Glycative Stress Research

Online edition : ISSN 2188-3610
Print edition : ISSN 2188-3602
Received : September 30, 2023
Accepted : October 20, 2023
Published online : December 31, 2023
doi:10.24659/gsr.10.4_145

Original article

Glycative stress: Molecular impacts and defense mechanisms

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***Abstract*:** Glycative stress has drawn attention in recent years as a factor that significantly affects tissues and organs of the human body and is directly related to health maintenance and aging-related diseases. Glycative stress refers to the excessive formations of carbohydrate-derived aldehydes or fatty-acid-derived aldehydes in the body. These aldehydes impact diverse substances such as proteins, lipids, and bases in cells, and subcellular organelles. Proteins in the body are modified by non-enzymatic procedure and abnormal proteins, *i.e.*, carbonylated proteins, advanced glycation end-products (AGEs), are formed and stored intra- and extra-cellularly. AGEs induce endoplasmic reticulum stress, causing the deterioration of cellular functions Although glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glyoxalase, and aldehyde dehydrogenase (ALDH) are prepared for protection in the body as a defensive measure against aldehydes, a co-enzyme, nicotinamide adenine dinucleotide (NAD) is consumed to a large extent through metabolic process. As a result, a tricarboxylic acid cycle (TCA cycle) in mitochondria, where NAD is needed, is unable to function smoothly. Fumaric acid is elevated and important proteins such as GAPDH and adiponectin undergo succinylation, which causes the deterioration of functional proteins. The present study reviewed the involvement of glycative stress in the insulin secretion of pancreatic β cell and Alzheimer's dementia (AD). For AD protection, it is important to inhibit peroxidation of lipids which exist abundantly in the brain and inhibit glycative modification of amyloid β (Aβ) and tau proteins. We hope that further extensive research on anti-glycation will be undertaken, and deeper understanding and increased participation will be encouraged. Studies on glycative stress are necessary to lead to a social implementation as a practical science.

***KEY WORDS*:** glycative stress; carbohydrate-derived aldehydes; fatty-acid-derived aldehydes; nicotinamide adenine dinucleotide (NAD); glyceraldehyde-3-phosphate dehydrogenase (GAPDH); insulin resistance; Aβ clearance; melatonin; exercise resistance

1. Introduction

Glycative stress is a condition in which carbohydrate-derived aldehydes and fatty-acid-derived aldehydes are formed in excess in the body: the former is derived from reducing sugar and alcohols and the latter from lipids. When these aldehydes react to proteins, carbonylated proteins and advanced glycation end-products (AGEs) are formed within organisms, and further, bind with RAGE (receptor for AGEs). Consequently, proinflammatory cytokine is produced, and degenerative changes and tissue disorders are caused [1-3]. The present study reviewed impacts of glycative stress at the molecular level and defensive mechanisms against it.

2. Impacts of glycative stress

2.1. Reason and mechanism

Major causative factors for excessive formation of aldehyde are i) glucose spikes (associated with diabetes, impaired glucose tolerance and insulin resistance), ii) high fat diet (HFD), and iii) a high rate of alcohol consumption. Reducing sugars such as glucose and fructose have cyclic structures with 99% or higher ratio. Some part of reducing sugar is in a ring opening with a straight-chain structure. Aldehyde group (-CHO) and keto group (-C=O) are exposed. As a result, reactivities are elevated. A rapid rise in blood glucose (glucose spike) triggers a chain reaction that simultaneously produces a variety of aldehydes [4-6]. We have termed this phenomenon "aldehyde spark.

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Aldehydes are highly reactive and undergo chemical reactions readily with proteins, lipids, and bases. Structural changes and functional changes are induced. Alcohol-consumption-induced acetaldehyde (AA), postprandial-hyperglycemia-induced glyceraldehyde (GA), 3-deoxyglucosone (3DG), glyoxal (GO), and methylglyoxal (MGO) are carbohydrate-derived aldehydes[7]. Fatty-acid-derived aldehydes, which are caused by the oxidation of lipids, are malondialdehyde (MDA), hydroxynonenal (HNE), acrolein (Acro), and methylglyoxal (MGO). MGO is produced from both carbohydrate and fatty acid. Findings indicate that in organs with high lipid composition, fatty-acid-derived aldehydes are deeply related to pathological mechanisms. Impacts of glycative stress at the molecular level are shown in ***Fig. 1***.

2.2. *High fat diet (HFD) and fatty-acid-derived aldehyde*

In experiments of mice fed a HFD, a fatty liver was generated and oxidative stress induced the increase of lipid peroxidation and the decrease of cysteine[8]. Furthermore, another important issue is the decline of the protein level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the liver. GAPDH in the glycolysis system provides another phosphate group to glyceraldehyde-3-phosphate (G3P). Decreased activities of GAPDH promote the increase of GA an MGO in the blood[7]. When lipids are oxidized due to oxidation stress to form lipid peroxide, the formation of fatty-acid-derived aldehydes is caused simultaneously[9-11]. Aldehydes pass through cell membranes easily, transfer into hepatic cells and further, permeate through mitochondrial inner membranes. Abnormal proteins, which undergo non-physiological post-translational modifications of proteins lead to carbonylation, AGE modification, and succinylation (the formation of S-(2-succinyl) cysteine), resulting in an increase in abnormal proteins[12-14]. Therefore, endoplasmic reticulum (ER) stress increases. When AGEs bind with RAGE, which express in Kupffer cells and microphages, AGE/RAGE signals are activated to form proinflammatory cytokine and cause inflammatory responses[1]. Increased diverse factors include proinflammatory cytokine (IL-1 [interleukin 1], IL-6 and TNF-α [tumor necrosis factor-α]), chemokine (MCP-1[monocyte chemoattractant protein 1]), cell adhesion molecule, and matrix metalloproteinase[15-16]. That is, when inflammation is added to a non-alcoholic fatty liver disease, this can be a potential trigger for progression to steatohepatitis.

It has been verified that not all molecular entities of AGE ligands bind with RAGE. N^{ε}-(carboxymethyl) lysine

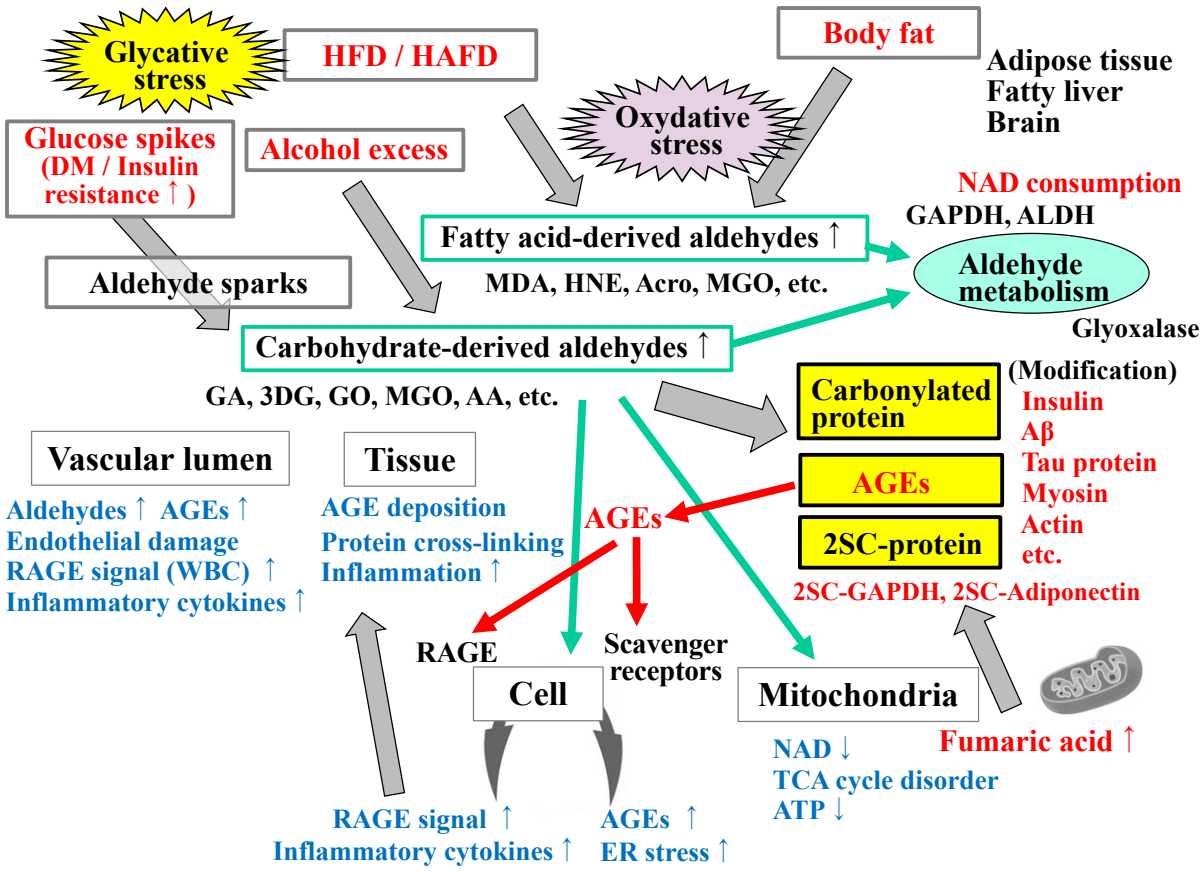

Fig. 1. **Impacts of glycative stress.**

HFD, high fat diet; HAFD, high animal-fat diet; DM. diabetes mellitus; NAD, nicotinamide adenine dinucleotide; GA, glyceraldehyde; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ALDH, aldehyde dehydrogenase; MDA, malondialdehyde; HNE, hydroxynonenal; Acro, acrolein; MGO, methylglyoxal; 3DG, 3-deoxyglucosone; GO, glyoxal; AA, acetaldehyde; AGEs, advanced glycation endproducts; RAGE, Receptor for AGEs; WBC, white blood cell; Aβ, amyloid β; 2SC,S-(2-succinyl)cysteine; ER, endoplasmic reticulum; TCA, tricarboxylic acid.

(CML), GA, and AGEs that are modified by glycolaldehyde can bind with RAGE [17].

Among HFD, excessive consumption of animal fat due to a high animal-fat diet (HAFD) is a considerable concern nowadays from the viewpoint of addiction. HAFD attenuates the function of leptin, an adipocyte-derived hormone. Leptin resistance is induced, and the body constitution is changed to have a difficulty in losing weight [18-21]. The arcuate-nucleus located in the appetite center, the hypothalamus, is considered a major site for the regulation of homeostasis of appetite, which hormones and the autonomous nervous system are responsible for. This is called the metabolic/hunger control system. HAFD causes an inflammation of the hypothalamus as well as ER stress, inducing the disorder of the metabolic/hunger control system. Therefore, the judgement of the brain is impaired regarding caloric requirement [19]. The body not only craves animal fat but also become lazy, detesting exercise.

In HAFD-fed mice, inflammatory activated microglia infiltrate the hypothalamus within a short period of time, causing progressive brain damage and leukocyte migration, eventually leading to a chronic inflammatory state [20]. We speculate that lipids abundant in brain tissue, once oxidized, may enhance fatty-acid-derived aldehyde production, resulting in increased susceptibility to protein modification, which in turn may induce ER stress in brain neurons.

3. Molecular impacts of glycative stress

3.1. Molecular impacts of glycative stress on insulin

In pancreatic β cells, insulin is synthesized via pre-proinsulin and proinsulin (*Fig. 2*) [3]. Proinsulin is cleaved due to the function of proprotein convertase at the ends of Arg 31–Arg 32, which connect insulin B-chain and C peptide, as well as at the ends of Lys 64–Arg 65, which connect C peptide and A-chain. These basic amino acids (Arg and Lys) are removed due to the function of carboxypeptidase E. Consequently, insulin and C peptide are produced. During elevated glycative stress, short-chain aldehydes in the blood increase and transfer into β cells to attack proinsulin and form carbonylated proinsulin. Arg and Lys have two amino bases ($-NH_2$) are susceptible to carbonylation modification resulting in a conformational change at the cleavage sites that causes resistance against carboxypeptidase E, resulting in a decrease in insulin formation [22-27]. Some of the insulin stored in β cells undergo modifications to be glycated insulin: N-terminus (Phe), Arg and Lys residues and amino bases ($-NH_2$). Glycated insulin is secreted due to a glucose stimulation as (non-glycated) insulin is secreted [27].

IRI includes insulin (glycation/non-glycation) and proinsulin (glycation/non-glycation). Healthy persons have 0.05–0.4 ng/mL of proinsulin in fasting time, and ratios to IRI values are 5–48% [28]. In diabetic patients, it has been reported that 9% of insulin is glycated insulin [25]. When glycative stress is accelerated, compositional ratios are altered. Ratios of glycated insulin increase and that of active insulin decrease. However, decreased glycative stress via exercise loading leads to the decrease of glycated proinsulin and glycated insulin in β cells. As a result, it is suggested that the formation of insulin increases, the compositional ratio of glycated insulin decreases, and that of insulin increases. *Fig. 3* shows an assumed schematic diagram where IRI changes when glycative stress is accelerated and improved [29]. Through physical exercise, proinsulin ratios in IRI decline and IRI values also decline. Ratios of glycative modifications in proinsulin and insulin decrease. As a result, it is assumed that even though IRI values decrease, insulin activities increase and therefore, insulin resistances improve. To prove this hypothesis, measurements in IRI are required regarding insulin and proinsulin, and with or without glycation. This is a further task and challenge.

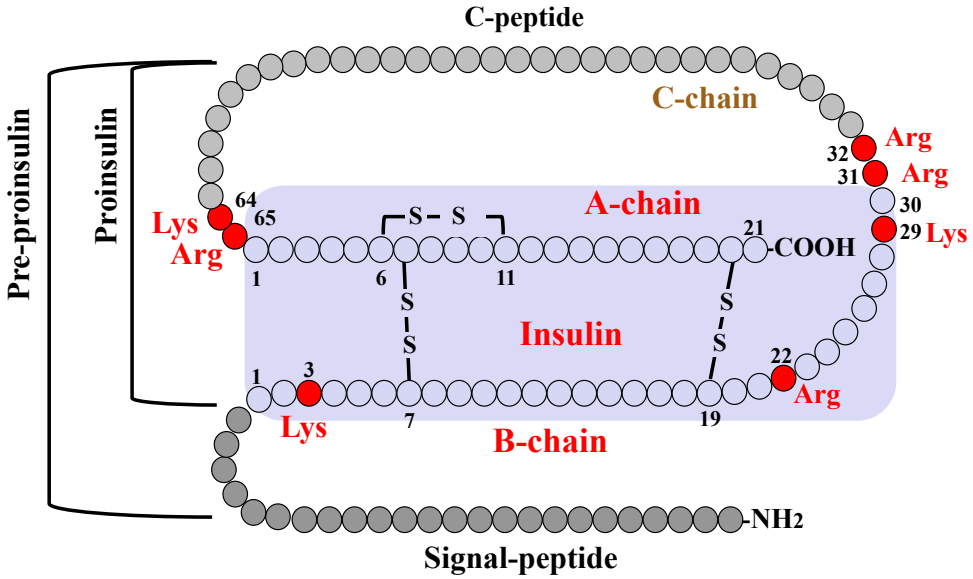

Fig. 2. **Insulin synthesis in pancreatic β cells.**

The figure quoted from Reference 3. The values of IRI (immunoreactive insulin), which we usually use in clinical sites, are measured using antigenicity against specific parts of insulin peptide. Immunological cross-reactivity is shown. There are cross-reactions in proinsulin and intermediate products in biosynthesis systems, and the obtained measurement values do not represent only insulin.

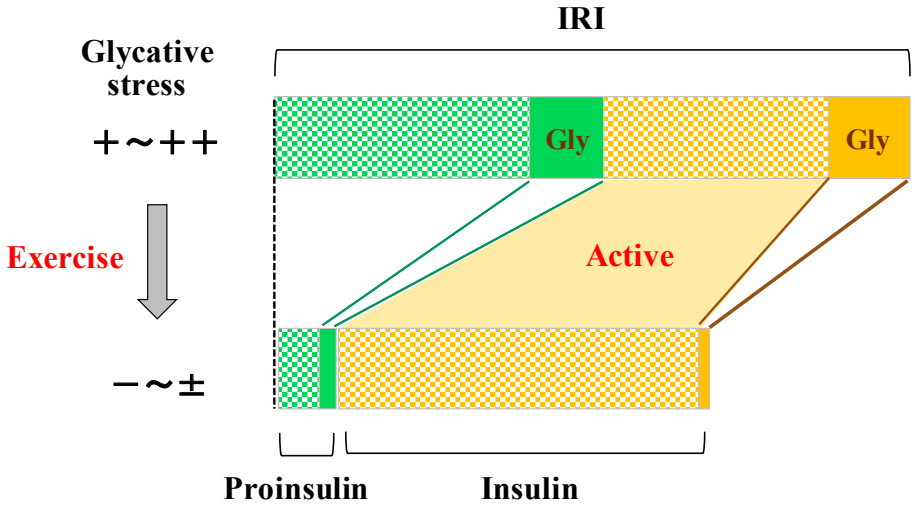

Fig. 3. **IRI compositional changes by exercise (speculated schematic).**

If physical exercise decreases glycative stress, expectedly insulin action would increase despite the decrease in IRI. IRI, immune reactive insulin; Gly, glycated proinsulin or insulin. The figure quoted from Reference 29.

3.2. Involvement of glycative stress in Alzheimer-type dementia (AD)

Neuropathological characteristics of the brain of AD patients consist of cerebral atrophy, senile plaque and neurofibrillary tangle. Senile plaques mainly consist of Aβ. Aβ is a peptide with a molecular weight of 4,300−4,500, consisting of approximately forty amino acids(Aβ40, Aβ42). Aβ is produced via cleave from amyloid-β precursor protein (APP) through the excision with β- and γ-secretase (***Fig. 4***)[30].

The brain is a rich organ in terms of lipid content, and proteins conjugated with fatty-acid-derived aldehydes such as Acro and MGO are observed in brains of patients with AD[31-41].

When Aβ is incubated under the presence of glucose, it is glycated and undergoes AGE modification (AGE-Aβ). Aβ has lysine residues at the numbers 16 and 18 in the amino acid sequence of glucose (Lys; K) and arginine residue at the number 5 (Arg; R). These amino acid sequence positions are related to glycation. Furthermore, Aβ is incubated under the existence of glucose and is aggregated. It is possible that AGE-Aβ is a "seed" that promotes the aggregation of Aβ[42].

It is reported that regarding protein in frontal lobe tissues containing senile plaque protein, AD patients have three times as much AGEs as healthy aged people[43]. Whereas, when Aβ-glucose reaction system is incubated with the addition of aminoguanidine, which is an inhibitor against glycation, the formation of Aβ aggregation is reduced[43]. The existence of AGEs such as pentosidine, pyrraline and CML *N*ε-arboxymethyl lysine is observed in senile plaque[44,45]. There is a possibility that AGE-Aβ, where Aβ is altered due to glycation, is related to factors for the formation of protein cross-linking and the promotion of Aβ aggregation.

Tau protein of paired helical filaments (PHF), which was collected from brains of AD patients, was examined and tau protein that was modified by AGEs was observed, while soluble tau protein collected from people without Alzheimer-type dementia or dementia did not show AGE modification. It is reported that tau protein has thirteen lysine residues in amino acid sequence and six lysine residues are glycated out of thirteen[45]. As glycation positions of tau proteins are located in binding sites with microtubules, binding functions are damaged. Glycated tau protein induces oxidative stress such as active oxygen and IL-6, and nerve cell functions are damaged[46].

In an *in vitro* experiment system of tau protein, it is demonstrated that glycated tau protein generates PHF-like fibers but non-glycated tau protein does not generate fibers[47]. AGEs such as pentosidine, pyrraline and CML are observed in PHF. Glycated tau protein induces the formation of APP and Aβ. Intracellular transport is reduced due to the phosphorylation and glycation of tau protein, and APP cannot be secreted. As a result, APP is deposited in cells. The glycation of Aβ and tau protein facilitatively affects the deposit and aggregation of Aβ, and at the same time, exacerbates the deposit of tau.

Normally, Aβ is rapidly degraded and discharged as brain waste (Aβ clearance). However, when abnormal Aβ is generated due to modification of Aβ structure by oxidative and glycative stress, insoluble fibers are formed and deposited[48,49]. Specifically, protein cross-linking is formed, which induces Aβ-peptide polymerization. This formation of abnormal Aβ polymer causes refractory, aggregation of neurotoxin and decline of Aβ clearance. Furthermore, when Aβ is not discarded and is accumulated in the brain, Aβ senile plaques (amyloid pathology) are generated. Abnormal Aβ with intensive neurotoxin clings to healthy nerve cells, which affects nerve cells. With this as a trigger, tau protein, which is a microtubule connected protein, induces the formation of neurofibrillary tangle with filamentation and condensation in cytoplasm. As a result, the brain gradually atrophies and Alzheimer-type dementia progresses. This is the concept that unites glycative stress with the "amyloid cascade hypothesis."[50-52]. In this concept, the importance of Aβ clearance is emphasized.

We conducted an experiment to examine impacts of glycative stress and sleep quality on phagocytic activity of microglia, using the primary microglia cultured cell (***Fig. 5***)[30].

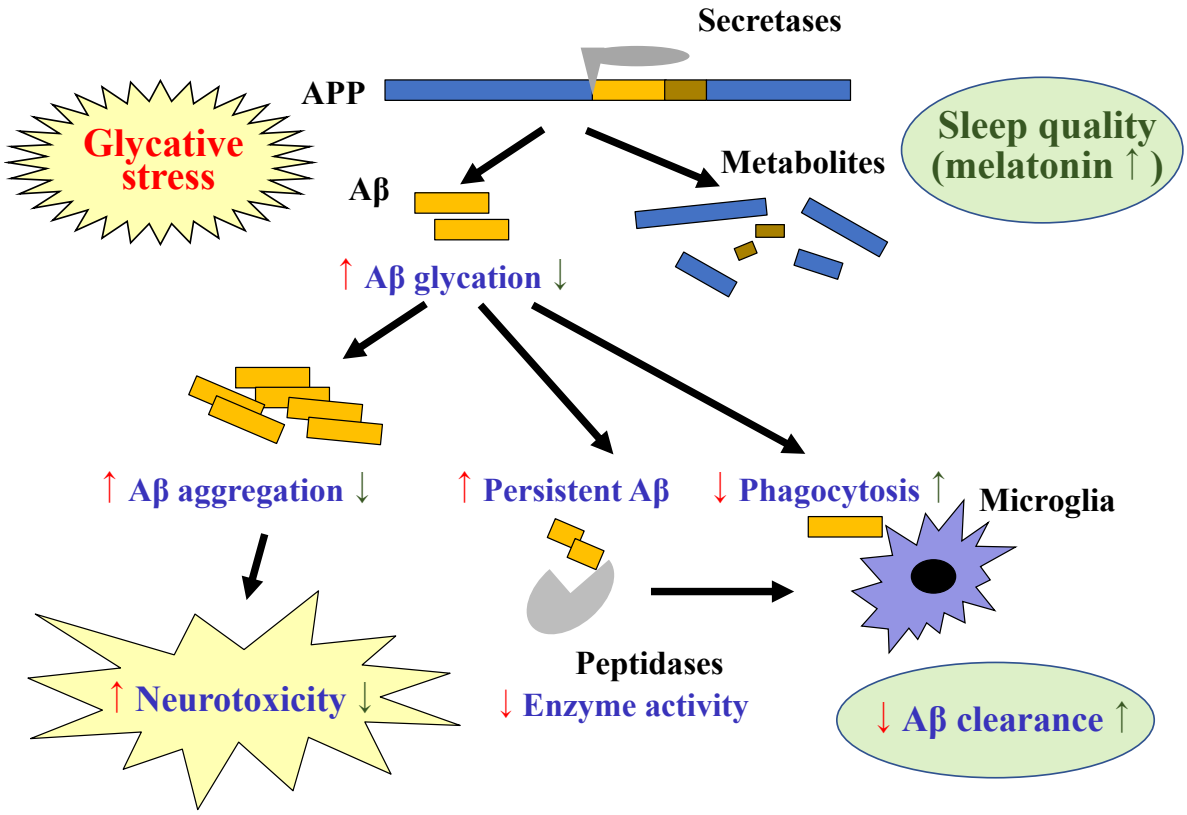

Fig. 4. **Aβ cascade: Impacts of glycative stress and protection by sleep quality/melatonin.**

The figure based on data from Reference 30. Aβ, amyloid β; APP, amyloid-βprecursor protein.

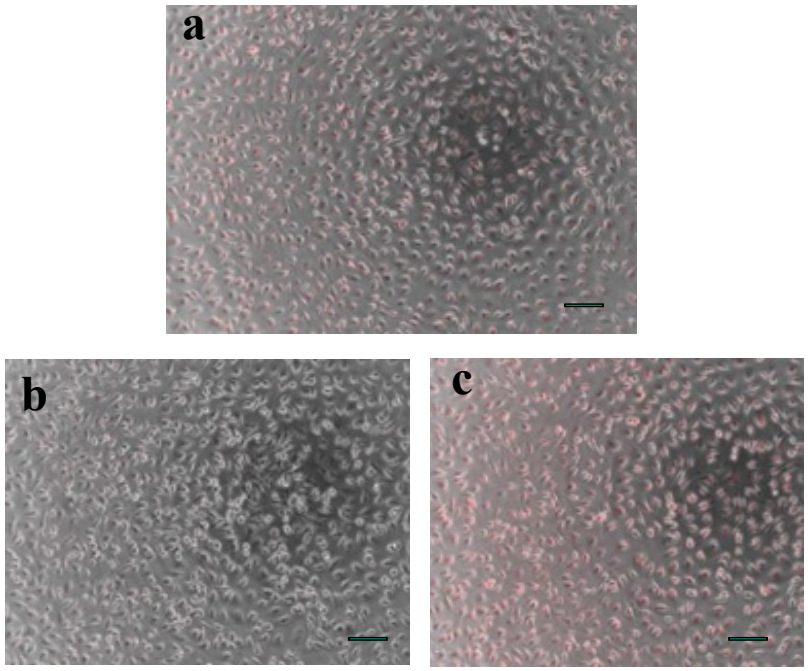

Fig. 5. **Aβ phagocytosis by microglia: Fluorescence microscopy image.**

a) Aβ phagocytosis, **b**) Glycated-Aβ phagocytosis, **c**) Aβ phagocytosis with melatonin (100 μM). Images (x 100) of cultured cells 10 days after seeding (8 days after addition of Aβ). The bar indicates 200 μm. Fluorescence-labelled Aβ (TAMRA-Aβ, Cosmo Bio, Japan) and rat-derived microglia primary culture cells (Cosmo Bio, Japan) were used. Red color portions indicate Aβ-derived fluorescence uptaken by microglia. Note that Aβ phagocytosis was inhibited by Aβ glycation and accelerated by the addition of melatonin. Glycated-Aβ was prepared by MGO treatment (10 mM, one day). Figures quoted from Reference 30. Aβ, amyloid β; MGO, methylglyoxal.

Glycated Aβ was prepared via MGO or Acro treatment. This is an example case of impacts at the molecular level. These findings confirmed that phagocytic activities of microglia remarkably deteriorated toward glycated Aβ. Contrarily, microglial phagocytosis for glycated Aβ are reinforced when we added melatonin, which is related to the quality of sleep. However, since the amount of melatonin used in the experiment was higher than the physiological range in the middle-aged or elderly, the melatonin action may be limited. Further verification of this point is needed.

In Aβ cascade, the importance of Aβ clearance has been an increasing interest in recent years. This finding of our study has provided a distinct possibility that "glycative stress decreases Aβ clearance, and melatonin, which is related to the improvement of sleep quality, promotes Aβ clearance." Furthermore, this finding has indicated that one of the mechanisms where glycative stress reduces Aβ clearance is the deterioration of microglial phagocytosis.

4. Defensive mechanism against glycative stress

4.1. Defensive mechanism

The body prepares their own defense mechanisms against glycative stress. However, once AGEs have formed, the body has difficulties in degradation of protease and proteosome as well as individual countermeasures for the diversity of AGEs. Therefore, degradation and removal mechanisms cannot provide effective approaches. It is reported that oxidized protein hydrolase (OPH), which is a type of serine protease, functions for degradation of AGEs [53-55]. Components that promote OPH activities are explored [56].

In the body, major defenses function in the stage of aldehydes. Typical components are ALDH, glyoxalase, and GAPDH.

GAPDH exists in abundance, accounting for 10–20% of intracellular proteins. In a glycolysis system, GAPDH, with NAD as a coenzyme, converts G3P into D-1,3-diphosphonooxypropanoic acid (1,3-BPG) [7,57]. This reaction requires energy that is obtained from reduction process from coenzyme NAD to NADH.

Livers and kidneys are fortresses for the defense system against glycative stress. Notably, GAPDH are contained abundantly in hepatocytes and podocytes of the kidney. When this enzyme is inhibited, intracellular and extracellular GA is increased, cellular necrosis is induced, and defense activities against aldehydes are deteriorated, resulting in increase of GA blood concentration (***Fig. 6***) [7]. Exploratory research had started to identify components for the elevation of GAPDH activity. A report is presented on potentiation effects of a chlorella extract to promote GAPDH activities [58].

Glyoxalase system, with an assistance of reduced glutathione (GSH), has a function of metabolization from MGO to lactic acid. This enzyme is largely contained in livers and kidneys playing an important role for the defense against glycative stress [59, 60].

Aldehyde dehydrogenase (ALDH) is known for 19 types of isoenzymes. ALDH2 has the most expression

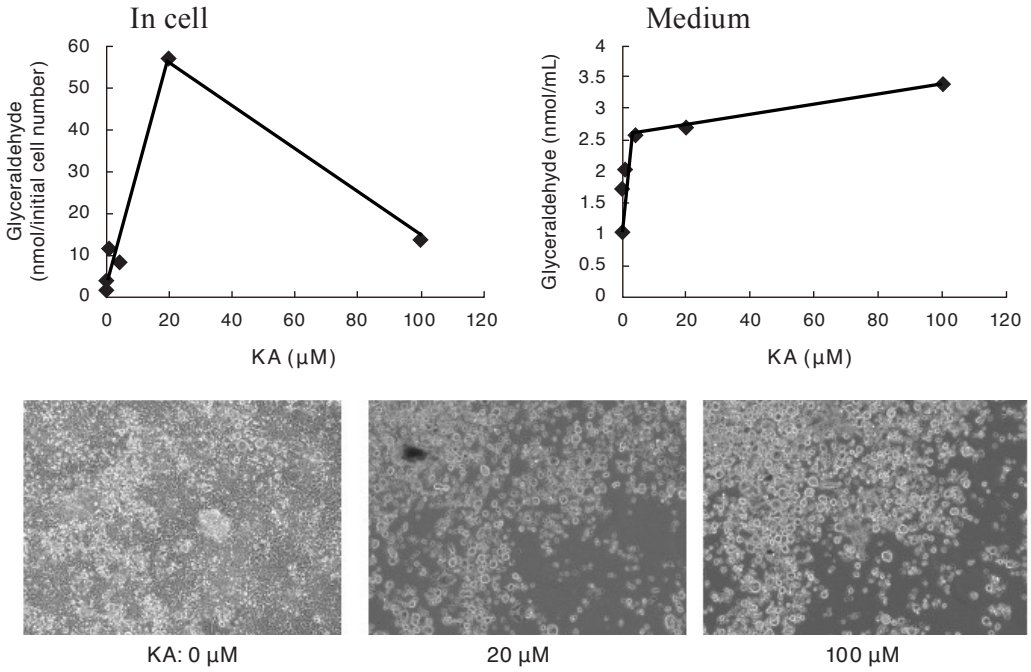

Fig. 6. **Effect of KA on HCT116 cell intracellular and medium GA.**

HCT116 cells (1 x 10^5) were inoculated on a 24 well plate and incubated in a high glucose DMEM medium containing antibiotics and 10% fetal bovine serum. KA was added to the medium one day after inoculation. Cells were harvested 7 days after inoculation and washed with the medium. Intracellular GA was extracted with 75% ethanol. The intracellular (**a**) and medium (**b**) GA was quantified by the method of Martin-Morales *et al*. (Reference 8). Cells after 6-day incubation with KA (**c**: 0 μM, **d**: 20 μM, and **e**: 100 μM) were photographed (lower). Figures quoted from Reference 7. KA, koningin acid; GA, glyceraldehyde.

quantity, and is contained in livers mostly [61, 62]. ALDH2 not only metabolizes acetaldehyde to acetic acid but also has detoxification for diverse aldehydes. To name a few, there are metabolizations from GA to glyceric acid, from glycol aldehyde to glycolic acid, and from MGO to pyruvic acid. ALDH2 activities are classified into low, moderate, and high activity. ALDH2 with high activity has a stronger defense function against glycative stress.

We are interested in the phenomenon that some individuals are more prone to aldehyde spikes and others less when compared to those who exhibit the same level of blood glucose spikes. If an aldehyde-trapping substance, which readily reacts with aldehydes, exists in the blood in large quantity, aldehydes can be detoxified. For instance, GO is involved in pathogenesis of hepatopathy induced by methimazole, a thyroid inhibitor. There is a possibility that hepatopathy could be prevented by GO trapping. We pay attention to amino acid in blood, holding and verifying a hypothesis, "Persons whose concentration of total amino acid are high tend not to have aldehyde spark".

4.2. Involvement of glycative stress in NAD

TCA cycle of mitochondria is inhibited in the state of high blood glucose/high aldehyde, and fumaric acid increases, which induces the formation of S-(2-succinyl) cysteine [2SC] of thiol group in cysteine, which comprises peptide [12-14]. This is a type of unphysiological post-translational modification of protein.

In mitochondria, NAD is involved in reactions of TCA cycle at three points. In the state of high blood glucose/high aldehyde, among aldehyde metabolizing enzymes, GAPDH and ALDH metabolize aldehydes utilizing NAD. Consequently, NAD is depleted in the state of excessive aldehyde. Therefore, it is considered that the cycle cannot function without hindrance.

It is reported that examples of succinylated proteins are 2SC-GAPDH [13, 63], 2SC-adiponectin [11], 2SC-heat shock protein (2SC-HSP) [10], kelch-like ECH-associated protein 1 (KEAP1) [64], and mitochondrial aconitase (ACO2) [64]. It is clarified that they largely affect intracellular metabolism.

Disorders of TCA cycle induce the formation of 2SC-GAPDH. Metabolism activities of GA are deteriorated, and blood concentrations of GA increasingly elevate [7, 13, 63]. This results in a vicious circle; the high aldehyde state by glycative stress induces the decline of GAPDH activity, which induces the increase of aldehydes [3].

In visceral fat, TCA cycle disorder induces the formation of 2SC-adiponectin (trimer). When Cys-39 thiol group (-SH) of adiponectin (trimer) is succinylated, disulfide linkages are not formed. Thus, hexamer, which has high membrane permeability, are not formed, and the blood concentration of high-molecular-form adiponectin (hexamer) decreases [11]. Adiponectin may represent a therapeutic strategy for insulin resistance. When glycative stress is intense, blood concentration of adiponectin decreases, insulin resistance is exacerbated, and subsequently glucose tolerance decreases. A vicious cycle is induced where glycative stress is increasingly aggravated.

These findings indicated that intervention at an early stage is necessary for prevention of onset and progression of glycative-stress-induced diseases.

We conducted a clinical trial with an oral administration of 300 mg/day of nicotinamide mononucleotide (NMN), which is a precursor of NAD, to seventeen post-menopausal women (mean age: 55.0) for eight weeks. Research data of glycative-stress-related indicators showed that HbA1c and intensity of AGE-derived skin autofluorescence (SAF) significantly decreased, and high molecular weight adiponectin significantly increased [65]. SAF, which is a non-invasive measurement by an AGE reader for skin fluorescence intensity, assumed AGE accumulation, is frequently utilized as an indicator for glycative stress [66, 67]. The research participants were in good health, but their metabolism waned in comparison with the younger generation in their thirties. The finding of this examination indicated a possibility that due to the supply of NMN, NAD in mitochondria reached sufficient quantity, which smoothed the TCA cycle, and the succinylation of GAPDH and adiponectin were reduced. It was suggested that the supply of NMN is an effective measure for a state of insufficient NAD.

4.3. Significance of improvement of "quality of sleep"

Core measures against glycative stress are dietetic therapy, exercise therapy and "quality of sleep". The diet is classified into the three categories according to the steps of glycative reaction: i) the blood glucose spike control/aldehyde trap, ii) the inhibition of AGE formation and iii) the promotion of AGE degradation. We recommend efficient ingestion of food ingredients that promote these effects. For carbohydrates, which account for approximately 60 % of total caloric intake, we recommend whole-grain foods. To counter animal fat dependency, γ-oryzanol, contained in unpolished brown rice, could provide positive effects [21, 68-70].

"Quality of sleep" and glucose tolerance affect interactivity. Forty percent of diabetes patients with intensive glycative stress have a sleep disorder. Patients with sleep apnea syndrome (SAS) and a lowered quality of sleep have complications of obesity and diabetes with a high frequency [71]. A glycative stress indicator of SAF is affected in terms of long or short sleep time. SAF values of persons with short sleep period shift upward. [72] To reduce glycative stress, it is important to simultaneously consider the independent disorders of carbohydrate metabolism and sleep disorders for prevention and treatment.

Metabolism of melatonin is gaining attention as a key factor to link "quality of sleep" and glucose tolerance [73]. *In vitro* experiments have shown that melatonin does not inhibit AGE formation [74], but it does promote the breakdown of AGEs [75]. When melatonin is administered at night, postprandial blood glucose level is subdued on the following morning [76]. These integrated activities reduce glycative stress.

Fig. 7 [77] compiles mechanisms that melatonin improved glucose and lipid metabolism, reducing glycative stress.

First, melatonin has antioxidation activity *in vitro* and *in vivo* [78-88]. This is based on two actions: a direct action of removing reactive oxygen species (ROS), and an action of increasing antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase [89,90]. Furthermore, metabolites, such as N-acetyl-N-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK), have antioxidation activities *in vitro*.

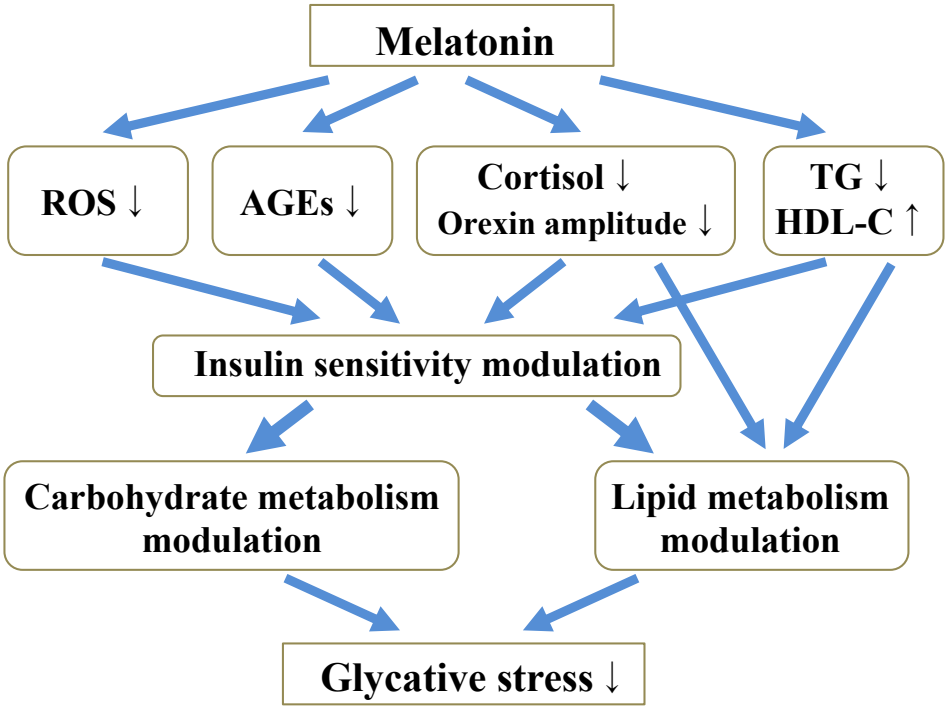

Fig. 7. **Melatonin and glycative stress.**

The figure quoted from Reference 77. ROS, reactive oxygen species; AGEs, advanced glycation endproducts; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol.

Second, melatonin promotes degradation of AGEs *in vitro* [91]. In abnormal glucose metabolism such as diabetes, ER stress in pancreatic islets β cells is exacerbated and insulin secretion is lowered [92]. AGEs increase ER stress in β cells and decrease the production and secretion of insulin [93]. When AGEs are decreased via degradation, ER stress decreases. Consequently, it is expected that the lowered insulin secretion ability is recovered.

Third, melatonin has activities mediated by hormones. Melatonin reduces the secretion of glucocorticoid such as cortisol from the adrenal cortex. Glucocorticoid promotes the acceleration of protein catabolism and gluconeogenesis, and at the same time, accelerates insulin resistance. This causes high blood glucose. Cortisol holds 95% of glucocorticoid activities and has a direct action for lipid metabolism. Cortisol, which is secreted due to acute stress, has an activity for lipolysis and promotes mitochondria usage of glucose, lipids, and amino acid. However, in a chronic elevated state of cortisol, the accumulation of lipids is caused with the inhibition of peroxisome proliferator-activated receptor γ (PPARγ) in white lipids [94].

Orexin, which is a type of brain hormone, has a sleep arousal effect. Orexin is in low values during sleep at night and is secreted from dawn to daytime (orexin amplitude). Orexin secretion is affected by blood glucose. Low blood glucose promotes secretion, and high blood glucose inhibits secretion [95-97]. Orexin amplitude is decreased due to aging. Orexin and blood glucose alternation affect interactively. Therefore, it is important to maintain the orexin amplitude with the purpose of a healthy alternation of blood glucose. Melatonin lowers orexin secretion which is elevated during night and regains the amplitude of orexin.

Fourth, melatonin plays a role in the improvement of lipid metabolism *in vivo*. In an experiment with animals with hyperlipidemia, melatonin administration decreased triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C). An experiment with normal rats indicated that high-density lipoprotein cholesterol (HDL-C) was increased [98-100]. Melatonin administration improves insulin resistance and some are mediated by insulin activity. Rats whose pineal body was removed suggested that melatonin secretion was diminished and abnormal glucose metabolism was exhibited [101]. It is confirmed that melatonin plays an important role to maintain homeostasis of glucose metabolism. When melatonin is ingested before bedtime, postprandial blood glucose level after breakfast is subdued the next morning [76].

Four mechanism paths of melatonin are mentioned above. Some are mediated by insulin activities and others directly act to glucose metabolism.

Activities toward pancreatic β cells are complicated *in vivo*. In a state of hyperinsulinemia such as prediabetes and early-stage type II diabetes, melatonin decreases insulin while fasting [102]. However, insulin activities are intensified in skeletal muscles, and intake of glucose is activated [103, 104]. It can be considered that insulin resistance decreases, where the homeostasis model assessment of insulin resistance

(HOMA-IR) decreases. In a state of intense oxidative stress and glycative stress, the formation of AGEs increases, ER stress in β cells increases, and the formation and secretion of insulin decreases. In these conditions, melatonin, which has anti-oxidation and anti-glycation activities (to promote the degradation of AGEs), expectedly functions to complement insulin secretion in pancreatic β cells. The deterioration of insulin secretion is a risk factor for onset of type II diabetes [105]. Melatonin is facilitative in the hypertrophy and activation of brown adipose tissue *in vivo* [106]. It is considered that melatonin is involved in not only glucose metabolism but also energy metabolism.

Impairment of "quality of sleep" and circadian rhythm disorders increase the risk of onset of AD [107]. Melatonin, which is secreted during the night, has activities of anti-oxidation [108] and anti-glycation [90] (to promote the degradation of AGEs). Melatonin plays a role protecting the brain during sleep from oxidation and glycative stress. Furthermore, melatonin has *in vitro* activities to inhibit the formation of Aβ [109], to inhibit the aggregation of Aβ [110, 111], to decrease neurotoxicity [112, 113], to improve Aβ clearance [114], and *in vivo* activities to maintain memory [115, 116], as is reported. Melatonin, by regulating the secretase expression control network, inhibits APP processing and Aβ formation [109]. From these perspectives, melatonin attracts attention as a factor that links "quality of sleep" to AD [117]. Therefore, educational programs for lifestyle regarding "quality of sleep" must be included in the list of preventive measures of AD, improving "quality of sleep" with increased melatonin secretion.

4.4. Physical exercise: impacts of glycative stress on skeletal muscles and exercise-resistance

Physical exercise for type II diabetes is recognized to play a role of improvements in insulin resistance, glucose tolerance, and lipid metabolism.

When insulin receptors on the surface of myocyte bind with insulin, glucose transporter 4 (GLUT-4) in cytoplasm transfer to membranes (translocation), and glucose is taken into cells via GLUT-4. When skeletal muscle motions (sliding of myosin and actin) occur, gene expression of GLUT-4 increases and intracellular GLUT-4 in cells increases [118]. At the time of insulin stimulation, the number of GLUT-4 on membranes increases. This raises the rate of glucose assimilation, which is a mechanism of the improvement of insulin resistance through exercise. As it is an exceedingly effective as a glycative stress countermeasure, this is employed as a part of therapeutic plans for type II diabetes.

Approximately 70% of glucose is uptaken into skeletal muscles and is utilized. However, muscle mass of persons who live a normal daily life is deprived by one percent per year. A pathological condition is designated as sarcopenia. However, there is a case that is designated as dynapenia, where the loss of muscle strength is not caused by muscle mass decrease. Some patients with diabetes maintain their skeletal muscle mass but lose muscle strength.[119-121]. It is considered that muscle proteins (myosin and actin) undergo glycative modification and then, muscle contractile function is impaired. There is an increasing interest of glycative stress as a causative factor of the deterioration of skeleton muscular functions [122].

The first report was that increased blood CML level was a risk factor of the deterioration of muscle strength and walking ability [123-125]. Ensuing epidemiological studies on middle-aged and older persons have suggested that there is a correlation between the increase of blood AGEs and SAF and the decrease of muscle strength and motor function. It is indicated that younger persons with high SAF level have decreased muscle strength and physical endurance. These findings suggest that impacts of glycative stress on motor functions and skeleton muscle functions occur in spite of age [122, 126, 127].

Primary factor of AGE-accumulation-related deterioration of decreased motor functions and skeleton muscle functions is glycative modification of muscle contractile proteins such as myosin, actin, and tropomyosin decreasing muscle contractile functions [128-131]. Further, involvement is suggested regarding the structural alternations of extracellular matrix, the decrease of ATPase activity [129, 132], and the decrease of motor nerve transmission function [133].

In an experiment, where mice were fed a diet containing a large quantity of AGEs for sixteen weeks, which is five-times-volume, the following is recognized: the decrease of expressions in myogenic factor 5 (Myf5) and myogenic differentiation 1 (MyoD), which plays a role to promote the formation of muscular tissue, and the decrease of signal transduction of insulin-like growth factor 1 (IGF-1), which plays a role to promote the formation of protein, thus eventually causing the decrease of muscle mass [134, 135]. An experiment on mice fed with MGO for twenty weeks suggested that along with muscle mass decrease, the gene expressions of inflammatory cytokines, *i.e.*, IL-1β, IL-6, were confirmed. A possibility was suggested that glycative stress induces protein catabolism via inflammatory signals [136].

There are differences among individuals regarding health promotion effects. Patients with diabetes and older adults are individually different. It is reported that one out of five people do not gain sufficient exercise benefits [137-140]. Glycated muscle proteins, which are modified due to glycative stress, result in the accumulation of AGEs. This decreases skeletal muscle contractile functions and at the same time, muscular tissue formation. Consequently, "exercise-resistance" is induced. This state means that benefits of exercise are unlikely to be gained [122]. Therefore, it is essential to start an appropriate exercise habit before exercise resistance is established.

5. Conclusion

Glycative stress comprehensively affects the body, from head to toe, diverse tissues and organs. Maintenance of the human body and prevention of aging-related diseases are significantly related to glycative stress. Lifestyle habits are important for practical measures against glycative stress. We can uncover a clue from lifestyle habits, in dietary education (nutrition, functional food and chrono-nutrition), physical education (loaded muscle exercise and aerobic exercise) and intellectual education (sleep and stress management). The point is that untreated long-term glycative stress leads to a vicious cycle of progression of aging-related diseases. Exercise-resistance is induced due to impacts on skeleton muscles. Thus, it is difficult in gaining benefits of exercise.

As an example of AD, it is difficult to treat advanced AD and to restore health. Thus, preventive measures are necessary. To prevent lipid peroxide of the brain due to oxidation stress and similarly, to prevent Aβ glycation from the perspective of countermeasures against glycative stress, we should pay attention to diverse countermeasures. In addition to physical exercise, aldehyde control, nutrition therapies, improvement of "sleep quality", and understanding of melatonin are required. We hope that further extensive research on anti-glycation will be undertaken, and deeper understanding and increased participation will be encouraged. Studies on glycative stress are required to lead to a social implementation as a practical science.

Funding

The present study was supported by Ministry of Education, Culture, Sports, Science and Technology with Grants-in-Aid for Scientific Research. (JSPS KAKENHI #26350917, #17K01880, 20K11593)

Conflicts of Interest

The authors declare that they have no conflict of interest.

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