Glycative Stress Research

Online edition : ISSN 2188-3610
Print edition : ISSN 2188-3602
Received : July 9, 2025
Accepted : December 1, 2025
Published online : December 31, 2025
doi:10.24659/gsr.12.4_156

Original articles

From fatty liver to steatohepatitis: Prevention by Glycated Stress Care

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Abstract

In recent years, the prevalence of metabolic syndrome in Japan has increased due to the Westernization of dietary habits and decreased physical activity. The progression of fatty liver to MASLD (metabolic dysfunction-associated steatotic liver disease) and its inflammatory and fibrotic variant, MASH (metabolic dysfunction-associated steatohepatitis), has become a growing health concern. Excessive or inappropriate intake of carbohydrates and lipids causes rapid postprandial hyperglycemia "blood glucose spikes", which in turn induces the production of various sugar- and lipid-derived aldehydes. We refer to this phenomenon as the "aldehyde spark". These short-chain aldehydes not only damage vascular endothelial cells but also modify proteins in pancreatic β-cells and hepatocytes, resulting in the formation of advanced glycation end-products (AGEs) and impaired protein function. Glycative stress (GS) describes a pathological condition in which the body is overloaded with aldehydes. Metabolism of excess aldehydes by dehydrogenases (*e.g.*, ALDH, GAPDH) requires NAD+, thereby reducing the NAD+/NADH ratio. NAD+ depletion impairs β-oxidation of lipid droplets in hepatocytes, while mitochondrial dysfunction in the TCA cycle leads to the overproduction of fumaric acid, a metabolite capable of modifying proteins. Furthermore, AGEs bind to receptors for AGEs (RAGE) expressed on macrophages and Kupffer cells, which subsequently induces chronic inflammation. This cascade is thought to contribute significantly to the transition from simple steatosis to steatohepatitis. This report proposes a preventive strategy centered on GS care. Specifically, we advocate a three-pillar intervention consisting of nutritional education, physical activity promotion, and behavioral support to suppress postprandial glucose and aldehyde spikes. In addition, supplementation with NAD+ precursors and correction of protein insufficiency may help enhance the aldehyde-trapping capacity of amino acids, promote insulin biosynthesis, and improve insulin resistance. We hope that such comprehensive metabolic approaches will help prevent the progression of steatohepatitis and support liver metabolic health.

***KEY WORDS*:** Steatohepatitis (MASH: Metabolic Dysfunction-Associated Steatohepatitis), aldehyde production chain after blood glucose spike (aldehyde spark), insulin resistance, NAD+ deficiency, fatty acid beta-oxidation, glycative stress

Introduction

In recent years, the prevalence of metabolic syndrome (MetS) in Japan has increased due to the Westernization of dietary habits and reduced physical activity. The progression of fatty liver to metabolic dysfunction-associated steatotic liver disease (MASLD) and its inflammatory and fibrotic variant, metabolic dysfunction-associated steatohepatitis (MASH), has emerged as a major public health problem. MASLD and MASH were previously termed non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). However, these terms have been replaced because they are defined passively as "fatty liver in people who do not consume alcohol". In reality, most patients with NAFLD/NASH have underlying metabolic disorders such as obesity, type 2 diabetes mellitus (T2DM), dyslipidemia, and hypertension; therefore, metabolic dysfunction is the fundamental pathology. In addition, the term "non-alcoholic" may lead to misunderstanding or stigma toward patients.

According to data from the Ministry of Health, Labour and Welfare, from the 1960s to the 1970s, overall energy

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intake increased, accompanied by rises in protein and fat intake, while carbohydrate intake declined (***Fig. 1-a***)[1]. In the 1990s, the proportion of energy derived from fat continued to increase, whereas that from carbohydrates decreased markedly. Although total energy intake has shown a downward trend since 1986, this decline coincides with a steady increase in the number and proportion of older adults with lower energy requirements. Thus, age adjustment may alter the conclusion that energy intake has consistently decreased since 1986.

Since 2010, total energy intake, the dietary fat ratio, and the proportion of animal fat have all increased again (***Fig. 1-b***), while physical activity levels have declined substantially (***Fig. 1-c***). Consequently, the number of individuals with MetS continues to rise (***Fig. 2***)[2]. Considering hepatic outcomes, the incidence of fatty liver and its progression to steatohepatitis is also presumed to be increasing. Addressing these issues and improving population health are critical for reducing public healthcare expenditures and securing a stable future workforce[3].

MetS represents a state of severe glycative stress (GS). Based on previous findings[4], this paper proposes a preventive strategy for suppressing the progression of steatohepatitis, with a focus on alleviating glycative stress ("GS care").

Metabolic syndrome (MetS) and glycative stress (GS)

MetS is characterized by visceral obesity, dyslipidemia (particularly hypertriglyceridemia), reduced adiponectin secretion, increased insulin resistance, impaired glucose tolerance, and postprandial hyperglycemia, and is a major risk factor for atherosclerosis[5-7]. MetS represents a typical pathological condition accompanied by severe glycative stress (GS). A reduction in adiponectin—which enhances insulin sensitivity—leads to further insulin resistance, impaired glucose tolerance, and postprandial hyperglycemia.

Yagi *et al*. demonstrated that postprandial hyperglycemia promotes the production of highly reactive aldehyde-type dicarbonyl compounds, including 3-deoxyglucosone (3DG), glyoxal (GO), and methylglyoxal (MGO)[8]. Subsequent studies confirmed that additional aldehydes, such as glyceraldehyde and acetaldehyde, are also generated[9]. The term "aldehyde spark" was introduced to describe this chain reaction of aldehyde generation triggered by a postprandial blood glucose spike (≥ 140 mg/dL)[10].

Blood in patients with MetS contains elevated levels of triglycerides and free fatty acids. These fatty acids undergo glycative and oxidative reactions or interact with environmental and behavioral factors, such as smoking and alcohol intake, to produce lipid-derived aldehydes[11-23].

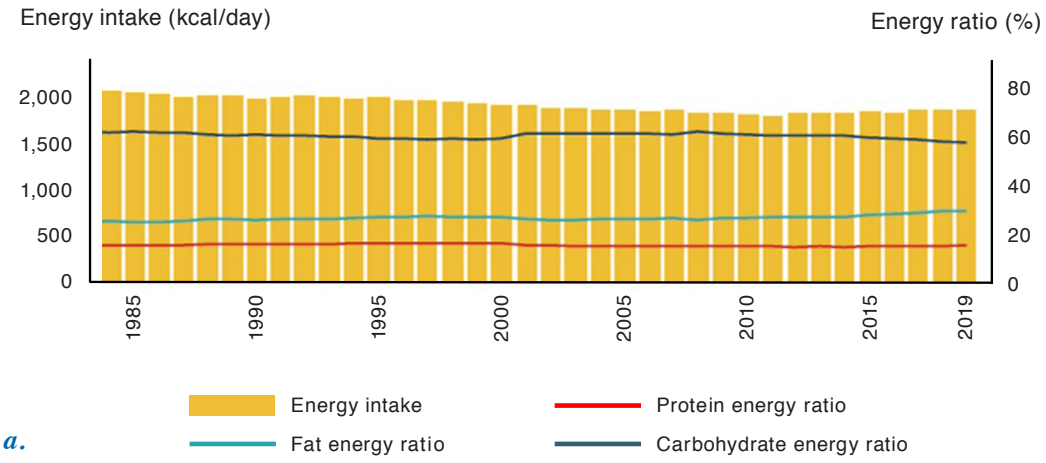

Fig. 1-a.

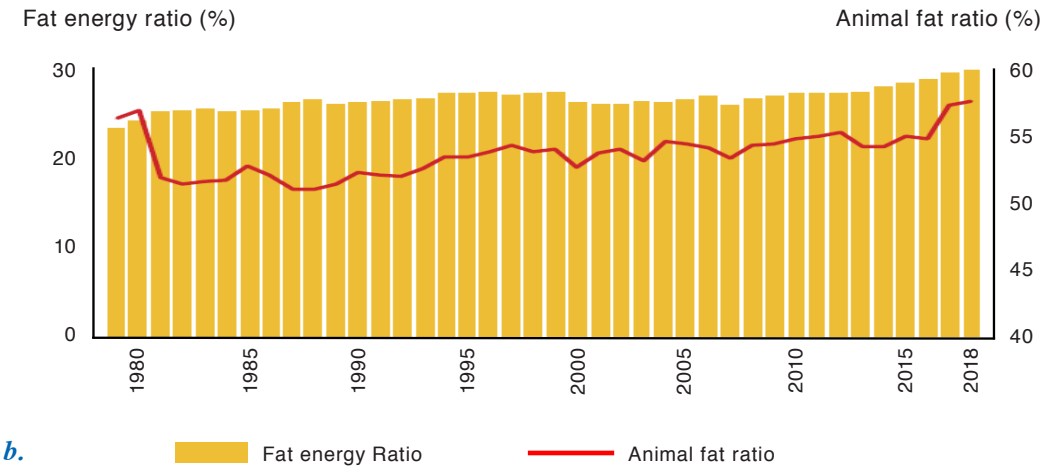

Fig. 1-b.

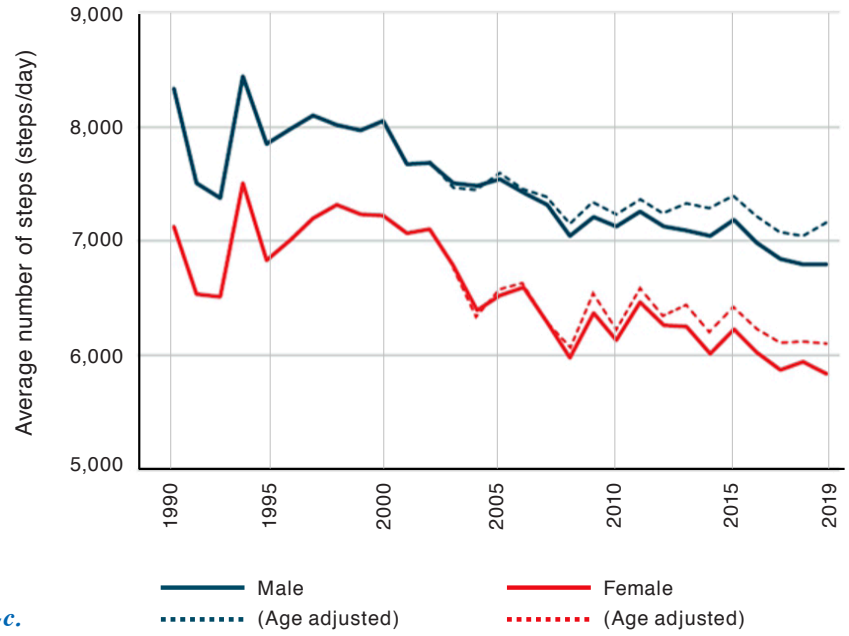

Fig. 1-c.

Fig. 1. **Nutritional and health trends in Japan.**

a) Trends in energy intake and nutrient composition (ages ≥ 1 year). Since 2008, both total energy intake and the fat-derived energy ratio have increased. **b)** Trends in average daily step counts (ages ≥ 20 years). "Healthy Japan 21 (Second Edition)" sets targets of 9,000 steps/day (men) and 8,500 steps/day (women) aged 20–64 years, and 7,000 steps/day (men) and 6,000 steps/day (women) aged ≥ 65 years. Current average step counts continue to decline and remain below targets. **c)** Trends in fat-derived energy ratio and animal-fat ratio (ages ≥ 1 year). Both the fat energy ratio and the proportion of animal fat have steadily increased. The recommended dietary fat energy ratio is 20–30% according to the "Dietary Reference Intakes for Japanese (2020 Edition)." Source: Ministry of Health, Labour and Welfare, "Changes in nutrition and health in Japan." (Ref. 1)

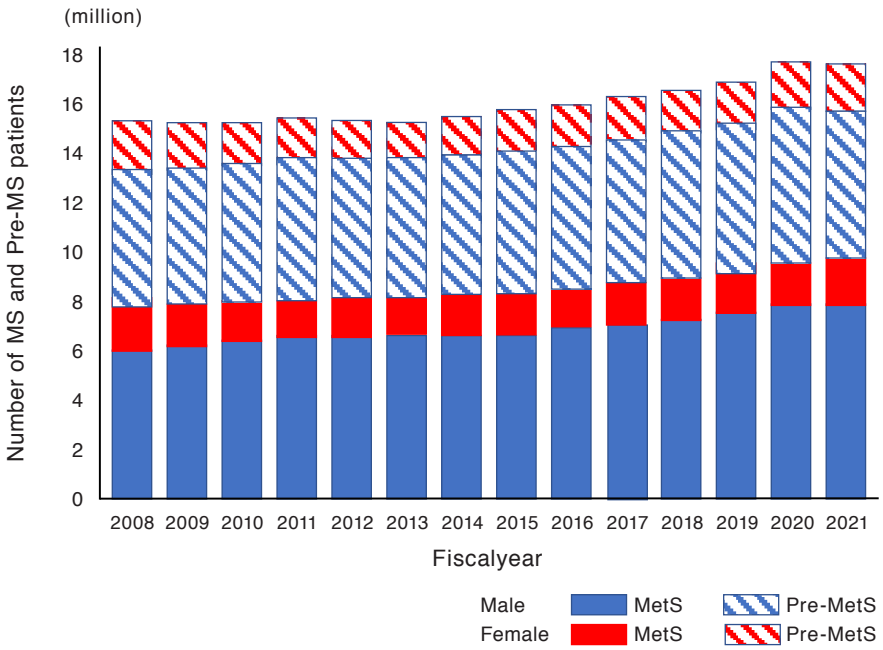

Fig. 2. **Trends in the number of individuals with metallic syndrome (including those at risk).**

Source: Compiled based on the Ministry of Health, Labor and Welfare's "Status of Specific Health Checkups and Specific Health Guidance," the Ministry of Internal Affairs and Communications' Statistics Bureau's "Population Estimates (as of October 1, 2024)," and the Dai-ichi Life Research Institute report (Reference 2). MetS, metabolic syndrome.

Consequently, intracellular and extracellular proteins are modified by aldehydes, leading to the formation of advanced glycation end-products (AGEs). Not only proteins but also lipids and genomic DNA are susceptible to aldehyde-induced damage.

GS is fundamentally a condition in which aldehydes are overproduced. Sugar-derived aldehydes include GO, MGO, 3DG, and glyceraldehyde (GA)[8]. Lipid-derived aldehydes include malondialdehyde (MDA), MGO, and acrolein[23]. Alcohol consumption and smoking additionally contribute acetaldehyde and tobacco-related aldehydes. Importantly, MGO is produced from both carbohydrates and lipids, making it a central mediator of glycation toxicity.

Thus, excessive intake of carbohydrates and lipids markedly increases GS, promoting degenerative changes throughout the body—making the phrase "sugars and fats destroy the body" quite fitting.

Dicarbonyl compounds are defined as molecules containing two carbonyl groups (C = O). This class includes aldehydes, ketones, carboxylic acids, and related derivatives. Among these, short-chain dicarbonyl aldehydes are highly reactive and particularly toxic in vivo[23]. Because major sugar-derived aldehydes (GO, MGO, 3DG) belong to this category, they are often collectively referred to as "dicarbonyl compounds" in the literature. However, since aldehydes that are not strictly dicarbonyl compounds also contribute significantly to GS-induced biological reactions, the more comprehensive term "aldehydes" is adopted in this report.

Glycative Stress (GS) and the Progression to Steatohepatitis

Steatohepatitis develops when simple fatty liver (steatosis) is accompanied by inflammation and fibrosis[24-26]. In animal studies, acetaldehyde has been identified as a major contributor to "alcoholic liver injury without steatosis", leading first to alcoholic hepatitis with inflammation, then to alcoholic liver fibrosis, and ultimately to alcoholic cirrhosis if the condition progresses further. When combined with a high-fat diet or physical inactivity, alcoholic fatty liver can develop. Similarly, it is plausible that aldehydes other than acetaldehyde may also induce inflammation, fibrosis, and cirrhosis.

We propose a hypothesis regarding the role of GS in the progression from simple steatosis to steatohepatitis (***Figs. 3, 4***). The receptor for advanced glycation end-products (RAGE), which specifically recognizes AGEs, is expressed on macrophages and Kupffer cells in the liver[27-29]. Binding of AGEs to RAGE triggers intracellular signaling pathways that promote the production and release of inflammatory cytokines[30,31], thereby contributing to hepatic inflammation.

Excessive aldehydes overload aldehyde-metabolizing enzymes, consuming nicotinamide adenine dinucleotide (NAD+) and reducing the NAD+/NADH ratio[32,33]. NAD+ depletion impairs the tricarboxylic acid (TCA) cycle and fatty acid β-oxidation, which is expected to play a critical role in the development and progression of steatohepatitis.

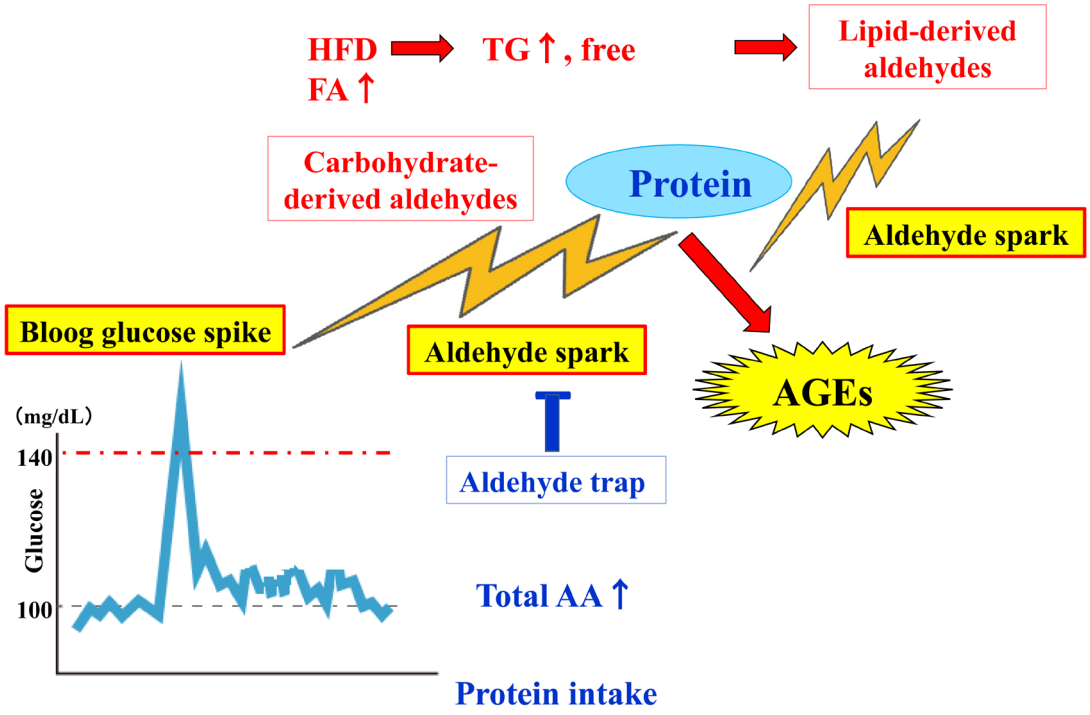

Fig. 3. **Formation of sugar-derived and lipid-derived aldehydes.**
Postprandial hyperglycemia ("blood glucose spikes") induces a chain reaction producing short-chain sugar-derived aldehydes. These aldehydes further react with free fatty acids, generating lipid-derived aldehydes ("aldehyde sparks"). Amino acids trap aldehydes and mitigate toxicity. HFD, high fat diet; TG, triglycerides; AGEs, advanced glycation endproducts; AA, amino acids; FA, fatty acids

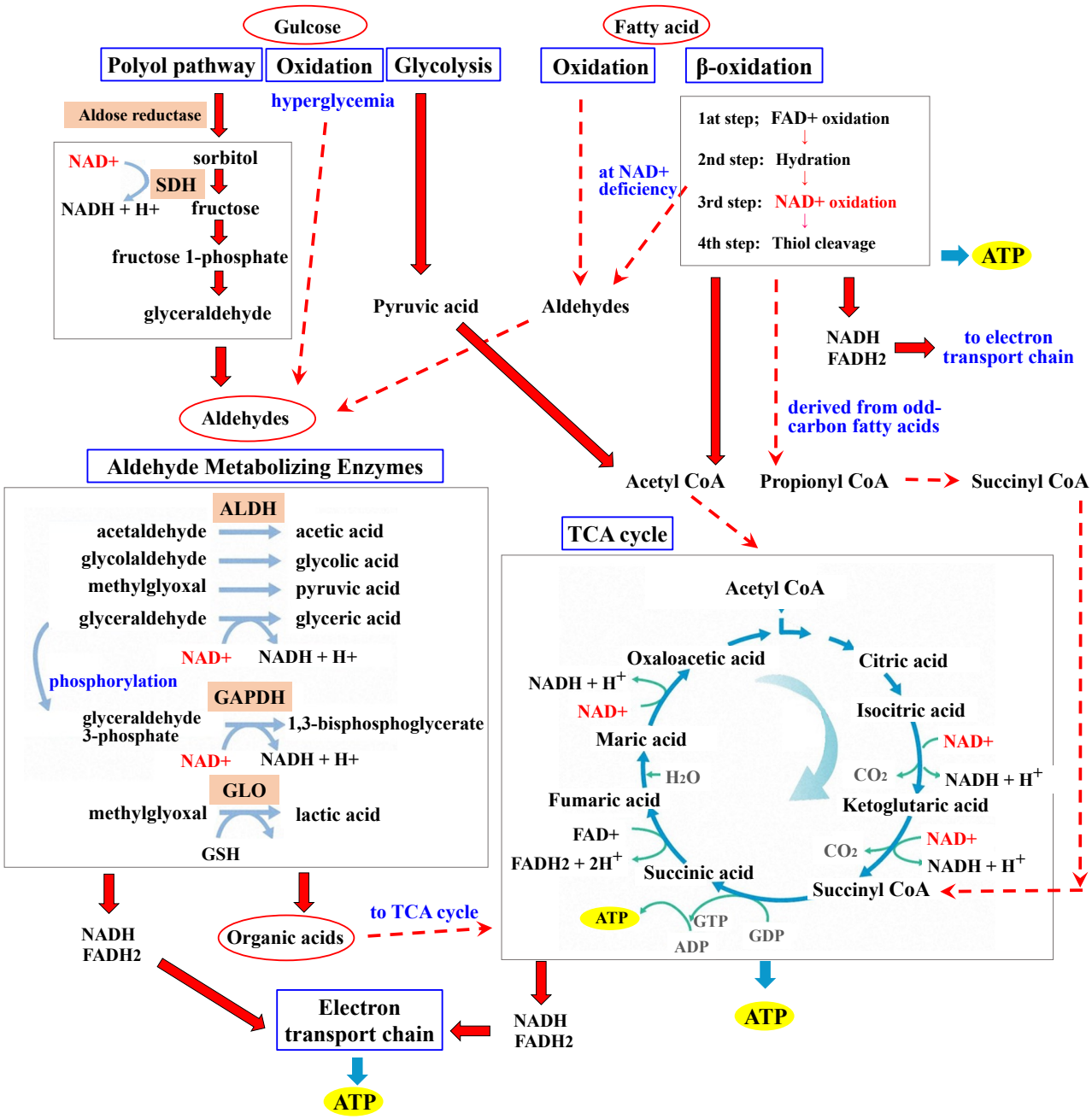

Fig. 4. **NAD+ consumption and its effects in glucose and lipid metabolism.**

Excess carbohydrates activate the polyol pathway, while fatty acid β-oxidation and glycative stress overload metabolic enzymes (ALDH, GAPDH), accelerating NAD+ consumption and decreasing the NAD+/NADH ratio. This disrupts the TCA cycle and increases the NADH burden on the electron transport chain, enhancing ROS production. NAD, nicotinamide adenine dinucleotide; NADH, reduced NAD+; FAD, flavin adenine dinucleotide; FADH2, reduced FAD; SDH, sorbitol dehydrogenase; TCA, tricarboxylic acid; ALDH, aldehyde dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLO, glyoxalase; GSH, glutathione; ATP, adenosine triphosphate; ADP; adenosine diphosphate; GTP, guanosine triphosphate; GDP, guanosine diphosphate; acetyl CoA, acetyl coenzyme A; ROS, reactive oxygen species.

NAD+ deficiency (a reduction in the NAD^+/NADH ratio) can profoundly impair the tricarboxylic acid (TCA) cycle (*Fig. 5*)[34-36]. First, reduced NAD^+ availability decreases TCA cycle efficiency, leading to lower ATP production, which may manifest clinically as fatigue. Second, impaired TCA cycle flux causes an accumulation of fumaric acid, a metabolite implicated in accelerated aging processes.

For example, succinate derived from dietary sources—such as the "umami" components in shellfish—enters the TCA cycle and is metabolized. Succinyl-CoA is also produced from β-oxidation of odd-chain fatty acids, yielding acetyl-CoA and propionyl-CoA; the latter is converted to succinyl-CoA through several enzymatic steps before entering the TCA cycle. A high-fat diet can therefore lead to increased flux through the "succinyl-CoA → succinate → fumarate → malate" segment of the cycle.

Succinate dehydrogenase, which converts succinate to fumarate, functions as Complex II in the electron transport chain. Although a reverse reaction has been reported in certain organisms, it depends on the FAD^+/FADH2 ratio rather than feedback from elevated fumarate, and thus is unlikely to occur unless under severe hypoxia or FADH2 depletion.

Fumarate is normally converted to malate by fumarase. However, the subsequent step—conversion of malate to oxaloacetate—requires NAD+; therefore, when NAD+ is depleted, malate is not efficiently metabolized[37,38]. Because the fumarase reaction is reversible, excess malate can be converted back to fumarate, resulting in marked fumarate accumulation under GS conditions.

Effects on Adiponectin

Mature adiponectin (244 amino acids) contains 16 lysine residues, 10 arginine residues, and 8 cysteine residues (with sulfhydryl groups), all of which are susceptible to glycation and related modifications. Fumaric acid, a metabolite of the TCA cycle, can react with cysteine residues to form S-(2-

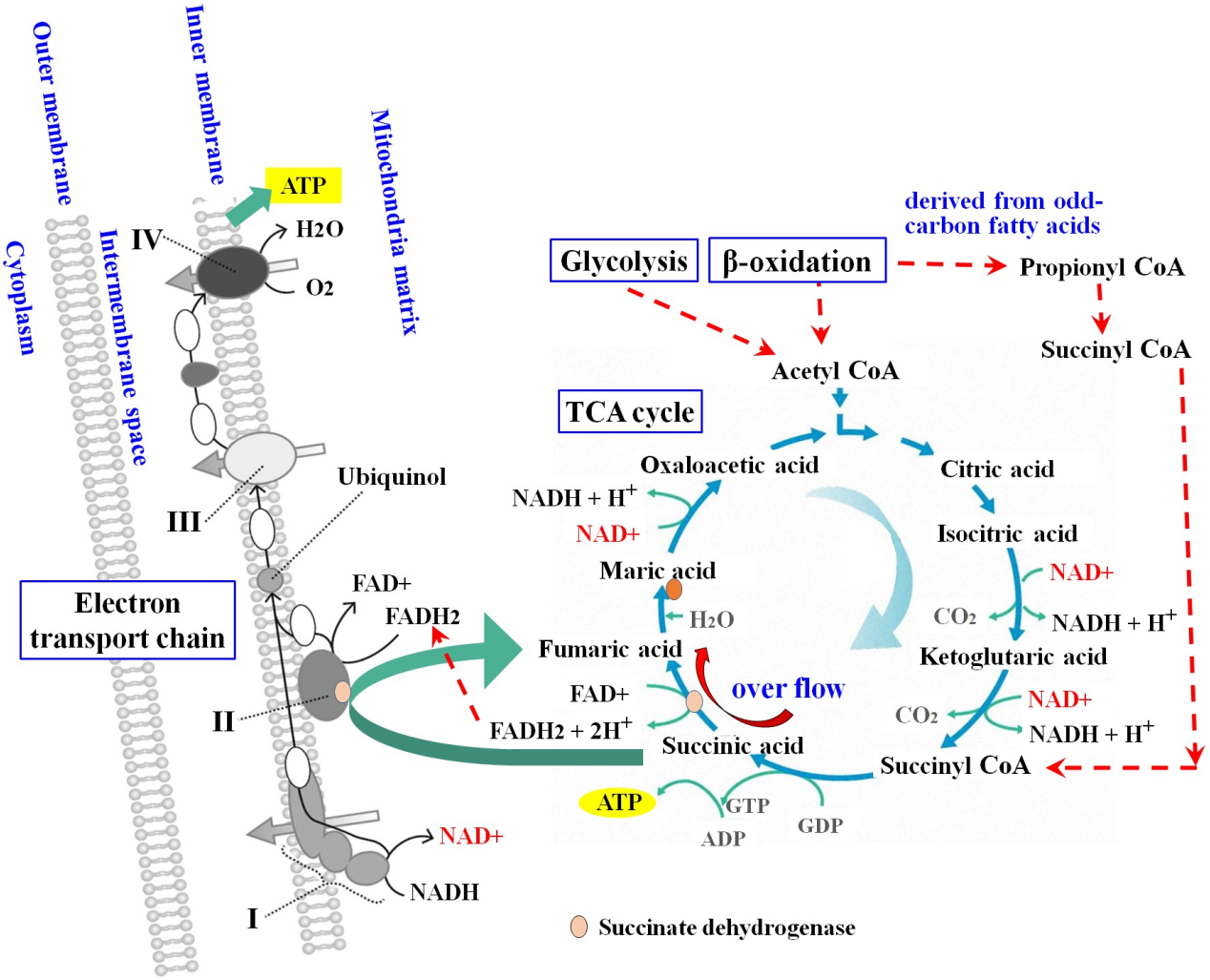

Fig. 5. **TCA cycle dysfunction and fumarate accumulation.**

Even-chain fatty acids produce acetyl-CoA, and odd-chain fatty acids produce propionyl-CoA and succinyl-CoA, which enter the TCA cycle. Succinate dehydrogenase (Complex II) in the inner mitochondrial membrane converts succinate to fumarate while producing FADH2, which donates electrons to ubiquinone. Under NAD^+ deficiency, succinate dehydrogenase remains active as long as FADH2 is available, leading to fumarate accumulation. Abbreviations as in **Fig. 4**.

succinyl)cysteine (2SC). In adipocytes, adiponectin monomers assemble into trimers and hexamers primarily through disulfide bond formation, followed by multimerization into high-molecular-weight (HMW) adiponectin (12-mer or larger), which possesses the highest biological activity and secretion efficiency.

However, when cysteine residues are modified by 2SC, low-molecular-weight forms of adiponectin (monomers and trimers) become misfolded and are targeted for degradation. As a result, the production and secretion of medium-molecular-weight (MMW) hexamers and HMW multimers (≥12-mer) are reduced (***Fig. 6***)[39]. Glycated forms of adiponectin are also expected to be recognized as abnormal and similarly degraded.

Consequently, the proportion of HMW adiponectin in the bloodstream decreases. In healthy individuals, HMW adiponectin—which is essential for insulin-sensitizing and anti-inflammatory functions—constitutes the majority of circulating adiponectin. In contrast, obese individuals and those with type 2 diabetes mellitus (T2DM) exhibit a selective reduction in the HMW fraction. Thus, a decrease in circulating HMW adiponectin is considered a potential indicator of progression from simple steatosis to steatohepatitis.

Although fatty acid β-oxidation was not addressed in our previous report[4], the NAD+-dependent oxidation step plays an essential role in this process[40, 41]. When β-oxidation is halted at the second dehydrogenation step due to NAD+ deficiency, intermediate fatty acid metabolites either accumulate or undergo oxidation to form new and different aldehydes. Accumulation of these intermediates may contribute to abnormal lipid droplet morphology and distribution in hepatocytes.

While the mechanisms of fibrosis are not yet fully understood, studies have reported that DNA damage and glutathione (GSH) depletion contribute to fibrotic progression[42-44].

MGO, a highly cytotoxic aldehyde derived from both carbohydrates and lipids, is detoxified via the glyoxalase (GLO) system—comprising GLO1 and GLO2—which converts MGO into lactate using GSH as an essential cofactor[45, 46]. Excess MGO consumption of GSH results in GSH depletion. Because GSH functions as a major antioxidant, it is also consumed during detoxification of reactive oxygen species (ROS) and free radicals. Consequently, GSH deficiency in liver tissue enhances oxidative stress, thereby contributing to the progression of steatohepatitis.

Involvement of increased fumarate in steatohepatitis

Post-translational modification analyses in adipocytes—which play an important role in MetS progression—have revealed that excess fumarate production due to mitochondrial dysfunction can react with cysteine residues to form S-(2-

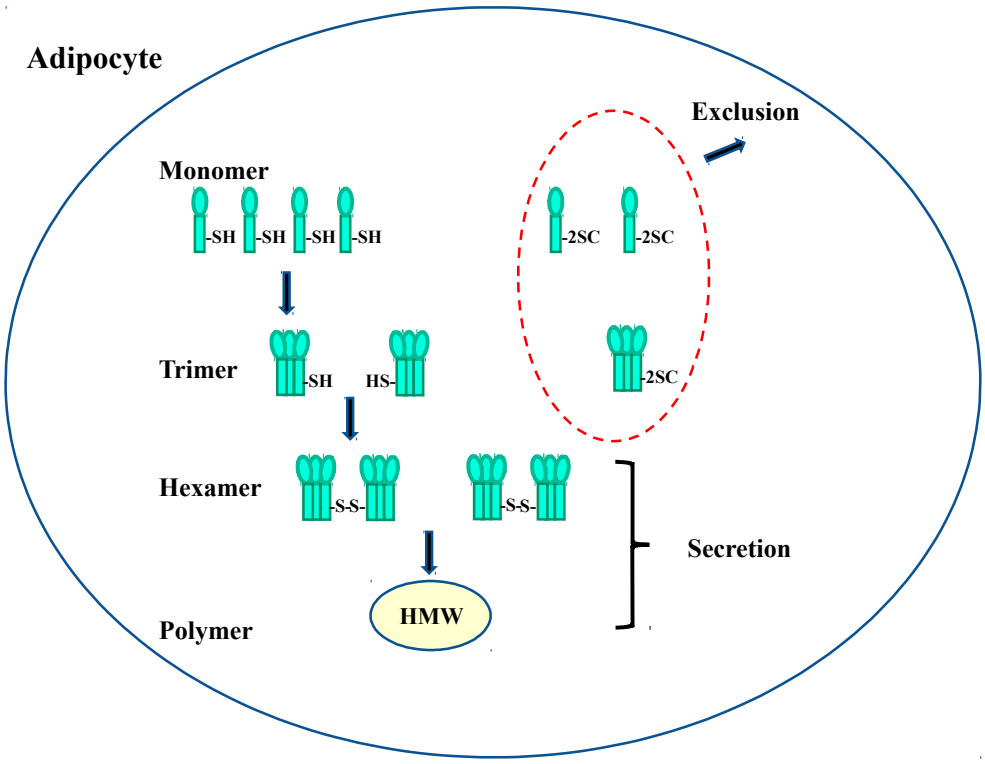

Fig. 6. **Fumarate-mediated modification of adiponectin and reduced HMW multimer secretion.**
Adiponectin monomers fold in the ER to form trimers, which assemble into hexamers and high-molecular-weight (HMW) multimers for secretion. Increased fumarate modifies cysteine residues to 2SC, preventing multimer formation and leading to impaired secretion of HMW adiponectin. Modified from Nagai R et al., J Biol Chem 2007 (Ref. 47). ER, endoplasmic reticulum; 2SC, S-(2-succinyl)cysteine; HMW, high-molecular-weight.

succinyl)cysteine (2SC), a process termed succination[47-49]. Fumarate is known to trigger immunometabolic inflammatory responses by stabilizing hypoxia-inducible factor 1α (HIF-1α) and promoting the expression of inflammatory cytokines[50]. However, because fumarate is rapidly metabolized and readily diffuses, it is difficult to use directly as a clinical biomarker.

Accumulation of 2SC-modified proteins has been observed in skeletal muscle and adipose tissue in diabetic rat models[51]. In addition, 2SC-modified proteins have been implicated in chronic airway inflammatory diseases, such as chronic obstructive pulmonary disease (COPD) and asthma, in humans[52].

Furthermore, growing evidence suggests that TCA cycle intermediate metabolites (e.g., fumarate and succinate) and mitochondrial dysfunction contribute to the progression of fatty liver to steatohepatitis. Therefore, therapeutic strategies targeting mitochondrial metabolic pathways represent an important challenge for future interventions[53].

Causes of NAD+ deficiency

A reduction in the body's capacity to synthesize NAD+ primarily results from age-related declines in the activity and expression of key biosynthetic enzymes, such as nicotinamide phosphoribosyltransferase (NAMPT) and nicotinamide mononucleotide adenylyltransferase (NMNAT)[54,55]. As NAD+ is generated from precursors including vitamin B3 (niacin), insufficient dietary intake can also contribute to reduced NAD+ levels.

Additionally, increased NAD+ consumption contributes to NAD+ deficiency. NAD+ is required for aldehyde metabolism by dehydrogenases, fatty acid β-oxidation, and glucose metabolism via the polyol pathway; these reactions convert NAD+ to NADH, thereby lowering the NAD+/NADH ratio[4,53]. Thus, strategies that alleviate glycative stress (GS care) are considered important for maintaining NAD+ homeostasis.

Previous animal studies using nicotinamide mononucleotide (NMN) have demonstrated improvements in hepatic steatosis, inflammation, and fibrosis, suggesting that NMN supplementation may help prevent the progression from simple steatosis to steatohepatitis. Multiple reports show that NMN increases hepatic NAD+ levels in experimental models of fatty liver, leading to improved lipid metabolism, restoration of mitochondrial function, and attenuation of inflammation[56-60].

For example, Shi *et al.* reported that NMN reduced hepatic lipid accumulation and improved oxidative metabolism in obese mice[56]. Li *et al.* demonstrated reduced hepatic inflammation and steatosis alongside improvements in liver function markers[57]. Hong *et al.* showed that NMN enhanced mitochondrial biogenesis and ameliorated metabolic abnormalities[58]. Yoon *et al.* reported improvements in insulin resistance, hepatic steatosis, and inflammatory markers[59]. Similarly, Long *et al.* observed restored hepatic NAD+ metabolism and improvements in high-fat diet–induced insulin resistance and fatty liver[60].

Collectively, these findings indicate that NMN supplementation exerts multifaceted protective effects on lipid accumulation, inflammation, and insulin resistance—key drivers of fatty liver onset and progression—supporting its potential to prevent the progression of ßsteatohepatitis.

Insulin resistance and glycative stress

In the early stages of T2DM, pancreatic β-cells secrete excess insulin to compensate for elevated blood glucose levels. This results in hyperinsulinemia, which temporarily maintains euglycemia. However, with chronic overeating or obesity, insulin resistance worsens, and the secretory burden placed on β-cells eventually exceeds their capacity, leading to a gradual decline in insulin output. During this process, β-cells are exposed to glycative stress (excessive aldehydes), which can impair insulin biosynthesis.

Insulin biosynthesis begins with preproinsulin, which contains an N-terminal signal peptide that directs its translocation into the endoplasmic reticulum (ER). Removal of the signal peptide converts preproinsulin into proinsulin. Within the ER, proinsulin folds into its active tertiary structure through the formation of three disulfide bonds between the A- and B-chains—an essential step for insulin's biological function. Proinsulin then transits to the Golgi apparatus and is packaged into secretory granules. In these granules, prohormone convertases PC1/3 and PC2, followed by carboxypeptidase E (CPE), cleave C-peptide to produce mature insulin (***Fig. 7***)[61,62].

Immature secretory granules contain large amounts of proinsulin, whereas the proportion of insulin increases during granule maturation; in mature granules, the majority of contents is insulin[63]. Lysine and arginine residues near proinsulin cleavage sites are particularly susceptible to glycation. When these residues are modified, proinsulin becomes resistant to peptidase cleavage, resulting in increased proinsulin retention and reduced insulin production[64]. Consequently, the number of immature secretory granules rises.

In response to glucose stimulation, β-cells release secretory granules into the bloodstream. Under normal conditions, most released granules are mature. However, severe glycative stress increases the proportion of immature granules secreted, leading to a higher proinsulin-to-insulin ratio in circulation. Reported fasting plasma proinsulin concentrations in Japanese individuals are:

- Healthy: 5.8 ± 3.3 pmol/L
- Impaired glucose tolerance: 9.5 ± 6.9 pmol/L
- T2DM: 12.6 ± 7.5 pmol/L[65]

Routine clinical assays measure immunoreactive insulin (IRI), which cross-reacts with proinsulin and therefore does not directly reflect true insulin levels. Proinsulin represents 10–20% of fasting IRI in healthy individuals but can reach ~50% in T2DM[66].

Insulin itself is also subject to glycation. In T2DM patients, ~9% of circulating IRI is glycated insulin in the fasting state, increasing to ~28% after meals[67]. Glycated insulin exhibits little to no biological activity, as it fails to effectively promote cellular glucose uptake[67,68]. An estimated time course of glucose-stimulated insulin secretion is shown in ***Fig. 8*** and ***9***. Thus, glycative stress likely plays a major role in the development of insulin resistance.

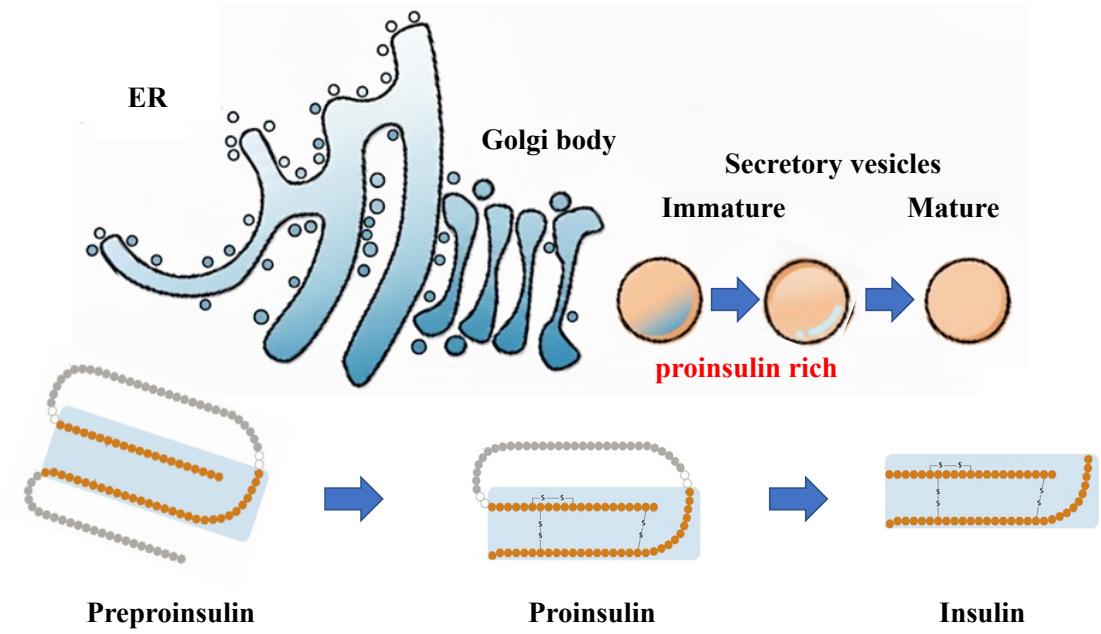

Fig. 7. **Insulin biosynthesis in pancreatic β cells.**

Under severe glycative stress, insulin undergoes aldehyde modification during biosynthesis. This modification impairs the conversion of proinsulin to insulin within secretory granules, resulting in an increased proportion of immature granules rich in proinsulin during glucose-stimulated secretion. ER, endoplasmic reticulum.

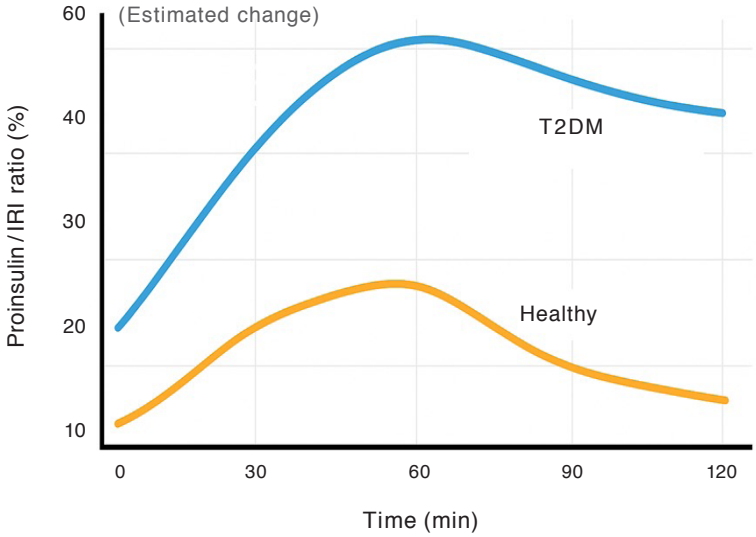

Fig. 8. **Estimated time course of glucose-stimulated insulin and proinsulin secretion.**

Based on previous reports [65-67], this graph was estimated to predict what would happen if approximately 75g of glucose was ingested. As insulin secretion approaches its peak, immature secretory granules increase, and the proportion of proinsulin increases. Even after the peak, immature secretory granules are expected to remain dominant, with proinsulin remaining at a high level in T2DM. Support was provided by ChatGPT and Gemini for the creation of this graph. T2DM, type 2 diabetes mellitus.

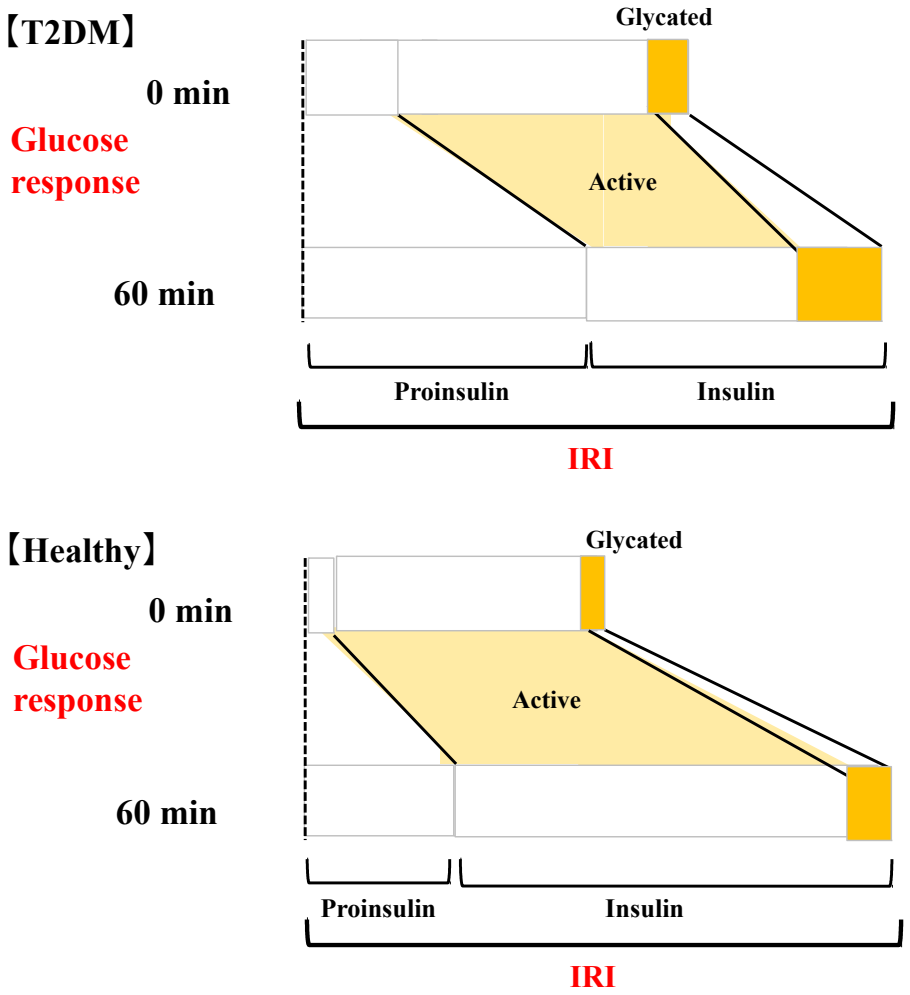

Fig. 9. **Conceptual changes in glucose-stimulated insulin secretion.**

Predictions at baseline and 60 minutes after glucose ingestion are shown. The proinsulin/IRI ratio was assumed to increase from ~20% to ~40% in T2DM and from ~10% to ~20% in healthy individuals. The glycated-insulin/insulin ratio was assumed to be 9% vs. <3%, respectively. The 60-minute IRI was expressed as the same value for both T2DM and healthy controls. In early T2DM, the glucose-stimulated response of β cells is predicted to show excessive insulin secretion in the early stages, but to show hypoinsulin secretion in the mid- to late stages. IRI, immunoreactive insulin, T2DM, type 2 diabetes mellitus.

For guidance

Regarding body shape, it is desirable to focus on physical exercise rather than excessive dietary restriction to improve body composition[69-71]. Because postprandial hyperglycemia (blood glucose spikes) induces aldehyde sparks, it is important to combine dietary education—such as chewing food thoroughly, eating slowly, and avoiding "skipping breakfast"—with adjustments to food quantity and nutritional balance[72-76]. Medications may be used as needed to correct dyslipidemia. If protein deficiency is suspected based on blood albumin levels, appropriate protein intake should be targeted. Blood amino acids have been reported to have an aldehyde-trapping effect[77-79]. Therefore, even if the peak value of blood glucose spikes is similar, individuals with higher blood total amino acid concentrations may experience reduced aldehyde sparks. Thus, for MetS prevention, care should be taken to avoid protein deficiency. For MetS treatment, an adequately high-protein diet (protein: 15–22% of total energy) is recommended, aiming for plasma total amino acid concentrations similar to those of healthy individuals (2–3 mmol/L)[80-82].

Proposed prevention method

Various approaches have been attempted to control the progression of simple fatty liver to steatohepatitis. Experimental and clinical results suggest that antioxidant administration alone cannot prevent this progression[83-85]. Here, we propose a new preventive method incorporating the latest concepts of glycative stress. Recommended numerical targets are shown in ***Table 1***.

As an indicator of glucose tolerance, we focused on suppressing postprandial hyperglycemia. It is important to keep in mind that blood glucose spikes generate aldehyde

Table 1. GS care numerical goals.

GS Care Numerical Goals	
[Body Type]	
Waist circumference:	Men: Less than 85 cm; Women: Less than 90 cm
BMI	21-24
[Blood Tests]	
Postprandial Hyperglycemia:	Less than 140 mg/dL
ΔCmax	Less than 40 mg/dL
Insulin Resistance:	
Fasting insulin (IRI)	Less than 5 μU/mL
Proinsulin/IRI ratio	Less than 15%
Adiponectin (HMW)	Men: 6 μg/mL or greater; Women: 9 μg/mL or greater
Dyslipidemia:	
TG	Less than 150 mg/dL
LDL-C	Less than 140 mg/dL
Free fatty acids (NEFA)	0.2-0.5 mmol/L
Protein Intake Goal:	
Plasma total amino acids	3.0-4.0 mmol/L

These values represent levels of free fatty acids (FFA or NEFA) transported bound to albumin in the blood. These fatty acids are released when triglycerides are hydrolyzed by lipase in adipose tissue and are used as an energy source in the liver and muscles. The targets reflect the fact that MetS patients exhibit chronically elevated NEFA concentrations (0.7–1.0 mmol/L or higher). While the general reference range for plasma total amino acids is 2.3–3.5 mmol/L, the table shows values based on the top quartile (estimated). GS, glycative stress; MetS, metabolic syndrome; BMI, body mass index; ΔCmax, increment from the preprandial blood glucose level to maximum blood glucose concentration (Cmax) after starting a meal; IRI, immunoreactive insulin; HMW, high-molecular-weight form; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; FFA, free fatty acids; NEFA, non-esterified fatty acids.

sparks, which are promoted by fatty acids and alleviated by amino acids. It is expected that insulin resistance will naturally improve with the implementation of GS care. For each target, it is advisable to consider whether it is upstream or downstream in the pathological cascade, as well as the sequence and timeline of interventions.

Controlling disease progression through the Trinity of "Physical Education," "Dietary Education," and "Intellectual Education"

I. Dietary education—A Lifestyle with Balanced Carbohydrate and Lipid Intake

Glycative stress (GS) is considered a major driver of the transition from simple steatosis to steatohepatitis. Excessive or imbalanced consumption of carbohydrates and lipids leads to aldehyde overproduction, which not only induces inflammation and fibrosis in hepatic tissue but also exacerbates key pathophysiological features related to steatohepatitis, such as reduced secretion of HMW adiponectin and increased insulin resistance.

Aldehyde metabolism via ALDH and GAPDH requires NAD+, and its increased consumption lowers the NAD+/NADH ratio. This impairs fatty acid β-oxidation, disrupts TCA cycle function, and reduces electron transport chain efficiency. A decrease in the NAD+/NADH ratio overloads the respiratory chain, thereby enhancing mitochondrial ROS production[86,87].

Elevated oxidative stress leads to excessive GSH utilization through the glyoxalase (GLO) system, ultimately causing GSH depletion. Therefore, supplementation with antioxidants alone is insufficient; NAD+ replenishment through precursors such as NMN or niacin is also necessary.

Most endogenous aldehydes originate from blood glucose spikes that trigger cascade production of sugar-derived aldehydes ("aldehyde sparks"). Elevated circulating free fatty acids due to a high-fat diet further intensify aldehyde generation. Conversely, maintaining adequate total amino acid levels through sufficient protein intake can help "trap aldehydes", thereby mitigating aldehyde sparks.

The reaction of aldehydes with amino acid residues in proteins promotes AGE formation. Certain foods contain bioactive compounds capable of inhibiting AGE production. The amount of aminoguanidine (AG)-like activity in foods is expressed as AG equivalents, and dietary recommendations focused on anti-glycation foods have been proposed[88].

The foundation of prevention lies in appropriate caloric intake and nutritional balance. For an adult with a standard body weight of 60 kg, a daily energy intake of approximately 2,100 kcal is recommended, with the following macronutrient distribution:

- Protein: 15–22%
  Diversify sources beyond meat (e.g., seafood, soy products, nuts, dairy); reduce animal fat.
- Fat: 25–30%,
  Increase polyunsaturated fatty acids, including marine-derived omega-3 fatty acids.
- Carbohydrates: 55–60%
  Increase dietary fiber and whole grains; limit sugar-sweetened beverages; calories from alcohol should also be counted as carbohydrate-related intake.

Dietary recommendations based on Global Burden of Disease (GBD) epidemiological data suggest intake levels that minimize excess mortality risk (***Table 2***)[89].

Table 2. Proportion of excess mortality related to diet: Results of the GBD epidemiological survey.

Risky Foods	Recommended intake	Excess mortality rate (estimated %: Japan)			
Risks of inadequate intake		Cardiovascular	Cancer	T2DM	Total
Fruits	200 ～ 200 g/day	3.6	1.2	6.0	1.4
Vegetables	280 ～ 320 g/day	0.9	0.1		0.2
Legumes	90 ～ 100 g/day	2.4			0.6
Whole grains	140 ～ 160 g/day	5.6	2.1	4.6	2.2
Nuts & seeds	10 ～ 19 g/day	3.2		2.8	0.8
Milk	360 ～ 500 g/day		2.4		0.8
Calcium	1.06 ～ 1.1 g/day		2.0		0.6
Dietary fiber	21 ～ 22 g/day	2.7	0.3	3.0	0.8
PUFA	7 ～ 9 % of total energy intake	0.9			0.2
Ω-3 Fatty acids	430 ～ 470 mg/day	1.4			0.4
Risks of excessive intake					
Sodium	1 ～ 6 g/day, 24 hour urine	7.9	1.1		2.7

Both men and women, ages 25 and older, 2019 estimates. GBD, Global Burden of Disease. Source: Shoichiro Tsugane. What is a diet that contributes to maintaining health? Current evidence. Food and Science 2022. Reference 89.

To prevent postprandial glucose spikes, individuals should chew thoroughly, eat slowly, and avoid "skipping breakfast". In addition, minimizing alcohol consumption and eliminating smoking are strongly advised, as these behaviors increase aldehyde burden.

II. Intellectual education — Cognitive Support for Motivation and Behavioral Change

Motivation-driven behavioral change is essential for sustaining lifestyle improvements. Accordingly, motivational support constitutes a central pillar of this trinity. The most significant threats to motivation are mental and physical stress; thus, ensuring adequate rest and sleep is crucial. Successful intervention requires healthcare professionals to support patients and build trusting relationships. Helping individuals understand their disease trajectory and visualize future health outcomes can be particularly effective [90-92].

People who are dependent on animal fats may crave them more and resist exercise—possibly due to neurobiological changes rather than simple willpower. Importantly, animal fat preference is reinforced by neural adaptations within the brain's reward and metabolic pathways [93-95].

Saturated fatty acids strongly activate the dopaminergic reward system (VTA–NAc–PFC pathway), reinforcing eating behavior [96, 97]. Chronic excessive intake reduces dopamine D2 receptor sensitivity, raising "reward thresholds", which promotes further intake and facilitates animal fat addiction [98, 99].

Progressive leptin resistance and insulin resistance further disrupt satiety signaling, leading to overeating, obesity, and fatty liver disease [100-102]. Therefore, difficulties in adhering to dietary guidance or engaging in physical activity should be recognized as biologically driven consequences of "reward system dysregulation" rather than a lack of willpower.

Pathway of the vicious cycle in the metabolic reward system

Step Mechanism

High-fat diet → Dopamine release → Pleasure and reinforcement

Excess dietary fat → Increased NAD^+ consumption → Impaired β-oxidation → Fat accumulation

Hepatic fat accumulation → Production of lipid-derived aldehydes → Oxidative & glycative stress

Inflammation & insulin resistance → Reward system sensitization → Increased food-seeking behavior

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Practical treatment and prevention measures

Improve dietary composition (increase plant-based fats and omega-3 fatty acids)

Reset dopaminergic signaling through exercise (enhance BDNF expression and restore D+ receptor sensitivity)

Supplement NAD+ precursors (e.g., niacin, NMN)

Supplement antioxidant and anti-glycation nutrients (e.g., GSH, vitamins C and E)

Utilize γ-oryzanol from rice bran oil (supports lipid metabolism and autonomic regulation)

Adopt dietary strategies that suppress AGE formation (anti-glycation recipes)

III. Physical Education - Exercise: breaking the "Vicious Cycle" by moving your muscles

Exercise is an essential therapeutic stimulus for resetting abnormal dopamine metabolism in the metabolic reward system. Exercise restores plasticity in mesolimbic dopaminergic neurons, primarily in the ventral tegmental area (VTA)–nucleus accumbens (NAc) pathway, thereby reactivating the reward system [103, 104].

Moderate-intensity aerobic exercise promotes brain-derived neurotrophic factor (BDNF) expression and induces remodeling of dopaminergic neuronal dendrites in the VTA and NAc[105,106]. BDNF supports synapse formation, reduces hypersensitivity of reward circuits, and helps improve lipid-preferring behavior and compulsive eating[107,108].

Exercise-enhanced dopamine release restores dopamine D2 receptor sensitivity and normalizes the elevated reward threshold caused by a high-fat diet or excess sugar intake[104–106]. This process involves improving mitochondrial metabolism, correcting the NAD+/NADH ratiso, and activating the AMPK and PGC-1α pathways. Exercise is not simply a means of burning calories; it is a physiological readjustment that re-trains the metabolic reward system.

On the other hand, delayed exercise intervention allows the progression of glycative and oxidative modification of muscle proteins, resulting in reduced muscle contractile force due to stiffening of myosin and actin via abnormal cross-links[109-111]. This leads to the progression of "dynapenia" (muscle weakness), reducing the metabolic benefits of exercise[111-114].

Declining muscle function exacerbates insulin resistance, further increasing glycative stress, creating a vicious cycle known as "exercise resistance". The accumulation of AGEs in muscles causes mitochondrial dysfunction and impairs calcium handling, inhibiting muscle regeneration.

To prevent this progression, early adoption of moderate-intensity, sustained exercise (30–40 minutes/day, about 5 times/week) is recommended[69,115]. Such exercise enhances the secretion of BDNF[105,116] and irisin[117,118] and optimizes metabolic signaling to the liver and brain via muscle-derived myokines[116,119].

Additionally, the anti-glycation and antioxidant response (activation of the Nrf2 pathway) associated with exercise helps alleviate glycative stress, which underlies the progression of steatohepatitis.

Therefore, preventing steatohepatitis requires more than dietary restriction; it necessitates a comprehensive approach through a trinity of nutritional, intellectual, and physical education. Understanding glycative stress and maintaining a balanced relationship among sugar, fat, exercise, and cognition is key to preserving liver and brain health and supporting healthy longevity.

The existence of a vicious cycle and its control

There is a vicious cycle that accelerates the progression from simple fatty liver to steatohepatitis. Understanding this vicious cycle is essential for developing strategies to interrupt it. We propose that focusing on aldehydes—previously overlooked as merely contributors to "ROS production"—can lead to significant advances in pathophysiological understanding.

I. Fat Accumulation → Lipotoxicity → Hepatocellular Injury → Inflammation → Fibrosis

When excess triglycerides and free fatty acids accumulate in the liver, oxidative degradation and disposal cannot keep pace, resulting in the production of lipotoxic metabolites (*e.g.*, diacylglycerol, ceramide)[120-123]. This induces mitochondrial stress, ROS production, and cell injury (apoptosis/necroptosis), triggering inflammatory signaling from hepatocytes. ROS increase the production of sugar- and lipid-derived aldehydes, which promote inflammation via AGEs → RAGE → inflammatory cytokine production[30,31].

In parallel, a decrease in the NAD+/NADH ratio impairs the TCA cycle and drives fumarate accumulation, which sustains chronic inflammation[34-36]. Inflammation subsequently activates hepatic stellate cells (HSCs), resulting in excessive extracellular matrix deposition, including collagen[124-126]. These processes lead to liver fibrosis, which further deteriorates metabolic function.

Additionally, aldehydes play a major role in alcoholic liver fibrosis[127-130]. Pure steatohepatitis in patients who completely abstain from alcohol appears to be relatively uncommon in clinical practice[131-134].

II. Insulin resistance/Metabolic dysfunction

Obesity, increased visceral fat, and insulin resistance promote hepatic fat accumulation through enhanced fatty acid influx, upregulated *de novo* lipogenesis, and accelerated adipose lipolysis[135-138]. Accumulated fat and free fatty acids increase glycative stress, impairing insulin responsiveness and glucose metabolism in hepatocytes, which further exacerbates insulin resistance[139]. One major cause of insulin resistance is disrupted insulin biosynthesis due to aldehyde exposure during β-cell function[140-142]. A reduced NAD+/NADH ratio impairs β-oxidation, leading to accumulation of dysfunctional lipids in the liver[143-145].

Collectively, these metabolic abnormalities form a vicious cycle that reinforces hepatic fat accumulation.

III. Gut-Liver Axis/Visceral Fat-Liver Axis

Visceral adipose tissue exhibits chronic low-grade inflammation (adipokine imbalance, macrophage infiltration), sending inflammatory signals to the liver[146-148].

Intestinal barrier damage and dysbiosis allow endotoxin (LPS) translocation into the bloodstream, stimulating Kupffer cells and enhancing intrahepatic inflammation[149-151]. Inflammation disrupts hepatic lipid metabolism, accelerating steatosis and fibrosis progression.

Glycative stress elevates tissue AGE levels, activating RAGE and amplifying cytokine production[152-156]. Elevated fumarate drives chronic inflammation[157-161]. Aldehydes also contribute to intestinal barrier disruption, promoting "leaky gut"[162-166].

IV. Mitochondria/Oxidative stress → Hepatocellular injury → Fibrosis

Excessive fatty acid and carbohydrate intake increases the metabolic load on hepatic mitochondria, leading to electron leakage from the respiratory chain and enhanced ROS generation[144,167-170]. ROS induce lipid peroxidation, producing aldehydes and causing DNA damage, ER stress, and hepatocyte death. Increased aldehyde metabolic load via dehydrogenases reduces the NAD+/NADH ratio[127,171-174], further impairing the TCA cycle and oxidative phosphorylation. When electrons overload complexes I–IV, excess electrons react with oxygen, elevating ROS generation, especially at complexes I and III[86,175-178]. These pathological loops perpetuate mitochondrial damage, fibrosis, and metabolic failure.

Practical treatment and prevention measures

Methods to interrupt this vicious cycle are needed. The vicious cycle driven by glycative stress is reinforced by molecular "oxidative and glycative reactions", in combination with adverse lifestyle and metabolic factors. Therefore, a multi-layered intervention strategy incorporating biochemical, nutritional, and behavioral approaches is required to disrupt disease progression. The main interventions and supporting evidence are summarized below.

Integrated intervention is required to break the vicious cycle of fatty liver progression. Evidence indicates that moderate weight loss, improvements in insulin resistance, and restoration of healthy liver metabolism are key therapeutic goals.

1. Exercise intervention

Aerobic and resistance exercise significantly reduce visceral fat, liver fat content, and insulin resistance[179-183]. Frequent physical activity (reducing prolonged sitting) further supports metabolic improvement. Importantly, such interventions should be initiated early, before "exercise resistance" develops.

2. Weight management

A moderate 5–10% weight reduction—not excessive dieting—improves hepatic steatosis and slows inflammation and fibrosis progression[184-188]. Combining balanced calorie adjustment with physical activity enhances liver function outcomes.

3. Improvement of dietary patterns

Optimizing the quality of lipid intake (reducing animal fats, increasing unsaturated/omega-3 fatty acids) and improving carbohydrate sources (limiting sweetened beverages, increasing whole grains and fiber) reduces fatty liver risk[189-193]. Alcohol consumption should be minimized, and alcohol calories counted as carbohydrate-related energy intake.

4. Correction of insulin resistance and metabolic dysfunction

Metabolic disorders (T2DM/MetS) are strong risk factors for progression to steatohepatitis. Appropriate pharmacological intervention should be combined with lifestyle modification to optimize glucose tolerance, lipid profiles, amino acid levels, and waist circumference[194-197].

5. Modulation of the gut–liver axis and fibrosis

Dysbiosis may increase the risk of steatohepatitis progression. Interventions including probiotics, prebiotics, and lipid metabolism-modifying drugs have been proposed as supportive strategies[151,198-201].

6. Regular monitoring and early intervention

Early identification of high-risk individuals (obesity, impaired glucose tolerance, dyslipidemia, visceral fat accumulation, markers of glycative stress) and timely follow-up of liver enzymes, liver fat content, and fibrosis indicators are essential[194,202-204].

Medication for steatohepatitis

Since steatohepatitis is a "metabolic disease of the liver", treatment requires lifestyle modification and pharmacotherapy to correct metabolic dysfunction. If steatohepatitis fails to improve despite appropriate lifestyle intervention, drug therapy should be used in combination.

Table 3 summarizes the current approval and clinical trial status of drugs for the treatment of steatohepatitis (MASLD/MASH)[205-211]. While no drugs have yet been approved for MASH in Japan as 2025, GLP-1 receptor agonists and dual GIP/GLP-1 receptor agonists—already approved for obesity and diabetes—have shown clinically meaningful benefits in improving steatohepatitis. These drugs are particularly promising for preventing and improving liver fibrosis.

The recommended strategy is to combine pharmacotherapy with lifestyle modification and metabolic correction. Internationally, resmetirom was approved by the US Food and Drug Administration (FDA) in 2024 as the first therapy for MASH, and regulatory procedures are underway in Japan.

Table 3. Development status of drugs for treating steatohepatitis (MASLD/MASH).

Drug Class/Name	Main Effects and Characteristics	Overseas Approval/ Clinical Trial Stage	Key Literature
GLP-1 receptor agonist (semaglutide)	Reduces liver fat and inflammation through appetite suppression, weight loss, and improved insulin sensitivity.	Improvement of MASH was confirmed in a Phase 3 study (ESSENCE trial). Applications for MASH indication are currently being submitted in the US and Europe.	Sanyal AJ, et al. N Engl J Med. 2025.
GIP/GLP-1 dual receptor agonist (tirzepatide)	The combined effects of GIP and GLP-1 have been shown to significantly improve weight loss and metabolism.	Efficacy confirmed in the SYNERGY-NASH trial (2024). Phase 3 completed, currently under review by the US FDA.	Loomba R, et al. N Engl J Med. 2024.

SGLT2 inhibitors (e.g., empagliflozin, dapagliflozin)	Promotes urinary glucose excretion, resulting in weight loss, liver fat reduction, and improved insulin resistance.	Numerous Phase 2 trials have reported improvements in liver fat and ALT. Global trials are ongoing.	Cho KY, et al. J Diabetes Investig. 2021.
Thiazolidinedione (pioglitazone)	Improves insulin resistance and has anti-inflammatory effects.	Multiple RCTs have reported improvement in NASH tissue structure. Overseas, it is sometimes recommended for patients with metabolic disorders.	Sumida Y, et al. J Gastroenterol. 2018.
Vitamin E (tocopherol)	Reduces hepatocellular damage through antioxidant effects.	Effective for non-diabetic NASH in the PIVENS trial. Limited recommendation in international guidelines.	Sanyal AJ, et al. N Engl J Med. 2010.
THR-β agonist (resmetirom)	Promotes hepatic fatty acid oxidation via thyroid hormone receptor β.	Approved by the US FDA in 2024 as the world's first treatment for MASH. Application preparations are underway in Japan.	Harrison SA, et al. N Engl J Med. 2024.
FXR agonist (obeticholic acid/OCA)	Improves bile acid metabolism and has anti-fibrotic effects.	An international Phase 3 trial (REGENERATE) reported a trend toward improved fibrosis. Approval was postponed due to side effects (pruritus, elevated LDL-C).	Sanyal AJ, et al. J Hepatol. 2023.

GLP-1, glucagon-like peptide-1; GIP, gastric inhibitory polypeptide ; MASLD, metabolic dysfunction-associated steatotic liver disease ; MASH, metabolic dysfunction-associated steatohepatitis ; NASH, non-alcoholic steatohepatitis ; FXR, farnesoid X receptor; RCT, randomized controlled trial; OCA, obeticholic acid, activating FXR ; SGLT, sodium-glucose co-transporter; ALT, alanine aminotransferase; LDL-C, low-density lipoprotein cholesterol .

Conclusion

Steatohepatitis progression is closely associated with elevated glycative stress, driven by excessive and inadequate sugar and lipid intake and insufficient physical activity. The resulting aldehyde overload and NAD+ deficiency disrupt mitochondrial metabolism, inflammation control, and insulin sensitivity—constituting a central mechanism underlying disease advancement.

Based on this pathophysiological insight, we propose a comprehensive “GS care” strategy that integrates nutritional, physical, and cognitive interventions. This framework has the potential to enhance the therapeutic effectiveness of pharmacologic treatment and to broaden preventive approaches against steatohepatitis.

Future clinical studies are warranted to validate “GS care” as a targetable axis for preventing progression to fibrosis and improving metabolic health outcomes in affected populations.

Acknowledgments

This study was supported by the Isyoku-Dogen Research Foundation, a public interest incorporated foundation.

Conflict of Interest Declaration

None applicable.

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