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Efficacy of a Brown Algae–Based Nutraceutical Combined with the Mediterranean Diet on Metabolic Dysfunction in Hepatic Steatosis: The Gdue® Study

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1. Introduction

1. Definition and nomenclature of MASLD

Metabolic dysfunction–associated steatotic liver disease (MASLD) is the current umbrella term encompassing a spectrum of liver conditions characterized by excessive hepatic fat accumulation in the presence of metabolic dysfunction. This condition includes metabolic dysfunction–associated steatotic liver (MASL), corresponding to isolated steatosis in the absence of significant inflammation; metabolic dysfunction–associated steatohepatitis (MASH), defined by the presence of lobular inflammation and hepatocellular ballooning; and progressive disease stages that may evolve toward liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC)(1, 2).

In particular, MASLD is also described as the "hepatic manifestation of metabolic syndrome (MS)"(3), as its diagnosis requires the presence of hepatic steatosis—typically identified by abdominal ultrasonography—in combination with metabolic abnormalities classically included in MS definitions, in individuals in whom significant alcohol consumption has been excluded (4).

Historically, in 1980 Ludwig and colleagues introduced the term *non-alcoholic steatohepatitis* (NASH) to describe a severe form of fatty liver disease observed predominantly in overweight individuals who denied alcohol intake, thereby highlighting an apparently "unknown" etiology (5). Subsequently, the broader term *non-alcoholic fatty liver disease* (NAFLD) was adopted to describe hepatic steatosis affecting more than 5% of hepatocytes in the absence of significant alcohol consumption—defined as <20 g/day for women and <30 g/day for men—and other secondary causes (6).

However, the NAFLD terminology has progressively been considered inadequate, as it defines the disease by exclusion rather than by its underlying metabolic drivers. Given the strong

association between hepatic steatosis and metabolic conditions such as obesity, type 2 diabetes mellitus (T2DM), and dyslipidemia, a shift toward the term *metabolic dysfunction–associated fatty liver disease* (MAFLD) was proposed to emphasize the central role of metabolic dysfunction (7). This change was formalized through an international expert consensus led by Eslam et al.(7), with diagnostic criteria requiring hepatic steatosis in combination with overweight/obesity (BMI ≥25 kg/m²), T2DM, or at least two additional metabolic abnormalities to better describe the involvement of metabolic status in this condition (6).

Despite its conceptual advances, the MAFLD definition was later reconsidered , as it was also deemed insufficient to fully capture the complexity of the disorder. In particular, the scientific community sought to further elucidate the underlying pathophysiological processes by adopting a unified diagnostic framework aimed at facilitating disease recognition and minimizing the risk of misclassification of the affected population. The new nomenclature proposed through a multisocietal Delphi consensus process—engaging more than 200 participants from over 50 countries, including representatives of scientific societies and patient advocacy organizations—also seeks to promote a potentially less stigmatizing diagnosis, given that the term “fatty” has repeatedly been perceived as discriminatory (4).

As claimed by the latest joint guidelines issued by the European Association for the Study of the Liver (EASL), the European Association for the Study of Diabetes (EASD) and the European Association for the Study of Obesity (EASO)(8), MASLD is now the term universally recognized by scientific community to describe the presence of excess hepatic triglyceride storage in addition with at least one cardiometabolic risk factor. MASLD is insert into the newly established consensus definition of steatotic liver disease (SLD).

2. Epidemiological data

According to a recent systematic review and meta-analysis of 92 population-based studies, MASLD is nowadays recognized as the most frequent liver disease worldwide, with its prevalence affecting more than one-third of the general adult population, including about 80%

of people with obesity and 70% of those with type 2 diabetes (9). The trend analysis showed a 50% increase in prevalence in the period from 2016–2019 compared to 1990-2006. Findings also confirm that the prevalence of MASLD may vary by ethnicity and it is higher in Latin American (44%) than in Western Europe (25%) (10). Specifically, a previous meta-analysis of 34 included studies shows that the prevalence of MASLD in USA vary from 22.9% in Hispanic people, of 14.4% in white people and 13.0% in black people. However, outside USA, data appear to be very limited (11).

Regarding the incidence of MASLD, a meta-analysis covering the period from 2000 to 2016 (12) reported an overall incidence of 4,613 new cases per 100,000 person-years. Specifically, the analysis showed that between 2000 and 2015 the incidence of MASLD in Asia increased substantially, rising from approximately 2,000 to nearly 7,000 cases per 100,000 person-years. Among individuals with overweight or obesity, the incidence of MASLD was almost threefold higher compared with individuals with normal weight, reaching 8,417 versus 3,358 cases per 100,000 person-years, respectively.

Consistently, data from the Global Burden of Disease Study(13), conducted between 2010 and 2021 across more than 200 countries, highlighted a progressive increase in both prevalence and annual incidence of MASLD. The estimated annual incidence was 608 new cases per 100,000 population (95% uncertainty interval: 599–618), with a higher prevalence observed in men compared with women (15,731 vs. 14,310 per 100,000 population). The highest point of occurrence in men is at 45-49 years of age while for women is at 50-54 years. Another American study confirmed the upward trend in the incidence of MASLD. Between 1997 and 2014, the annual incidence rose from 62 to 329 cases per 100,000 inhabitants (14).

Based on this evidence, the scientific community agrees that the incidence of MASLD has increased over time. This trend is partially explained by the rising prevalence of metabolic comorbidities as well, such as obesity and type 2 diabetes mellitus (T2DM) (15, 16).

Consequently, it has been estimated that, driven by these conditions, the prevalence of MASLD will exceed 55% by 2040 (17).

3. Pathophysiology of MASLD

Insulin resistance, overweight, genetic predisposition, and alterations in the gut microbiota—arising from both environmental and genetic determinants—play a central role in the development and progression of MASLD. Consequently, therapeutic strategies aimed at addressing major metabolic comorbidities, including appropriate pharmacological interventions, may contribute to limiting the progression and overall burden of MASLD (18).

However, despite significant advances in research, the pathogenesis of MASLD remains incompletely understood. The disease is thought to result from multiple interrelated mechanisms, in which excessive dietary energy intake, adipocyte dysfunction, and insulin resistance—promoting increased free fatty acid release and enhanced de novo hepatic lipogenesis—play pivotal roles (3). These processes lead to an imbalance between lipid synthesis, influx, oxidation, and export, ultimately resulting in triglyceride accumulation within hepatocytes. In physiological conditions, lipolysis is suppressed by insulin, but in insuline resistance conditions, which is really common in patients with MASLD, this regulation is impaired, resulting in excessive free fat acids accumulation in liver (19). This intracellular lipid overload induces cellular injury and apoptosis, thereby triggering hepatic inflammation and fibrogenesis, contributing potentially to the several form of MASLD, i.e. MASH (20).

Several dietary factors, tipically belonging to Western diet, including diets high in sugars—particularly glucose and fructose—or rich in fats, can contribute to low-grade inflammation in the liver and other organs (21). Adipose tissue further amplifies inflammatory responses through the production of pro-inflammatory cytokines and the recruitment and activation of macrophages, thereby promoting the progression of MASLD (21, 22).

High fructose levels are also associated with progression to MASH, increasing intestinal permeability, activating a cascade of inflammatory cytokines and causing microbiota dysbiosis (23). This altered mechanism is supported by increased expression of hepatic toll-like receptors

(TLRs) and alterations in bile acid metabolism, exacerbating inflammation and tissue damage for new therapies (24).

4. Clinical Presentation and Diagnostic criteria of MASLD

Since MASLD is considered the hepatic manifestation of metabolic syndrome (MS), its diagnosis integrates evidence of hepatic steatosis—most commonly detected by abdominal ultrasonography—with the presence of at least one of the metabolic criteria defining MS, including central obesity (waist circumference WC ≥102 cm in men and ≥88 cm in women, or ethnicity-specific cut-offs), arterial hypertension (systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg, or current antihypertensive treatment), hypertriglyceridemia (fasting triglycerides ≥150 mg/dL or lipid-lowering therapy), impaired glycemic control (fasting plasma glucose >100 mg/dL or previously diagnosed type 2 diabetes mellitus), or reduced high-density lipoprotein cholesterol (HDL-C) levels (<40 mg/dL in men and <50 mg/dL in women), in individuals without significant alcohol consumption (<2 standard drinks/day for women and <3 standard drinks/day for men) (3).

In the absence of cirrhosis, patients with MASLD are usually asymptomatic or may report mild, nonspecific symptoms such as fatigue, malaise, or vague abdominal discomfort, particularly in the right upper quadrant (25). Consequently, hepatic steatosis is frequently diagnosed incidentally during abdominal imaging performed for unrelated reasons or following further investigations prompted by mild to moderate elevations in serum aminotransferase levels, especially alanine aminotransferase (ALT) (26). However, ALT levels do not reliably correlate with disease severity, as up to two-thirds of patients—including those with advanced fibrosis or cirrhosis—may exhibit normal aminotransferase values (27). Mild to moderate hepatomegaly is observed in approximately 50% of patients with MASLD, whereas splenomegaly is relatively uncommon, occurring in about 5% of cases (26). Epidemiological data indicate that nearly 80% of individuals with MASLD have a body mass index (BMI) greater than 25 kg/m^2; approximately 70% present with atherogenic dyslipidemia, characterized

by low HDL-C levels and/or elevated plasma triglycerides; around 60% have prediabetes or overt type 2 diabetes mellitus; and nearly half are affected by arterial hypertension (28).

Moderate-to-severe hepatic steatosis is commonly detected using conventional abdominal ultrasonography, which demonstrates a sensitivity ranging from 80% to 89% and a specificity between 87% and 90% (26, 29-31). In contrast, the detection of mild steatosis—defined as hepatic fat content below 20%—remains challenging with ultrasonography due to its limited sensitivity, which is reported to be below 50% (32). These limitations overdescripte could be overcome by the use of the controlled attenuation parameter (CAP) feature. American Association for the Study of Liver Diseases (AASLD) recommends the use of the CAP to assess and quantify the severity of hepatic steatosis, as CAP shows greater sensitivity for detecting mild steatosis compared with conventional ultrasonography (33). According to current guideline recommendations (8), CAP assessment is a non-invasive feature, typically performed in concert with vibration-controlled transient elastography, which evaluates liver stiffness by measuring the propagation speed of ultrasound waves through hepatic tissue. CAP values are expressed in decibels per meter (dB/m), and hepatic steatosis is generally identified at values exceeding 248 dB/m, with increasing values reflecting greater histological severity of steatosis. Based on established cut-off thresholds (8), steatosis is classified as absent (S0) at CAP values ≤248 dB/m, mild (S1) between 248 and 268 dB/m, moderate (S2) between 268 and 280 dB/m, and severe (S3) at values >280 dB/m. Furthermore, CAP is considered a fast, validated and clinically useful tool, as it enables point-of-care assessment of hepatic steatosis, including mild forms (34).

However, beyond its metabolic and hepatic implications, MASLD is increasingly recognized as a condition associated with a substantial burden on patients' quality of life and overall health status (35). Individuals with MASLD not only exhibit a broader spectrum of comorbidities compared with those without the disease, but also experience significant impairments in both physical and mental well-being (36). According to the AASLD practice guidance, MASLD is frequently accompanied by multiple extrahepatic conditions that contribute to disease burden and negatively affect daily functioning (6). Consistently, a study conducted by the United States Nonalcoholic Steatohepatitis Clinical Research Network demonstrated that 713 patients with

MASLD reported significantly lower physical and mental health scores compared with population in general (37). These findings are further supported by evidence indicating that individuals with MASLD are at increased risk of developing mental health disorders, a vulnerability largely attributed to disease-related stigma and obesity. Such aspects underscore the importance of a holistic, patient-centered approach that healthcare professionals should consider in the clinical management of MASLD (6).

5. Treatment of MASLD : the evidences for therapeutic approach

Variability in the clinical presentation and natural history of MASLD is strongly driven by the convergence of genetic susceptibility, epigenetic regulation, and environmental exposures. The ultimate manifestation of the disease therefore arises from the combined and interactive effects of these determinants, which collectively shape disease trajectory (1). As a result, optimal management of MASLD will depend on tailored therapeutic strategies and, more importantly, on integrated multidisciplinary care models that address the complexity and synergistic influence of these contributing factors (2, 38).

Despite ongoing advances in scientific research aimed at elucidating the pathophysiological mechanisms underlying MASLD, the therapeutic strategies currently supported by strong expert consensus and guideline recommendations remain predominantly focused on lifestyle modification (39). In particular, the modern Western lifestyle—characterized by excessive caloric intake, high consumption of refined carbohydrates, and physical inactivity—plays a central role in the development of metabolic disorders such as obesity, type 2 diabetes mellitus, MS and MASLD (40). These conditions share common pathophysiological features, including chronic low-grade inflammation and impaired glucose metabolism (39). Within this metabolic framework, behavioral interventions promoting dietary balance, alcohol avoidance and increased physical activity are widely recognized as effective first-line strategies, exerting pleiotropic benefits across multiple metabolic pathways (20).

6. Diet therapy

In the management of MASLD, caloric restriction—typically involving a daily energy deficit of 500–1000 kcal—combined with regular physical activity represents the cornerstone of treatment for the improvement of hepatic steatosis (41). In clinical practice, daily energy intakes of approximately 1200 kcal/day for women and 1500 kcal/day for men have been shown to be effective in significantly reducing hepatic lipid content, decreasing visceral adipose tissue, and promoting histological improvement (42, 43). However, dietary interventions must be individualized, and caloric targets should be adjusted according to the patient's clinical condition, metabolic profile, and energy expenditure (41).

Current clinical guidelines on MASLD treatment (44) indicate that a weight loss of approximately 5% of total body weight is associated with a reduction in hepatic fat accumulation, whereas a weight loss of 7–10% leads to significant improvements in hepatocellular ballooning, hepatic steatosis, and normalization of biochemical parameters. Sustained weight loss exceeding 10% may confer additional benefits, including improvement in hepatic fibrosis.

Beyond caloric restriction alone, the qualitative composition of the diet plays a critical role in the development and progression of metabolic disturbances and, consequently, MASLD. The Western dietary pattern represents a paradigmatic example of an unfavorable nutritional model, being characterized by a high intake of foods rich in simple sugars, which may account for more than 15% of total daily energy intake. This pattern is particularly enriched in fructose, mainly consumed as high-fructose corn syrup and sucrose, commonly ingested as table sugar (45).

Fructose is rapidly absorbed and preferentially taken up by the liver, where it bypasses key regulatory steps of carbohydrate metabolism and strongly stimulates de novo lipogenesis (46). At the cellular level, excessive fructose metabolism is associated with a marked reduction in intracellular phosphate and ATP availability, leading to an energy-depleted state that impairs protein synthesis and promotes oxidative damage alongside mitochondrial dysfunction (45)

(47). Unlike other carbohydrates, fructose phosphorylation proceeds largely unchecked by feedback inhibition, resulting in extensive hepatic phosphorylation and channeling of substrates toward lipogenic pathways (48).

In contrast to Western dietary habits, a growing body of studies have evaluated the Mediterranean model as the most suitable for the treatment of MASLD, as the Mediterranean diet (MD). MD is recognized as the first-line dietary choice also for cardiovascular diseases and other metabolic disorders, such as type 2 diabetes mellitus, all risk factors closely related to MASLD (49-51).

Specifically in MASLD, adherence to the MD has been associated with reductions in key markers of liver injury, including AST and GGT levels, as well as with decreased hepatic fat accumulation (52).

The MD promotes the consumption of minimally processed food, where food sources such as whole grains, legumes, nuts, fish or extra virgin oil are favored, and, on the other hand, transformed meat or sweetened and packaged drinks are minimized (27).

From a nutritional standpoint, the MD represents a balanced dietary pattern, as its macronutrient distribution aligns with established nutritional recommendations. Prospective observational studies further indicate that high-quality dietary patterns, such as the MD, are associated with a lower prevalence of MASLD (53, 54), whereas unhealthy dietary behaviors rich in processed foods and sugar-sweetened beverages appear to promote disease development in a dose-dependent manner mechanism (55). Accordingly, the most recent EASL–EASD–EASO clinical practice guidelines confirm the MD as the dietary approach of choice for the treatment of non-alcoholic metabolic and liver disorders (8).

Other dietary patterns have also been investigated as potential strategies for the management of MASLD, particularly with respect to the reduction of hepatic steatosis and associated biochemical abnormalities. Among these, low-carbohydrate diets have received increasing attention (56).
In low-carbohydrate dietary regimens, the intake of starch and rapidly absorbable carbohydrates

is markedly reduced. The proposed therapeutic rationale is grounded in specific metabolic mechanisms. High carbohydrate consumption stimulates insulin secretion and increases circulating insulin-like growth factor-1 (IGF-1) levels, which may promote inflammatory signaling pathways implicated in the progression of hepatic steatosis. Persistent activation of these pathways has also been linked to the stimulation of pro-tumorigenic mechanisms, including those involved in hepatocarcinogenesis (57, 58). Nevertheless, the comparative effectiveness of low-carbohydrate diets versus other dietary approaches in MASLD remains a matter of debate.

A recent meta-analysis showed no significant differences between low-carbohydrate and low-fat diets in reducing hepatic fat content or improving liver enzyme profiles in patients with NAFLD (56). Notably, ten of the eleven studies included reported significant weight loss in both dietary groups, suggesting that overall caloric restriction and weight reduction—rather than macronutrient distribution per se—may represent the primary drivers of hepatic improvement. Earlier meta-analyses similarly evaluated low-carbohydrate diets mainly through single-arm designs, limiting direct comparisons with alternative dietary patterns and precluding definitive conclusions regarding their superiority (59).

7. Healthy life-style with physical activity

According to the available literature, lifestyle and behavioral modifications fully comply with first-line therapeutic recommendations for MASLD, owing to their broad and well-documented beneficial metabolic effects (60). Among non-pharmacological strategies, regular physical activity plays a pivotal and independent role.

Engagement in structured exercise programs has been associated with a reduction in systemic inflammation and oxidative stress, together with measurable improvements in liver biochemical parameters (61). In addition, physical activity contributes to increased energy expenditure, thereby facilitating the achievement of a sustained negative energy balance. When combined

with dietary interventions, this mechanism supports weight loss and improves overall metabolic control (43). International guidelines emphasize the importance of both increasing physical activity levels and limiting sedentary behavior, as prolonged inactivity has been linked to MASLD progression through a dose-dependent relationship (62). To this end, recommendations suggest a minimum of 150 minutes per week of moderate-intensity aerobic exercise, or alternatively 75–150 minutes per week of vigorous-intensity activity, as an effective threshold to induce metabolic and hepatic benefits (8). Importantly, accumulating evidence indicates that physical exercise may reduce hepatic fat content even in the absence of substantial weight loss or strict dietary caloric restriction, highlighting a direct effect on intrahepatic lipid metabolism (63). Nevertheless, current data regarding the potential antifibrotic effects of physical activity remain limited, and further studies are required to clarify its impact on fibrosis regression (64).

To enhance long-term adherence to lifestyle interventions, behavioral support strategies have been increasingly integrated into clinical care. Techniques such as motivational interviewing and cognitive behavioral therapy have demonstrated efficacy in facilitating sustained behavioral change, improving treatment compliance, and supporting increases in physical activity levels (65, 66).

Finally, a comprehensive lifestyle-based approach to MASLD management should also address additional modifiable risk factors. Smoking cessation and the limitation or avoidance of alcohol consumption are strongly encouraged, given their adverse effects on metabolic health and their contribution to liver disease progression(27).

8. Nutraceutical approach as a therapeutic option

At present, no approved pharmacological therapy is specifically available for the treatment of MASLD. Given the increasing prevalence and clinical burden of this condition, there is a growing need for safe and effective strategies to mitigate disease progression. Alongside lifestyle interventions, recent research has increasingly focused on nutraceutical approaches with documented beneficial effects on metabolic disorders such as type 2 diabetes and obesity,

which may represent a complementary, non-pharmacological strategy in the management of MASLD (67).

In this context, accumulating evidence supports the use of nutraceutical products containing bioactive molecules, like polyphenols, a broad class of phytochemicals characterized by phenolic structures, and a kind of polysaccharides called fucoidans, for the improvement of metabolic alterations including insulin resistance and hepatic steatosis as well (68, 69).

Meta-analytical data derived from twenty-seven randomized controlled trials (70) indicate that polyphenol-based nutraceuticals are associated with improvements in key MASLD-related parameters, such as serum transaminase levels, glycemic control, and inflammatory markers.

The beneficial effects of polyphenols in MASLD appear to be mediated by the modulation of key regulatory pathways involved in hepatic lipid metabolism (71). In particular, these compounds have been shown to influence cellular energy sensing mechanisms, notably through the activation of AMPK, which in turn downregulates lipogenic transcription factors such as SREBP-1c and limits fatty acid synthesis (72). Collectively, these molecular events contribute to the attenuation of hepatic lipid accumulation (71). In addition, Evidence suggests that the hepatoprotective properties of polyphenols involve the attenuation of pro-inflammatory signaling cascades. By downregulating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-Kb) activation, these compounds may limit oxidative stress and inflammatory damage, thereby mitigating mechanisms implicated in MASLD progression (73).

In addition to polyphenols, increasing attention has been directed toward sulfated polysaccharides, such as fucoidans, rich in fucose composed of 44% fucose and 26% sulphate, found in various marine plant species which have been investigated for a wide range of biological activities, including antioxidant, anti-inflammatory, antidiabetic, and lipid-lowering effects (74). Intestinal absorption of fucoidans has been scientifically proven. Nagamine et al. also found significant absorption at the level of liver cells, which was then confirmed in experimental in vivo models of rats with NAFLD fed fucoidans. Rats fed a high-fat diet are a widely used experimental model for studying NAFLD in humans, as they readily show

alterations in lipid metabolism, transaminase function, inflammation and, of course, intrahepatic lipid accumulation (75). In this context, fucoidans appear to act as a functional tool in the treatment of NAFLD and related complications, reducing the degree of inflammation and oxidative stress while improving lipid metabolism (76). In particular, fucoidans, administered for 12 weeks at a dose of 100 mg/kg to rats fed a high-fat diet, led to an improvement in BMI, the amount of fat accumulated in the liver, transaminases, lipid profile (total cholesterol and triglycerides) and blood glucose compared to the control group treated with the high-fat diet alone (76). There was also a reduction in highly reactive aldehydes produced as a result of lipid peroxidation, which act as amplifiers of oxidative stress, such as malondialdehyde, but also nitric oxide; at the same time, there was an increase in glutathione, an endogenous antioxidant molecule. Concomitantly, a downregulation of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), has been consistently reported (76).

Additional mechanistic insights have been provided by studies investigating gene expression related to lipid metabolism. In the study by Yokota et al (77), supplementation with fucoidans in mice with apolipoprotein E deficiency and fed a high-fat diet led to an improvement not only in anthropometry but also in blood lipid levels, particularly total cholesterol, non-HDL cholesterol, and triglycerides, as well as glucose, compared to the control group that did not receive supplementation. In addition, HDL-C and plasma lipoprotein lipase activity increased considerably. These effects were associated with the downregulation of genes involved in hepatic lipogenesis, such as sterol regulatory element-binding protein-1 (SREBP-1), and cytochrome P450 enzymes (CYP7A1 and CYP8B1), alongside the upregulation of pathways promoting fatty acid transport and oxidation, including peroxisome proliferator–activated receptor-α (PPARα) and fatty acid-binding protein (77). Collectively, these findings suggest that fucoidans may exert a multifaceted protective role in MASLD by simultaneously modulating lipid metabolism, reducing hepatic inflammation, and attenuating oxidative stress (76). Such properties support their inclusion within nutraceutical formulations aimed at complementing dietary and lifestyle interventions in metabolic liver disease.

Among the most extensively investigated natural sources of both polyphenols and sulphated polysaccharides, marine algae have gained increasing attention due to their richness in bioactive secondary metabolites with recognized pharmacological potential (68, 78).

Within the international research landscape, brown algae in particular have been extensively studied, with *Ascophyllum nodosum* and *Fucus vesiculosus* representing well-characterized species rich in a unique class of polyphenolic compounds known as phlorotannins and fucoidans (79, 80). These compounds have been associated with a broad spectrum of biological activities, including antioxidant, anti-inflammatory, antibacterial and enzyme-inhibitory properties (74, 81-84) .

The role of a nutraceutical formulation containing polyphenols and fucoidans derived from brown seaweed will be discussed in detail in the following chapter.

9. GDUE®: a nutraceutical based on brown algae and chromium picolinate

Gdue® is a nutraceutical composition formed by two brown seaweeds, Ascophyllum nodosum and Fucus vesiculosus in ration 95/5, enriched with picholinate chromium. Specifically, each capsule of Gdue® (466 mg) contains 237.5 mg of Ascophyllum nodosum, 12.5 mg of Fucus vesiculosus, and 7.5 µg of picolinate chromium. Recent evidence suggests that the combined use of Ascophyllum nodosum and Fucus vesiculosus may exert beneficial effects on metabolic dysfunctions. These effects are mainly linked to the presence of biologically active compounds in the extracts, including polyphenols such as bromophenols, phlorotannins and sulfated polysaccharides like fucoidans (85).

Preclinical and clinical evidence suggests that these seaweed-derived extracts are able to inhibit the activity of intestinal α-glucosidase and α-amylase, resulting in a delayed digestion and absorption of carbohydrates and then contributing to the regulation of postprandial blood

glucose levels. In addition, these extracts may affect intestinal cholesterol absorption by increasing luminal viscosity, thereby enhancing the fecal excretion of dietary cholesterol (86).

Carbohydrate digestion occurs through a sequence of enzymatic reactions, that allow starch to be metabolized and absorbed. α-Amylase, released by the salivary glands and pancreas, initiates this process by hydrolyzing complex polysaccharides—such as starch, amylopectin, glycogen, and amylose—into smaller carbohydrate fragments. The final digestive step is mediated by α-glucosidase, a brush-border enzyme of the intestinal epithelium, which converts these intermediates, including complex carbohydrates and disaccharides, into absorbable monosaccharides (87, 88).

In addition to seaweed-derived bioactives, chromium represents in Gdue® a relevant component of the formulation. Chromium is recognized as an essential trace element involved in the regulation of carbohydrate, lipid, and protein metabolism. Despite a relatively low dietary bioavailability, chromium plays a critical role in glycemic control by acting as a cofactor in insulin signaling (89). In particular, it enhances insulin receptor activity in insulin-sensitive tissues, including skeletal muscle, adipose tissue, and liver, thereby facilitating glucose uptake and contributing to improved glucose tolerance (90).

Among trivalent chromium compounds, chromium picolinate is considered one of the most bioavailable forms and has been associated with potential health benefits in humans, particularly in relation to glucose metabolism and blood pressure regulation (91).

In vivo animal studies conducted in experimental models of diet-induced NAFLD and NASH suggest beneficial effects of Gdue® under overnutrition and insulin resistance states when associated with hepatic steatosis (92). In these experimental models, male rats were exposed to a high-fat diet combined with 30% fructose in drinking water for 12 weeks to induce NAFLD or for 18 weeks to promote disease progression toward NASH. Under these conditions, daily oral administration of Gdue® was associated with an overall improvement in systemic metabolic parameters, including body weight regulation, fasting glycemia, circulating triglyceride levels, and inflammatory markers. Model-specific effects were also observed, with

improved postprandial glycemic responses in NASH animals and a reduction in total cholesterol levels in NAFLD rats, suggesting a potential role of seaweed-derived compounds in the modulation of cholesterol metabolism and/or excretion. Liver function was also positively affected, as indicated by the normalization of plasma biochemical markers commonly altered in steatotic liver disease, including ALT, alkaline phosphatase, and total bilirubin. Histological evaluation further demonstrated a reduction in hepatic lipid accumulation, with an improvement in both the size and number of lipid droplets within the liver parenchyma in treated NAFLD and NASH rats.

To gain mechanistic insight into these effects, molecular pathways involved in hepatic lipid storage and metabolism were examined. Gdue® administration was associated with the downregulation of genes implicated in triglyceride synthesis and lipid droplet formation, including diacylglycerol acyltransferase isoforms (DGAT-1 and DGAT-2), in both disease models compared with untreated controls, supporting a mechanistic role for the nutraceutical formulation in limiting hepatic lipid accumulation (92).

Consistent with these preclinical findings, data derived from a real-world observational study in humans indicate that six months of Gdue® supplementation is associated with an overall improvement in metabolic alterations related to MS (93). In this study, 505 subjects presenting at least one diagnostic criterion of metabolic syndrome were enrolled during routine clinical visits by healthcare professionals, including physicians and nutritionists. Daily intake of Gdue® (2–3 tablets per day) was associated with improvements in multiple metabolic syndrome components, particularly those related to carbohydrate metabolism, such as fasting plasma glucose, insulin levels, and insulin resistance assessed by the HOMA-IR index. Additional benefits were observed on systolic blood pressure, inflammatory markers, and waist circumference. Notably, these improvements were already evident after three months of supplementation and were further confirmed after six months of treatment (93).

In line with other evidence available in the literature**,** these clinical observations complement the preclinical findings obtained in experimental models, supporting the potential role of Gdue® as a nutraceutical strategy in metabolic dysfunction. On the basis of these premises, a

randomized, double-blind, placebo-controlled clinical trial was designed to evaluate the effects of Gdue®, in combination with a standardized hypocaloric diet, on hepatic steatosis and anthropometric parameters in patients with metabolic syndrome associated with MASLD.

2. Materials and Methods

1. Study design

A six-month, randomized, double-blind, controlled clinical trial was conducted at the Department of Internal Medicine and Medical Sciences, University of Genoa (Genoa, Italy), in collaboration with the Unit of Dietetics and Clinical Nutrition (UOSD), IRCCS Policlinico San Martino (Genoa, Italy).

The study protocol was approved by the local Institutional Ethics Committee and the trial was conducted in accordance with the principles of the Declaration of Helsinki and the guidelines of the Council for International Organizations of Medical Sciences (CIOMS).

All study procedures were thoroughly explained to eligible participants prior to enrollment, and written informed consent was obtained from all subjects before inclusion in the study.

2. Patients

101 Patients with MASLD were included in the study, according to the following eligibility criteria: male or female subjects aged ≥18 years; presence of metabolic syndrome (MS), defined according to the International Diabetes Federation (IDF) criteria, namely waist circumference ≥94 cm in males and ≥80 cm in females, plus at least two of the following conditions: triglyceride levels >150 mg/dL; HDL-C <40 mg/dL in males and <50 mg/dL in females; systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg or current antihypertensive treatment; impaired fasting glucose (IFG; 110–125 mg/dL) or established type

2 diabetes mellitus (T2DM). Hepatic steatosis of moderate (S2) to severe grade (S3) was required and confirmed by Controlled Attenuation Parameter (CAP) assessment.

Exclusion criteria were: age <18 years; inability to understand the study procedures or to provide written informed consent; use of nutraceutical supplements within the previous six months; renal insufficiency, defined as serum creatinine values above the upper reference limit; presence of cancer, chronic inflammatory diseases (including rheumatic or infectious conditions), or psychiatric disorders; severe cardio- or cerebrovascular disease (e.g., New York Heart Association class II–IV heart failure, history of myocardial infarction or stroke), or cerebrovascular events occurring within six months prior to study enrollment; and current smoking status.

3. Ultrasound and Elastography Assessment

Hepatic steatosis was assessed non-invasively using the CAP, obtained by vibration-controlled transient elastography (FibroScan®), simultaneously with liver stiffness measurement. CAP provides a quantitative estimate of liver fat content and has been validated for the assessment of steatosis in patients with metabolic-associated liver disease.

Measurements were performed according to standardized quality criteria, and only valid examinations were included in the analysis. CAP values corresponding to moderate steatosis (S2: 268–280 dB/m) and severe steatosis (S3: >280 dB/m) were considered.

4. Treatment

To ensure an appropriate balance of baseline characteristics, participants were randomly allocated into two parallel arms to receive either Gdue®, a nutraceutical formulation containing

extracts of *Ascophyllum nodosum*, *Fucus vesiculosus*, and chromium picolinate, or a matching placebo, for a period of six months, according to a randomized, double-blind, placebo-controlled study design. Blinding was maintained by packaging both the nutraceutical and placebo in indistinguishable dosage forms, identical in appearance, color, and size. The capsules were provided in identical blister packs and stored in unlabeled containers bearing only a coded identification, thereby preventing recognition of treatment allocation by both participants and investigators involved in data collection.

Gdue® and placebo were self-administered three times daily, approximately 30 minutes before the main meals (breakfast, lunch, and dinner). Participants were instructed to begin the assigned supplementation on the day following study enrollment. Both interventions were provided at no cost and were administered in addition to any ongoing standard medical therapy. All subjects also received individualized dietary counseling and followed a personalized hypocaloric Mediterranean diet tailored to their clinical condition.

Treatment adherence was monitored by pill count at each scheduled follow-up visit, during which all unused study medication was collected and recorded to assess compliance.

5. Diet intervention and lifestyle

All participants received individualized dietary counseling by a qualified dietitian and were prescribed a hypocaloric Mediterranean diet aimed at achieving a body weight reduction of approximately 5–15% over six months. Daily energy intake was reduced by 600 to 1000 kcal relative to individual energy requirements, in accordance with current guidelines for the management of overweight and obesity.

Basal metabolic rate was estimated using the Harris–Benedict equation. The prescribed diet provided approximately 45–50% of total energy from carbohydrates, 35–40% from fats (with <7% from saturated fatty acids, up to 10% from polyunsaturated fatty acids, and up to 20%

from monounsaturated fatty acids), and 15–20% from proteins. Intake of simple sugars was limited to 10–12% of total daily energy. In addition to dietary intervention, participants received individualized lifestyle counseling aimed at increasing habitual physical activity, in accordance with their clinical condition and baseline physical fitness. Alchool consumption was not permitted.

6. Assessment

At baseline (start of Gdue® treatment) and after 90 days (3 months) and 180 days (6 months) of treatment, the following data were collected: age, sex, body weight, height, waist circumference, fasting plasma glucose, glycated hemoglobin (HbA1c), fasting insulin levels, homocysteine levels, total cholesterol (TC), HDL-C cholesterol, low-density lipoprotein (LDL-C) cholesterol, triglycerides (TGL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Insulin resistance was estimated using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), calculated according to the formula: HOMA-IR = (fasting glucose × fasting insulin) / 22.5. A physical examination was performed at each visit, including measurement of blood pressure. Information regarding comorbidities and concomitant therapies was also collected, with particular attention to the presence of type 2 diabetes mellitus, hypertension, and dyslipidemia. Adherence to the Mediterranean diet was assessed using the validated 14-item PREDIMED questionnaire for adults (94). Each item was scored as 0 or 1, yielding a total score ranging from 0 to 14. According to established cut-off values, adherence to the MD was classified as low (≤5 points), moderate (6–9 points), or high (≥10 points).

Dietary habits were further evaluated through nutritional assessment to estimate basal metabolic rate, total daily energy expenditure, and habitual daily caloric intake. In addition, macronutrient intake was analyzed, and the relative contribution of proteins, lipids, and carbohydrates to total energy intake was calculated, with particular attention to their percentage distribution. At baseline and after 6 months of treatment, body composition was assessed by bioelectrical

impedance analysis (BIA). Hepatic steatosis was evaluated using transient elastography with controlled attenuation parameter (FibroScan® CAP).

7. Statistical Analysis

Descriptive analysis of continuous variables are expressed as median (IQR), given the non-normal distribution of the data. Patient characteristics at baseline were compared by Placebo and Gdue® groups using the Mann-Whitney U-test for continuous variables and the Pearson's chi-squared test for categorical variables. Changes from baseline in percentage (Δ) were calculated by comparing the baseline T0 and T6 median values, using the following formula:

$$\Delta\,(\%) = \frac{\text{Median}_{\text{T6}} - \text{Median}_{\text{T0}}}{\text{Median}_{\text{T0}}} \times 100$$

All tests are two-sided, and a P value < 0.05 was considered for statistical significance. Statistical analyses were performed using the SPSS software ver. 23.0

3. Results

Recruitment started in March 2023, and completion of the six-month follow-up occurred in June 2025. A total of 101 patients were enrolled and randomly allocated into two parallel groups.

As reported in Table 1, at baseline participants assigned to the placebo group were predominantly male (57%) and had a median age of 60 years (IQR 46–65). Median body mass index (BMI) was 30.6 kg/m² (IQR 28.2–34.6), with a median weight of 91 kg (IQR 79.7-101) and a median value of WC of 108 cm (IQR 100–117). The median phase angle was 6.1 (IQR

5.6–6.6), and the median Controlled Attenuation Parameter (CAP) value was 312 dB/m (IQR 282–336). Hypertension was present in 73% of subjects, 33% had a diagnosis of type 2 diabetes mellitus (T2DM), and cardiovascular disease (CAD) was reported in 12%.

Participants in the Gdue® group were also mainly male (69%), with a median age of 58.5 years (IQR 55.5–64). Median weight value was 87.5kg (IQR 79.1-98), median value of BMI was 29.9 kg/m² (IQR 27.9–31.5), and median WC was 105 cm (IQR 100–111). The median phase angle was 6.2 (IQR 5.9–6.9), while the median CAP value was 299 dB/m (IQR 273–319). Hypertension was observed in 64% of patients, T2DM in 19%, and CAD in 8%. Regarding family history, dyslipidemia was reported in 71% of patients in the Gdue® group and 63% in the placebo group. A family history of liver disease was observed in 14% of participants in both groups, while a family history of T2DM was reported in 52% of the Gdue® group and 53% of the placebo group. MD adherence score with Predimed test reaches a median value in both of groups of 7, indicating a medium adherence to MD.

Baseline biochemical parameters for each group are reported in Table 2. No statistically significant differences were observed at baseline between the placebo and nutraceutical groups for glycemic parameters, lipid profile, liver enzymes, homocysteine levels, or insulin resistance indices, confirming adequate comparability between groups.

Table 3 summarizes the longitudinal changes in the parameters of interest after 3 months (T3) and 6 months (T6) of treatment. After three months of intervention, a significant reduction in BMI was observed in the Gdue® group compared with placebo ($p = 0.029$), and this difference remained statistically significant at six months ($p = 0.049$), as showed by Figure 1. WC decreased over time in both groups, without statistically significant between-group differences at either follow-up.

After six months of intervention, percentage changes from baseline were analyzed to further evaluate the effects of Gdue® supplementation compared with placebo on anthropometric, metabolic, and hepatic parameters (Table 4). With respect to anthropometric outcomes, both groups experienced similar reductions in body weight–related measures; however, a greater

median decrease in BMI was observed in the Gdue® group compared with placebo (−5.4% vs −4.5%). Similarly, WC showed a more pronounced reduction in the Gdue® group (−5.7% vs −5.5%) than in the placebo group, indicating a more favorable effect on central adiposity.

Regarding body composition, fat mass percentage decreased substantially in both groups. Although the median reduction was comparable between groups (−20% in the Gdue® group vs −4% in the placebo group), a wider interindividual variability was observed among Gdue®-treated subjects, with a significantly greater reduction compared with placebo ($p = 0.018$) (Figure 2).

Improvements in glucose metabolism were also detected. Fasting plasma glucose and HbA1c levels showed modest improvements over time in both groups, with no significant between-group differences and but a more favorable trend for Gdue® group (-4.8% vs -3.2%,). However, fasting insulin levels were significantly lower in the Gdue® group at three months compared with placebo (-9.3% vs 7.0%, $p = 0.033$). At T6, fasting insulin levels decreased in both arms, with median reductions of −6.7% in the Gdue® group and in the placebo group −7.8%, although wide interindividual variability was observed. HOMA-IR showed a favorable trend over time without reaching statistical significance.

In terms of lipid metabolism, HDL-C increased in both groups, with a significantly greater median percentage increase in the Gdue® group (+13% vs +2%) compared with placebo at T6 ($p = 0.041$), indicating a more favorable modulation of the lipid profile associated with nutraceutical supplementation (Figure 3). Total cholesterol, LDL-cholesterol, and triglyceride levels did not differ significantly between groups.

Plasma liver enzymes (AST, ALT, and ALP) remained within normal ranges throughout the study in both groups, with no significant between-group differences.

A clinically meaningful improvement in hepatic steatosis was observed, as reflected by the reduction in CAP values, as showed by Figure 4. At T6, the median percentage decrease in CAP was greater in participants receiving Gdue® (−13.5% vs −12.8%) compared with those

receiving placebo], with a statistically significant between-group difference (p = 0.039), suggesting an enhanced reduction in liver fat content in the nutraceutical-treated group.

Overall, the analysis of percentage changes highlights a trend toward greater improvements in anthropometric parameters, hepatic steatosis, and selected metabolic markers in patients receiving Gdue®, supporting its potential additive benefit when combined with a hypocaloric Mediterranean diet in individuals with metabolic syndrome–associated MASLD.

Overall, the nutraceutical intervention was well tolerated. Mild gastrointestinal adverse events were reported in 2.9% of participants (n = 3), all occurring in the placebo group and consisting mainly of transient aerophagia. No moderate or severe gastrointestinal adverse events, such as diarrhea or clinically relevant gastrointestinal discomfort, were reported in either group during the study period. No participant discontinued the study because of adverse events.

	Placebo	**Gdue®**	***P***
GENDER	M 28 57%	M 36 69%	NS
	F 21 43%	F 16 31%	
AGE	60	58,5	NS
	(IQR 46-65)	(IQR 55,5-64)	

WEIGHT (KG)	91 (IQR 79,7-101)	87,5 (IQR 79,1-98)	NS
BMI (kg/m^2)	30,6 (IQR 28,2-34,6)	29,9 (IQR 27,9-31,5)	NS
WC (cm)	108 (IQR 100-117)	105 (IQR 100-111)	NS
BIA (°)	6,1 (IQR 5,6-6,6)	6,2 (IQR 5,9-6,9)	NS
CAP (dB/m)	312 (IQR 282- 336)	299 (IQR 273-319)	NS
Hypertension (%)	73	64	NS
T2DM (%)	33	19	NS
CAD (%)	12	8	NS

Table 1. Patients characteristic at baseline. Values are expressed as median and interquartile range or %.

Table 2. Baseline blood chemistry values for patients expressed as median and interquartile range

Parameter	Placebo Baseline	Gdue® Baseline	*P*
Fasting blood glucose (mg/dL)	92 (84–102)	94 (84.5–103.5)	NS
HbA1c (mmol/mol)	5.7 (5.4–6.0)	5.7 (5.4–5.9)	NS
AST (U/L)	32 (27–39)	28 (24.5–34)	NS
ALT (U/L)	40 (28–51)	34 (24.5–46.5)	NS
ALP (U/L)	73 (61–86)	67 (57–80)	NS

Parameter	Placebo Baseline	Gdue® Baseline	*P*
TC (mg/dL)	173 (137–216)	213.5 (152–243)	NS
HDL-C (mg/dL)	44 (38–50)	45 (39.5–49.5)	NS
TGL (mg/dL)	154 (94–209)	156.5 (121–228.5)	NS
LDL-C (mg/dL)	99 (65.5–147.5)	129.9 (88.6–155.0)	NS
Homocysteine (µmol/L)	14.8 (12.1–18.1)	13.6 (10.9–16.9)	NS
Fasting insulin (µu/mL)	12.7 (8.9- 17.2)	11.2 (8.4-14.3)	NS
HOMA-IR	2.7 (1.9–3.7)	2.63 (1.93–3.49)	NS

T0 Placebo	T0 Gdue®	T3 Placebo	T3 Gdue®	*P* T3	T6 Placebo	T6 Gdue®	*P* T6
			BMI				
			(kg/m^2)				
30.6 (28.2-34.6)	29.9 (27.9-31.5)	29.9 (27.8-32.8)	28.2 (26.8-30.9)	0.029	29.2 (27.8-32.5)	28.3 (26.3-30.5)	**0.049**
			WC				
			(cm)				
108 (100-117)	105 (100-111)	102 (96-111)	101 (95-106)	NS	102 (96-109)	99 (95-103.5)	NS
			Fasting blood glucose				
			(mg/dL)				
92 (84–102)	94 (84.5–103.5)	92 (83–98)	92 (84.5–102)	NS	89 (81–98)	89 (81–97)	NS
			HbA1c				
			(mmol/mol)				
5.7	5.7 (5.4–5.9)	5.6 (5.4–6.0)	5.6 (5.2–5.8)	NS	5.5 (5.3–5.8)	5.4 (5.3–5.8)	NS

T0 Placebo	T0 Gdue®	T3 Placebo	T3 Gdue®	*P* T3	T6 Placebo	T6 Gdue®	*P* T6
(5.4–6.0)							
			AST (U/L)				
32 (27–39)	28 (24.5–34)	27 (24–38)	25 (21–33.5)	NS	26 (24–33)	26 (22–32)	NS
			ALT (U/L)				
40 (28–51)	34 (24.5–46.5)	37 (22–49)	27(20.5–40)	NS	28 (20–40)	30 (20–38)	NS
			ALP (U/L)				
73 (61–86)	67 (57–80)	70 (61–92)	67 (56.5–80)	NS	74 (60–88)	67 (55–80)	NS
			TC				
			(mg/dL)				
173 (137–216)	213.5 (152–243)	161 (124–199)	183 (138–217.5)	NS	169 (138–203)	183 (147–209)	NS
			HDL-C (mg/dL)				

T0 Placebo	T0 Gdue®	T3 Placebo	T3 Gdue®	*P* T3	T6 Placebo	T6 Gdue®	*P* T6
44 (38–50)	45 (39.5–49.5)	45 (39–51)	49 (41.5–53)	NS	45 (38–53)	51 (43–55)	**0.041**
			TGL (mg/dL)				
154 (94–209)	156.5 (121–228.5)	122 (98–165)	128 (95–166)	NS	112 (88–176)	120 (95–166)	NS
			LDL-C (mg/dL)				
99 (65.5–147.5)	129.9 (88.6–155.0)	95.9 (57.6–128)	102.6 (60–144.4)	NS	107 (66–134)	109 (67–131)	NS
			Homocysteine (µmol/L)				
14.8 (12.1–18.1)	13.6 (10.9–16.9)	14.2 (11.8–17.3)	13.6 (11.–16.1)	NS	14 (12–17)	13 (11–15)	NS
			Fasting insulin (µu/mL)				
12.7 (8.9-17.2)	11.2 (8.4-14.3)	11.8 (9.4-17.3)	10.1 (6.8-13.9)	0.033	11.7 (8.3-15.6)	10.5 (7.1-13.2)	NS
			HOMA-Index				

T0 Placebo	T0 Gdue®	T3 Placebo	T3 Gdue®	*P* T3	T6 Placebo	T6 Gdue®	*P* T6
2.69 (1.9–3.7)	2.63 (1.9–3.4)	2.6 (1.9–4.2)	2.4 (1.3–3.4)	NS	2.6 (1.9–3.6)	2.2 (1.4–3.2)	NS
			FM (%)				
36.0 (31.2-42.2)	35.9 (31.0-40.8)				34.5 (28.5-40.4)	28.8 (25.3-35.8)	0.018
			CAP (dB/m)				
312 (282-336)	299 (273-319)				275 (253-308)	258.5 (225.5-292.5)	0.039

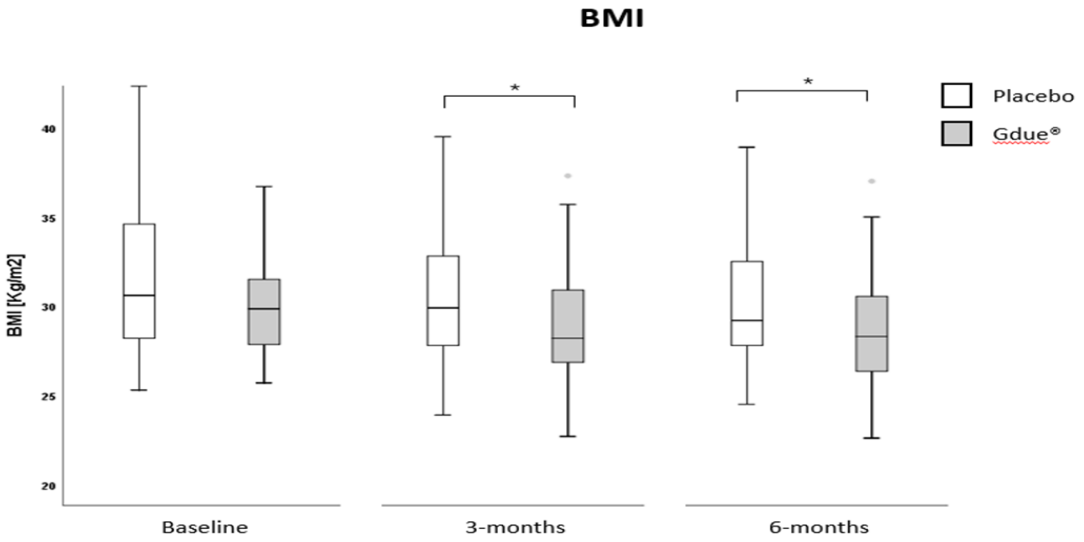

Figure 1. Boxplot showing changes in body mass index (BMI) from baseline (T0) to 3 months (T3) and 6 months (T6) of intervention in placebo and Gdue® group.

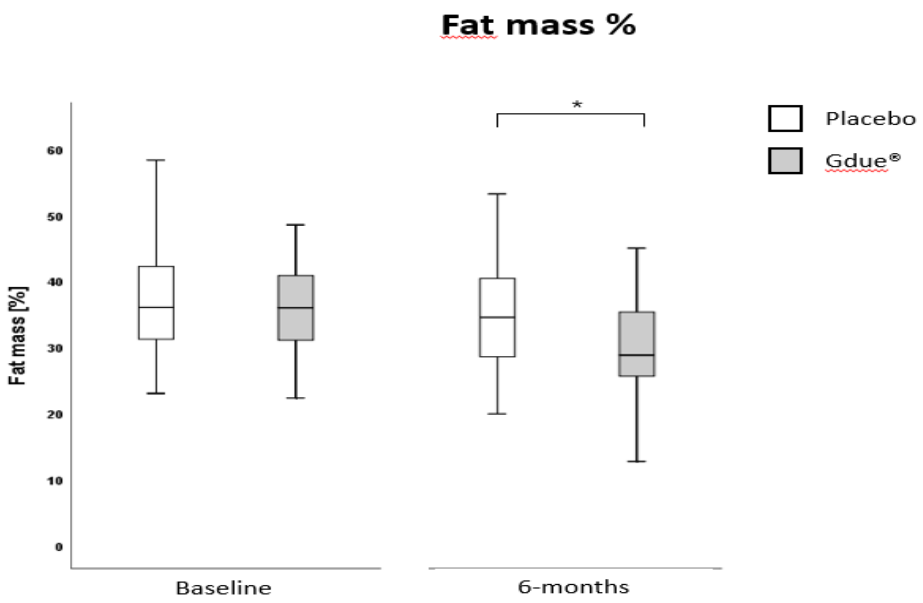

Figure 2. Boxplot showing changes in Fat Mass percentage (FM%) assessed with BIA from baseline (T0) to months (T6) of intervention in placebo and Gdue® group

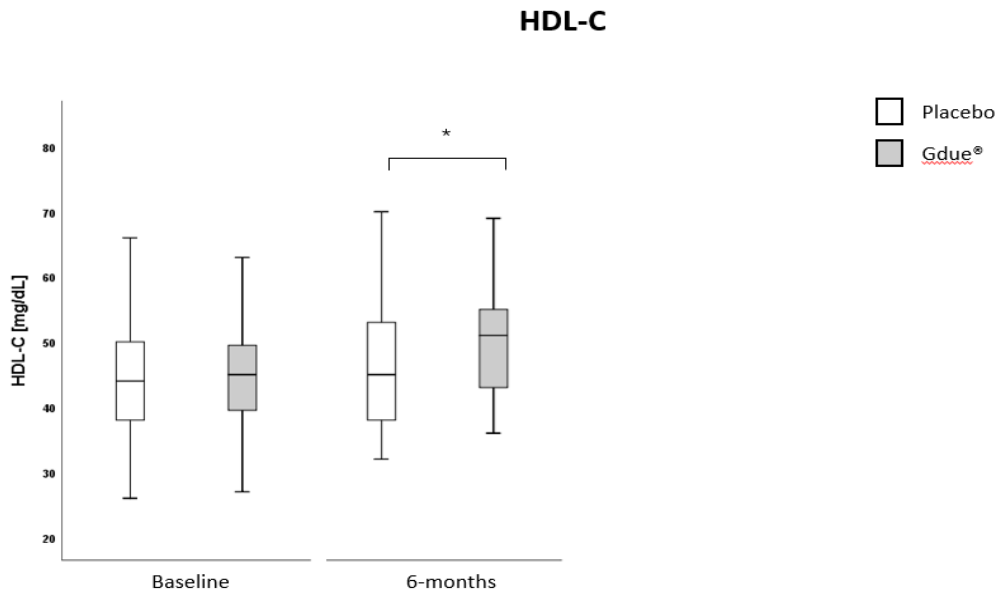

Figure 3. Boxplot showing changes in HDL-C from baseline (T0) to months (T6) of intervention in placebo and Gdue® group

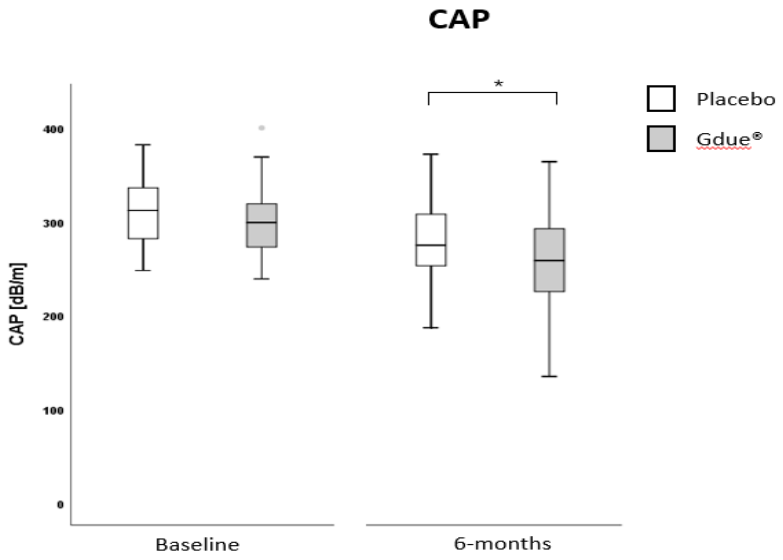

Figure 4. Boxplot showing changes in CAP from baseline (T0) to months (T6) of intervention in placebo and Gdue® group

Table 4. Percentage change from baseline after 6 months (Δ%)

Parameter (Δ%)	Placebo	Gdue®	*P*
WEIGHT	−4.2%	−4.2%	NS
BMI	−4.5%	−5.4%	0.049
WC	−5.5%	−5.7%	NS
HDL-C	+2%	+13%	0.041
CAP	−11.8%	−13.5%	0.039

Parameter (Δ%)	Placebo	Gdue®	*P*
Fat Mass (%)	−4%	−20%	0.018
Fasting glucose	−3.2%	−4.8%	NS
Fasting insulin	−7.8%	−6.7%	NS

4. Discussion

To date, no approved pharmacological therapy is specifically available for the treatment of hepatic steatosis and metabolic dysfunction–associated steatotic liver disease (MASLD). Consequently, current therapeutic strategies rely primarily on lifestyle modification, with dietary intervention and regular physical activity representing the cornerstone of disease management (8). These approaches have consistently demonstrated efficacy in improving the metabolic alterations underlying MASLD, including insulin resistance, dyslipidemia, and excess hepatic fat accumulation (49).

In this context, nutraceuticals have emerged as adjunctive interventions that lie along a nutritional continuum between conventional diet therapy and pharmacological treatment (95). Defined as food supplements containing concentrated doses of bioactive compounds originally derived from foods (96), nutraceuticals are intended to integrate within dietary and lifestyle interventions rather than act independently. By reinforcing specific metabolic pathways targeted by diet and physical activity, nutraceutical supplementation may enhance the overall efficacy of lifestyle-based approaches in improving glycemic control, lipid profiles, and low-grade inflammation. This integrated perspective supports the use of nutraceuticals as complementary tools within a comprehensive nutritional strategy for metabolic dysfunction

(95, 97). Within this framework, the hypocaloric Mediterranean diet represented the essential background intervention upon which the effects of Gdue® were evaluated.

The present randomized, double-blind, placebo-controlled trial was designed to evaluate the effectiveness of Gdue®, a seaweed-based nutraceutical rich in pholyphenols and fucoidans, administered for six months in combination with a hypocaloric Mediterranean diet, on hepatic steatosis and anthropometric parameters in patients with MASLD, compared with diet alone.

Marine algae extracts are recognized as a rich reservoir of biologically active compounds, including polyphenols and sulfated polysaccharides, with well-documented inhibitory activity on digestive enzymes (98). In particular, α-glucosidase and α-amylase inhibitors, commonly present in the polyphenolic fraction of brown algae—especially phlorotannins—have been shown to reduce intestinal carbohydrate digestion and glucose absorption, thereby modulating postprandial glycemic excursions (99). Beyond their effects on carbohydrate metabolism, these compounds may influence hepatic lipid metabolism through the modulation of intracellular signaling pathways involved in inflammation and lipogenesis, such as nuclear factor kappa B (NF-κB), ultimately leading to reduced lipid synthesis and enhanced fatty acid oxidation (100).

Fucoidans, the principally studied sulfated polysaccharides in literature, amplify metabolic improvement of polyphenols on NAFLD, presumably acting on inflammation and oxidative stress, implementing fatty acid oxidation rather than accumulation (77).

In our study, both treatment arms experienced reductions in BMI and WC, highlighting the central role of dietary intervention as a form of structured dietotherapy.

In this regard, it can be hypothesized that patients with MASLD may exhibit a particularly high level of adherence to dietary prescriptions, possibly related to an increased awareness of liver dysfunction and the perception of the liver as a vital organ. Additionally, the diagnosis of a chronic metabolic liver condition may induce a sense of concern or perceived health vulnerability, which could further motivate patients to comply more strictly with lifestyle recommendations.

However, patients receiving Gdue® supplementation exhibited a significantly greater reduction in BMI compared with placebo (-5.4%vs -4.5%) at six months, suggesting an additive effect of the nutraceutical when integrated into a dietary intervention. WC, a surrogate marker of visceral adiposity closely associated with hepatic fat content and cardiometabolic risk, also showed a slightly more pronounced median reduction in the Gdue® group, although between-group differences did not reach statistical significance (-5.7%vs -5.5%). In addition to anthropometric changes, body composition analysis performed by bioelectrical impedance analysis (BIA) revealed a statistically significant reduction in fat mass percentage (FM%) in patients receiving Gdue® supplementation compared with placebo (-20%vs -4%). This finding suggests that the observed BMI improvement in the Gdue® group was primarily driven by a decrease in adipose tissue rather than lean mass loss. The reduction in FM% is clinically relevant, as excess adiposity—particularly visceral and ectopic fat—plays a central role in the pathogenesis of MASLD and its associated metabolic complications (101). A preferential loss of fat mass may contribute to improvements in insulin sensitivity, lipid metabolism, and hepatic fat accumulation, thereby reinforcing the metabolic benefits observed in the Gdue® group. These results further support the hypothesis that nutraceutical supplementation, when integrated into a hypocaloric dietary intervention, may enhance qualitative changes in body composition beyond those achieved through caloric restriction alone, particularly when targeting abdominal fat distribution, which is strongly implicated in MASLD pathophysiology (102).

Improvements in glucose metabolism were observed in both groups, consistent with the effects of weight loss and dietary modification (103).Notably, fasting insulin levels were significantly reduced in the Gdue® group at three months, suggesting an early beneficial effect on insulin sensitivity. Although reductions in fasting insulin and HOMA-IR were observed at six months in both arms without statistically significant between-group differences, the overall trend supports a potential modulatory role of Gdue® on insulin dynamics, according to previous studies (92).

These effects are biologically plausible, as algae-derived polyphenols, including bromophenols and phlorotannins, are known inhibitors of α-amylase and α-glucosidase, leading to reduced intestinal carbohydrate absorption and attenuation of postprandial glycemic excursions.

Additionally, these compounds may influence hepatic lipid metabolism through modulation of signaling pathways involved in lipogenesis and fatty acid oxidation (102).

With respect to lipid metabolism, a significant increase in HDL-C was observed in the Gdue® group compared with placebo at six months. This finding is consistent with previous clinical evidence suggesting a favorable effect of Gdue® supplementation on lipid homeostasis (93). Although the precise mechanisms remain speculative, potential pathways include modulation of cholesterol biosynthesis or enhanced cholesterol efflux, ultimately contributing to a reduction in cardiometabolic risk components associated with MASLD. However, it would appear that the fraction of fucoidans contained in Gdue® plays a significant role in improving the lipid profile. According to previous studies, fucoidans are able to reverse the transport of cholesterol from peripheral tissues to the liver by inhibiting key enzymes in the synthesis of cholesterol triacylglycerols such as HMG CoA reductase or fatty acid synthase and acetyl-CoA carboxylase or activitaing lipoprotein lipase (69, 104) .

One of the most clinically relevant findings of the present study is the significant reduction in hepatic steatosis, as assessed by Controlled Attenuation Parameter (CAP). Patients receiving Gdue® exhibited a greater median percentage decrease in CAP values compared with placebo (-13.5 vs -11.8), indicating a more pronounced reduction in liver fat content. This result aligns with preclinical evidence showing partial regression of steatosis and normalization of transaminases in overnourished animal models treated with Gdue® (99).

Importantly, the improvement in CAP observed in our cohort occurred in the context of lifestyle intervention in both groups, reinforcing the concept that nutraceuticals act as coadjuvants within a comprehensive dietary strategy, rather than as substitutes for diet and physical activity.

The hypocaloric Mediterranean diet prescribed in this study was individually tailored to each patient’s clinical condition and estimated energy expenditure. A robust body of literature supports the Mediterranean dietary pattern as one of the most effective strategies for reducing cardiovascular risk and improving metabolic disorders that share common pathophysiological features with MASLD, including insulin resistance, obesity, and ectopic fat accumulation (105).

Dietary patterns rich in unsaturated fats and characterized by controlled carbohydrate intake appear particularly effective in mobilizing ectopic fat depots, including hepatic and myocardial fat, compared with low-fat dietary approaches (10, 41).

Consistent with this evidence, BMI improvement was observed in both study arms, underscoring the effectiveness of hypocaloric dietary intervention in reducing hepatic adiposity and improving liver-related outcomes (106). Scientific evidence consistently demonstrates a strong association between excessive caloric intake and hepatic fat accumulation (107), and patients with MASLD frequently exhibit an unfavorable macronutrient distribution and caloric excess (41).

In line with this evidence, baseline dietary assessment in our cohort revealed an average daily caloric intake exceeding estimated energy requirements by more than 300 kcal, confirming the presence of caloric surplus as a relevant and modifiable pathogenic factor. This observation is clinically relevant, confirming the role of energy balance correction as a key determinant of hepatic improvement in MASLD.

Importantly, the magnitude of weight loss plays a key role in determining hepatic benefit. Weight reductions exceeding 7% have been associated with significant improvements in hepatic inflammation and hepatocellular ballooning, while greater weight loss may also positively impact fibrosis progression (108). Accordingly, both the EASL–EASD–EASO clinical practice guidelines and the current ESPEN guidelines on the management of liver disease (8, 109) strongly recommend weight reduction through hypocaloric diet and physical activity as first-line therapy in patients with MASLD and overweight or obesity.

In this context, our findings suggest that the integration of nutraceutical supplementation such as Gdue® into a structured hypocaloric Mediterranean dietary approach may potentiate the beneficial effects of lifestyle intervention on hepatic steatosis and selected metabolic parameters (110). This concept is supported by previous clinical evidence showing that the combination of a Mediterranean diet with nutraceutical supplementation leads to greater improvements in anthropometric outcomes, including body weight, WC, and hip

circumference, compared with diet alone. In particular, Chiaruzzi et al. demonstrated that a low-calorie Mediterranean diet combined with nutraceutical supplementation over three months resulted in significantly greater reductions in anthropometric measures in patients with MASLD and obesity compared with placebo-treated controls (111).

Notably, the mild gastrointestinal symptoms reported during the study occurred exclusively in the placebo group, suggesting that Gdue® supplementation itself was not associated with adverse effects. This finding further supports the excellent tolerability and safety profile of the nutraceutical, which is particularly relevant for long-term use in chronic metabolic conditions such as MASLD.

The present study has several strengths. First, the randomized, double-blind, placebo-controlled design ensures a high level of internal validity and minimizes potential bias. Second, the six-month duration allows for the evaluation of medium-term effects of nutraceutical supplementation, which is particularly relevant for chronic metabolic conditions such as MASLD.

Hepatic steatosis was assessed using Controlled Attenuation Parameter (CAP), a validated and non-invasive technique that provides quantitative information on liver fat content. Fourth, the integration of Gdue® supplementation within a standardized hypocaloric Mediterranean diet reflects real-world clinical practice and supports the translational relevance of the findings.

Finally, the favorable safety and tolerability profile observed in this study strengthens the potential applicability of Gdue® as an adjunctive nutritional strategy in patients with MASLD.

Some limitations should be acknowledged. First, although the sample size was adequate to detect differences in key metabolic and hepatic outcomes, larger multicenter studies are warranted to confirm these findings and improve generalizability.

Liver fat content was assessed using CAP rather than histological evaluation, since it may be influenced by operator expertise and technical factors. However, all examinations in the present study were performed according to standardized procedures and quality criteria, thereby

minimizing measurement variability. In addition, liver biopsy is not feasible in large clinical trials and CAP represents a validated and widely accepted non-invasive alternative. Furthermore, although BIA represents a practical and widely used method for body composition assessment, it may be influenced by hydration status; however, standardized measurement conditions were applied to minimize variability.

Dietary adherence and physical activity were assessed through counseling and follow-up visits but were not objectively quantified, which may have introduced some variability in lifestyle-related outcomes.

Finally, the study did not include a post-intervention follow-up period, thus limiting conclusions regarding the long-term persistence of the observed benefits after discontinuation of supplementation.

5. Conclusion and future perspectives

MASLD represents a major and growing global health burden, closely intertwined with obesity, insulin resistance, and MS in general. To date, the absence of approved pharmacological therapies specifically targeting hepatic steatosis has placed lifestyle modification—namely dietary intervention and physical activity—at the center of disease management. However, the long-term adherence and effectiveness of lifestyle measures alone remain challenging, highlighting the need for complementary strategies capable of enhancing metabolic and hepatic outcomes within a nutritional framework.

In this context, the present randomized, double-blind, placebo-controlled clinical trial was designed to evaluate the efficacy and safety of Gdue®, a seaweed-based nutraceutical,

administered for six months in combination with a hypocaloric Mediterranean diet, in patients with MASLD. The findings of this study demonstrate that the integration of Gdue® into a structured dietary intervention provides additional benefits beyond diet alone, particularly with respect to anthropometric parameters, lipid metabolism, and hepatic steatosis.

Both treatment arms experienced significant improvements in body weight–related measures, underscoring the pivotal role of caloric restriction and diet quality as effective therapeutic tools in MASLD. Nevertheless, patients receiving Gdue® supplementation exhibited a significantly greater reduction in BMI at six months compared with placebo, suggesting an additive effect of the nutraceutical when combined with dietary intervention. WC, a surrogate marker of visceral adiposity and a key driver of hepatic fat accumulation, also showed a more pronounced median reduction in the Gdue® group, further supporting a potential role in modulating central fat distribution.

Improvements in glucose metabolism were observed in both groups, consistent with the metabolic benefits of weight loss and dietary modification. Notably, fasting insulin levels were significantly reduced in the Gdue® group at three months, indicating an early improvement in insulin sensitivity. Although between-group differences in insulin resistance indices were not maintained at six months, the overall trend supports a favorable modulatory effect of Gdue® on insulin dynamics, in line with the known inhibitory effects of algae-derived polyphenols on carbohydrate digestion and postprandial glycemic excursions.

With regard to lipid metabolism, Gdue® supplementation was associated with a statistically significant increase in HDL-C at six months compared with placebo. This finding is clinically relevant, as HDL-C plays a protective role in cardiometabolic risk and is often reduced in patients with MASLD. The observed improvement suggests a potential effect of the nutraceutical on lipid homeostasis, possibly mediated through modulation of cholesterol synthesis or enhanced cholesterol efflux, although further mechanistic studies are warranted.

One of the most clinically meaningful outcomes of the present trial is the significant reduction in hepatic steatosis, as assessed by Controlled Attenuation Parameter (CAP). Patients treated

with Gdue® showed a greater median percentage decrease in CAP values compared with those receiving placebo (-13.5% vs -11.8%), indicating a more pronounced reduction in liver fat content. This finding is particularly relevant given the central role of hepatic steatosis in MASLD pathogenesis and progression. Importantly, the observed improvement occurred in the context of lifestyle intervention in both groups, reinforcing the concept that nutraceuticals act as coadjuvants rather than substitutes for dietary and lifestyle modification.

The favorable hepatic effects observed in this study are consistent with preclinical evidence demonstrating reduced lipid accumulation and modulation of lipogenic pathways in experimental models treated with Gdue®. Collectively, these data support a biological plausibility for the observed clinical benefits, likely mediated by the combined actions of polyphenols and other bioactive compounds present in brown seaweed extracts.

From a safety perspective, Gdue® demonstrated an excellent tolerability profile. Mild gastrointestinal adverse events were infrequently reported and occurred exclusively in the placebo group, with no moderate or severe adverse effects observed in either arm. This finding further supports the suitability of Gdue® for long-term use in patients with chronic metabolic conditions such as MASLD.

Several limitations should be acknowledged. The duration of the study, although sufficient to detect meaningful metabolic and hepatic changes, does not allow conclusions regarding long-term outcomes or disease progression. Additionally, while CAP represents a validated and widely used non-invasive method for the assessment of hepatic steatosis, it remains partially operator-dependent, although standardized acquisition protocols were applied to minimize variability. Future studies incorporating longer follow-up periods and additional imaging or histological endpoints may further clarify the long-term impact of nutraceutical supplementation on MASLD progression.

In conclusion, the results of this doctoral research support the integration of Gdue® as a complementary nutritional strategy within lifestyle-based interventions for MASLD. When combined with a hypocaloric Mediterranean diet, Gdue® appears to enhance improvements in

anthropometric parameters, lipid metabolism, and hepatic steatosis, while maintaining an excellent safety profile. These findings contribute to the growing evidence supporting nutraceuticals as valuable adjuncts in the nutritional management of metabolic liver disease and provide a rationale for further large-scale, long-term clinical investigations.

The findings of the present study open several avenues for future research in the nutritional management of MASLD. While the results support the role of Gdue® as a complementary intervention to lifestyle modification, further investigations are needed to better define its long-term clinical impact, optimal duration of treatment, and target patient populations.

Larger randomized controlled trials with longer follow-up periods are warranted to evaluate whether the observed improvements in hepatic steatosis and metabolic parameters translate into sustained benefits over time and whether nutraceutical supplementation may influence disease progression, including inflammation and fibrosis. The inclusion of additional non-invasive markers of liver injury and fibrosis, as well as advanced imaging techniques, could provide a more comprehensive assessment of liver disease evolution.

Future studies should aim to identify subgroups of patients who may derive the greatest benefit from nutraceutical supplementation. Factors such as baseline metabolic profile, degree of insulin resistance, severity of steatosis, and dietary adherence may modulate individual responses to treatment. A personalized nutrition approach, integrating nutraceuticals, could therefore represent a promising strategy in MASLD management.

Mechanistic studies are needed to further elucidate the molecular pathways through which seaweed-derived bioactive compounds exert their metabolic and hepatoprotective effects in humans. In particular, the impact of Gdue® on hepatic lipid metabolism, inflammatory signaling pathways, gut microbiota composition, and intestinal permeability represents an area of growing scientific interest. In particular, it emerges from recent studies that dysbiosis can exacerbate MASLD through several mechanisms. Understanding these microbial signatures pathways could help refine nutraceutical formulations and guide their rational use in clinical practice.

Finally, from a translational perspective, nutraceuticals such as Gdue® may represent a practical and well-tolerated option to support lifestyle interventions in real-world settings, where adherence to dietary and physical activity recommendations is often suboptimal. Their integration into structured nutritional programs could enhance patient engagement and improve long-term metabolic outcomes, particularly in the early stages of MASLD.

Overall, future research should continue to explore the role of nutraceuticals within a comprehensive lifestyle-based framework, with the ultimate goal of developing effective, safe, and sustainable strategies for the prevention and management of metabolic dysfunction–associated steatotic liver disease.

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