT was significantly shorter than before Ramadan (**Table 5**). The graphic profile of H_2 -dependent OCTT in all subjects and with individual H_2 ppm changes is reported in **Figure 6A-B**. AUC was greater after- than before Ramadan (**Table 5**).

Gastrointestinal symptoms

The self-reported data of gastrointestinal symptoms before and after Ramadan are depicted in **Table 6**. Vomiting was invariably absent before and after Ramadan. Before Ramadan, the most evident symptom was gastric fullness, although with mild severity (i.e., low VAS score), and with low frequency and duration. After Ramadan, gastric fullness, epigastric pain, nausea, and heartburn significantly improved.

Discussion

In the present study, we performed a comprehensive survey on the effects of Ramadan as an example of intermittent fasting in a cohort of highly compliant adult Muslim individuals. To our knowledge, this is the first study combining data on anthropometric profile, lifestyle, blood analyses, gastrointestinal kinetics, and symptoms altogether.

In general, the effects of intermittent fasting must be interpreted in the light of the broader context of fed-fast circadian cycle which influences gene expression, neurohormonal control in various organs [18]. In this respect, overnight fasting or fasting during sleeping hours induces a nocturnal rise in plasma free fatty acids to meet the metabolic demands of the liver and muscle tissue. The overnight fasting is also associated with rise of ghrelin, growth hormone, and increased hepatic gluconeogenesis [19].

Additional forms of intermittent fasting [20] can include periodic prolonged fasting or intermittent calorie restriction, i.e., fasting for up to 24 hours once or twice a week with *ad libitum* food intake for the remaining days [21], time-restricted feeding i.e., eating for only 8 hours then fasting for the other 16 hours of the day, alternate-day fasting [22,23], i.e., alternating feast with *ad libitum* intake and fast days with 25% of energy needs or no caloric intake [21]. Ramadan is an example of intermittent fasting due to religious obligation once a year which includes alternate-day fasting and time-restricted feeding [1]. In time-restricted feeding the intermittent fasting implies the cessation of eating by a certain time each day with the prolonged period of fasting will persist until the next day.

Effect on body weight

Our findings show that Ramadan induces transient, mild nocturnal modifications of caloric intake, mainly from proteins (-28%), fats (-30.5%), and fiber (-10%), and confirms that total caloric intake overall is not drastically reduced (-13%) [1]. This effect appears to be transient since after Ramadan the caloric intake was resumed to levels comparable or even higher than before Ramadan, at least in the short-term. Nevertheless, body weight decreased by about -4% and -3.5% in males and female, respectively. Our study supports evidence that body weight tends to decrease during Ramadan irrespective of gender [24,25] likely due to reduced caloric intake, as shown in pregnant women [26]. A point is that reduced fluid intake can partly explain weight reduction during Ramadan [27-29]. Due to the study design, we did not assess the potential effects of Ramadan on dehydration or stress [1]. In accord with the observed body weight and BMI reduction, however, we also found that waist circumference and ultrasonographic thickness of both subcutaneous and visceral fats also decreased, pointing to a true, although modest, fat reduction. Of note, we observed a consistent decrease of overweight in one male, while one woman shifted from overweight to normal weight, suggesting a valid, likely transient, metabolic gain in a subgroup of individual.

The ultimate effect of intermittent fasting on weight loss is partly understood, although studies report a range of 2.5-9.9% in weight loss [19,30]. In a study lasting 12 weeks which included 32 individuals, alternate-day fasting (25% of energy consumed on "fast" days alternating with *ad libitum* "feast" days) provided a -5.2 Kg weight loss compared with a control group without caloric restriction [31].

In another study lasting 12 months, 100 individuals with obesity underwent alternate-day fasting, i.e., fast days with intake of 25% of total energy consumed and feast day with 125% of total energy consumed. This group was compared with a group on calorie restriction, i.e., 75% of total energy needs consumed daily, or a no-intervention control. Mean weight loss

was similar in the alternate-day fasting group and in the daily calorie restriction group compared with controls at 6 and 12 months [32].

Short-term trials using time-restricted feeding suggest that a feeding period aligned with circadian rhythms can promote weight loss and improve metabolic parameters [20,33,34]. Other longer-term trials do not support such beneficial effects. In a randomized trial lasting 12 months, obese adults (N=139) were randomly assigned to calorie-restriction plus time-restricted feeding for 8 hours/day or calorie restriction alone [35]. Weight loss was not significantly different between groups, i.e., respectively -8 kg and -6.4 kg.

Studies failing to demonstrate the positive effect of intermittent fasting on weight loss might have included subjects with low compliance or high dropout rates [20]. We can rule out this possibility in the present study, based on a highly motivated and compliant cohort during the 28 days Ramadan.

It has been reported that individuals during Ramadan often reduce their levels of activity during the day and are more active at night [1]. Apparently, this was not the case in our study since the level of physical activity, accurately measured as METs did not change before, during and after Ramadan. This finding suggests that Ramadan was associated with weight loss irrespective of sedentary lifestyle in the cohort of young individuals investigated.

Effects on metabolic profile

Overall, we observed that Ramadan ameliorated glucose metabolism. In both genders, the effect was associated with a significant reduction of serum insulin levels, and HOMA-IR (-24%). Men showed decreased fasting glucose during Ramadan. With other human models of intermittent fasting, however, studies show either stable or decreased fasting insulin with no change in fasting glucose [36-41]. In a previous study by hyperinsulinemic euglycemic clamp involving 8 healthy men, intermittent fasting by alternate-day fasting (20 hrs) for 15 days decreased insulin resistance, despite body weight was unchanged [42]. Others failed to confirm such change [43]. Other studies confirmed the improved insulin sensitivity after intermittent fasting using HOMA-IR [44]. Whether Ramadan provides more benefits to the metabolically unhealthy subjects was not investigated in the present study and deserves additional research.

We also observed a trend for increased HDL-cholesterol after Ramadan, especially in men. Although the effect was moderate, it may point to improved cardiometabolic risk, with or without weight change [45,46].

Additional effect of Ramadan, as a model of intermittent fasting include fasting-induced elevation of free fatty acids (i.e., rather pro-inflammatory), ketone bodies, and beta-hydroxybutyrate (β OHB) elevation with β OHB anti-inflammatory effects via modulation of the innate immunity and inhibition of the NLRP3 inflammasome [47]. Such effects could develop along with decreased oxidative stress involving mitochondria, increased production of antioxidant enzymes, peroxisome remodelling [48], as well as anti-inflammatory effects. Thus, depending on the context, intermittent fasting (including Ramadan), can contribute to inhibit the pro-inflammatory metabolic pathways involved in obesity-induced inflammation [49].

Although prolonged fasting is associated with acute hepatic steatosis and increased insulin resistance with further lipotoxicity [50], this was not the case in the present study, where liver transaminases remained normal, and ultrasonography did not show evidence of bright liver [51]. If Ramadan has beneficial effects on inflammation and oxidative stress in humans, is still a matter of debate, since free fatty acids and ketone bodies can have discrepant effects, and most studies have been performed in the animal models or cell lines [20].

Gastrointestinal motility and symptoms

Data about gastrointestinal motility are novel and open the way to pathophysiologically relevant speculations. Following the Ramadan period, we noticed a type of adaptation at three levels of investigations.

Following the RIF, the stomach showed a smaller fasting and maximal postprandial antral area, and the emptying speed was consistently increased (i.e., more rapid postprandial emptying). These results are in line with the rodent model of intermittent fasting, in which rats undergoing alternate-day fasting showed increased amplitude of gastric contractions and accelerated gastric emptying [52].

We speculate that this finding reflects an adaptive response to prolonged intermittent fasting with a likely carry-over effect still evident immediately after Ramadan. Although this study was not designed for a long follow-up, it is likely that gastric kinetics will resume to initial behaviour in the following weeks.

The decreased burden of visceral fat following RIF might also play a critical role. In fact, previous evidence documented a delayed postprandial gastric emptying in obese [53]. Furthermore, studies in rats fed a high-fat diet demonstrated that the increased visceral fat mass was linked with the development of a delayed gastric emptying, with attenuated bethanecol and sodium nitroprusside-induced motor responses in antral and fundic isolated tissues. According to this model, gastric dysmotility linked with the over-storage of visceral fat could depend on secondary alterations in the non-adrenergic non-cholinergic inhibitory neurotransmission [54].

Finally, an improvement of postprandial gastric emptying might also depend on varied levels of specific entero-hormones affecting gastric motility. Glucagon-like peptide 1 (GLP-1) is secreted from the enterocytes following the luminal bile acid activation of the membrane receptor G protein-coupled receptor (GPBAR-1) [55]. GLP-1 slows gastric emptying [56]. In humans decreased levels of GLP-1 have been documented following RIF [57].

Data about the gallbladder also showed a type of adaptation with smaller fasting volume and greater postprandial contraction, meaning more bile flowing in intestine, and faster postprandial emptying time. In accordance with the emptying curve, the faster refilling rate was able to bring the final volume to even greater values than before Ramadan. This evidence might be secondary to adaptative changes in serum concentration of the gut hormone cholecystokinin (CCK) following RIF. Of note, as shown in humans, RIF is linked with a significant pre-to-post increase in CCK levels [57].

For the first time, we were able to study the gut microbiota-associated fermentation of carbohydrates before and after Ramadan. The test provides a rough estimate of orocecal transit time, i.e. when the non-adsorbable lactulose substrate enters the colon and meets the local microbiota. The test provides info on the overall fermentative capacity of the resident colonic microbiota, irrespective of specific microbial populations. The RIF was associated with earlier H₂ peaks and greater H₂ levels. These results can be partly explained by the faster gastro-cholecysto-kinetic and by varied serum concentrations of entero-hormones [57], although we cannot exclude qualitative/quantitative changes of gut microbiota due to intermittent fasting. This possibility is supported by findings in mice undergoing intermittent fasting, which induced a shift of white adipose tissue (WAT) to beige fat, weight loss, and microbial changes, with increased *Firmicutes:Bacteroides* ratio [58]. In humans, a recent systematic review confirmed the increase in *Firmicutes:Bacteroides* ratio, also documenting increased abundance of some genera linked with weight change, abdominal circumference, and metabolic homeostasis [59].

The study of symptoms deserves some consideration. Intermittent fasting was able to decrease some domains linked to fullness, epigastric pain, and heartburn. This finding is in line with the improved gastrointestinal kinetics occurring at the same time and to less daily stimulation of digestive functions. By contrast, we observed a slight increase of duration of nausea, likely due to food resumption after Ramadan. We speculate that this might also be a transient effect of the month of intermittent fasting. The variations in gastrointestinal symptoms recorded in the present study might also be secondary to changes in specific gastrointestinal hormones (mainly GLP-1 and CCK) affecting both upper gastrointestinal motility and symptoms [57].

Unfortunately, the present study was limited by the lack of measurement of the main entero-hormones involved in gastrointestinal kinetics. Further studies in health and diseased subjects should better explore the link between the reported changes in gastrointestinal dynamics and time-dependent variations in gastrointestinal hormones.

Another limitation in the present study is that we did not monitor cardiac function before and after RIF. In fact, according to previous observations, RIF can generate adverse health effects, mainly in terms of increased risk of cardiovascular events [60]. Future studies should comprehensively explore multi-level, systemic effects of RIF in health and diseased subjects.

In conclusion, it appears that Ramadan, as an example of intermittent fasting closely monitored in a cohort of individuals, induces several multi-organic consequences with beneficial outcomes of fat distribution, metabolic profile, and even gastrointestinal motility. Major gastrointestinal symptoms also improved, although nausea could temporary increase due to the food resumption. Further studies are required to investigate the metabolic inflammatory status following Ramadan, as well as the ultimate clinical implications of this model of intermittent fasting. Whether the intermittent fasting, like Ramadan or other religious-regulated type of modified periodic intermittent fasting will ultimately become a form of lifestyle in metabolically healthy or unhealthy individuals deserves attention and further studies, in accord with geographical, cultural, social, and medical policies.

References

1. van Ewijk, R.J.; Painter, R.C.; Roseboom, T.J. Associations of prenatal exposure to Ramadan with small stature and thinness in adulthood: results from a large Indonesian population-based study. *Am J Epidemiol* **2013**, *177*, 729-736, doi:10.1093/aje/kwt023.
2. Faris, A.E.; Jahrami, H.A.; Alsibai, J.; Obaideen, A.A. Impact of Ramadan diurnal intermittent fasting on the metabolic syndrome components in healthy, non-athletic Muslim people aged over 15 years: a systematic review and meta-analysis. *Br J Nutr* **2020**, *123*, 1-22, doi:10.1017/S000711451900254X.
3. Jahrami, H.; Trabelsi, K.; Alhaj, O.A.; Saif, Z.; Pandi-Perumal, S.R.; BaHammam, A.S. The impact of Ramadan fasting on the metabolic syndrome severity in relation to ethnicity and sex: Results of a systematic review and meta-analysis. *Nutr Metab Cardiovasc Dis* **2022**, *32*, 2714-2729, doi:10.1016/j.numecd.2022.09.001.
4. Faris, M.A.; Kacimi, S.; Al-Kurd, R.A.; Fararjeh, M.A.; Bustanji, Y.K.; Mohammad, M.K.; Salem, M.L. Intermittent fasting during Ramadan attenuates proinflammatory cytokines and immune cells in healthy subjects. *Nutr Res* **2012**, *32*, 947-955, doi:10.1016/j.nutres.2012.06.021.
5. Molina-Molina, E.; Shanmugam, H.; Di Ciaula, A.; Grattagliano, I.; Di Palo, D.M.; Palmieri, V.O.; Portincasa, P. ((13)C)-Methacetin breath test provides evidence of subclinical liver dysfunction linked to fat storage but not lifestyle. *JHEP Rep* **2021**, *3*, 100203, doi:10.1016/j.jhepr.2020.100203.
6. Shanmugam, H.; Molina Molina, E.; Di Palo, D.M.; Faienza, M.F.; Di Ciaula, A.; Garruti, G.; Wang, D.Q.H.; Portincasa, P. Physical Activity Modulating Lipid Metabolism in Gallbladder Diseases. *Journal of Gastrointestinal and Liver Diseases* **2020**, *29*, 99-110, doi:10.15403/jgld-544.
7. Shanmugam, H.; Di Ciaula, A.; Di Palo, D.M.; Molina-Molina, E.; Garruti, G.; Faienza, M.F.; vanErpecum, K.; Portincasa, P. Multiplying effects of COVID-19 lockdown on metabolic risk and fatty liver. *European journal of clinical investigation* **2021**, *51*, e13597, doi:10.1111/eci.13597.
8. Di Ciaula, A.; Covelli, M.; Berardino, M.; Wang, D.Q.; Lapadula, G.; Palasciano, G.; Portincasa, P. Gastrointestinal symptoms and motility disorders in patients with systemic scleroderma. *BMC gastroenterology* **2008**, *8*, 7, doi:10.1186/1471-230X-8-7.
9. Gallagher, D.; Visser, M.; Sepulveda, D.; Pierson, R.N.; Harris, T.; Heymsfield, S.B. How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? *American journal of epidemiology* **1996**, *143*, 228-239, doi:10.1093/oxfordjournals.aje.a008733.
10. Gnatiuc, L.; Alegre-Diaz, J.; Wade, R.; Ramirez-Reyes, R.; Tapia-Conyer, R.; Garcilazo-Avila, A.; Chiquete, E.; Gonzales-Carballo, C.; Solano-Sanchez, M.; Clarke, R.; et al. General and Abdominal Adiposity and Mortality in Mexico City: A Prospective Study of 150 000 Adults. *Ann Intern Med* **2019**, *171*, 397-405, doi:10.7326/M18-3502.
11. Jensen, M.D.; Ryan, D.H.; Apovian, C.M.; Ard, J.D.; Comuzzie, A.G.; Donato, K.A.; Hu, F.B.; Hubbard, V.S.; Jakicic, J.M.; Kushner, R.F.; et al. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. *Circulation* **2014**, *129*, S102-138, doi:10.1161/01.cir.0000437739.71477.ee.

12. Matthews, D.R.; Hosker, J.P.; Rudenski, A.S.; Naylor, B.A.; Treacher, D.F.; Turner, R.C. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **1985**, *28*, 412-419, doi:10.1007/BF00280883.
13. Di Ciaula, A.; Grattagliano, I.; Portincasa, P. Chronic alcoholics retain dyspeptic symptoms, pan-enteric dysmotility, and autonomic neuropathy before and after abstinence. *J Dig Dis* **2016**, *17*, 735-746, doi:10.1111/1751-2980.12415.
14. Portincasa, P.; Colecchia, A.; Di Ciaula, A.; Larocca, A.; Muraca, M.; Palasciano, G.; Roda, E.; Festi, D. Standards for diagnosis of gastrointestinal motility disorders. Section: ultrasonography. A position statement from the Gruppo Italiano di Studio Motilità Apparato Digerente. *Dig Liver Dis* **2000**, *32*, 160-172, doi:10.1016/s1590-8658(00)80404-1.
15. Caponio, G.R.; Lorusso, M.P.; Sorrenti, G.T.; Marcotrigiano, V.; Difonzo, G.; De Angelis, E.; Guagnano, R.; Ciaula, A.D.; Diella, G.; Logrieco, A.F.; et al. Chemical Characterization, Gastrointestinal Motility and Sensory Evaluation of Dark Chocolate: A Nutraceutical Boosting Consumers' Health. *Nutrients* **2020**, *12*, 939, doi:10.3390/nu12040939.
16. Portincasa, P.; Moschetta, A.; Berardino, M.; Di-Ciaula, A.; Vacca, M.; Baldassarre, G.; Pietrapertosa, A.; Cammarota, R.; Tannoia, N.; Palasciano, G. Impaired gallbladder motility and delayed orocecal transit contribute to pigment gallstone and biliary sludge formation in beta-thalassemia major adults. *World journal of gastroenterology : WJG* **2004**, *10*, 2383-2390.
17. Hintze, J. *NCSS 2023 Statistical Software (2023)*. NCSS, LLC. Kaysville, Utah, USA, [ncss.com/software/ncss.](https://www.ncss.com/software/ncss/), Number Cruncher Statistical System (NCSS): Kaysville, Utah, 2023.
18. Bass, J.; Lazar, M.A. Circadian time signatures of fitness and disease. *Science* **2016**, *354*, 994-999.
19. Nørrelund, H. The metabolic role of growth hormone in humans with particular reference to fasting. *Growth Hormone & IGF Research* **2005**, *2*, 95-122.
20. Stockman, M.C.; Thomas, D.; Burke, J.; Apovian, C.M. Intermittent Fasting: Is the Wait Worth the Weight? *Curr Obes Rep* **2018**, *7*, 172-185, doi:10.1007/s13679-018-0308-9.
21. St-Onge, M.-P.; Ard, J.; Baskin, M.L.; Chiuve, S.E.; Johnson, H.M.; Kris-Etherton, P.; Varady, K. Meal Timing and Frequency: Implications for Cardiovascular Disease Prevention: A Scientific Statement From the American Heart Association. *Circulation* **2017**, *135*, e96-e121, doi:doi:10.1161/CIR.0000000000000476.
22. Collier, R. Intermittent fasting: the science of going without. **2013**.
23. Varady, K.A.; Bhutani, S.; Church, E.C.; Klempel, M.C. Short-term modified alternate-day fasting: a novel dietary strategy for weight loss and cardioprotection in obese adults. *The American journal of clinical nutrition* **2009**, *90*, 1138-1143, doi:<https://doi.org/10.3945/ajcn.2009.28380>.
24. Lamri-Senhadj, M.; El Kebir, B.; Belleville, J.; Bouchenak, M. Assessment of dietary consumption and time-course of changes in serum lipids and lipoproteins before, during and after Ramadan in young Algerian adults. *Singapore medical journal* **2009**, *50*, 288.

25. Ziaee, V.; Razaei, M.; Ahmadinejad, Z.; Shaikh, H.; Yousefi, R.; Yarmohammadi, L.; Bozorgi, F.; Behjati, M.J. The changes of metabolic profile and weight during Ramadan fasting. *Singapore Med J* **2006**, *47*, 409-414.
26. Arab, M. Ketouria and serum glucose of fasting pregnant women at the end of a day in Ramadan. *Acta Medica Iranica* **2004**, 209-212.
27. Sweileh, N.; Schnitzler, A.; Hunter, G.R.; Davis, B. Body composition and energy metabolism in resting and exercising muslims during Ramadan fast. *J Sports Med Phys Fitness* **1992**, *32*, 156-163.
28. Ennigrou, S.; Zenaidi, M.; Ben Slama, F.; Zouari, B.; Nacef, T. [Ramadan and customs of life: investigation with 84 adult residents in the district of Tunis]. *Tunis Med* **2001**, *79*, 508-514.
29. Afifi, Z.E. Daily practices, study performance and health during the Ramadan fast. *J R Soc Health* **1997**, *117*, 231-235, doi:10.1177/146642409711700406.
30. Heilbronn, L.K.; Smith, S.R.; Martin, C.K.; Anton, S.D.; Ravussin, E. Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. *The American journal of clinical nutrition* **2005**, *81*, 69-73.
31. Varady, K.A.; Bhutani, S.; Klempel, M.C.; Kroeger, C.M.; Trepanowski, J.F.; Haus, J.M.; Hoddy, K.K.; Calvo, Y. Alternate day fasting for weight loss in normal weight and overweight subjects: a randomized controlled trial. *Nutr J* **2013**, *12*, 146, doi:10.1186/1475-2891-12-146.
32. Trepanowski, J.F.; Kroeger, C.M.; Barnosky, A.; Klempel, M.C.; Bhutani, S.; Hoddy, K.K.; Gabel, K.; Freels, S.; Rigdon, J.; Rood, J.; et al. Effect of Alternate-Day Fasting on Weight Loss, Weight Maintenance, and Cardioprotection Among Metabolically Healthy Obese Adults: A Randomized Clinical Trial. *JAMA internal medicine* **2017**, *177*, 930-938, doi:10.1001/jamainternmed.2017.0936.
33. Longo, V.D.; Panda, S. Fasting, Circadian Rhythms, and Time-Restricted Feeding in Healthy Lifespan. *Cell Metab* **2016**, *23*, 1048-1059, doi:10.1016/j.cmet.2016.06.001.
34. Lowe, D.A.; Wu, N.; Rohdin-Bibby, L.; Moore, A.H.; Kelly, N.; Liu, Y.E.; Philip, E.; Vittinghoff, E.; Heymsfield, S.B.; Olgin, J.E.; et al. Effects of Time-Restricted Eating on Weight Loss and Other Metabolic Parameters in Women and Men With Overweight and Obesity: The TREAT Randomized Clinical Trial. *JAMA internal medicine* **2020**, *180*, 1491-1499, doi:10.1001/jamainternmed.2020.4153.
35. Liu, D.; Huang, Y.; Huang, C.; Yang, S.; Wei, X.; Zhang, P.; Guo, D.; Lin, J.; Xu, B.; Li, C.; et al. Calorie Restriction with or without Time-Restricted Eating in Weight Loss. *The New England journal of medicine* **2022**, *386*, 1495-1504, doi:10.1056/NEJMoa2114833.
36. Harvie, M.N.; Pegington, M.; Mattson, M.P.; Frystyk, J.; Dillon, B.; Evans, G.; Cuzick, J.; Jebb, S.A.; Martin, B.; Cutler, R.G. The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers: a randomized trial in young overweight women. *International journal of obesity* **2011**, *35*, 714-727.
37. Eshghinia, S.; Mohammadzadeh, F. The effects of modified alternate-day fasting diet on weight loss and CAD risk factors in overweight and obese women. *Journal of Diabetes & Metabolic Disorders* **2013**, *12*, 1-4.

38. Harvie, M.; Wright, C.; Pegington, M.; McMullan, D.; Mitchell, E.; Martin, B.; Cutler, R.G.; Evans, G.; Whiteside, S.; Maudsley, S. The effect of intermittent energy and carbohydrate restriction v. daily energy restriction on weight loss and metabolic disease risk markers in overweight women. *British Journal of Nutrition* **2013**, *110*, 1534-1547.
39. Johnson, J.B.; Summer, W.; Cutler, R.G.; Martin, B.; Hyun, D.-H.; Dixit, V.D.; Pearson, M.; Nassar, M.; Tellejohan, R.; Maudsley, S. Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma. *Free Radical Biology and Medicine* **2007**, *42*, 665-674.
40. Wegman, M.P.; Guo, M.H.; Bennion, D.M.; Shankar, M.N.; Chrzanowski, S.M.; Goldberg, L.A.; Xu, J.; Williams, T.A.; Lu, X.; Hsu, S.I. Practicality of intermittent fasting in humans and its effect on oxidative stress and genes related to aging and metabolism. *Rejuvenation research* **2015**, *18*, 162-172.
41. Heilbronn, L.K.; Civitarese, A.E.; Bogacka, I.; Smith, S.R.; Hulver, M.; Ravussin, E. Glucose tolerance and skeletal muscle gene expression in response to alternate day fasting. *Obesity research* **2005**, *13*, 574-581.
42. Halberg, N.; Henriksen, M.; Soderhamn, N.; Stallknecht, B.; Ploug, T.; Schjerling, P.; Dela, F. Effect of intermittent fasting and refeeding on insulin action in healthy men. *J Appl Physiol (1985)* **2005**, *99*, 2128-2136, doi:10.1152/jappphysiol.00683.2005.
43. Soeters, M.R.; Lammers, N.M.; Dubbelhuis, P.F.; Ackermans, M.; Jonkers-Schuitema, C.F.; Fliers, E.; Sauerwein, H.P.; Aerts, J.M.; Serlie, M.J. Intermittent fasting does not affect whole-body glucose, lipid, or protein metabolism. *The American journal of clinical nutrition* **2009**, *90*, 1244-1251.
44. Trepanowski, J.F.; Kroeger, C.M.; Barnosky, A.; Klempel, M.; Bhutani, S.; Hoddy, K.K.; Rood, J.; Ravussin, E.; Varady, K.A. Effects of alternate-day fasting or daily calorie restriction on body composition, fat distribution, and circulating adipokines: Secondary analysis of a randomized controlled trial. *Clinical Nutrition* **2018**, *37*, 1871-1878.
45. Antoni, R.; Johnston, K.L.; Collins, A.L.; Robertson, M.D. Investigation into the acute effects of total and partial energy restriction on postprandial metabolism among overweight/obese participants. *British Journal of Nutrition* **2016**, *115*, 951-959.
46. Horne, B.D.; Muhlestein, J.B.; May, H.T.; Carlquist, J.F.; Lappé, D.L.; Bair, T.L.; Anderson, J.L.; Group, I.H.C.S. Relation of routine, periodic fasting to risk of diabetes mellitus, and coronary artery disease in patients undergoing coronary angiography. *The American journal of cardiology* **2012**, *109*, 1558-1562.
47. Youm, Y.-H.; Nguyen, K.Y.; Grant, R.W.; Goldberg, E.L.; Bodogai, M.; Kim, D.; D'agostino, D.; Planavsky, N.; Lupfer, C.; Kanneganti, T.D. The ketone metabolite β -hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. *Nature medicine* **2015**, *21*, 263-269.
48. Ristow, M.; Schmeisser, K. Mitohormesis: promoting health and lifespan by increased levels of reactive oxygen species (ROS). *Dose-response* **2014**, *12*, dose-response. 13-035. Ristow.

49. Kosteli, A.; Sugaru, E.; Haemmerle, G.; Martin, J.F.; Lei, J.; Zechner, R.; Ferrante, A.W. Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. *The Journal of clinical investigation* **2010**, *120*, 3466-3479.
50. Chalasani, N.; Younossi, Z.; Lavine, J.E.; Diehl, A.M.; Brunt, E.M.; Cusi, K.; Charlton, M.; Sanyal, A.J. The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* **2012**, *55*, 2005-2023.
51. Portincasa, P. NAFLD, MAFLD, and beyond: one or several acronyms for better comprehension and patient care. *Internal and emergency medicine* **2023**, doi:10.1007/s11739-023-03203-0.
52. Beckmann, A.P.S.; Gama, L.A.; Machado, M.P.R.; Alves, W.D.L.; Cora, L.A.; Americo, M.F. Alternate-day fasting induces metabolic and morphofunctional changes in the gastrointestinal tract of male rats. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme* **2023**, doi:10.1139/apnm-2022-0356.
53. Di Ciaula, A.; Wang, D.Q.; Portincasa, P. Gallbladder and gastric motility in obese newborns, pre-adolescents and adults. *J Gastroenterol Hepatol* **2012**, *27*, 1298-1305, doi:10.1111/j.1440-1746.2012.07149.x.
54. Kaya, S.D.; Sinen, O.; Bulbul, M. Gastric motor dysfunction coincides with the onset of obesity in rats fed with high-fat diet. *Clinical and experimental pharmacology & physiology* **2021**, *48*, 553-562, doi:10.1111/1440-1681.13448.
55. Di Ciaula, A.; Garruti, G.; Lunardi Baccetto, R.; Molina-Molina, E.; Bonfrate, L.; Wang, D.Q.; Portincasa, P. Bile Acid Physiology. *Annals of hepatology* **2017**, *16*, s4-s14, doi:10.5604/01.3001.0010.5493.
56. Dhillon, W.S.; Bloom, S.R. Gastrointestinal hormones and regulation of food intake. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme* **2004**, *36*, 846-851, doi:10.1055/s-2004-826174.
57. Zouhal, H.; Bagheri, R.; Triki, R.; Saeidi, A.; Wong, A.; Hackney, A.C.; Laher, I.; Suzuki, K.; Ben Abderrahman, A. Effects of Ramadan Intermittent Fasting on Gut Hormones and Body Composition in Males with Obesity. *International journal of environmental research and public health* **2020**, *17*, doi:10.3390/ijerph17155600.
58. Li, G.; Xie, C.; Lu, S.; Nichols, R.G.; Tian, Y.; Li, L.; Patel, D.; Ma, Y.; Brocker, C.N.; Yan, T. Intermittent fasting promotes white adipose browning and decreases obesity by shaping the gut microbiota. *Cell metabolism* **2017**, *26*, 672-685. e674.
59. Perez-Gerdel, T.; Camargo, M.; Alvarado, M.; Ramirez, J.D. Impact of Intermittent Fasting on the Gut Microbiota: A Systematic Review. *Adv Biol (Weinh)* **2023**, e2200337, doi:10.1002/adbi.202200337.
60. NasrAllah, M.M.; Osman, N.A. Fasting during the month of Ramadan among patients with chronic kidney disease: renal and cardiovascular outcomes. *Clin Kidney J* **2014**, *7*, 348-353, doi:10.1093/ckj/sfu046.

Table 1: Baseline characteristics of the 21 Muslim adult subjects starting Ramadan.

	All subjects (N=21)	Males (N=10)	Females (N=11)	P value (Males vs. Females)
Number	21	10	11	0.75
Age (yrs)	30.6±1.4	35.4±1.9	26.3±1.1	0.0004
BMI (kg.m⁻²)	23.3±0.8	24.8±1.1	22.0±0.9	0.06
Normal weight N. (%)	18 (85.7%)	8 (80.0%)	10 (90.9%)	0.5
Overweight N. (%)	2 (9.5%)	1 (10.0%)	1 (9.1%)	0.9
Obese N. (%)	1 (4.8%)	1 (10.0%)	0 (0.0%)	0.3

BMI, Body mass index, N: Number of subjects, yrs, Years. Data are mean±SEM (Standard Error of the Mean), differences between means by paired t-Test and between percentages by Chi-square test.

Table 2: Lifestyle behaviour across the Ramadan period

Lifestyle	Before Ramadan	During Ramadan	After Ramadan	P value
Dietary caloric intake (Kcal)				
Total	2069 (1677-2641)*	1798 (1289-3126)*#	2000 (1309-3485)#	0.04
Carbohydrates	1361 (819-1768)	1329 (786-1997)	1475 (1087-1987)	0.18
Proteins	240 (155-724)	172 (92-691)#	312 (101-809)#	0.03
Fat	361 (146-761)	251 (88-593)	332 (121-689)	0.04
Fiber (g)	22.2 (9-60)	20.1 (8.4-34.8)#	28.1 (19.7-48.8)#	0.0007
Physical activity (METs)	2400 (900-18720)	2500 (820-18720)	2520 (1120-18720)	0.9

Kcal, kilocalories; METs, metabolic equivalent tasks. Data are expressed as Median (Min-Max), p value is calculated by Kruskal-Wallis One way ANOVA, differences between groups was checked by Kruskal-Wallis Multiple-Comparison Z-Value Test (Dunn's Test). Similar symbols indicate significant difference between groups.

Table 3. Effect of Ramadan on anthropometric data and fat distribution in all subjects and according to gender.

	All subjects (N=21)			Males (N=10)			Females (N=11)		
	Before	After	P	Before	After	P	Before	After	P
Weight (kg)	66.2±3.1	63.7±3.1	0.001	77.0±3.8	74.2±3.9	0.04	56.4±2.3	54.3±2.1	0.01
BMI (kg/m²)	23.3±0.8	22.4±0.7	0.0005	24.8±1.1	23.9±1.1	0.03	22.9±0.9	21.1±0.8	0.01
Waist circumference (cm)	83.5±2.5	78.4±2.4	0.002	90.1±3.9	85.0±3.3	0.03	77.5±2.1	72.5±2.3	0.02
Subcutaneous fat (SAT)									
Pre-hepatic (mm)	12.4±0.9	8.9±0.6	0.00002	12.0±1.3	8.7±1.0	0.02	12.8±1.2	9.1±0.9	0.0002
Pre-pancreatic (mm)	12.4±0.8	9.8±0.6	0.01	12.5±1.3	10.4±1.0	0.3	12.3±1.1	9.3±0.8	0.002
Pre-aortic (mm)	15.8±1.2	13.8±2.1	0.4	18.6±1.5	17.7±3.9	0.9	13.3±1.4	10.3±0.9	0.04
Visceral fat (VAT)									
Pre-hepatic (mm)	13.8±1.2	12.1±1.1	0.2	14.8±1.8	12.6±1.7	0.4	12.8±1.5	11.5±1.3	0.36
Pre-pancreatic (mm)	42.7±3.4	40.5±3.4	0.3	48.9±3.1	49.0±3.6	0.96	37.1±5.5	32.7±4.6	0.2
Pre-aortic (mm)	69.0±5.8	56.3±5.2	0.01	81.1±6.5	64.6±8.2	0.1	58.1±8.2	48.7±6.7	0.04
Hepato-renal (mm)	14.5±3.5	12.7±3.6	0.14	12.4±1.9	11.8±2.7	0.76	16.5±6.5	13.6±6.5	0.08
Spleno-renal (mm)	15.8±3.8	15.2±3.5	0.7	12.0±1.5	15.6±2.3	0.09	19.2±7.0	14.8±6.6	0.009

BMI, Body mass index; N, Number of subjects. Data are mean±SEM (Standard Error of the Mean); differences between Before and After period tested by paired t-Test.

Table 4. Effect of Ramadan on metabolic serum biomarkers and according to gender

	All subjects (N=21)			Males (N=10)			Females (N=11)		
	Before	After	P value	Before	After	P value	Before	After	P value
Glycemia (mg/dL)	87.0±2.4	84.2±2.1	0.17	92.7±3.0	86.0±3.7	0.02	81.9±2.9	82.6±2.1	0.8
Insulin (uM/mL)	6.8±0.8	5.4±0.6	0.03	7.3±1.2	5.9±1.1	0.02	6.3±1.0	5.4±0.6	0.29
HOMA-IR	1.5±0.2	1.2±0.2	0.03	1.73±0.3	1.3±0.3	0.01	1.3±0.2	1.1±0.1	0.34
Triglycerides (mg/dL)	75.5±7.1	73.5±7.1	0.46	93.3±12.5	89.8±12.7	0.42	59.4±3.0	59.6±3.6	0.9
Total-Cholesterol (mg/dL)	206.8±7.0	202.3±8.2	0.32	227.6±10.6	222.1±11.4	0.09	188.0±4.8	184.4±9.2	0.67
HDL-cholesterol (mg/dL)	46.8±1.5	53.5±4.6	0.15	50.4±1.9	52.6±1.4	0.051	43.5±1.8	54.4±8.7	0.2
LDL-cholesterol (mg/dL)	147.7±6.1	145.4±6.2	0.46	162.8±9.6	160.9±8.9	0.58	133.9±5.4	131.3±6.2	0.6
Non – HDL-cholesterol (mg/dL)	161.1±6.2	152.2±9.8	0.28	177.8±9.4	174.9±9.2	0.34	146.3±5.2	131.5±14.4	0.36
AST (U/l)	13.7±1.2	14.0±1.4	0.6	15.3±2.3	15.7±2.7	0.6	12.3±0.8	12.4±0.8	0.9
ALT (U/l)	13.0±1.4	16.4±3.3	0.4	15.1±2.8	16.2±3.9	0.44	11.1±0.8	11.5±1.0	0.5
GGT (U/l)	20.2±2.4	28.0±7.9	0.3	24.7±4.5	24.0±4.2	0.44	16.1±1.3	16.7±1.4	0.5
Creatinine (mg/dL)	0.7±0.02	0.7±0.2	0.3	0.8±0.2	0.7±0.2	0.2	0.7±0.02	0.6±0.02	0.3

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; GGT, gamma-glutamyltransferase; HDL, High-density lipoprotein; HOMA-IR,

Homeostatic Model Assessment for Insulin Resistance; LDL, Low-density lipoprotein; N, Number of subjects.

Data are mean±SEM (Standard Error of the Mean), differences between Before and After period tested by paired t-Test.

Table 5. Gastrointestinal motility before and after Ramadan

	Before Ramadan	After Ramadan	P value
Stomach			
Basal antral area (cm ²)	4.3±0.2	3.8±0.2	0.004
Maximal antral area (cm ²)	12.3±0.4	11.5±0.5	0.04
Minimal antral area (cm ²)	7.6±0.4	6.3±0.3	0.002
Minimal antral area (%)	41.8±3.3	34.9±3.6	0.14
tT _{1/2} (min)	67.1±2.7	57.8±2.1	0.007
eT _{1/2} (min)	110.2±5.3	91.7±4.5	0.02
AUC (cm ² x 120 min)	1235±51	1057±53	0.0005
AUC (% x 120 min)	8469±227	7576±245	0.01
Gallbladder			
Fasting volume (mL)	22.4±1.0	20.9±0.9	0.006
Residual volume (mL)	9.3±0.5	6.8±0.5	0.0002
Residual volume (%)	41.4±0.9	32.5±1.8	0.0006
Time to residual volume (min)	42.9±2.6	36.4±1.7	0.046
Ejection volume (mL)	13.1±0.6	14.1±0.7	0.003

Ejection volume (%)	58.6±0.9	67.5±1.8	0.0006
Emptying rate (mL/min)	-0.36±0.04	-0.5±0.07	0.03
Emptying rate (%/min)	-1.6±0.18	-2.3±0.23	0.006
Emptying tT _{1/2} (min)	17.4±0.7	15.2±0.6	0.02
Emptying eT _{1/2} (min)	32.5±1.9	24.1±1.2	0.0005
Refilling rate (mL/min)	0.10±0.01	0.14±0.01	0.00000
Refilling rate (%/min)	0.47±0.03	0.67±0.02	0.00000
Refilling tT _{1/2} (min)	78.7±3.0	78.3±2.8	0.88
Refilling eT _{1/2} (min)	61.3±4.2	65.1±3.7	0.54
Final volume (mL)	17.5±1.1	18.7±0.8	0.11
Final volume (%)	77.6±2.4	89.7±1.9	0.0004
AUC (mL x 120 min)	1012±52	1015±75	0.1
AUC (% x 120 min)	4553±174	4816±274	0.43
Small intestine			
H ₂ baseline (p.p.m.)	2.8±0.3	2.9±0.2	0.66
H ₂ max (p.p.m.)	18.9±2.3	24.3±2.6	0.0002
OCTT (min)	105.7±3.6	80.0±4.5	0.00004
AUC (p.p.m. x 120 min)	1024±94	1347±108	0.001

AUC, area under curve; eT_{1/2}, Estimated half-time; OCTT, oro-cecal transit time; p.p.m, parts-per million; tT_{1/2}, True half-time . Data are mean±SEM (Standard Error of the Mean), differences between Before and After period tested by paired t-Test.

Table 6. Gastrointestinal symptom severity in the study group.

	Before Ramadan	After Ramadan	P value
Gastric fullness			
Intensity (mm)	45.0±8.0	15.0±3.0	0.002
Frequency (score)	1.4±0.2	0.7±0.1	0.02
Duration (score)	1.3±0.2	0.9±0.2	0.07
Epigastric pain			
Intensity (mm)	20.0±7.0	7.0±3.0	0.03
Frequency (score)	0.8±0.2	0.3±0.1	0.025
Duration (score)	0.8±0.2	0.3±0.1	0.01
Nausea			
Intensity (mm)	12.0±6.0	6.0±3.0	0.22
Frequency (score)	0.6±0.2	0.4±0.1	0.5
Duration (score)	0.5±0.2	1.1±0.2	0.028
Heartburn			
Intensity (mm)	25.0±9.0	11.0±4.0	0.15
Frequency (score)	0.9±0.2	0.5±0.1	0.025
Duration (score)	0.7±0.1	0.9±0.2	0.5

Data are mean±SEM (Standard Error of the Mean) differences between Before and After period tested by paired t-Test

Figure Legends

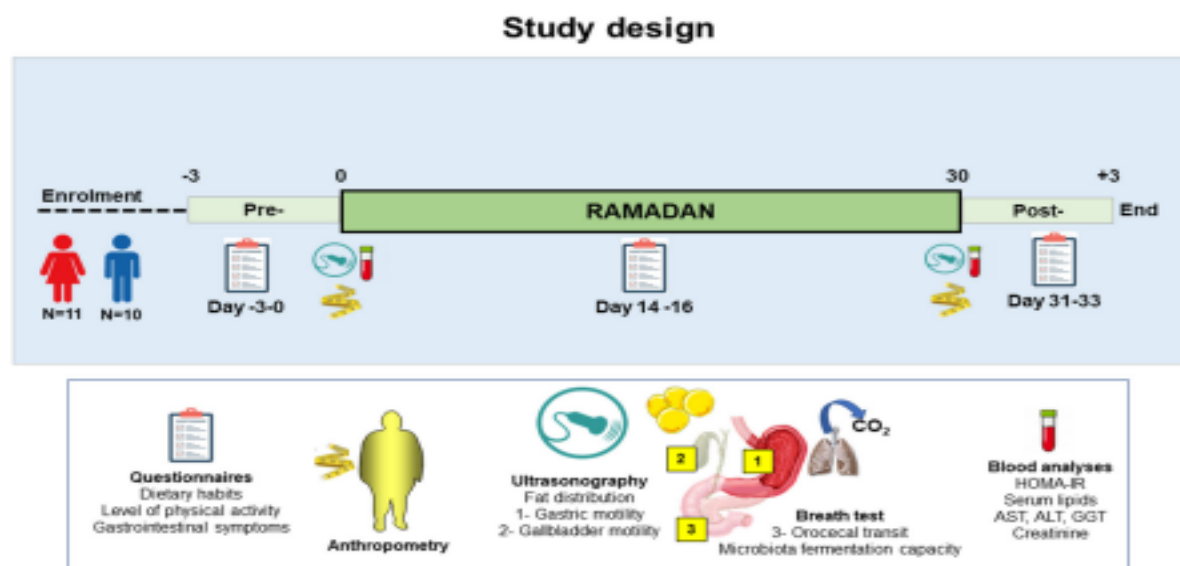


Figure 1

Study design. Arbitrarily, the terms “Before”, “During”, and “After” Ramadan refer respectively to the 3 days before the start, at days 14-16 during Ramadan, and the 3 days after the end of Ramadan.

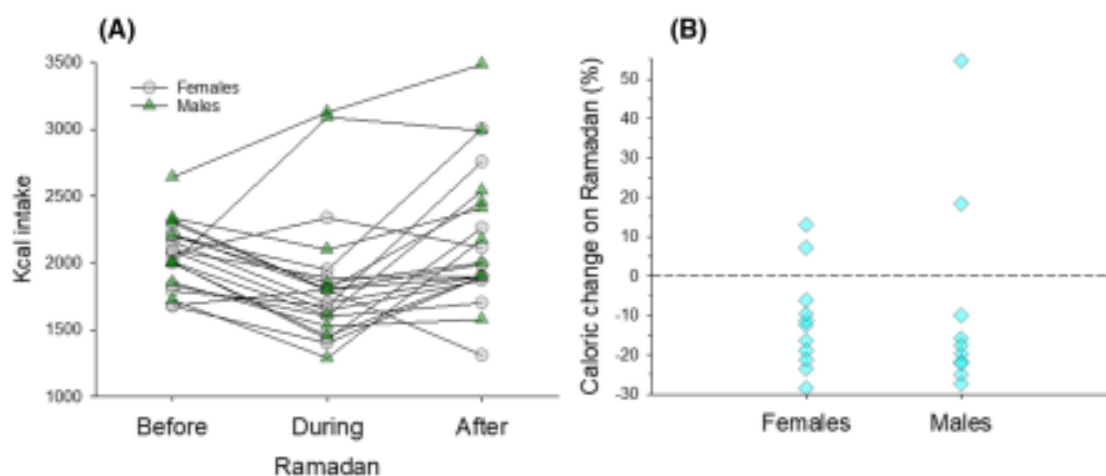


Figure 2

A) Individual changes of total caloric intake across Ramadan according to gender.

B) Change of total caloric intake during Ramadan according to gender. Comparison is expressed as percentage change from during Ramadan to before Ramadan.

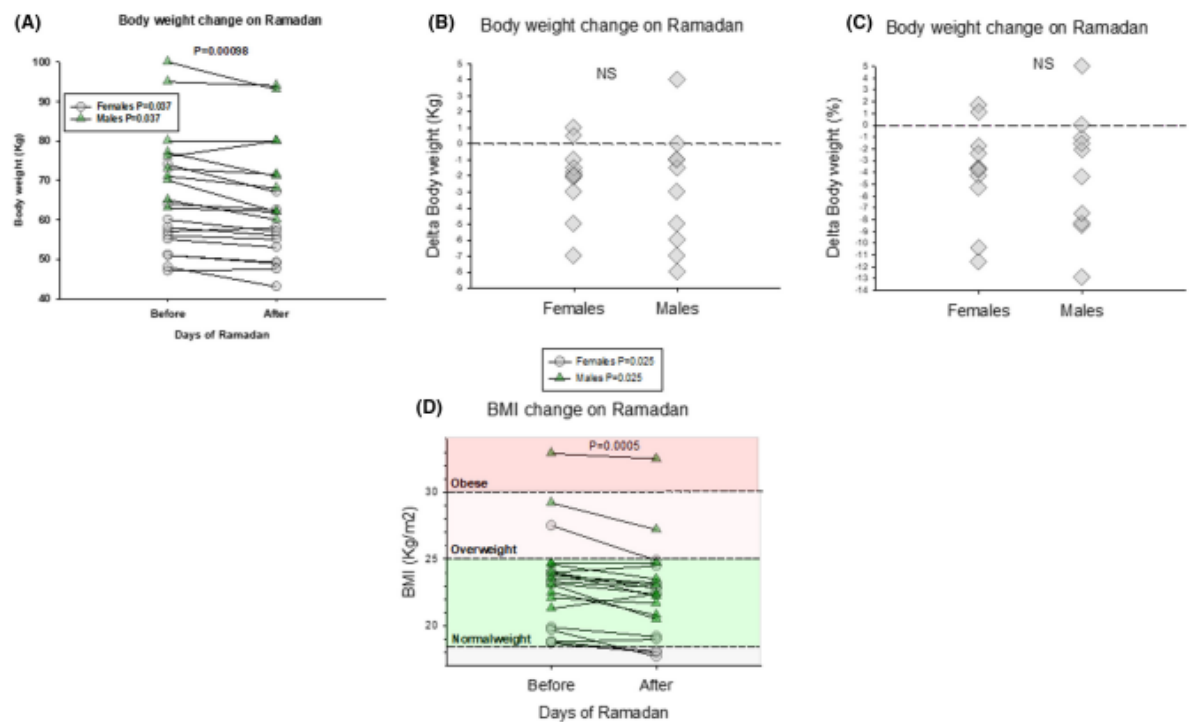


Figure 3

Changes of body weight and BMI during Ramadan according to gender.

- A)** The analysis of individual data shows a significant decrease of body weight in the whole study groups and according to gender.
- B)** The decrease of body weight occurs in most subjects and ranges from -1 to -8 Kg.
- C)** Ramadan induces a 1-13% decrease of body weight.
- D)** According to BMI, the study group before Ramadan was composed by 18 normal weight (NW) subjects (8 Males, 10 Females), 2 overweight (OW) subjects (1 Males and 1 Females)), and 1 male obese (OB). Ramadan was associated with a significant decrease of BMI in both genders, meaning that such changes shifted body size in 2 females from normal weight to underweight and from overweight to normal weight.

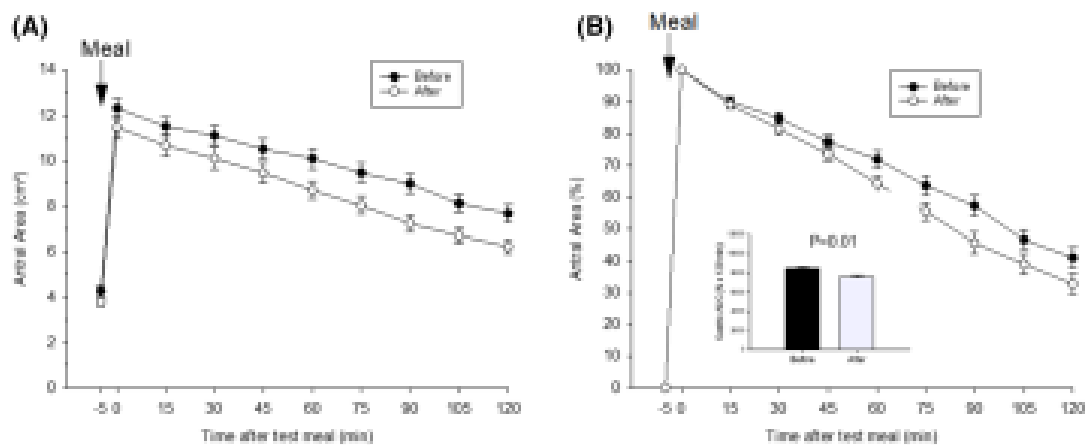


Figure 4

Gastric emptying curves in response to the ingestion of the test meals. Time-dependent changes of **(A)** Absolute antral area (cm²) and **(B)** Percentage antral area (%), are represented as mean $\pm$ SEM (Standard Error of the Mean).

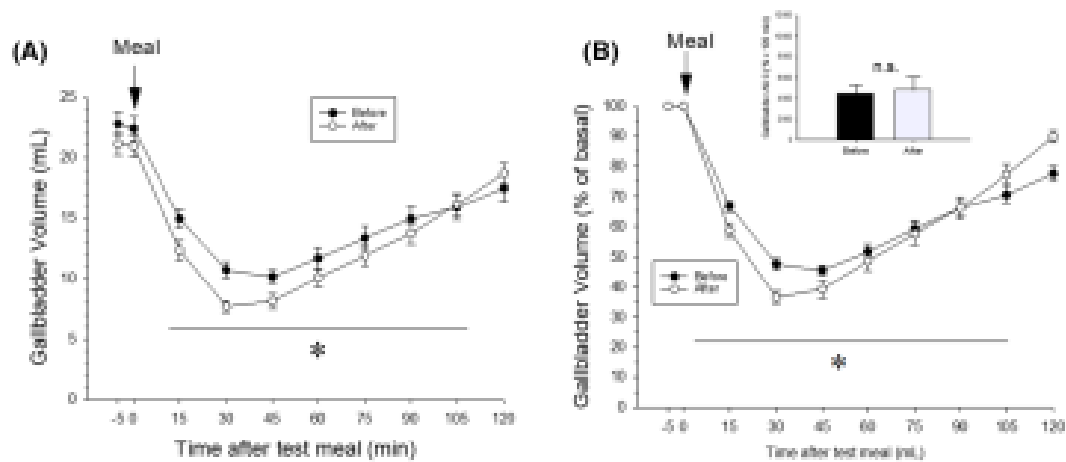


Figure 5

Gallbladder emptying curves in response to the ingestion of the test meal. Time-dependent changes of **(A)** Absolute fasting volume (mL) and **(B)** Percentage fasting volume (%)

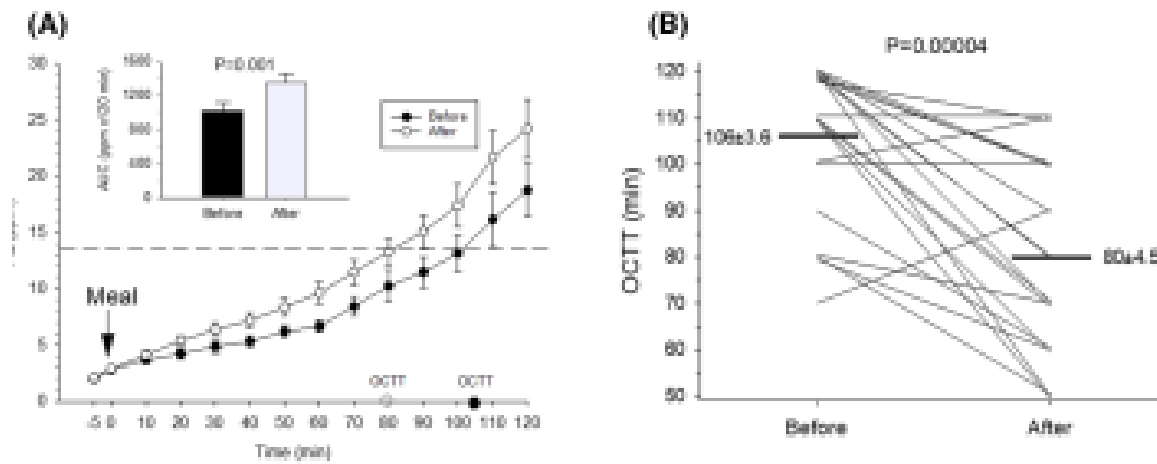


Figure 6

Oro-cecal transit time (OCTT). **(A)** Time-dependent curves of H_2 concentration (ppm) in exhaled air. **(B)** changes of 21 subjects of OCTT between Before and After Ramadan. Differences by paired t-Test , * $p=0.0006$.

9. Ramadan or traditional intermittent fasting?

Distinct biophysiological effects of Ramadan fasting and traditional intermittent fasting on markers of body fat storage. A real-life study.

Mohamad Khalil ¹, Hala Abdallah ², Nour Jaber ², Gabriella Garruti ³, Agostino Di Ciaula ⁴, Piero Portincasa ⁵

1Clinica Medica "A. Murri", Department of Precision and Regenerative Medicine and Ionian Area (DiMePre-J), University of Bari "Aldo Moro", Piazza Giulio Cesare 11, Bari 70124, Italy. Electronic address: mohamad.khalil@uniba.it.

2Clinica Medica "A. Murri", Department of Precision and Regenerative Medicine and Ionian Area (DiMePre-J), University of Bari "Aldo Moro", Piazza Giulio Cesare 11, Bari 70124, Italy.

3Section of Endocrinology, Department of Precision and Regenerative Medicine and Ionian Area (DiMePre-J), University of Bari "Aldo Moro", Bari, Italy. Electronic address: gabriella.garruti@uniba.it.

4Clinica Medica "A. Murri", Department of Precision and Regenerative Medicine and Ionian Area (DiMePre-J), University of Bari "Aldo Moro", Piazza Giulio Cesare 11, Bari 70124, Italy. Electronic address: agodiciaula@gmail.com.

5Clinica Medica "A. Murri", Department of Precision and Regenerative Medicine and Ionian Area (DiMePre-J), University of Bari "Aldo Moro", Piazza Giulio Cesare 11, Bari 70124, Italy; General Secretariat, Consortium of Mediterranean Universities, Italy. Electronic address: piero.portincasa@uniba.it.

Abstract

Background: Ramadan Intermittent fasting (RIF) has been linked with beneficial metabolic effects and improved gastrointestinal motility. However, a comparison between RIF and the traditional 16-hours intermittent fasting (16IF), a popular strategy for weight loss, is lacking.

Methods: A total of 34 subjects (median age 32.5 years, range 18-63 years; median BMI 24.5 Kg/m², range 18.6-37.6 Kg/m²) were assigned to RIF (N=18) or 16IF (N=16) for 30 days. We measured variations in anthropometric measures (BMI, waist, and abdominal circumference), serum insulin, glucose, cortisol, non-esterified fatty acid (NEFA), body fat composition (bioelectrical impedance analysis, BIA) and the ultrasonographic measurements of liver steatosis (Hepatorenal index, HRI) and thickness of subcutaneous (SAT) and visceral (VAT) fats.

Results: At baseline, BMI, rates of liver steatosis and distribution of normal weight, overweight, and obese subjects were comparable between the two groups. Body weight

significantly decreased at the end of fasting in both RIF (-4.2% vs baseline, $P=0.002$) and 16IF (-2.1% vs baseline, $P=0.002$). Waist and abdominal circumference significantly decreased in RIF but not in 16IF, as well, as the amount of body fat. In subjects with liver steatosis, SAT and VAT significantly decreased following RIF, but not 16h-IF, as well as the ultrasonographic HRI.

Conclusion: Both 16IF and RIF are able, during 1-month, to reduce body weight. However, RIF but not 16IF also generates marked beneficial effects in terms of reduced subcutaneous fat and liver steatosis. Further studies urge to verify the effects of different models of IF in weight-cycling and long-term management of obesity and related dysmetabolic conditions, such as ectopic fat over-storage.

Keywords: bioimpedenziometry, body composition, fasting, liver steatosis, metabolic syndrome, nutrition, ultrasonography, weight loss

Introduction

Obesity is a metabolic disease characterized by fat over-storage in multiple districts. The continuous increase of the obesity epidemic makes mandatory innovative interventions and new guidelines to manage the weight gain ¹.

Besides reduced energy intake increased physical activity, pharmacological interventions, and bariatric surgery or endoscopy, a critical role is played by the timing of daily food consumption. Intermittent fasting (IF), in particular, has been gaining popularity worldwide as an effective strategy for losing weight. It has been proposed due to its efficacy in reducing weight, improving cardiovascular diseases, and decreasing oxidative stress ².

However, different types of IF have been proposed, and a precise validation of each IF subtype still needs an ultimate assessment. Complete alternate-day fasting (CADF) includes alternate fasting days with eating days ³. Alternate-day fasting (ADF) is characterized by the consumption of only 20-25% of the needed energy during the fasting days ⁴. Time-restricted feeding (16IF) consists in 8 hours of eating and 16 hours of fasting per day ⁵. The various

subtypes of IF have been linked with about 0.8% to 13.0% reduced weight during 2 to 12 weeks [6](#).

Ramadan intermittent fasting (RIF), a religious form of IF practiced by adult Muslim people for the whole lunar month every year [7](#), is emerging as a further, interesting model of intermittent fasting [8](#). RIF consists of fasting from food and drink, including water, from dawn to sunset [8](#). Previous evidence from our group showed that RIF has beneficial effects in terms of reduced body weight, body mass index (BMI), waist circumference, fasting serum glucose, serum insulin and grade of insulin resistance, decreased subcutaneous and visceral fat, and improved gastrointestinal motility [8](#). However, a comprehensive comparison between the beneficial effects induced by RIF or other types of IF is still lacking. The increasing trend of non-alcoholic liver steatosis, recently re-named metabolic dysfunction-associated steatotic liver disease (MASLD)^{[9,10](#)}, has attracted our interest to study also the effect of IF regimens on the evolution of MASLD. The present study is therefore aimed to compare the effects of RIF with those recorded in subjects who undergo 16IF, a popular strategy for weight loss [11](#). It aims to compare the differences of caloric intake, macronutrient content, body weight, serum biomarkers, body fat, and liver steatosis between RIF and 16IF.

SUBJECTS AND METHODS

Subjects

A total of 34 subjects (17 females, median age 32.5 years, age range 18-63 years, IQR 14.5) joined the study on a voluntary basis. After enrolment, 18 Muslim subjects joined RIF, and 16 non-Muslim subjects underwent 16IF. Subjects were informed about the aims of the study protocol, and were asked to sign the detailed informed consent. Afterwards, they were screened for eligibility prior to enrolment.

Study design

The screening consisted of full clinical assessment by medical history and physical examination, with review of general blood analyses and medications not older than three months. This step excluded any organic or functional condition possibly interfering with the study protocol.

After enrolment, the follow up consisted of 30 days, and during the whole period of intermittent fasting, participants were asked maintain their usual habits, including daily activities and physical exercise.

The RIF period started on 22 March and ended on 21 April 2023 (Ramadan Islamic month, رمضان). For RIF, the fasting period is from dawn to sunset, and consists in the total deprivation from food, drinks, and smoking (صيام).

The 16IF period lasted 30 days from March to April 2023. The 16IF started at 8:00 pm until 12:00 pm next day and consisted of the deprivation from foods and any caloric drink, i.e., juice, coffee with sugar, milk etc. Water and sugar-free coffee or tea were allowed (**Figure 1**).

The initial screening of enrolled subjects and the subsequent follow-up assessment were conducted at the Clinical Medica “A. Murri”, University of Bari (Italy) and the study protocol was approved by the Hospital Ethical Board (Protocol n. 677, study n. 1352/CE).

The study design is depicted in **Figure 2** with respect to dietary habits, anthropometric measurements, ultrasonographic distribution of abdominal fat including liver steatosis, bioelectrical impedance analysis (BIA), electrocardiogram, and blood analyses.

Assessment of dietary habits

The individual caloric intake was assessed by a web-based questionnaire on day 0 (baseline), 15, 30 (end of fasting), and then at 33, 45, 60, and 90 days from enrolment.

A validated web-based questionnaire ⁸ served to assess 3-days dietary habits including type, amount, and portion size of meals. The assessment includes 2 weekdays and 1 weekend. The caloric intake, and the quantity of macronutrients and micronutrients were calculated by a trained clinical dietitian using the WinFood software (Medimatica, Martinsicuro, Teramo, Italy)¹².

Anthropometric measurements

Anthropometric measurements were performed on day 0 and 30 by measurements of weight (to the nearest 0.1 Kg), height (to the nearest 0.5 cm), body mass index BMI ($\text{BMI} = \text{Weight (Kg)} / \text{Height (m}^2\text{)}$)¹³, and body circumferences, i.e., waist, neck, abdominal by a flexible meter, and expressed as cm¹⁴.

Ultrasonographic assessment of fat distribution and liver steatosis

By ultrasonography we measured the distribution of abdominal subcutaneous fat (SAT), and visceral fat (VAT). Based on our previous study¹⁵, an artificial intelligence (AI)-based ultrasonographic algorithm was used to accurately detect liver steatosis by calculating the hepato-renal index (HRI). Exams were performed using an E2 machine equipped with a convex 3.4-5.0 MHz probe (Sonoscape/Fujifilm, Tokyo, Japan - Eurisko Technology srl, Bari, Italy). Liver steatosis was graded as absent, i.e., no difference in echogenicity between liver and kidney, and present, i.e., increase of liver echogenicity (i.e., brightness) as compared to kidney parenchyma, as detected by ultrasonography and calculated by HRI.

Bioelectrical Impedance Analysis (BIA)

A bioimpedance analysis was conducted by the Nutribox bioelectrical analyser to estimate body composition, in particular body water (Liter), body fat (Kg), lean mass (fat-free mass, in

Kg and percentage), and the body cell mass BCM (Kg). This methods gives an overall view about the difference of body composition, especially body fat and muscles before and after the fasting during Ramadan. Using a total of 4 electrodes (two in one hand and 2 in one foot), a weak electric current flow through the body and the voltage is measured to calculate impedance (resistance and reactance) of the body (Body Impedance Analyzer Nutribox, Pocking, Germany). All parameters were provided by the software according to <https://www.winfood.it/software-dieta-professionale/>.

Blood test analyses

Peripheral venous blood (2.5mL) was drawn into serum separating test tubes. Within 30 min, samples were centrifuged at 3000 rpm (1008 g) for 10 min at room temperature and about 1mL of serum was separated. Measurements included serum concentrations of insulin ($\mu\text{U/mL}$), plasma glucose (mg/dL), cortisol ($\mu\text{g/dl}$) and the non-esterified fatty acid NEFA (mM) according to standard laboratory procedures.

Electrocardiogram (ECG)

The 12-lead ECG was performed after a resting period for at least 5 minutes before the test. Electrodes were attached to the chest and limbs of the patient and connected to the monitor to detect the heart's electrical activity, cardiac rhythm disturbances, myocardial ischemia or infarction.

Data Analysis

Data analyses were performed using NCSS21 (NCSS LLC, Kaysville, UT, US) 16. Variables without normal distribution are expressed as median (IQR). Differences between baseline and end of the intermittent fasting (i.e., 30 days) period were analysed using Wilcoxon T-test, a

non-parametric test to compares two related samples. Differences between RIF and 16h-IF fasting group were analysed by Mann-Whitney T-test, a non-parametric test to compare two independent groups. Differences between proportions were analysed by chi-square test, to examine the association between categorical variables, checking if the observed frequencies differ from the expected frequencies. Differences between different times were tested by Kruskal Wallis test. A probability (P) equal or less than 5% was considered statistically significant.

Results

Baseline assessment

Table 1 shows the baseline characteristics of subjects enrolled according to the fasting period. In the RIF group, the prevalence of males (66.7% vs. 31.3%), Arab nationality (94.4% vs. 18.8%), and smokers (55.6% vs. 18.8%) was higher ($P<0.00001$), and the number of fasting hours (17.0 vs. 16.0) was slightly longer ($P=0.002$) than in the 16IF group. By contrast, in the 16IF group, the prevalence of Italian nationality (81.2% vs. 0.0%), alcohol consumers (66.7% vs. 0.0%), and the number of weekly drinks (1.5 vs. 0.0) were higher ($P<0.00001$) than in the RIF group. All other parameters, including age, body weight, and the prevalence of liver steatosis were comparable between groups.

Assessment of caloric intake, macronutrient content, and body weight

Figure 3 depicts the time-dependent changes in daily (total) caloric intake throughout the study period of 90 days, as absolute (**3A**) and normalized (percent) values (**3B**).

At baseline, the average caloric intake was significantly higher in the RIF than in 16IF group ($1987\pm SEM156$ Kcal/d and 1542 ± 116 Kcal/d, respectively, $P=0.047$). On intermittent fasting, caloric intake significantly decreased in both groups by day 15 (RIF -644 Kcal/d, $P<0.05$ and

16IF -306 Kcal/d, $P < 0.05$ both vs. baseline), and remained stable by day 30 (i.e., end of intermittent fasting period in both groups). On follow-up, caloric intake in both RIF and 16IF increased to a higher plateau already on day 33 (RIF 1672.0 ± 152.0 Kcal/d and 16IF 1370.0 ± 134.0 Kcal/d), 45, and 60, and approached the baseline value on day 90. Normalized data (**3B**) confirmed the same time-dependent changes of caloric intake.

Panels **3C-D** show the time-dependent changes of body weight throughout the study period of 90 days as absolute and normalized (percent) values, respectively. At baseline, the average body weight was 76.4 ± 4.6 Kg in RIF and 73.5 ± 4.1 Kg in 16IF group ($P = \text{N.S.}$). The body weight significantly ($P = 0.002$) decreased at the end of the fasting period, although the change tended to be more evident during Ramadan (RIF -1.3 Kg from baseline vs. 16IF -0.6 Kg from baseline, $P = \text{N.S.}$). Body weight increased again to reach the baseline values on day 90. Percent changes of body weight followed the same trend, as absolute changes at the end of fasting period (RIF -2.2% Kg from baseline, 16IF -1.9% from baseline, $P = \text{N.S.}$).

Individual changes in daily macronutrient intake (i.e., carbohydrates, proteins, lipids, and fibers) between baseline and day 15 of intermittent fasting in RIF and 16IF are shown in **Figure 4**. In RIF, a significant decrease ($0.0004 < P < 0.049$) occurred for all macronutrients with the exception of fibers. In 16IF only carbohydrates decreased significantly ($P = 0.003$). In both RIF and 16IF groups, **Table 2** provides detailed time-dependent changes of caloric intake, body weight and BMI, ratio of caloric intake to BMI, and macronutrients consumption during the study period of 90 days.

According to the BMI, in RIF group 10 subjects had normal weight (NW), 3 were overweight (OW) and 5 were with obesity (OB) ($P = \text{N.S.}$ vs. 16IF). NW subjects lost an average of -1.9 ± 0.4 Kg (-2.92%) of weight (Median= -2.1 (IQR 1.2) (-3.3%), while combined overweight and obese subjects lost -1.5 ± 0.8 Kg (-1.8%) (Median= -1.9 (IQR 4.4) (-2.2%). Moreover, 12 over

18 subjects (66.7%) lost more than 2% of their weight at day 30, i.e., 8 NW (-3.73%), and 3 OW+ 1 OB (-3.8%).

In the 16IF group, 8 subjects were NW, 4 OW and 4 OB. Overall, NW subjects lost an average of -1.0 ± 0.3 Kg (-1.6%) (Median= -1.2 (IQR 1.3) -2.1%), but overweight and obese subjects lost -1.7 ± 0.3 Kg (-2.2%) (Median=-1.0 (IQR 2.9) (-1.2%). Moreover, 8 over 16 subjects (50%) lost more than 2% of their weight at day 30, 5 NW (-2.6%), 2OW + 1 OB (-4.8%). Differences between groups in terms of BMI and weight loss were not significant.

Anthropometric measures, body composition, liver steatosis and serum biomarkers

Table 3 depicts changes in anthropometric measures, body circumferences, fat, composition, and serum biomarkers at baseline and the end of the intermittent fasting period in both groups.

Whereas both RIF (-2.9%, $P=0.002$ vs. baseline) and 16IF (-1.9%, $P=0.002$ vs. baseline) were associated with a decrease of weight and BMI, with a slight greater effect for RIF, only RIF and not 16IF was associated with a greater decrease of body circumferences (between -2.2% and -4.2%; $0.0004 < P < 0.02$).

Concerning subcutaneous and visceral fats measured by US, both RIF and 16IF were associated with a significant ($0.02 < P < 0.001$) decrease of fat thickness, with exception of subcutaneous pre-pancreatic and pre-aortic fat during 16IF. Of note, the percent decrease of pre-pancreatic fat was significantly greater with RIF than 16IF (-22.5% vs -4.2%, $P=0.01$). The analyses of body composition by BIA showed a significant reduction of body fat only in the RIF group (-8.7%, $P=0.03$).

Serum levels of glucose, insulin, and HOMA-index were not affected by both intermittent fasting models.

The levels of NEFA increased in both RIF (+29.0%, $P=0.01$) and 16IF (+16.8%, $P=0.03$), while serum levels of cortisol significantly decreased in RIF (-13.9%, $P=0.01$), but increased in the 16IF group.

As reported in **Table 4**, ECG parameters were comparable at baseline and the end of fasting period in both the RIF and 16IF groups.

Influence of IF on liver steatosis

Overall, HRI significantly decreased only in the RIF group (-11.3%, $P=0.04$) (**Table 3**).

Figure 5 depicts changes in body weight, BMI, subcutaneous and visceral fats, and HRI in both RIF and 16IF during the fasting period, according to diagnosis of liver steatosis.

At baseline, liver steatosis in the RIF group was associated with significantly higher weight, BMI, SAT, VAT, and HRI ($0.00001 < P < 0.01$). In the 16IF group, this was observed only for VAT and HRI ($0.01 < P < 0.03$).

In the RIF group, at the end of the fasting period, weight and BMI, SAT and VAT had significantly decreased in subjects without steatosis ($P < 0.00001$). By contrast, the presence of steatosis was associated with a significant decrease of SAT, VAT, and HRI ($0.03 < P < 0.046$).

In 16IF group, the fasting period was associated with a significant decrease of weight, BMI, and VAT in subjects without steatosis ($0.004 < P < 0.008$). Notably, the presence of steatosis was not associated with any significant change of weight, BMI and fats, including HRI.

Discussion

Results from the present study confirm that one-month intermittent fasting is associated with several anthropometric and likely beneficial metabolic changes, including a decrease in caloric intake, body weight, and visceral fats. Nevertheless, outcomes are distinct in subjects

undergoing RIF or 16IF. Notably, under certain circumstances liver steatosis decreases as well, especially on RIF.

In general, IF is a time-restricted diet in which subjects fast every day usually from 14 to 18 hours [7,17](#). IF has gained popularity due to potential beneficial effects on weight loss and metabolic profile [7,17,18](#). The outcome likely involves the innate circadian rhythm which is highly responsible for metabolic homeostasis maintenance, i.e., protein and lipid synthesis, glycolysis, gluconeogenesis, etc. [19](#). In this respect, any disparity between circadian timing and food intake may increase insulin resistance, oxidative stress, and disrupt hormonal secretion [20](#). Previous animal studies documented the beneficial effects of IF time-restricted eating on circadian rhythm disruption and on the prevention of dyslipidaemia, insulin resistance, and liver steatosis^{[21](#)}. In humans, IF reduced body weight, fasting glucose, triglyceride levels, inflammatory markers, and body fat^{[22,23](#)}.

Ramadan is the Islamic practice of fasting, when healthy Muslim people abstain from food, drinks, water, and smoking during one month every year. A study reported that RIF is associated with a change in body weight and metabolic parameters [24](#) but to our knowledge this is the first comprehensive “real-life” study comparing two types of intermittent fasting. The effects of RIF and 16IF were analysed on caloric intake, anthropometric indices, amount of abdominal fat, liver steatosis, body composition, serum analyses, and effects on ECG.

During one month, the caloric intake decreased in both RIF and 16IF subjects, along with body weight. During RIF the overall caloric restriction was paralleled by a significant decrease in intake of total carbohydrates, proteins, and lipids. On 16IF, however, only intake of total carbohydrates decreased significantly. In both models, fiber intake tended to decrease slightly, but not significantly. Distinctive food types and preparations between nationalities likely explains the greater effect in macronutrient reduction in RIF than 16IF group.

The decreased caloric intake during RIF and 16IF is likely responsible for the decreased body weight, body circumferences, and fat deposition, since physical activity remained unchanged. The higher reduction in the intake of total lipids and total proteins in the RIF group, as compared with 16IF, may explain the trend towards a higher reduction of body weight, and the significant reduction of body circumferences in subjects undergoing RIF, as compared to those on 16IF.

Previous study showed the effect of IF on increasing the NEFA levels ²⁵. However, there is no clear evidence regarding NEFAs during RIF, except of a study in which NEFA was decreased after 2 weeks of RIF in healthy subjects²⁶. However, in our study, NEFA increased in both groups after the fasting period (4 weeks). These findings may be due to the temporary increase of NEFA levels after the lipids catabolism, resulted by a decrease of body fat detected by BIA, and the decrease of visceral and subcutaneous fat.

We measured serum cortisol levels to find a possible relationship between intermittent fasting and individual stress. Serum cortisol levels decreased after RIF but not after the 16IF. Previous studies showed a decrease in the serum cortisol level on the third and fourth weeks of RIF compared to the first week of fasting²⁷. This evidence might explain variations in cortisol levels during RIF as due to the shift to night-time eating, alteration of sleeping habits, and staying awake most of the night, in addition to a normal performance of physical activity (i.e. work, study, gym) during the fasting period.

Decreased caloric intake and body weight were detected in both groups, although this outcome was more marked in subjects who underwent RIF. In this respect, a previous study showed that breakfast may improve weight loss and decrease caloric intake during the day²⁸. This finding is linked with the macronutrient content, since the morning intake of carbohydrates, protein, and fat at breakfast is associated with reduced intake of total carbohydrates, protein, and fat during the day, resulting in body weight loss²⁹. These findings conflict with results from the

present study, where the skip of breakfast in both groups was associated with a significant decrease of total carbohydrates, total protein, and total fat in the RIF group, and with a decrease in total carbohydrates in the 16IF group. Moreover, as also documented by results from the present study, the timing of meals during RIF (from sunset to sunrise) improves fasting glucose levels and the grade of insulin resistance, decreases body weight, body fat, and the risk of liver steatosis^{30,31}.

Furthermore, it has been documented that an irregular number of meals, by eating between the three initial meals of the day (i.e. breakfast, lunch, and dinner) is associated with insulin resistance and high serum levels of γ -glutamyl transferase³². A previous study reported that RIF can reduce fat mass (Kg), especially in overweight subjects and in subjects with obesity³³. In line with these results, and similarly to the higher decrease of weight and subcutaneous fat in the RIF group compared to the 16IF group, in the present series the body fat mass measured by BIA was significantly decreased only in the RIF group.

Previous studies on RIF showed its beneficial effect in terms of decreased total body fat mass and visceral adipose tissue³⁴. These findings are in line with results from the present study, showing a significant reduction of visceral fat during RIF. Another study in women with obesity showed that IF combined with caloric restriction was associated with a significant reduction of visceral fat³⁵. Another study on a time-restricted diet of 4 to 10h/day resulted in a mild decrease of body weight (1 to 4% from baseline), and in a decrease of subcutaneous and visceral fat after 1-12 weeks of fasting³⁶. Finally, previous results from our group exploring the effects of RIF on gastrointestinal motility and visceral fat, confirmed the significant effect of RIF in terms of decreased subcutaneous and visceral fat thickness⁸.

Metabolic dysfunction associated steatotic liver disease (MASLD) is the most frequent liver disease, and weight loss and increased physical activity remain at the moment the only approved treatment strategies³⁷. From a clinical point of view, MASLD has been recognized

as an independent risk factor for cardiovascular disease³⁸, possibly due to pathogenic pathways involving insulin resistance, endothelial dysfunction, chronic inflammation, and oxidative stress linked with fat-over-storage³⁹.

Previous evidence suggested that IF has beneficial effects on MASLD, with an improvement of liver enzymes, fat liver accumulation, and liver stiffness ^{37,40}. The burden of MASLD in terms potential progression, and association to increased cardiovascular risk has been well established^{41,42}. Our findings show that HRI, a semiquantitative and non-invasive ultrasonographic technique¹⁵ for the evaluation/quantification of liver steatosis, significantly decreased on RIF but not on 16IF. This finding is in line with a previous *in vivo* animal study, in which mice undergoing IF showed a white adipose tissues browning (i.e., conversion of white adipose tissues into brown adipose tissues), which decreased weight, insulin resistance, and hepatic steatosis through gut microbiota alterations ⁴³.

Tacking together, RIF significantly induced a decrease of the amount of both subcutaneous and visceral fat, even in the presence of liver steatosis, while 16IF only decreased the amount of visceral fat only in non steatotic subjects. Since the abdominal VAT deposition stimulates the production of inflammatory cytokines⁴⁴, a decreased VAT thickness through body weight loss may improve the inflammation outcomes, finally resulting in a reduction of metabolic alterations, including the risk of MASLD^{35,45,46}. We speculate that this might explain our results, where RIF contributed to a body higher weight loss, followed by a reduction of VAT deposition, and a decreased liver steatosis compared to traditional 16IF.

The timing of eating and fasting, in addition to the total caloric intake and the type of macronutrients, plays a critical role in weight loss and metabolic diseases ³⁰. These findings were confirmed by results from the present study, where the difference in the number of fasting hours (higher in RIF than in 16IF) may explain the more pronounced beneficial effects recorded

following RIF, in comparison to 16IF. We did not control for this factor, due to the “real life” design of the study.

Previous evidence documented that RIF could have negative effects on health increasing cardiovascular risks such as acute coronary syndrome, chronic peripheral vascular disease, heart failure, and minor non haemorrhagic stroke [47](#). In this respect, results from our study excluded any effect on ECG during either RIF or 16IF. However, further studies are needed to adequately explore possible cardiologic outcomes following IF in the short-medium term.

In conclusion, IF is able to decrease caloric intake, body weight, and visceral fat deposition. However, RIF a specific type of IF, has more pronounced effects than the traditional 16IF, also in terms of improvement of liver steatosis. Further studies should verify the effects of RIF on the long-term management of obesity and, possibly, in reducing the risk of noncommunicable diseases associated with fat over-storage.

References

1. Di Ciaula A, Portincasa P. Contrasting obesity: is something missing here? *Internal and emergency medicine*. 2024.
2. Malinowski B, Zalewska K, Wesierska A, et al. Intermittent Fasting in Cardiovascular Disorders-An Overview. *Nutrients*. 2019;11(3).
3. Colakoglu M, Ercal T, Karslioglu S. Commencement of normal menstrual cycles after 17 years in a patient with Sheehan's syndrome. *Am J Med*. 1987;82(3):578.
4. Stekovic S, Hofer SJ, Tripolt N, et al. Alternate Day Fasting Improves Physiological and Molecular Markers of Aging in Healthy, Non-obese Humans. *Cell metabolism*. 2019;30(3):462-476 e466.
5. Longo VD, Panda S. Fasting, Circadian Rhythms, and Time-Restricted Feeding in Healthy Lifespan. *Cell metabolism*. 2016;23(6):1048-1059.
6. Welton S, Minty R, O'Driscoll T, et al. Intermittent fasting and weight loss: Systematic review. *Canadian family physician Medecin de famille canadien*. 2020;66(2):117-125.

7. Nowosad K, Sujka M. Effect of Various Types of Intermittent Fasting (IF) on Weight Loss and Improvement of Diabetic Parameters in Human. *Curr Nutr Rep.* 2021;10(2):146-154.
8. Abdallah H, Khalil M, Farella I, et al. Ramadan intermittent fasting reduces visceral fat and improves gastrointestinal motility. *European journal of clinical investigation.* 2023;53(9):e14029.
9. Rinella ME, Sookoian S. From NAFLD to MASLD: updated naming and diagnosis criteria for fatty liver disease. *Journal of lipid research.* 2024;65(1):100485.
10. Portincasa P, Baffy G. Metabolic dysfunction-associated steatotic liver disease: Evolution of the final terminology. *European journal of internal medicine.* 2024.
11. Badran H, Elsabaawy M, Sakr A, et al. Impact of intermittent fasting on laboratory, radiological, and anthropometric parameters in NAFLD patients. *Clin Exp Hepatol.* 2022;8(2):118-124.
12. Shanmugam H, Di Ciaula A, Di Palo DM, et al. Multiplying effects of COVID-19 lockdown on metabolic risk and fatty liver. *European journal of clinical investigation.* 2021;51(7):e13597.
13. Gallagher D, Visser M, Sepulveda D, Pierson RN, Harris T, Heymsfield SB. How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? *American journal of epidemiology.* 1996;143(3):228-239.
14. Heymsfield SB, Wang Z, Baumgartner RN, Ross R. Human body composition: advances in models and methods. *Annual review of nutrition.* 1997;17:527-558.
15. Santoro S, Khalil M, Abdallah H, et al. Early and accurate diagnosis of steatotic liver by artificial intelligence (AI)-supported ultrasonography. *European journal of internal medicine.* 2024.
16. *NCSS 2023 Statistical Software (2023).* NCSS, LLC. Kaysville, Utah, USA, [ncss.com/software/ncss](https://www.ncss.com/software/ncss). [computer program]. Kaysville, Utah: Number Cruncher Statistical System (NCSS); 2023.
17. Anton SD, Moehl K, Donahoo WT, et al. Flipping the Metabolic Switch: Understanding and Applying the Health Benefits of Fasting. *Obesity.* 2018;26(2):254-268.
18. Vasim I, Majeed CN, DeBoer MD. Intermittent Fasting and Metabolic Health. *Nutrients.* 2022;14(3).
19. Swiatkiewicz I, Wozniak A, Taub PR. Time-Restricted Eating and Metabolic Syndrome: Current Status and Future Perspectives. *Nutrients.* 2021;13(1).
20. Xie Y, Tang Q, Chen G, et al. New Insights Into the Circadian Rhythm and Its Related Diseases. *Front Physiol.* 2019;10:682.

21. Chung H, Chou W, Sears DD, Patterson RE, Webster NJ, Ellies LG. Time-restricted feeding improves insulin resistance and hepatic steatosis in a mouse model of postmenopausal obesity. *Metabolism: clinical and experimental*. 2016;65(12):1743-1754.
22. Moro T, Tinsley G, Bianco A, et al. Effects of eight weeks of time-restricted feeding (16/8) on basal metabolism, maximal strength, body composition, inflammation, and cardiovascular risk factors in resistance-trained males. *Journal of translational medicine*. 2016;14(1):290.
23. Tinsley GM, Forsse JS, Butler NK, et al. Time-restricted feeding in young men performing resistance training: A randomized controlled trial. *Eur J Sport Sci*. 2017;17(2):200-207.
24. Patterson RE, Sears DD. Metabolic Effects of Intermittent Fasting. *Annual review of nutrition*. 2017;37:371-393.
25. Liu B, Hutchison AT, Thompson CH, Lange K, Wittert GA, Heilbronn LK. Effects of Intermittent Fasting or Calorie Restriction on Markers of Lipid Metabolism in Human Skeletal Muscle. *The Journal of clinical endocrinology and metabolism*. 2021;106(3):e1389-e1399.
26. Vardarli MC, Hammes HP, Vardarli I. Possible metabolic impact of Ramadan fasting in healthy men. *Turk J Med Sci*. 2014;44(6):1010-1020.
27. Riat A, Suwandi A, Ghashang SK, et al. Ramadan Fasting in Germany (17-18 h/Day): Effect on Cortisol and Brain-Derived Neurotrophic Factor in Association With Mood and Body Composition Parameters. *Frontiers in nutrition*. 2021;8:697920.
28. de Castro JM. The time of day and the proportions of macronutrients eaten are related to total daily food intake. *Br J Nutr*. 2007;98(5):1077-1083.
29. de Castro JM. When, how much and what foods are eaten are related to total daily food intake. *Br J Nutr*. 2009;102(8):1228-1237.
30. Marjot T, Tomlinson JW, Hodson L, Ray DW. Timing of energy intake and the therapeutic potential of intermittent fasting and time-restricted eating in NAFLD. *Gut*. 2023;72(8):1607-1619.
31. Aliasghari F, Izadi A, Gargari BP, Ebrahimi S. The Effects of Ramadan Fasting on Body Composition, Blood Pressure, Glucose Metabolism, and Markers of Inflammation in NAFLD Patients: An Observational Trial. *J Am Coll Nutr*. 2017;36(8):640-645.
32. Sierra-Johnson J, Unden AL, Linestrland M, et al. Eating meals irregularly: a novel environmental risk factor for the metabolic syndrome. *Obesity*. 2008;16(6):1302-1307.
33. Fernando HA, Zibellini J, Harris RA, Seimon RV, Sainsbury A. Effect of Ramadan Fasting on Weight and Body Composition in Healthy Non-Athlete Adults: A Systematic Review and Meta-Analysis. *Nutrients*. 2019;11(2).

34. Faris MAE, Madkour MI, Obaideen AK, et al. Effect of Ramadan diurnal fasting on visceral adiposity and serum adipokines in overweight and obese individuals. *Diabetes research and clinical practice*. 2019;153:166-175.
35. Klempel MC, Kroeger CM, Bhutani S, Trepanowski JF, Varady KA. Intermittent fasting combined with calorie restriction is effective for weight loss and cardio-protection in obese women. *Nutrition journal*. 2012;11:98.
36. Cienfuegos S, McStay M, Gabel K, Varady KA. Time restricted eating for the prevention of type 2 diabetes. *J Physiol*. 2022;600(5):1253-1264.
37. Lange M, Nadkarni D, Martin L, Newberry C, Kumar S, Kushner T. Intermittent fasting improves hepatic end points in nonalcoholic fatty liver disease: A systematic review and meta-analysis. *Hepatol Commun*. 2023;7(8).
38. Chan WK, Chuah KH, Rajaram RB, Lim LL, Ratnasingam J, Vethakkan SR. Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD): A State-of-the-Art Review. *J Obes Metab Syndr*. 2023;32(3):197-213.
39. Hutchison AL, Tavaglione F, Romeo S, Charlton M. Endocrine aspects of metabolic dysfunction-associated steatotic liver disease (MASLD): Beyond insulin resistance. *Journal of hepatology*. 2023;79(6):1524-1541.
40. Ali H, Shahzil M, Moond V, et al. Non-Pharmacological Approach to Diet and Exercise in Metabolic-Associated Fatty Liver Disease: Bridging the Gap between Research and Clinical Practice. *J Pers Med*. 2024;14(1).
41. Portincasa P, Khalil M, Mahdi L, et al. Metabolic Dysfunction–Associated Steatotic Liver Disease: From Pathogenesis to Current Therapeutic Options. *International journal of molecular sciences*. 2024;25:5640.
42. Younossi ZM, Paik JM, Stepanova M, Ong J, Alqahtani S, Henry L. Clinical profiles and mortality rates are similar for metabolic dysfunction-associated steatotic liver disease and non-alcoholic fatty liver disease. *Journal of hepatology*. 2024.
43. Lavallee CM, Bruno A, Ma C, Raman M. The Role of Intermittent Fasting in the Management of Nonalcoholic Fatty Liver Disease: A Narrative Review. *Nutrients*. 2022;14(21).
44. Meade RD, Vanderbilt D. Adatoms on Si(111) and Ge(111) surfaces. *Physical review B, Condensed matter*. 1989;40(6):3905-3913.
45. Heilbronn LK, Smith SR, Martin CK, Anton SD, Ravussin E. Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. *The American journal of clinical nutrition*. 2005;81(1):69-73.
46. Kim D, Chung GE, Kwak MS, et al. Body Fat Distribution and Risk of Incident and Regressed Nonalcoholic Fatty Liver Disease. *Clin Gastroenterol Hepatol*. 2016;14(1):132-138 e134.

47. NasrAllah MM, Osman NA. Fasting during the month of Ramadan among patients with chronic kidney disease: renal and cardiovascular outcomes. *Clin Kidney J.* 2014;7(4):348-353.

Tables

Table 1. Baseline characteristics of subjects enrolled for Ramadan intermittent fasting (RIF) and for 16-h intermittent fasting (16IF).

	RIF	16IF	P
N.	18	16	
Males/ Females N. (%)	12 (66.7%)/ 6 (33.3%)	5 (31.3%)/ 11 (68.7%)	<0.00001
Age (years)	33.5 (13.0)	30.0 (19.5)	0.8
Nationality N. (%)			
Arab	17 (94.4%)	3 (18.8%)	<0.00001
Italian	0 (0.0%)	13 (81.2%)	<0.00001
Others	1 (5.6%)	0 (0.0%)	0.01
Fasting hours	17.0 (1.6)	16.0 (0.5)	0.002
Weight (Kg)	68.7 (30.6)	70.7 (26.9)	0.6
BMI (Kg/m ²)	24.5 (10.5)	24.5 (8.5)	0.8
Normal weight N. (%)	10 (55.5%)	8 (50.0%)	0.4
Overweight N. (%)	3 (16.7%)	4 (25.0%)	0.1
Obese N. (%)	5.0 (27.8%)	4 (25.0%)	0.7
Smokers N. (%)	10 (55.6%)	3.0 (18.8%)	<0.00001
Alcohol consumers N. (%)	0 (0.0%)	10 (66.7%)	<0.00001
Alcohol consumption drinks/week	0.0 (0.0)	1.5 (1.0)	<0.00001
Diagnosis of liver steatosis N. (%)	6 (33.3%)	4 (25.0%)	0.2

Age, fasting hours, weight, BMI, and alcohol consumption expressed as median (IQR). The other parameters expressed as number and percentage. Differences between Median (IQR) assessed by Mann-Whitney. Differences between percentages were tested by the Chi-square test. Abbreviations: BMI, body mass index; N., number of subjects.

Table 2. Effect of Ramadan intermittent fasting (RIF) and 16-h intermittent fasting (16IF) on anthropometric parameters and macronutrient intake during the study period.

RIF	Baseline (Day 0)	Day 15	Day 30	Day 33	Day 45	Day 60	Day 90	P1 (Day 0-15, 30)	P2 (Day 30-33)	P3 (Day 0-90)
Total calories (Kcal)	2005.2 (896.0)*\$ 100%	1121.8 (913.0)* -44.0%	1095.6 (843.7)\$ -45.4%	1541.8 (1177.0) -23.1	1625.1 (589.0) -18.9%	1502.0 (873.0) -25%	1880.0 (643.0) -6.2%	0.001	0.002	0.5
Weight (Kg)	68.7 (30.6) 100%	67.7 (31.0) -1.5%	65.8 (31.5) -4.2%	65.8 (31.5) -4.2%	67.5 (31.3) -1.7%	66.8 (29.3) -2.8%	66.5 (30.8) -3.2%	0.8	1.0	0.03
Weight (Kg) (mean± SEM)	76.4± 4.6 100%	75.7± 4.6 -0.9%	74.7± 4.8 -2.2%	74.7± 4.8 -2.2%	75.6± 4.7 -1.0%	75.5± 4.6 -1.2%	75.3± 4.6 -1.4%	0.8	1.0	0.07
BMI (Kg/m2)	24.5 (10.5) 100%	24.2 (10.4) -1.2%	23.5 (10.4) -4.1%	23.5 (10.4) -4.1%	24.0 (10.2) -2.0%	24.0 (10.1) -2.0%	23.3 (10.1) -4.9%	0.8	1.0	0.02
Kcal/BMI	74.4 (41.7)	53.0 (26.6)	65.2 (30.0)	65.2 (30.0)	67.0 (33.1)	60.0 (18.6)	71.3 (25.3)			
Proteins (g/d)	84.3 (35.6) 100%	64.7 (46.5) -23.3%	62.8 (53.9) -25.5%	62.6 (63.1) -25.8%	54.8 (44.6) -35%	63.7 (28.7) -24.4%	64.6 (41.5) -23.4%	0.2	0.5	0.02
Lipids (g/d)	67.7 (29.0)*\$ 100%	40.8 (25.5)* -39.7%	36.3 (28.7)\$ -46.4%	55.9 (67.9) -17.4	62.4 (29.3) -7.8%	46.6 (38.4) -31.2%	50.8 (48.4) -25.0%	0.0009	0.05	0.1
Carbohydrates (g/d)	384.5 (225.7)*\$ 100%	294.5 (230.0)* -23.4%	296.5 (230.9)\$ -22.9%	380.8 (193.1) -1.0%	336.6 (135.5) -12.5%	296.3 (150.5) -22.9%	320.5 (157.8) -16.6%	0.03	0.1	0.03
Fibers (g/d)	14.9 (12.0) 100%	10.3 (8.8) -30.9%	11.3 (11.8) -24.2%	11.5 (10.1) -22.8%	13.0 (10.6) -12.8%	7.9 (10.9) -46.9%	9.8 (10.5) -34.2%	0.3	0.5	0.7
16IF	Baseline (Day 0)	Day 15	Day 30	Day 33	Day 45	Day 60	Day 90			
Total Kcal	1440.0 (863.0)*\$ 100%	1147.3 (695.0)* -20.3%	1185.5 (634.5)\$ -17.7%	1221.4 (595.0) -15.2%	1248.6 (620.0) -13.3%	1212.8 (946.0) -15.8%	1447.3 (796.0) +0.5%	0.05	0.5	0.9
Weight (Kg)	70.7 (26.9) 100%	70.4 (26.5) -0.4%	69.2 (26.4) -2.1%	69.2 (26.4) -2.1%	69.3 (26.3) -2.0%	69.5 (26.6) -1.7%	69.8 (28.2) -1.3%	0.8	1.0	0.07
Weight (Kg) (mean± SEM)	73.5± 4.1 100%	73.1± 4.1 -0.5%	72.1± 4.1 -1.9%	72.1± 4.1 -1.9%	72.3± 4.2 -1.6%	71.8± 4.2 -2.3%	72.1± 4.2 -1.9%	0.8	1.0	0.07
BMI (Kg/m2)	24.5 (8.5) 100%	24.4 (8.3) -0.4%	24.1 (8.6) -1.6%	24.1 (8.6) -1.6%	24.1 (8.5) -1.6%	23.8 (8.2) -2.9%	24.0 (8.3) -2.0%	0.8	1.0	0.04
Kcal/BMI	60.5 (23.9)	51.5 (23.0)	56.1 (26.8)	56.1 (26.8)	56.2 (28.4)	54.2 (36.6)	58.6 (33.8)			
Protein (g/d)	65.1 (39.1) 100%	67.3 (33.8) +3.4%	59.6 (31.2) -8.4%	59.8 (47.4) -8.1%	50.8 (24.6) -21.9%	65.0 (41.2) -0.2%	67.3 (24.6) +3.4%	0.8	1.0	1.0
Lipid (g/d)	53.2 (29.4) 100%	45.4 (20.5) -14.5%	44.3 (14.8) -16.7%	58.2 (23.6) +9.4%	60.7 (21.9) +14.1%	52.2 (28.7) -1.9%	55.2 (25.0) +3.8%	0.9	0.3	0.5
Carbohydrates (g/d)	323.7 (156.3)*\$ 100%	206.5 (190.6)* -36.2%	212.3 (195.2)\$ -34.4%	224.8 (186.8) -30.6%	238.0 (157.8) -26.5%	258.3 (175.2) -20.2%	284.0 (168.0) -12.3%	0.03	0.7	0.02
Fibers (g/d)	11.6 (6.1) 100%	11.5 (3.5) -0.9%	13.8 (8.0) +19.0%	10.4 (7.3) -10.3%	11.7 (10.8) -0.9%	10.5 (7.0) -9.5%	12.1 (8.6) +4.3%	0.6	0.1	0.9

Data are median (IQR), and % of change from baseline. Difference between groups are tested by Kruskal Wallis. BMI: body mass index, g/d: gram per day.

P1, ANOVA between days 0,15, 30 followed by post-hoc analyses. Similar symbols indicate differences between time groups; P2: Wilcoxon t-test days 30, 33; P3: Wilcoxon t-test days 0, 90

Table 3. Effect of fasting on anthropometric measures, body circumferences, body fat, body composition and blood biomarkers according to the fasting type for Ramadan intermittent fasting (RIF) and for 16-h intermittent fasting (16IF).

	RIF				16IF				
	Baseline (day 0)	End (day 30)	P	% change	Baseline	End	P	% change	P between % changes
Weight (Kg)	68.7 (30.6)	65.8 (31.5)	0.002	-2.9 (3.0)	70.7 (26.9)	69.2 (26.4)	0.002	-1.9 (2.9)	0.3
BMI (Kg/m ²)	24.5 (10.5)	23.4 (10.4)	0.002	-2.9 (3.0)	24.5 (8.5)	24.1 (8.6)	0.003	-1.9 (2.9)	0.3
Circumference (cm)									
Waist	82.5 (28.3)	81.5 (24.0)	0.0004	-4.2 (6.2)	76.0 (25.0)	74.0 (25.0)	0.3	-1.9 (5.1)	0.02
Abdominal	81.5 (27.8)	80.5 (28.8)	0.02	-2.2 (3.7)	75.0 (24.0)	75.0 (23.0)	0.4	-3.6 (11.3)	0.8
Neck	38.5 (9.0)	36.5 (7.8)	0.0008	-3.6 (8.0)	34.0 (8.5)	34.5 (6.5)	0.3	-1.2 (4.4)	0.02
Subcutaneous fat by US (mm)									
Pre-hepatic	10.5 (1.0)	8.5 (7.8)	0.01	-16.1 (43.7)	10.6 (6.2)	8.0 (5.1)	0.02	-13.8 (27.9)	0.5
Pre-pancreatic	12.3 (12.6)	11.6 (6.6)	0.002	-22.5 (35.0)	10.8 (5.5)	10.3 (7.5)	0.5	-4.2 (23.4)	0.01
Pre-aortic	15.1 (10.9)	11.6 (11.2)	0.005	-19.8 (49.0)	12.3 (8.8)	11.0 (10.2)	0.2	-5.4 (32.0)	0.1
Visceral fat by US (mm)									
Pre-hepatic	7.8 (8.0)	7.0 (7.6)	0.001	-22.9 (48.6)	10.0 (8.8)	8.0 (8.0)	0.02	-17.2 (51.0)	0.7
Pre-pancreatic	27.0 (10.8)	22.4 (18.6)	0.02	-17.2 (47.0)	32.7 (23.0)	29.4 (12.5)	0.001	-11.3 (21.1)	0.6
Pre-aortic	33.7 (19.0)	31.0 (21.0)	0.001	-12.1 (18.3)	33.0 (28.5)	27.0 (34.1)	0.04	-6.9 (16.5)	0.2
Hepato-renal	13.8 (6.8)	10.8 (8.2)	0.002	-19.7 (42.5)	16.0 (13.9)	13.4 (11.7)	0.002	-8.7 (32.8)	0.3
Spleno- renal	16.0 (7.2)	13.3 (9.0)	0.002	-18.6 (29.0)	16.5 (9.4)	11.0 (8.4)	0.007	-12.6 (28.9)	0.8
Liver									
Hepato-renal index	1.3 (0.7)	1.1 (0.4)	0.04	-11.3 (22.7)	1.12 (0.3)	1.1 (0.2)	0.3	-2.6 (25.8)	0.4
Bioimpedance analysis (BIA)									
Basal metabolism (Kcal)	1565.0 (396.0)	1530.0 (462.5)	0.3	-2.1 (5.6)	1455.0 (255.0)	1410.0 (350.0)	1.0	0.35 (6.0)	0.3
Phase angle (quality of lean mass) °	6.5 (1.0)	6.7 (1.5)	0.9	-0.7 (11.7)	6.4 (1.0)	6.4 (1.2)	1.0	0.7 (13.0)	0.9
Body water (Litre)	38.6 (15.5)	37.9 (14.7)	0.3	-1.5 (6.9)	36.0 (11.0)	34.8 (13.6)	0.7	-0.5 (5.8)	0.5
Lean mass (fat-free mass, BCM plus ECM) (Kg)	52.7 (21.2)	51.7 (20.1)	0.3	-1.2 (6.4)	49.1 (15.1)	47.6 (18.7)	0.7	-0.6 (5.8)	0.8
ECM (interstizi d. cellule, ossa, tess. Connett.) (Kg)	24.4 (10.3)	24.0 (6.9)	0.4	-0.5 (9.9)	24.0 (8.2)	23.2 (9.0)	0.9	0.5 (8.9)	0.5
BCM (Organ muscle and cell mass) (Kg)	29.9 (13.4)	29.1 (14.6)	0.4	-1.9 (10.1)	26.6 (8.2)	25.1 (11.2)	0.9	0.6 (9.5)	0.4
ECM/BCM Index (External/Internal Cell Ratio)	0.85 (0.1)	0.8 (0.2)	1.0	0.0 (14.0)	0.9 (0.1)	0.9 (0.2)	0.9	0.0 (15.8)	0.9
Percentage of cells (%)	54.1 (4.4)	55.1 (6.5)	1.0	0.0 (6.7)	53.7 (4.4)	53.7 (5.4)	1.0	0.0 (6.9)	0.9
Body fat (Kg)	18.4 (9.2)	16.8 (9.8)	0.03	-8.7 (31.5)	15.1 (20.6)	15.0 (22.1)	0.2	-2.8 (11.5)	0.3
Blood analyses									
NEFA (mmol/L)	550.0 (178.0)	641.0 (286.2)	0.01	29.0 (51.9)	654.0 (177.0)	749.0 (573.0)	0.03	16.8 (61.0)	0.9
Fasting glucose (mg/dl)	104.0 (20.8)	103.0 (16.0)	0.7	0.65 (23.3)	95.0 (14.0)	97.0 (17.0)	0.3	4.3 (15.6)	0.8
Cortisol (ng/ml)	119.0 (40.8)	105.5 (46.8)	0.01	-13.9 (20.7)	155.0 (76.0)	162.0 (84.0)	0.5	3.2 (40.4)	0.04
Insulin (μU/ml)	11.7 (11.2)	12.1 (10.8)	0.5	-1.2 (65.7)	9.0 (8.2)	11.3 (8.0)	0.1	11.2 (61.3)	0.1
Homa-index	3.2 (3.0)	2.8 (2.4)	0.9	0.1 (2.2)	2.1 (1.7)	2.7 (2.1)	0.2	0.1 (1.4)	0.3

Data are expressed as median (IQR). Differences between before and after were tested by Willcoxon. Differences between % of change was tested by Mann Whitney. Abbreviations: BCM, body cell mass; BMI, body mass index; ECM, extracellular mass; NEFA; non-esterified fatty acids.

Table 4. Electrocardiogram parameters change after the fasting period in Ramadan intermittent fasting (RIF) and 16-h intermittent fasting (16IF).

	RIF			16IF		
	Baseline	End	P	Baseline	End	P
Frequency of heart beats (bpm)	71.5 (15.0)	69.5 (20.0)	0.9	70.4 (15.8)	71.0 (16.3)	0.8
RR (ms)	839.0 (194.5)	873.0 (250.0)	0.9	851.5 (197.3)	845.0 (221.0)	0.7
PR interval (ms)	156.5 (55.8)	168.0 (44.5)	0.2	141.0 (25.5)	144.5 (19.5)	0.8
QRSD interval (ms)	90.1 (9.3)	91.5 (6.0)	1.0	91.5 (10.8)	91.5 (7.5)	0.6
QT interval (ms)	379.0 (31.3)	379.5 (35.5)	0.7	391.5 (59.8)	380.0 (58.8)	0.7
QTcB interval (ms)	418.0 (29.3)	414.0 (45.3)	0.5	430.0 (22.5)	422.0 (30.3)	0.2
QTcF interval (ms)	401.0 (25.5)	405.0 (30.3)	0.6	416.0 (39.5)	414.0 (27.0)	0.2
P wave interval (ms)	48.5 (37.3)	60.0 (28.5)	0.3	49.0 (53.8)	43.0 (54.3)	0.9
QRS complex interval (ms)	37.5 (63.5)	41.0 (61.0)	0.08	55.5 (44.8)	67.0 (43.0)	0.1
T wave interval (ms)	42.5 (14.3)	39.0 (26.3)	0.4	36.0 (26.8)	39.0 (27.0)	0.3

Data are Median (IQR). Differences between baseline and the end are tested by Wilcoxon t-test. The P wave denotes the depolarization of the atria. The QRS complex denotes depolarization of the ventricles. The T wave represents repolarising ventricle.

Figures

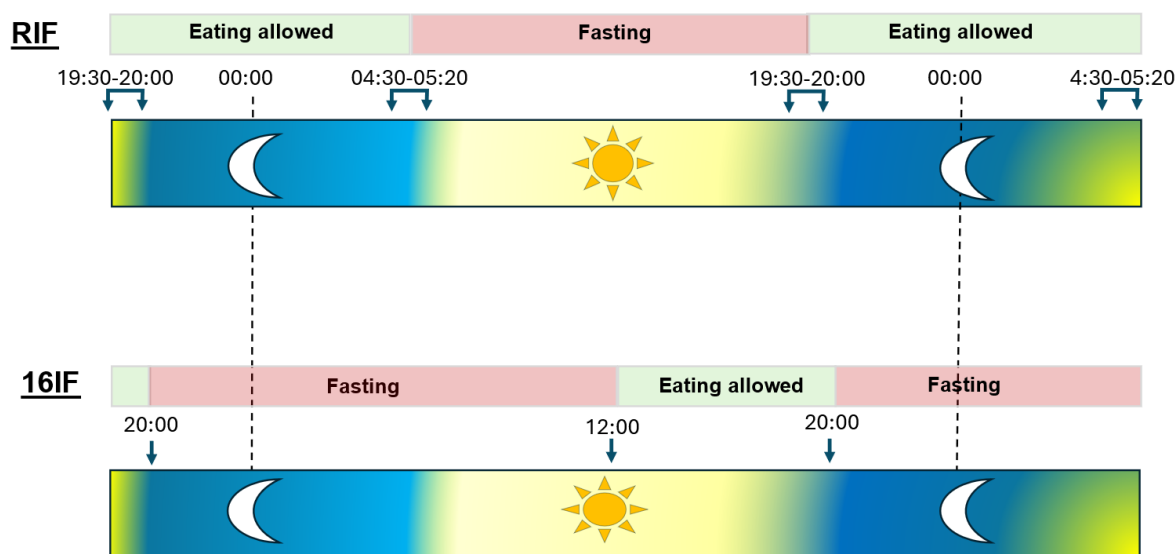


Fig. 1

Figure 1. Eating and fasting time during 30 days in RIF and 16-IF.



A

Total Kcal/day

Days

Intermittent fasting Follow-up

ANOVA $P=0.001$ $P=0.002$ $P=0.5$ $P=0.9$ $P=0.03$ $P=0.03$ $P=1.0$ $P=0.8$

● RIF
▼ 16IF

B

Total Kcal/day (%)

Days

Intermittent fasting Follow-up

● RIF
▼ 16IF

C

Weight (kg)

Days

Intermittent fasting Follow-up

ANOVA $P=0.8$ $P=1.0$ $P=0.03$ $P=0.8$ $P=0.03$ $P=1.0$ $P=0.8$

● RIF
▼ 16IF

D

Weight (%)

Days

Intermittent fasting Follow-up

● RIF
▼ 16IF

83

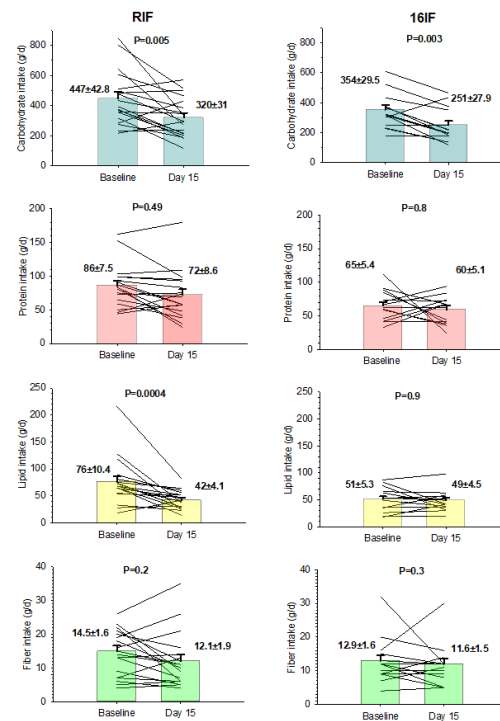


Figure 4. Changes in daily macronutrient intake including total carbohydrates, total proteins, total lipids, and total fibers between baseline and day 15 of intermittent fasting in RIF and 16IF. Lines represent individual changes while bars indicate the average changes (mean ± SE). Differences between baseline (day 0) and day 15 were tested by the Wilcoxon test

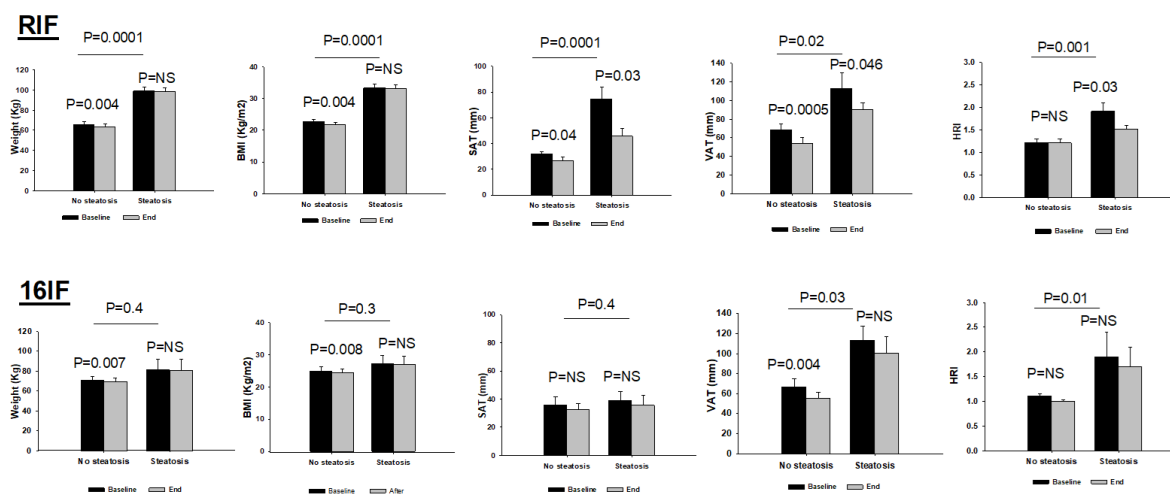


Fig. 5

Figure 5. Effect of RIF and 16IF on body weight, BMI, SAT, and VAT according to diagnosis of liver steatosis. SAT is the sum of the pre-hepatic, pre-pancreatic, and pre-aortic subcutaneous fats, and VAT is the sum of the pre-hepatic, pre-pancreatic and pre-aortic visceral fats. HRI is the AI-based hepatorenal index. Data were expressed as mean ± SE. The difference between baseline and end (day 30) was tested by Wilcoxon. The difference between no steatosis and steatosis at baseline was tested by Mann-Whitney.

10. Liver steatosis and metabolic dysfunction

Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD): Assessing Metabolic Dysfunction, Cardiovascular Risk Factors, and Lifestyle habits

Hala Abdallah^{1, *}, Mohamad Khalil^{1, *}, Ewa Awada¹, Elisa Lanza¹, Agostino Di Ciaula¹, Piero Portincasa^{1,2}

¹ Clinica Medica “A. Murri” and Department of Precision and Regenerative Medicine and Ionian Area (DiMePre-J), University of Bari "Aldo Moro", Bari, Italy.

² General Secretariat, Consortium of Mediterranean Universities.

ABSTRACT

Background: Steatotic liver disease (SLD), a key component of metabolic dysfunction, is associated with both hepatic and extra-hepatic complications, particularly cardiovascular disease (CVD). This study aimed to characterize a large cohort of dysmetabolic patients based on the presence of liver steatosis and the novel classification term of Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), assessing gender differences, metabolic risk factors, and the role of lifestyle habits.

Methods: A total of 406 patients (age 58.3±0.9 years; BMI 28.9±0.3 Kg/m²; Males/Females=224/182) were consecutively enrolled at the outpatient clinic for metabolic/CVD. Patients underwent physical examination, anthropometric measurements, and blood analyses. Lifestyle factors including Mediterranean diet (MD) and physical activity were assessed via validated questionnaires. Liver steatosis and atherosclerotic plaques were evaluated using abdominal and carotid ultrasounds. Metabolic and liver indices were calculated, and a 10-years cardiovascular disease (CVD) risk was estimated using the ASCVD Risk Calculator.

Results: Among subjects, 34.5% were diabetic, 58.6% were hypertensive, 40.6% with obesity, and 83.0% were dyslipidemic. SLD was detected in 268 (66.0%) subjects (CrypSLD 2.2%; MASLD 91.8%; MetALD 6.0%) with higher prevalence in males, who also exhibited higher rates of smoking, alcohol consumption, and CVD events compared to females. Among SLD patients, MASLD and MetALD patients shared similar dysmetabolic profiles compared to CrypSLD patients, though MetALD patients exhibited a higher prevalence of atherosclerotic plaques and ASCVD score, while MD score remained comparable.

Conclusion: This study highlights the complex interplay between liver steatosis, metabolic dysfunction, and CVD, emphasizing gender disparities and the importance of lifestyle factors, particularly diet. Screening for CVD risk factors in MASLD patients is highly recommended. Future studies should explore targeted interventions to mitigate CV complications in steatotic patients.

Keywords: Liver steatosis; metabolic-associated steatotic liver diseases (MASLD); cardiovascular diseases (CVD); Mediterranean diet

INTRODUCTION

The term non-alcoholic fatty liver disease (NAFLD) was coined in 1986 for liver steatosis, a condition that has become the most prevalent liver disorder worldwide. NAFLD was considered a diagnosis of exclusion until 2020. Due to the frequent coexistence with metabolic disorders, a group of international experts primarily from the Asia-Pacific region suggested to classify this specific condition as “metabolic dysfunction-associated fatty liver disease” (MAFLD) [1]. MAFLD is defined as liver steatosis identified through serum biomarkers, imaging, or liver histology, in conjunction with one of the following: overweight/obesity, type 2 diabetes, or at least two cardiometabolic risk factors. This new definition links MAFLD to metabolic abnormalities, regardless of other causes of chronic liver disease like alcohol use or viral hepatitis [2-4]. MAFLD describes the association of liver steatosis with metabolic dysfunctions, independently from alcohol intake [5,6].

In 2023, a multi-step, modified Delphi consensus process led by three major liver associations from Europe, North America, and Latin America, introduced the “metabolic dysfunction-associated steatotic liver disease” (MASLD), a condition characterized by liver steatosis along with at least one cardiometabolic risk factor. It falls under the broader category of “steatotic liver disease” (SLD), which includes all liver conditions associated with abnormal fat accumulation [7].

Previous evidence documented a higher risk of fatal and/or non-fatal cardiovascular events in patients with MASLD after adjusting for other cardiovascular risk factors [8-10], and liver steatosis has been identified by the American Heart Association as an independent factor for cardiovascular diseases [11]. Thus, primary and secondary prevention measures oriented to reduce cardiovascular risk in patients with MASLD (including changes in dietary habits and lifestyle) are urgently needed.

Mediterranean diet (MD) is a popular healthy diet, characterized by a high intake of plant-based food and healthy fats. The main components of the MD are vegetables, fruits, whole grains, fish, and extra virgin olive oil as a primary source of additional fat, with low-alcohol intake [12]. A high adherence to MD may help to reduce liver fat accumulation, increase insulin sensitivity, and improve liver function overall, subsequently leading to improvement of liver steatosis [13]. Beneficial effects of the MD include a reduction of development and progression of cardiovascular diseases (CVD). Thus, the aim of this study is to classify patients according to the MASLD criteria, to compare the life style, blood parameters, comorbidities, and to explore the CVD risk between patients with a diagnosis of MASLD or without, in addition to the role of individual dietary habits and lifestyle.

SUBJECTS AND METHODS

Subjects

A total of 406 adult subjects with metabolic diseases (224 males, 182 females; mean age 58.3 years, age range 19-89 years) were consecutively enrolled at the outpatient clinic for gastrointestinal and metabolic disorders (Clinica Medica “A. Murri” University of Bari, Italy). Subjects underwent full medical history assessment and physical examination to rule out major functional or organic diseases. Exclusion criteria included a history of current or prior diseases able to interfere with the study results, i.e., inflammatory, neoplastic diseases, major gastrointestinal surgery, including bariatric procedures, pregnancy, use of medications that are associated with liver steatosis, such as glucocorticoids, methotrexate, amiodarone, tamoxifen, or anti-obesity drugs such as tirzepatide and semaglutide. In accord with the Code of Ethics of the World Medical Association (Declaration of Helsinki), the study received the

approval from Local Ethics Committee (study n. 0030591, study number 7506). Subjects were fully informed about the study protocol and signed a detailed informed consent.

Study design

The study design is depicted in **Figure 1**. After initial screening, subjects underwent a full clinical assessment by medical history, including current therapies and physical exam with anthropometric measurements, blood pressure, blood analyses.

The presence of metabolic syndrome (MetS) was assessed according to the The National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) [14]. Specific and validated questionnaires served to assess lifestyle, including alcohol consumption, smoking status, adherence to MD, and extent of physical activity.

The presence of liver steatosis and of atherosclerotic plaques were assessed by abdominal and carotid ultrasound (US).

The following metabolic and liver indices were calculated: homeostatic model assessment for insulin resistance (HOMA-IR) [15], triglyceride-glucose index (TyG) [16], fatty liver index (FLI) [17], and hepatic steatosis index (HSI) [18].

The CVD risk was calculated by the Atherosclerotic Cardiovascular Disease (ASCVD) Risk Calculator determining the 10-years risk of heart disease or stroke ([ASCVD Risk Estimator +](#)).

Anthropometric measurements

Anthropometric measurements included weight (to the nearest 0.1 Kg), height (to the nearest 0.5 cm), body mass index BMI ($BMI = \text{Weight (Kg)} / \text{Height (m}^2\text{)}$) [19]. Body circumferences (as cm) were measured by a flexible meter at the neck, abdomen, waist, and hip [20].

Questionnaires

The individual adherence to MD was assessed using a validated questionnaire [21], with a total score ranging from 0 to 18. The questionnaire included registration of the intake of several components of the MD diet i.e., fruits, vegetables, dairy products, cereal, fish, meat, and alcohol consumption, according to daily/weekly intake. Each answer had a corresponding number of points ranging from 0 to 2, according to adherence to a certain component. Based on the sum of all points, a score of adherence was calculated, further classified as low adherence (score 0-6), moderate adherence (score 7-12), high adherence (score 13-18).

The level of physical activity was calculated by the International Physical Activity Questionnaire (long-format version) which provides a simple and rapid evaluation of physical activity during leisure, occupational, and sedentary time on a weekly basis. Levels of physical activity were calculated based on metabolic equivalent tasks (METs), with 1 MET equal to 3.5 ml of O₂/Kg/min. MET thresholds constitute valid indicators of physical activity intensity with relatively high accuracy; low physical activity: less than 600 MET-minutes/week, moderate physical activity: at least 600 MET-minutes/week, high physical activity: at least 3000 MET-minutes/week (https://www.physiopeedia.com/images/c/c7/Quidelines_for_interpreting_the_IPAQ.pdf)[22].

Assessment of liver steatosis

Abdominal ultrasonography served to diagnose the presence of fatty liver. The US scans were performed by using E2 machine equipped with a convex probe (3.4-5.0 Mhz) (Sonoscape/Fujifilm, Tokyo, Japan) provided by Eurisko Technology srl (Bari, Italy). A skilled operator (ADC) used a parasagittal scanning plane to obtain a combined view of the right lobe of the liver and the right kidney. Subjects were classified as non-SLD subjects when no difference in echogenicity (i.e., brightness) between liver and kidney parenchima was detected. SLD subjects were those with increased liver echogenicity, as compared to kidney parenchima.

Carotid ultrasound imaging

Carotid ultrasound imaging was performed using a high-resolution B-mode ultrasound system equipped with a 7–12 MHz linear-array transducer. Participants were positioned supine with their necks slightly extended and rotated contralateral to the side being imaged. Longitudinal and transverse views of the common carotid artery (CCA), carotid bulb, and internal carotid artery (ICA) were acquired bilaterally. Measurements of intima-media thickness (IMT) were conducted on the distal wall of the CCA over a 1 cm segment proximal to the bulb using semi-automated edge-detection software. The presence of atherosclerotic plaques was also recorded. All imaging and measurements were conducted by a trained sonographer blinded to participant clinical data.

Blood test analyses

Blood samples were collected in serum-separating test tubes, centrifuged at 3000 rpm for 10 minutes at room temperature within 30 minutes, and 1mL of serum was separated. Measurements included serum concentrations of plasma glucose (mg/dL), insulin (μ U/mL), liver enzymes (U/L) i.e., Alanine transaminase (ALT), Aspartate transferase (AST), Gamma- Glutamyl transferase (GGT), and lipid profile (mg/dL) i.e. total cholesterol (TC), High-density lipoprotein (HLD-cholesterol), Low-density lipoprotein (LDL-cholesterol), and triglycerides (TG), according to standard laboratory procedures. All measurements were performed at the central hospital laboratory during routine workup of patients.

SLD classification

Subjects with SLD were classified according to MASLD criteria[1,23], based on clinical and demographic data. The diagnosis of MASLD required liver steatosis plus at least one metabolic risk factor such as BMI ≥ 25 Kg/m² [23 Asia] or waist circumference > 94 cm (males), 80 cm (females), fasting serum glucose ≥ 5.6 mmol/L [100 mg/ dL] or 2-hours post-load glucose levels ≥ 7.8 mmol/L [140 mg/dL] or HbA1c $\geq 5.7\%$ [39 mmol/L], or type 2 diabetes or treatment for type 2 diabetes; blood pressure $\geq 130/85$ mmHg or specific antihypertensive drug treatment; plasma triglycerides ≥ 1.70 mmol/L [150 mg/dL] or lipid-lowering treatment; plasma HLD-cholesterol ≤ 1.0 mmol/L [40 mg/dL] (males) and ≤ 1.3 mmol/L [50 mg/dL] (females) or lipid-lowering treatment. In addition, alcohol consumption must be either absent or less than 30 g/day (or less than 210 g/week) for males or less than 20 g/day (or less than 140 g/week) for females.

The diagnosis of MetALD required a higher alcohol intake, i.e., 30-60 g/day (or 210–420 g/week) for males or 20-50 g/day (or 140–350 g/week) for females.

The diagnosis of CrypSLD included lean patients with liver steatosis but no other evident causes of steatosis (i.e. viral, drug-induced, autoimmune) and no metabolic risk factors or significant alcohol consumption (<https://www.aasld.org/>),[24].

ASCVD risk score

ASCVD risk (%) was assessed by ASCVD Risk Calculator [25], determining 10-years risk of CVD occurrence such as myocardial infarction, stroke, or heart failure in individuals aged 40-79 years old. Parameters used for risk estimation included age, sex, smoking status, systolic blood pressure, total cholesterol (mg/dL), HLD-cholesterol (mg/dL), T2DM diagnosis or treatment, hypertension diagnosis or treatment. Excluded from the estimation are patients <40 or >79 years old or with a previous diagnosis of CVD [26].

Data Analysis

Data analyses were performed using NCSS21 (NCSS LLC, Kaysville, UT, US) [27]. Variables with normal distribution are expressed as mean (SEM). Differences between two groups were tested by T-test. Differences between three groups were tested by ANOVA followed by post-hoc multi-comparison test. Differences between proportions were tested by chi-square test. A probability (P) of less than 5% was considered statistically significant.

RESULTS

Baseline assessment

Table 1 shows the baseline characteristics of the whole group of 406 dysmetabolic subjects, divided according to gender (224 males and 182 females). Overall, males and females were comparable for age, BMI and rates of normal weight, overweight and obesity. Comparable was also the prevalence of additional risk factors as dyslipidemia, hypertension, T2DM, MetS. Compared to females, males had significantly higher prevalence of smokers and alcohol consumers, as also of CVD history and SLD. However, the prevalence of carotid atherosclerosis was comparable among subjects with different gender.

The impact of liver steatosis

Table 2 shows subjects classified according to the presence of steatosis and gender.

Overall, age was comparable between subjects with or without SLD, but was higher in non-SLD males than females (P=0.02). Male patients with SLD tended to be younger than females.

BMI, body circumferences, waist/height ratio, and waist/hip ratios were higher in subjects with, than in those without SLD. By gender, neck and waist/hip ratio were significantly higher in males than females in both SLD and non-SLD groups.

In addition, in non-SLD group, abdominal circumference, waist and waist/height ratio were higher in males than females, while hip circumference was higher in females of SLD group compared to males.

The prevalence of smokers was comparable in both SLD and non-SLD groups, but invariably higher in males than females ($0.0007 < P < 0.002$).

The prevalence of alcohol consumers was lower in subjects with, than in those without SLD, and higher in males with SLD than in females ($P = 0.0005$).

Overall, the adherence to MD was medium, and was lower in subjects with, than in those without SLD, with no gender difference. The level of physical activity was comparable and invariably low across all groups.

The prevalence of hypertension and T2DM (but not of dyslipidemia) was higher in subjects with, than in those without SLD, with no gender difference.

Serum levels of glucose, insulin, ALT, AST, GGT and triglycerides were higher in subjects with, than in those without SLD, with higher ALT in SLD males, than in females. HDL-cholesterol was lower in subjects with SLD, and invariably lower in males of both SLD and non-SLD subgroups. Levels of total and LDL-cholesterol were comparable in subjects with or without SLD, with lower total cholesterol in males than females.

The rate of cardiovascular events was lower in subjects with, than in those without SLD, but was higher in males with SLD than in females. The prevalence of carotid atherosclerosis and the ASCVD score were comparable in subjects with or without SLD, with a higher ASCVD score in males than in females in both subgroups.

SLD subgroups

The majority of patients with liver steatosis were classified as MASLD ($N = 246$, 91.8%), followed by MetALD ($N = 16$, 6.0%), and CrypSLD ($N = 6$, 2.2%) (**Figure 2A**). Patients with CrypSLD were younger (**Figure 2B**), and had lower BMI (**Figure 2C**), than patients with MASLD or MetALD.

Table 3 shows indices of lifestyle, body circumferences and blood indices in patients with CrypSLD, MASLD, and MetALD.

As expected, the prevalence of alcohol consumers was higher in MetALD, than in MASLD and CrypSLD. The rate of current smokers was comparable in subjects with MASLD or MetALD, and smokers were absent among subjects with CrypSLD.

The level of physical activity tended to be higher in CrypSLD and MetALD than in MASLD, but a high variability was noticed between groups. Adherence to MD was comparable between the three subgroups.

All body circumferences (except hip circumference) were significantly lower in subjects with CrypSLD but comparable between MASLD and MetALD.

Average serum glucose levels progressively increased from CrypSLD to MASLD and MetALD, respectively. ALT levels were significantly lower in CrypSLD than in MASLD and MetALD.

levels of insulin, AST, GGT, total cholesterol, HDL, LDL, were comparable between the three subgroups, with a trend towards higher triglycerides levels in MASLD and MetALD, as compared with CrypSLD.

Results concerning indices of insulin resistance (HOMA-IR and TyG) and liver steatosis (HSI and FLI) in CrypSLD, MASLD, and MetALD are depicted in **Figure 3A-D**. Both HOMA-IR and TyG indexes tended to be higher in subjects with MASLD and MetALD, as compared to CrypSLD. HIS and FLI were significantly higher in MASLD and MetALD, as compared to CrypSLD.

Details about CVD in the three subgroups are depicted in **Figure 4A-D**. A family history for CVD was absent in subjects with CrypSLD and the rate increased significantly and progressively in subjects with MASLD (27.2%) or MetALD (75%). A similar trend occurred for the rate of CVD events, carotid atherosclerotic, and ASCVD score.

DISCUSSION

The present series allowed to fully characterize a large group of dysmetabolic subjects divided according to the presence of liver steatosis and different steatosis classification.

Results showed several gender- and steatosis- related differences. In particular, we confirmed that the prevalence of smokers, alcohol consumers, rate of cardiovascular disease, and presence of steatosis was invariably higher in males than in females. In line with previous evidence, we confirmed that even at a selected level of referral center, liver steatosis occurs more often in males than females [28], and that males consumed more alcohol and experienced more alcohol-related harms than females [29].

The analysis of anthropometric indices, lifestyle, comorbidities, serum examinations and rate of CVD pointed to a more severe expression of abnormalities in the presence of SLD. Based on recent studies, a high association between liver steatosis and metabolic dysfunction is well established, where metabolic risk factors such as overweight/obesity, hypertension, T2DM, and dyslipidemia have been identified as a cornerstone of liver steatosis [30]. Of note, liver steatosis has the potential to progress into more severe conditions which include both hepatic complications (as steatohepatitis, liver cirrhosis, and HCC [31]) and extra-hepatic diseases as CVD, extrahepatic cancers, chronic kidney disease, psoriasis, hypothyroidism, or polycystic ovarian syndrome [32]. We observed that patients with steatosis presented higher values of BMI and all body circumferences, which lies in line with the literature, since liver steatosis is highly associated with obesity [33] manifesting as increases in obesity indicators[34]. Among all extra-hepatic complications, the most significant is CVD, a leading cause of morbidity and mortality in steatotic patients globally[35]. As suggested by current evidence, patients with liver steatosis have an increased incidence rate of CVD[36]. Indeed, in our study, we observed that more than a quarter of SLD patients were previously diagnosed with CVD. In this scenario, fat over-storage into the liver plays a critical role, since high liver fat content is able to increase the cardiovascular risk independently of age, gender, ethnicity and BMI [37].

Overall, in the present series, it looks like that not only the presence of steatosis increased metabolic abnormalities, but such abnormalities are more evident in males than in females, and that males are younger when SLD has developed. This finding is in line with previous evidence, where the prevalence of metabolic syndrome was 46.3% in males and 23.3% in females in a sample from southern China {Ye, 2023 #819} and might partly explain the earlier occurrence of SLD complications in males than females.

Results from the present study confirm that adherence to MD can play a critical role, since a lower adherence to MD has been detected in subjects with, than in those without SLD. Of note, this evidence was irrespective of gender, and emerged as more relevant, as compared with the level of physical activity. In fact, subjects with or without SLD showed, at least in the present series, comparable levels of physical activity.

A further sub analysis focused on differences within SLD according to the most recent classification of Rinella et al [38][39], based on a phenotype-oriented assessment. Despite the low number of subjects with CrypSLD, several differences appeared as compared with MASLD or MetALD, with less evident effect of age, BMI, homeostasis, expression of liver steatosis degree, and CVD, whose parameters are indicative of metabolic dysfunction [40,41].

By contrast, MASLD and MetALD were closer with respect to metabolic disorder, although MetALD confirmed an increased risk of cardiovascular manifestations, which emphasizes the harmful additive effect of alcohol consumption on CVD development, most likely through mechanisms such as increased oxidative stress, inflammation, [42] and prothrombotic effects of alcohol [43]. Previous evidence also documented as a concomitant alcohol consumption in MASLD (i.e., MetALD) can generate an increased all-cause mortality [44-46].

An additional finding was that the beneficial effect of MD was not evident when comparing subjects with CrypSLD, MASLD or MetALD, who showed comparable levels of adherence to MD and physical activity.

A limitation in the present study is the lack of characterization of liver fibrosis. In fact, the presence and extent of liver fibrosis can modulate the cardiovascular risk[47-51] and has been also related with the development of atherosclerosis [47,49], structural left ventricular remodeling [50], heart failure[52], and coronary artery disease [48]. Future and specifically designed studies should better

explore the complex relationships between liver fibrosis and CVD in different phenotypes of patients with MASLD, also considering possible individual confounders.

In conclusion, we confirmed the higher cardio-metabolic risk in patients with, than in those without SLD, pointing to the highest level of risk in patients classified as MetALD, and to a relevant gender-effect.

Thus, all patients diagnosed with MASLD and MetALD should undergo a comprehensive CVD screening, and the implementation of bidirectional screening in the routine clinical practice may be essential for the early diagnosis and prevention of both SLD and CVD, mainly in the male gender.

The well-known additional pathogenic impact of alcohol consumption on CVD development in conjunction with other cardiometabolic risk factors makes mandatory a comprehensive assessment of the individual alcohol intake and a complete withdrawal in all subjects with SLD. In this respect, the novel classification of MASLD provides more accurate insights into assessing CVD risk in patients with liver steatosis, as it takes into account both metabolic-associated pathogenesis and the additive impact of alcohol consumption on CVD risk.

Moreover, results from the present study show that MD has a protective role against liver steatosis irrespective of MASLD classification, pointing to this dietary habit as an effective strategy for managing patients with either MASLD and CVD, also in the presence of low levels of physical activity.

References

1. Portincasa, P.; Baffy, G. Metabolic dysfunction-associated steatotic liver disease: Evolution of the final terminology. *Eur J Intern Med* **2024**, *124*, 35-39, doi:10.1016/j.ejim.2024.04.013.
2. Baldini, F.; Khalil, M.; Bartolozzi, A.; Vassalli, M.; Di Ciaula, A.; Portincasa, P.; Vergani, L. Relationship between Liver Stiffness and Steatosis in Obesity Conditions: In Vivo and In Vitro Studies. *Biomolecules* **2022**, *12*, doi:10.3390/biom12050733.
3. Eslam, M.; Sanyal, A.J.; George, J.; International Consensus, P. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* **2020**, *158*, 1999-2014 e1991, doi:10.1053/j.gastro.2019.11.312.
4. Mendez-Sanchez, N.; Bugianesi, E.; Gish, R.G.; Lammert, F.; Tilg, H.; Nguyen, M.H.; Sarin, S.K.; Fabrellas, N.; Zelber-Sagi, S.; Fan, J.G.; et al. Global multi-stakeholder endorsement of the MAFLD definition. *Lancet Gastroenterol Hepatol* **2022**, *7*, 388-390, doi:10.1016/S2468-1253(22)00062-0.
5. Eslam, M.; Newsome, P.N.; Sarin, S.K.; Anstee, Q.M.; Targher, G.; Romero-Gomez, M.; Zelber-Sagi, S.; Wai-Sun Wong, V.; Dufour, J.F.; Schattenberg, J.M.; et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol* **2020**, *73*, 202-209, doi:10.1016/j.jhep.2020.03.039.
6. Younossi, Z.M.; Rinella, M.E.; Sanyal, A.J.; Harrison, S.A.; Brunt, E.M.; Goodman, Z.; Cohen, D.E.; Loomba, R. From NAFLD to MAFLD: Implications of a Premature Change in Terminology. *Hepatology* **2021**, *73*, 1194-1198, doi:10.1002/hep.31420.
7. Rinella, M.E.; Lazarus, J.V.; Ratziu, V.; Francque, S.M.; Sanyal, A.J.; Kanwal, F.; Romero, D.; Abdelmalek, M.F.; Anstee, Q.M.; Arab, J.P.; et al. A multi-society Delphi consensus statement on new fatty liver disease nomenclature. *J Hepatol* **2023**, doi:10.1016/j.jhep.2023.06.003.
8. Targher, G.; Byrne, C.D.; Lonardo, A.; Zoppini, G.; Barbui, C. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis. *J Hepatol* **2016**, *65*, 589-600, doi:10.1016/j.jhep.2016.05.013.
9. Meyersohn, N.M.; Mayrhofer, T.; Corey, K.E.; Bittner, D.O.; Staziaki, P.V.; Szilveszter, B.; Hallett, T.; Lu, M.T.; Puchner, S.B.; Simon, T.G.; et al. Association of Hepatic Steatosis With Major Adverse Cardiovascular Events, Independent of Coronary Artery Disease. *Clin Gastroenterol Hepatol* **2021**, *19*, 1480-1488 e1414, doi:10.1016/j.cgh.2020.07.030.
10. Lv, Q.; Zhao, H. The association of metabolic dysfunction-associated steatotic liver disease (MASLD) with the risk of myocardial infarction: a systematic review and meta-analysis. *Ann Med* **2024**, *56*, 2306192, doi:10.1080/07853890.2024.2306192.
11. Duell, P.B.; Welty, F.K.; Miller, M.; Chait, A.; Hammond, G.; Ahmad, Z.; Cohen, D.E.; Horton, J.D.; Pressman, G.S.; Toth, P.P.; et al. Nonalcoholic Fatty Liver Disease and Cardiovascular Risk: A Scientific Statement From the American Heart Association. *Arterioscler Thromb Vasc Biol* **2022**, *42*, e168-e185, doi:10.1161/ATV.0000000000000153.
12. Lacatusu, C.M.; Grigorescu, E.D.; Floria, M.; Onofriescu, A.; Mihai, B.M. The Mediterranean Diet: From an Environment-Driven Food Culture to an Emerging Medical Prescription. *Int J Environ Res Public Health* **2019**, *16*, doi:10.3390/ijerph16060942.
13. Leone, A.; Bertoli, S.; Bedogni, G.; Vignati, L.; Pellizzari, M.; Battezzati, A. Association between Mediterranean Diet and Fatty Liver in Women with Overweight and Obesity. *Nutrients* **2022**, *14*, doi:10.3390/nu14183771.
14. Alberti, K.G.; Eckel, R.H.; Grundy, S.M.; Zimmet, P.Z.; Cleeman, J.I.; Donato, K.A.; Fruchart, J.C.; James, W.P.; Loria, C.M.; Smith, S.C., Jr.; et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* **2009**, *120*, 1640-1645, doi:10.1161/CIRCULATIONAHA.109.192644.
15. Matthews, D.R.; Hosker, J.P.; Rudenski, A.S.; Naylor, B.A.; Treacher, D.F.; Turner, R.C. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **1985**, *28*, 412-419, doi:10.1007/bf00280883.
16. Salazar, J.; Bermúdez, V.; Calvo, M.; Olivar, L.C.; Luzardo, E.; Navarro, C.; Mencia, H.; Martínez, M.; Rivas-Ríos, J.; Wilches-Durán, S.; et al. Optimal cutoff for the evaluation of insulin resistance through triglyceride-glucose index: A cross-sectional study in a Venezuelan population. *F1000Res* **2017**, *6*, 1337, doi:10.12688/f1000research.12170.3.
17. Bedogni, G.; Bellentani, S.; Miglioli, L.; Masutti, F.; Passalacqua, M.; Castiglione, A.; Tiribelli, C. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC gastroenterology* **2006**, *6*, 33, doi:10.1186/1471-230X-6-33.
18. Lee, J.H.; Kim, D.; Kim, H.J.; Lee, C.H.; Yang, J.I.; Kim, W.; Kim, Y.J.; Yoon, J.H.; Cho, S.H.; Sung, M.W.; et al. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. *Dig Liver Dis* **2010**, *42*, 503-508, doi:10.1016/j.dld.2009.08.002.

19. Gallagher, D.; Visser, M.; Sepulveda, D.; Pierson, R.N.; Harris, T.; Heymsfield, S.B. How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? *Am J Epidemiol* **1996**, *143*, 228-239, doi:10.1093/oxfordjournals.aje.a008733.
20. Heymsfield, S.B.; Wang, Z.; Baumgartner, R.N.; Ross, R. Human body composition: advances in models and methods. *Annu Rev Nutr* **1997**, *17*, 527-558, doi:10.1146/annurev.nutr.17.1.527.
21. Sofi, F.; Dinu, M.; Pagliai, G.; Marcucci, R.; Casini, A. Validation of a literature-based adherence score to Mediterranean diet: the MEDI-LITE score. *Int J Food Sci Nutr* **2017**, *68*, 757-762, doi:10.1080/09637486.2017.1287884.
22. Abdallah, H.; Khalil, M.; Farella, I.; JohnBritto, J.S.; Lanza, E.; Santoro, S.; Garruti, G.; Portincasa, P.; Di Ciaula, A.; Bonfrate, L. Ramadan intermittent fasting reduces visceral fat and improves gastrointestinal motility. *European journal of clinical investigation* **2023**, *53*, e14029, doi:10.1111/eci.14029.
23. Rinella, M.E.; Lazarus, J.V.; Ratziu, V.; Francque, S.M.; Sanyal, A.J.; Kanwal, F.; Romero, D.; Abdelmalek, M.F.; Anstee, Q.M.; Arab, J.P.; et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *Hepatology* **2023**, *78*, 1966-1986, doi:10.1097/HEP.0000000000000520.
24. Moon, J.H.; Jeong, S.; Jang, H.; Koo, B.K.; Kim, W. Metabolic dysfunction-associated steatotic liver disease increases the risk of incident cardiovascular disease: a nationwide cohort study. *EClinicalMedicine* **2023**, *65*, 102292, doi:10.1016/j.eclinm.2023.102292.
25. Zibaenejad, F.; Mohammadi, S.S.; Sayadi, M.; Safari, F.; Zibaenezhad, M.J. Ten-year atherosclerosis cardiovascular disease (ASCVD) risk score and its components among an Iranian population: a cohort-based cross-sectional study. *BMC Cardiovasc Disord* **2022**, *22*, 162, doi:10.1186/s12872-022-02601-0.
26. Rosu, M.M.; Popa, S.G.; Mota, E.; Popa, A.; Manolache, M.; Guja, C.; Bala, C.; Mota, C.; Mota, M. Cardiovascular Risk Assessment in the Adult (Aged 40-79 Years) Romanian Population. *Acta Endocrinol (Buchar)* **2018**, *14*, 227-234, doi:10.4183/aeb.2018.227.
27. Hintze, J. *NCSS 2023 Statistical Software (2023)*. NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss, Number Cruncher Statistical System (NCSS): Kaysville, Utah, 2023.
28. Teng, M.L.; Ng, C.H.; Huang, D.Q.; Chan, K.E.; Tan, D.J.; Lim, W.H.; Yang, J.D.; Tan, E.; Muthiah, M.D. Global incidence and prevalence of nonalcoholic fatty liver disease. *Clin Mol Hepatol* **2023**, *29*, S32-S42, doi:10.3350/cmh.2022.0365.
29. White, A.M. Gender Differences in the Epidemiology of Alcohol Use and Related Harms in the United States. *Alcohol Res* **2020**, *40*, 01, doi:10.35946/arc.v40.2.01.
30. Chan, W.K.; Chuah, K.H.; Rajaram, R.B.; Lim, L.L.; Ratnasingam, J.; Vethakkan, S.R. Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD): A State-of-the-Art Review. *J Obes Metab Syndr* **2023**, *32*, 197-213, doi:10.7570/jomes23052.
31. Portincasa, P. NAFLD, MAFLD, and beyond: one or several acronyms for better comprehension and patient care. *Intern Emerg Med* **2023**, *18*, 993-1006, doi:10.1007/s11739-023-03203-0.
32. Li, A.A.; Ahmed, A.; Kim, D. Extrahepatic Manifestations of Nonalcoholic Fatty Liver Disease. *Gut Liver* **2020**, *14*, 168-178, doi:10.5009/gnl19069.
33. Fabbrini, E.; Sullivan, S.; Klein, S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology* **2010**, *51*, 679-689, doi:10.1002/hep.23280.
34. Golia, N.; Krishan, K.; Kashyap, J.R. Assessment of Obesity by Using Various Anthropometric Measurements among Patients with Coronary Heart Disease Residing in North India. *Cureus* **2020**, *12*, e7948, doi:10.7759/cureus.7948.
35. Platek, A.E.; Szymanska, A. Metabolic dysfunction-associated steatotic liver disease as a cardiovascular risk factor. *Clin Exp Hepatol* **2023**, *9*, 187-192, doi:10.5114/ceh.2023.130744.
36. Yoneda, M.; Yamamoto, T.; Honda, Y.; Imajo, K.; Ogawa, Y.; Kessoku, T.; Kobayashi, T.; Nogami, A.; Higurashi, T.; Kato, S.; et al. Risk of cardiovascular disease in patients with fatty liver disease as defined from the metabolic dysfunction associated fatty liver disease or nonalcoholic fatty liver disease point of view: a retrospective nationwide claims database study in Japan. *J Gastroenterol* **2021**, *56*, 1022-1032, doi:10.1007/s00535-021-01828-6.
37. Kuo, S.Z.; Cepin, S.; Bergstrom, J.; Siddiqi, H.; Jung, J.; Lopez, S.; Huang, D.Q.; Taub, P.; Amangurbanova, M.; Loomba, R. Clinical utility of liver fat quantification for determining cardiovascular disease risk among patients with type 2 diabetes. *Aliment Pharmacol Ther* **2023**, *58*, 585-592, doi:10.1111/apt.17637.
38. Rinella, M.E.; Lazarus, J.V.; Ratziu, V.; Francque, S.M.; Sanyal, A.J.; Kanwal, F.; Romero, D.; Abdelmalek, M.F.; Anstee, Q.M.; Arab, J.P.; et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *J Hepatol* **2023**, *79*, 1542-1556, doi:10.1016/j.jhep.2023.06.003.
39. Rinella, M.E.; Lazarus, J.V.; Ratziu, V.; Francque, S.M.; Sanyal, A.J.; Kanwal, F.; Romero, D.; Abdelmalek, M.F.; Anstee, Q.M.; Arab, J.P.; et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *Hepatology* **2023**, *78*, 1966-1986, doi:10.1097/HEP.0000000000000520.

40. Vasan, S.K.; Thomas, N.; Christopher, S.; Geethanjali, F.S.; Paul, T.V.; Sanjeevi, C.B. Anthropometric measurements for the prediction of the metabolic syndrome: a cross-sectional study on adolescents and young adults from southern india. *Heart Asia* **2011**, *3*, 2-7, doi:10.1136/ha.2009.001735.
41. Srikanthan, K.; Feyh, A.; Visweshwar, H.; Shapiro, J.I.; Sodhi, K. Systematic Review of Metabolic Syndrome Biomarkers: A Panel for Early Detection, Management, and Risk Stratification in the West Virginian Population. *Int J Med Sci* **2016**, *13*, 25-38, doi:10.7150/ijms.13800.
42. Tsermpini, E.E.; Plemenitas Iljes, A.; Dolzan, V. Alcohol-Induced Oxidative Stress and the Role of Antioxidants in Alcohol Use Disorder: A Systematic Review. *Antioxidants (Basel)* **2022**, *11*, doi:10.3390/antiox11071374.
43. Emberson, J.R.; Bennett, D.A. Effect of alcohol on risk of coronary heart disease and stroke: causality, bias, or a bit of both? *Vasc Health Risk Manag* **2006**, *2*, 239-249, doi:10.2147/vhrm.2006.2.3.239.
44. Hutchison, A.L.; Tavaglione, F.; Romeo, S.; Charlton, M. Endocrine aspects of metabolic dysfunction-associated steatotic liver disease (MASLD): Beyond insulin resistance. *J Hepatol* **2023**, *79*, 1524-1541, doi:10.1016/j.jhep.2023.08.030.
45. Israelsen, M.; Torp, N.; Johansen, S.; Hansen, C.D.; Hansen, E.D.; Thorhauge, K.; Hansen, J.K.; Villesen, I.; Bech, K.; Wernberg, C.; et al. Validation of the new nomenclature of steatotic liver disease in patients with a history of excessive alcohol intake: an analysis of data from a prospective cohort study. *Lancet Gastroenterol Hepatol* **2024**, *9*, 218-228, doi:10.1016/S2468-1253(23)00443-0.
46. Choe, H.J.; Moon, J.H.; Kim, W.; Koo, B.K.; Cho, N.H. Steatotic liver disease predicts cardiovascular disease and advanced liver fibrosis: A community-dwelling cohort study with 20-year follow-up. *Metabolism* **2024**, 155800, doi:10.1016/j.metabol.2024.155800.
47. Durak, H.; Cetin, M.; Emlek, N.; Ozyildiz, A.G.; Ergul, E.; Duman, H. Association of subclinical carotid artery atherosclerosis assessed by carotid ultrasound with nonalcoholic fatty liver disease fibrosis score in young and middle-aged men. *Int J Cardiovasc Imaging* **2024**, doi:10.1007/s10554-024-03193-w.
48. McNally, B.B.; Rangan, P.; Wijarnpreecha, K.; Fallon, M.B. Fibrosis-4 Index Score Predicts Concomitant Coronary Artery Diseases Across the Spectrum of Fatty Liver Disease. *Dig Dis Sci* **2023**, *68*, 3765-3773, doi:10.1007/s10620-023-07987-1.
49. Su, J.; Zhou, L.; Liu, J.; Wang, Y.; Wang, G. Noninvasive liver fibrosis markers are independently associated with carotid atherosclerosis risk in patients with nonalcoholic fatty liver disease. *Scand J Gastroenterol* **2024**, 1-11, doi:10.1080/00365521.2024.2364878.
50. Edin, C.; Ekstedt, M.; Karlsson, M.; Wegmann, B.; Warntjes, M.; Swahn, E.; Ostgren, C.J.; Ebberts, T.; Lundberg, P.; Carlhall, C.J. Liver fibrosis is associated with left ventricular remodeling: insight into the liver-heart axis. *Eur Radiol* **2024**, doi:10.1007/s00330-024-10798-1.
51. Dang, H.N.N.; Luong, T.V.; Tran, T.T.; Hoang, T.A. The correlation between liver fibrosis and the 10-year estimated risk of cardiovascular disease in adults with metabolic-associated fatty liver disease: A cross-sectional study in Vietnam. *Health Sci Rep* **2024**, *7*, e2102, doi:10.1002/hsr2.2102.
52. Hydes, T.J.; Kennedy, O.J.; Glyn-Owen, K.; Buchanan, R.; Parkes, J.; Cuthbertson, D.J.; Roderick, P.; Byrne, C.D. Liver Fibrosis Assessed Via Noninvasive Tests is Associated With Incident Heart Failure in a General Population Cohort. *Clin Gastroenterol Hepatol* **2024**, doi:10.1016/j.cgh.2024.03.045.

Table 1. General characteristics of 406 dysmetabolic subjects, divided according to gender.

	Total (N=406)	Males (N=224)	Females (N=182)	P value (Males vs. Females)
Age (years)	58.3±0.9	58.7±1.1	57.8±1.4	0.61
BMI, (Kg/m ²)	28.9±0.3	28.8±0.4	29.0±0.5	0.76
Obese, N (%)	165 (40.6%)	88 (39.3%)	77 (42.3%)	0.5
Overweight, N (%)	131 (32.3%)	78 (34.8%)	53 (29.1%)	0.2
Normal weight, N (%)	110 (27.1%)	58 (25.9%)	52 (28.6%)	0.5
Dyslipidemia, N (%)	337 (83.0%)	188 (83.9%)	149 (81.9%)	0.58
Hypertension, N (%)	238 (58.6%)	133 (59.4%)	105 (57.7%)	0.73
T2DM, N (%)	140 (34.5%)	76 (33.9%)	64 (35.2%)	0.80
MetS, N (%)	176 (43.3%)	89 (39.7%)	87 (47.8%)	0.10
Smokers, N (%)	108 (26.6%)	79 (35.3%)	29 (15.9%)	<0.00001
Alcohol consumers, N (%)	200 (49.3%)	127 (56.7%)	73 (40.1%)	0.0009
CVD events, N (%)	128 (31.5%)	80 (35.7%)	48 (26.4%)	0.04
SLD, N (%)	268 (66.0%)	161 (71.9%)	107 (58.8%)	0.006
Atherosclerotic plaques, N (%)	65 (16.0%)	40 (17.9%)	25 (13.7%)	0.3

Data are expressed as mean ± standard error of the mean (SEM) or number of subjects and percentage (%), as appropriate. Differences were tested by the T-test for mean and by Chi-square test for percentages.

Abbreviations: BMI, body mass index; CVD, cardiovascular diseases; MetS, metabolic syndrome; N, Number of subjects; SLD, steatotic liver disease; T2DM, type 2 diabetes mellitus.

Table 2. General characteristics of enrolled dysmetabolic subjects according to diagnosis of liver steatosis and gender.

	Steatotic Liver Disease (SLD)				Non-SLD				
	Total (N=268)	Males (N=161)	Females (N=107)	P value (Males vs. Females)	Total (N=138)	Males (N=63)	Females (N=75)	P value (Males vs. Females)	P value (Total SLD- vs Total non SLD)
Age (years)	58.6±0.8	57.2±1.2	60.8±1.4	0.05	57.7±1.8	62.5±2.4	53.6±2.7	0.02	0.6
BMI (Kg/m²)	30.6±0.3	29.9±0.4	31.7±0.6	0.01	25.7±0.4	26.2±0.7	25.2±0.5	0.2	< 0.00001
Body circumferences									
Neck (cm)	39.4±0.5	40.6±0.3	37.5±1.1	0.001	35.4±0.5	38.9±0.7	33.3±0.5	< 0.00001	< 0.00001
Abdomen (cm)	106.0±0.9	106.2±1.0	105.6±1.5	0.7	92.5±1.5	97.8±2.0	88.8±2.0	0.003	< 0.00001
Waist (cm)	100.0±0.7	101.0±1.0	98.6±1.1	0.1	87.6±1.3	92.8±1.6	83.4±1.8	0.0002	< 0.00001
Hips (cm)	106.1±0.8	104.2±1.0	108.9±1.1	0.002	101.9±1.2	103.0±2.0	101.2±1.5	0.5	0.003
Waist/Height ratio	0.60±0.005	0.6±0.01	0.6±0.01	0.06	0.52±0.01	0.54±0.01	0.5±0.01	0.04	< 0.00001
Waist/hip ratio	0.96±0.009	1.0±0.01	0.9±0.01	< 0.00001	0.85±0.01	0.9±0.01	0.8±0.02	0.00001	< 0.00001
Lifestyle									
Smokers, N (%)	67 (25.0%)	52 (32.3%)	15 (14.0%)	0.0007	41 (29.7%)	27 (42.9%)	14 (18.7%)	0.002	0.3
Alcohol consumers, N (%)	120 (44.8%)	86 (53.4%)	34 (31.8%)	0.0005	80 (57.9%)	41 (65.1%)	39 (52.0%)	0.1	0.01
MD adherence score	9.7±0.1	9.5±0.2	9.9±0.2	0.2	10.5±0.2	10.9±0.4	10.2±0.3	0.1	0.005
Physical activity, (METs)	3063±374.5	3022.2±511.9	3137.6±504.0	0.9	3115±433.2	4063.3±980.9	2551.9±351.1	0.09	0.2
Comorbidities									
Dyslipidemia, N (%)	226 (84.3%)	135 (83.9%)	91 (85.1%)	0.8	111 (80.4%)	53 (84.1%)	58 (77.3%)	0.3	0.3
Hypertension, N (%)	170 (63.4%)	97 (60.3%)	73 (68.2%)	0.2	68 (49.3%)	36 (57.1%)	32 (42.7%)	0.1	0.006
T2DM, N (%)	105 (39.2%)	61 (37.9%)	44 (41.1%)	0.6	35 (25.4%)	15 (23.8%)	20 (26.7%)	0.7	0.006
Blood parameter									
Glucose (mg/dL)	104.1± 2.0	104.8±2.5	103.0±3.3	0.7	93.7±1.8	95.5±2.5	92.1±2.6	0.4	0.0009
Insulin (micro UI/mL)	19.2± 2.5	15.9±1.8	13.4±1.2	0.3	8.0±0.5	7.6±0.8	8.4±0.6	0.4	0.0005
ALT (U/L)	32.6±1.6	35.3±2.1	28.3±2.5	0.03	18.7±0.9	20.1±1.3	19.2±1.7	0.7	0.00001
AST (U/L)	24.2±0.7	25.2±1.0	25.1±2.2	1.0	19.1±0.7	20.6±0.9	20.3±1.5	0.9	0.00004
GGT (U/L)	40.9±3.5	46.7±4.7	38.4±4.6	0.2	25.3±3.1	33.0±3.4	23.7±3.7	0.07	0.008
Triglycerides (mg/dL)	134.5±5.1	146.6±7.6	116.4±4.5	0.003	99.8±4.0	105.9±6.7	94.9±4.5	0.2	0.00001
Total cholesterol (mg/dL)	174.8±2.7	170.4±3.5	182.2±3.8	0.03	166.8±3.8	153.1±6.0	178.3±4.6	0.0009	0.14
LDL-cholesterol (mg/dL)	105.7±2.6	105.3±3.2	106.8±4.3	0.8	97.5±3.1	91.9±4.9	102.0±3.9	0.1	0.06
HDL-cholesterol (mg/dL)	46.0±0.9	42.6±1.1	51.1±1.5	0.00001	51.6±1.3	44.0±1.9	57.9±1.6	0.00001	0.00001
CVD									
CVD events, N (%)	71 (26.5%)	52 (32.3%)	19 (17.8%)	0.01	57 (41.3%)	28 (44.4%)	29 (38.7%)	0.5	0.002
Atherosclerotic plaque, N (%)	47 (17.5%)	34 (21.1%)	13 (12.1%)	0.06	18 (13.0%)	6 (9.5%)	12 (16.0%)	0.3	0.2
ASCVD score, (%)	14.9±1.0	18.4±1.4	9.8±1.1	0.00001	18.9±1.7	23.3±2.5	14.3±2.0	0.006	0.06

Data are expressed as mean ± standard error of the mean (SEM) or number of subjects and percentage (%), as appropriate. Differences between groups were tested by T-test for means and by Chi-square test for percentages.

Abbreviations: ALT, Alanine aminotransferase; ASCVD, Atherosclerotic cardiovascular disease; AST, Aspartate aminotransferase; BMI, Body Mass Index; CVD, Cardiovascular diseases; GGT, Gamma-glutamyl transferase; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; MD, Mediterranean diet; MetS, Metabolic syndrome; METs, Metabolic equivalents of tasks; N, Number of subjects; T2D, Type 2 diabetes.

Table 3. Lifestyle, body circumferences, and blood parameters in different subgroups of subjects with liver steatosis.

	CrypSLD (N=6)	MASLD (N=246)	MetALD (N=16)	P value
Lifestyle				
Alcohol (%)	1 (16.7%)*	103 (41.9%)#	16 (100%)*#	<0.00001
Smoking (%)	0 (0.0%)	62 (25.3%)	5 (31.3%)	0.3
Physical activity (METs)	6722±2668	2946±387.2	4785±1886.8	0.08
MD adherence score	9.2±0.9	9.8±0.2	8.7±0.5	0.09
Body circumferences (cm)				
Neck	34.5±1.3*#	39.5±0.5*	39.5±1.3#	0.01
Waist	78.5±3.3*#	100.6±0.7*	99.2±2.6#	0.0006
Abdomen	91.3±8.3*	106.6±0.9*	102.3±2.8	0.01
Hip	101.2±2.5	106.7±0.8	102.3±1.7	0.05
Waist/Height ratio	0.46±0.03*#	0.60±0.005*	0.59±0.02#	<0.00001
Waist/hip ratio	0.79±0.05*#	0.96±0.009*	0.97±0.03#	0.002
Blood parameter				
Glucose (mg/dL)	83.5±5.0*#	103.2±2.0*^	125.4±15.1#^	0.001
Insulin (micro UI/mL)	6.3±1.0	19.8±2.6	11.0±0.5	0.2
ALT (U/L)	16.0±3.3*#	33.2±1.8*	30.8±3.7#	0.03
AST (U/L)	17.2±1.6	24.5±0.8	23.7±2.2	0.1
GGT (U/L)	25.0±7.7	41.8±3.8	34.2±7.6	0.4
Total cholesterol (mg/dL)	165.8±8.3	176.1±2.8	159.4±12.5	0.3
HDL-cholesterol (mg/dL)	57.2±6.4	45.7±2.5	45.2±2.6	0.1
LDL-cholesterol (mg/dL)	85.2±2.9	108.2±2.5	93.0±10.0	0.09
Triglycerides (mg/dL)	99.7±14.0	136.2±5.4	122.8±17.3	0.4

Data are expressed as mean ± standard error of the mean (SEM) or number of subjects and percentage (%), as appropriate. Comparisons between groups were performed using one-way ANOVA, followed by post-hoc multiple comparisons. Similar symbols (*, #, ^) indicate statistically significant differences between groups (P<0.05).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MD, Mediterranean diet; METs, metabolic equivalents tasks; SLD, steatotic liver disease; MASLD, metabolic dysfunction-associated steatotic liver disease; MetALD, metabolic-associated alcoholic liver disease; W/H, waist-to-height ratio; W/Hip, waist-to-hip ratio

Figures

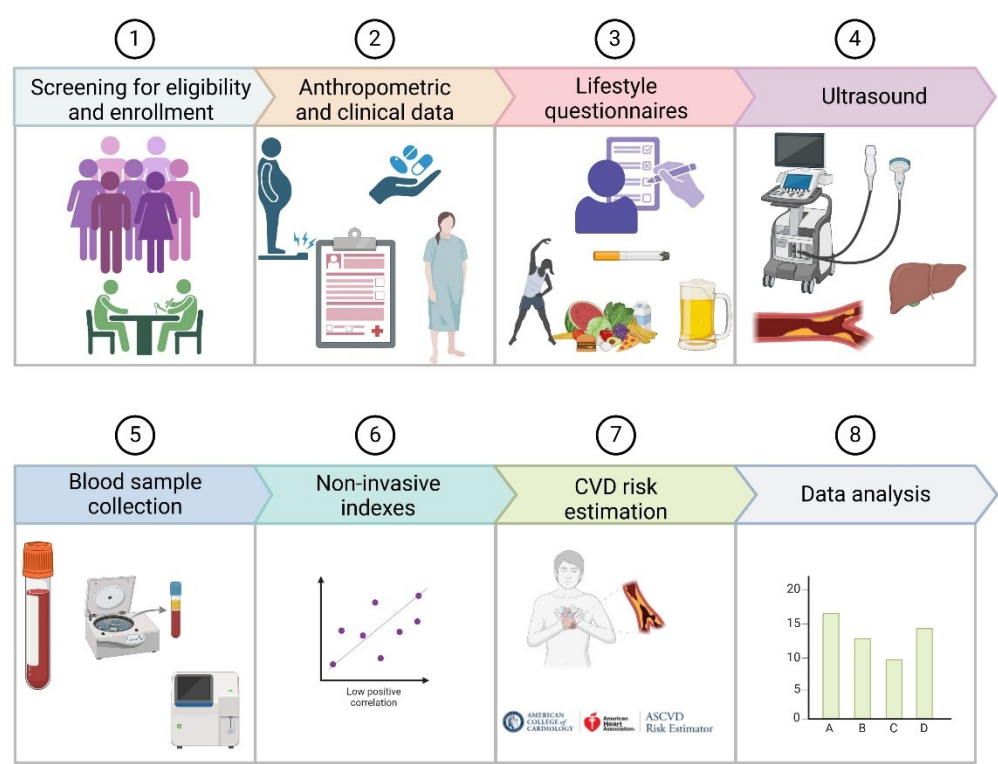


Figure 1. Study design.

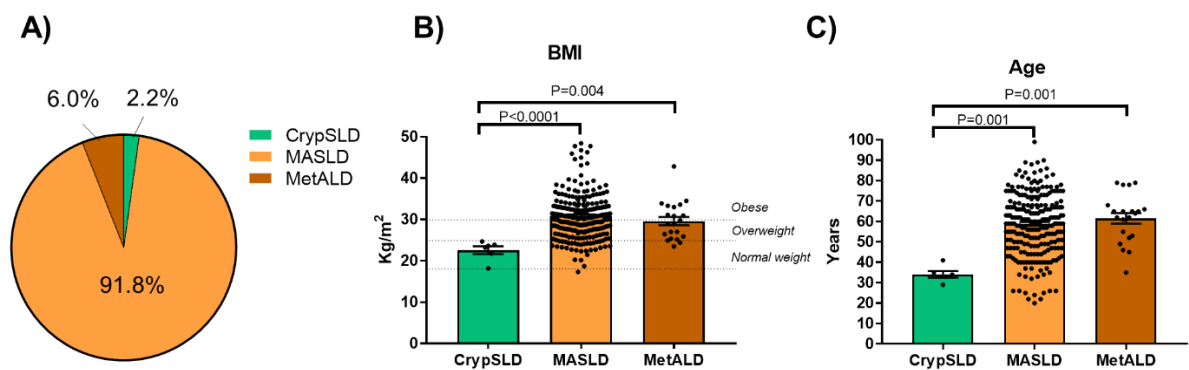


Figure 2: Prevalence (A), age (B), and BMI (C) in the SLD subgroups. Quantitative data of age and BMI are mean± SEM. Differences between subgroups were tested by ANOVA followed by multiple comparison test. Legend: CrypSLD; Cryptogenic Steatotic Liver Disease, MASLD; Metabolic dysfunction-associated Steatotic Liver Disease, and MetALD; Metabolic dysfunction-associated Alcoholic Liver Disease.

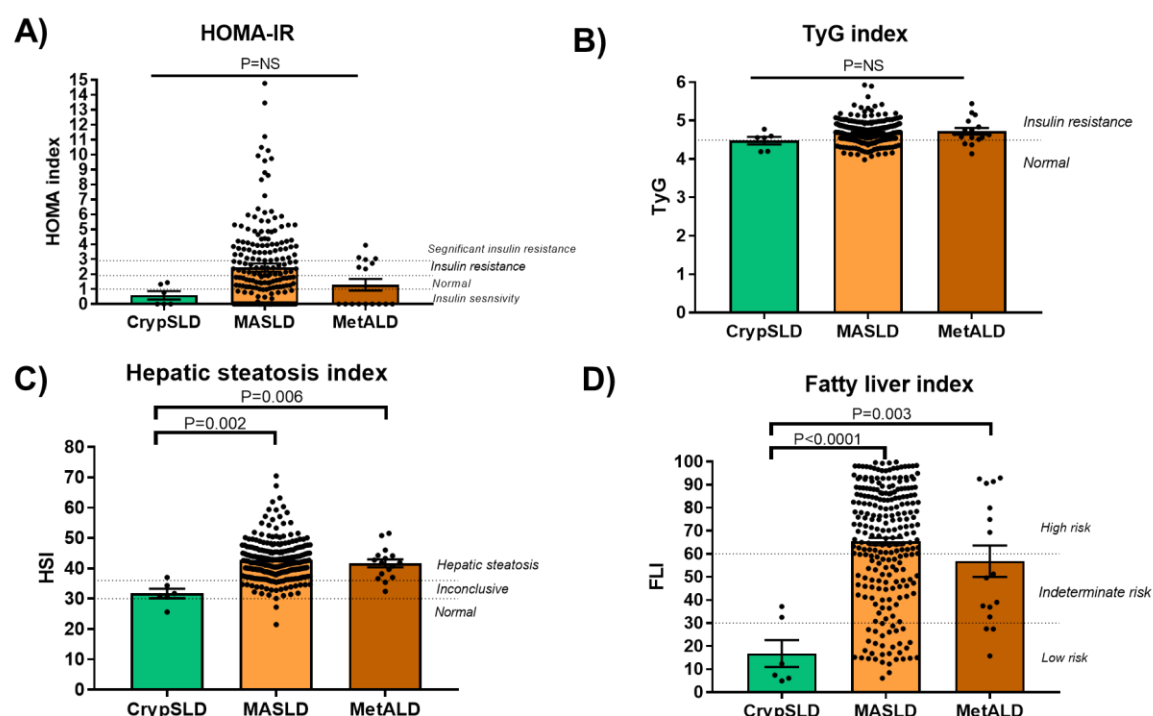


Figure 3

A: HOMA Index: The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was used to evaluate insulin sensitivity. An HOMA-IR score <1 indicates optimal insulin sensitivity, a score between 1 and 1.9 is considered normal, while a score between 2 and 2.9 suggests early insulin resistance. A score > 2.9 indicates significant insulin resistance.

B: TyG Index: The Triglyceride-Glucose Index (TyG) was used as an alternative marker for insulin resistance and metabolic risk. Calculated as $[\text{fasting triglycerides (mg/dL)} \times \text{fasting glucose (mg/dL)}] / 2$, a TyG index of ≥ 4.5 is typically considered indicative of insulin resistance and metabolic risk.

C: Hepatic Steatosis Index (HSI): The Hepatic Steatosis Index (HSI) is a diagnostic tool used to predict the presence of hepatic steatosis. The HSI score is calculated based on BMI, waist-to-hip ratio, and the presence of type 2 diabetes. A score <30 is normal, between 30 and 36 is inconclusive, and ≥ 36 is generally used to diagnose hepatic steatosis.

D: Fatty Liver Index (FLI): The Fatty Liver Index (FLI) is used to assess the likelihood of fatty liver disease. FLI scores is based on BMI, waist circumference, triglycerides, and GGT levels. An FLI score < 30 suggests that fatty liver should be ruled out. FLI scores between 30 and 60 remain inconclusive, while scores ≥ 60 indicate the presence of fatty liver.

Data are presented as mean $\pm$ standard error of the mean (SEM) for the three groups: Comparisons between groups were performed using one-way ANOVA, followed by post-hoc multiple comparisons.

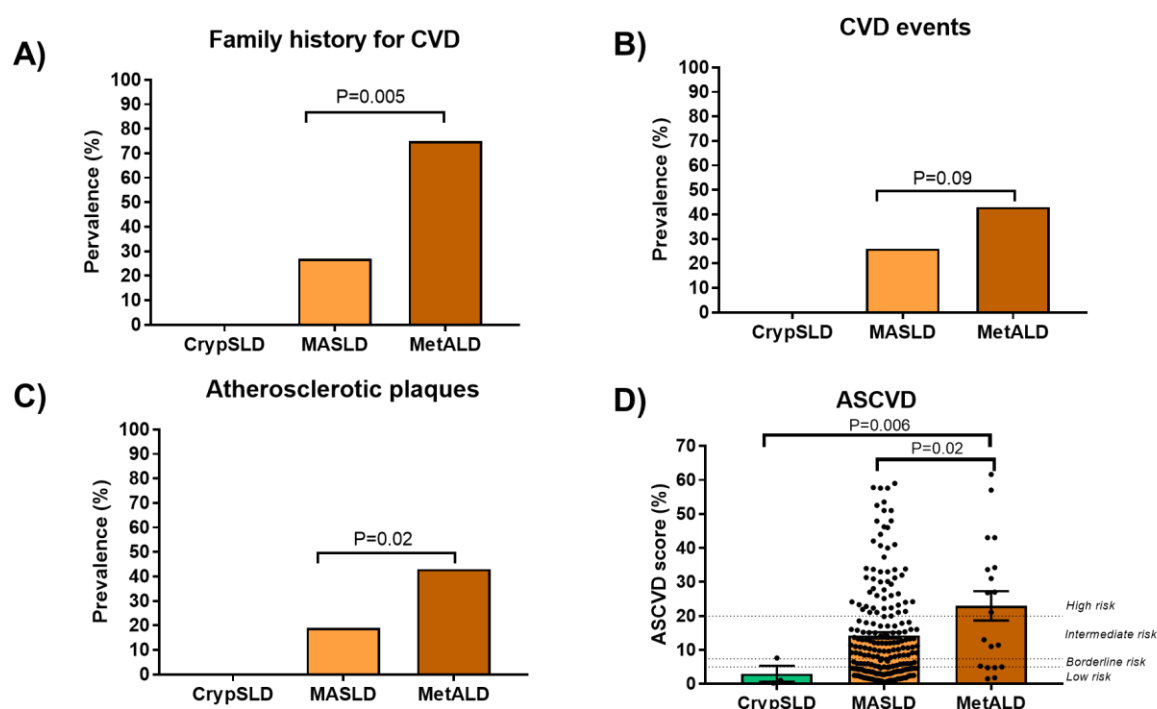


Figure 4: CVD aspects among CrypSLD, MASLD, and MetALD groups. **A)** family history of CVD, **B)** previous CVD events, **D)** presence of atherosclerotic plaques, and **D)** ASCVD score calculated for participants aged 40-79 years without previous CVD events.

11. Conclusion

In conclusion, Ramadan fasting, as a form of intermittent fasting, has beneficial effects on fat distribution, metabolic profile, and gastrointestinal motility. Further research is needed to explore its long-term metabolic and clinical implications. Intermittent fasting, including Ramadan fasting, can reduce caloric intake, body weight, and visceral fat, with Ramadan fasting showing more pronounced benefits, particularly for liver steatosis. Comprehensive cardiovascular screening is crucial for patients with steatotic liver disease (SLD), especially those with metabolic alcohol-associated liver disease (MetALD), due to their higher cardio-metabolic risk. The Mediterranean diet is protective against liver steatosis and beneficial for managing both MASLD and cardiovascular disease, even with low physical activity. Assessing and eliminating alcohol intake is essential for reducing cardiovascular risk in these patients.

12. Paper

1. **Distinct biophysiological effects of Ramadan fasting and traditional intermittent fasting on markers of body fat storage. A real-life study.**

Khalil M, **Abdallah H**, Jaber N, Garruti G, Di Ciaula A, Portincasa P.

2. **Early and accurate diagnosis of steatotic liver by artificial intelligence (AI)-supported ultrasonography.**

Santoro S, Khalil M, **Abdallah H**, Farella I, Noto A, Dipalo GM, Villani P, Bonfrate L, Di Ciaula A, Portincasa P.

3. **Comprehensive Strategies for Metabolic Syndrome: How Nutrition, Dietary Polyphenols, Physical Activity, and Lifestyle Modifications Address Diabetes, Cardiovascular Diseases, and Neurodegenerative Conditions.**

Martemucci G, Khalil M, Di Luca A, **Abdallah H**, D'Alessandro AG.

4. **Ramadan intermittent fasting reduces visceral fat and improves gastrointestinal motility.**

Abdallah H, Khalil M, Farella I, John Britto JS, Lanza E, Santoro S, Garruti G, Portincasa P, Di Ciaula A, Bonfrate L.

5. **Self-reported symptoms after COVID-19 vaccination. Distinct sex, age, and geographical outcomes in Lebanese and Italian cohorts.**

Khalil M, Bonfrate L, Di Ciaula A, Portincasa P; **Hala Abdallah**, Michela Capurso, Ilaria Galerati, Soukayna Hayek, Hala Khalifeh, Edoardo Mastandrea, Antonino Noto

6. **The Potential of the Mediterranean Diet to Improve Mitochondrial Function in Experimental Models of Obesity and Metabolic Syndrome.**

Khalil M, Shanmugam H, **Abdallah H**, John Britto JS, Galerati I, Gómez-Ambrosi J, Frühbeck G, Portincasa P.

7. **Herbal medicine in three different Mediterranean living areas during a COVID-19 pandemic: The role of polyphenolic-rich thyme-like plants**

Mohamad Khalil *, **Hala Abdallah**, Maria Calasso, Nour Khalil, Ahmad Daher, Jihen Missaoui, Farah Diab, Lama Zeaiter, Laura Vergani, Agostino Di

8. The Impact of Za'atar Antioxidant Compounds on the Gut Microbiota and Gastrointestinal Disorders: Insights for Future Clinical Applications

Mohamad Khalil^{1 2}, Hala Abdallah¹, Danute Razuka-Ebela³, Maria Calasso², Maria De Angelis², Piero Portincasa¹

9. Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD): Assessing Metabolic Dysfunction, Cardiovascular Risk Factors, and Lifestyle habits

Hala Abdallah^{1, *}, Mohamad Khalil^{1, *}, Ewa Awada¹, Elisa Lanza¹, Agostino Di Ciaula¹, Piero Portincasa^{1,2}

¹Clinica Medica “A. Murri” and Department of Precision and Regenerative Medicine and Ionian Area (DiMePre-J), University of Bari "Aldo Moro", Bari, Italy.

²General Secretariat, Consortium of Mediterranean Universities.