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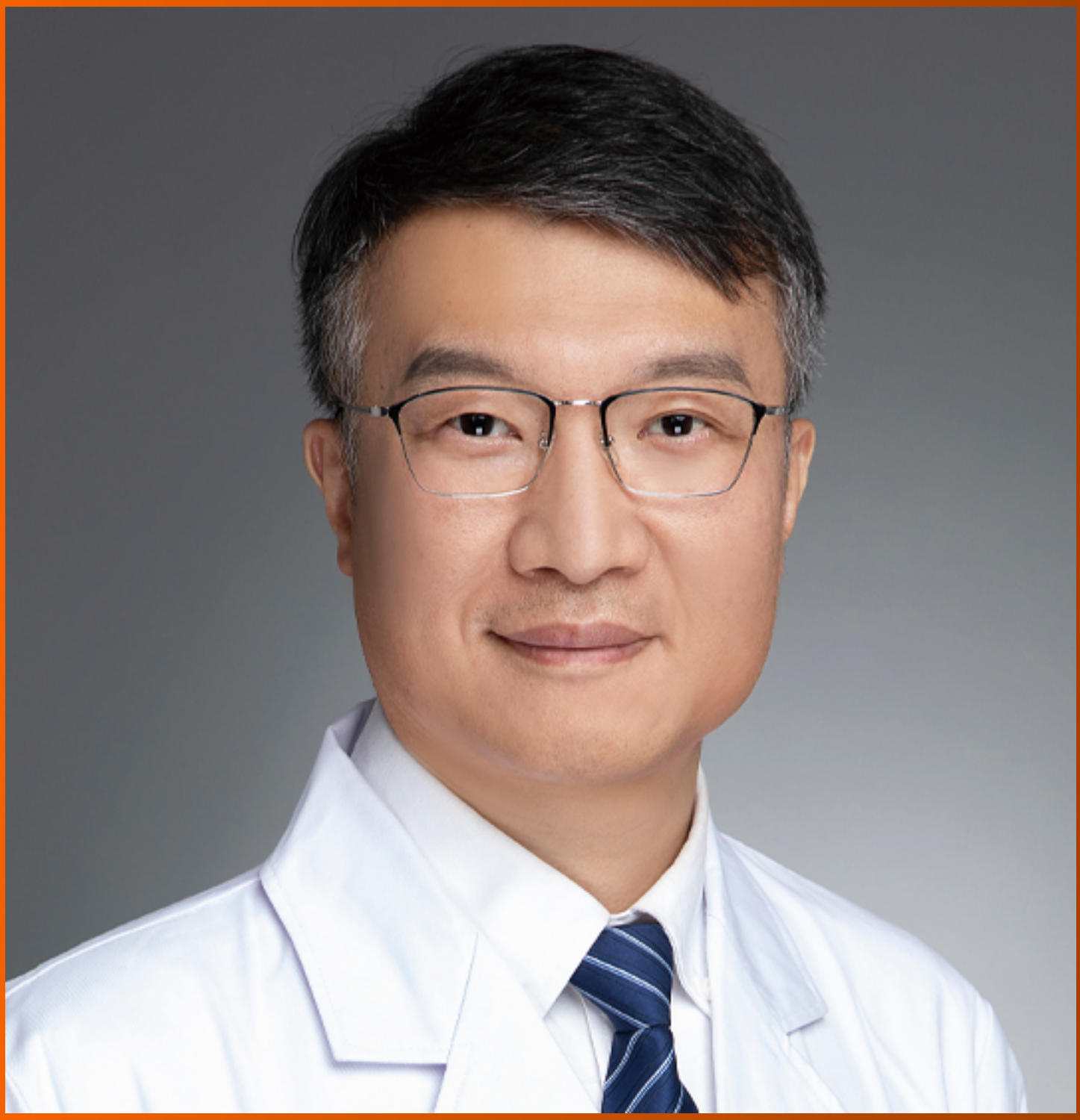

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REVIEW

Comprehensive overview of human serum albumin glycation in diabetes mellitus

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Abstract

The presence of excess glucose in blood is regarded as a sweet hurt for patients with diabetes. Human serum albumin (HSA) is the most abundant protein in human plasma, which undergoes severe non-enzymatic glycation with glucose in patients with diabetes; this modifies the structure and function of HSA. Furthermore, the advanced glycation end products produced by glycated HSA can cause pathological damage to the human body through various signaling pathways, eventually leading to complications of diabetes. Many potential glycation sites on HSA have different degrees of sensitivity to glucose concentration. This review provides a comprehensive assessment of the *in vivo* glycation sites of HSA; it also discusses the effects of glycation on the structure and function of HSA. Moreover, it addresses the relationship between HSA glycation and diabetes complications. Finally, it focuses on the value of non-enzymatic glycation of HSA in diabetes-related clinical applications.

Key Words: Diabetes mellitus; Human serum albumin; Non-enzymatic glycation; Advanced glycation end products; Glycation sites; Diabetic complications

Core Tip: In the case of hyperglycemia state, the glycation level of albumin in plasma is significantly increased, which alters the structure and function of albumin. Herein we review the different glycation sites and functional changes of glycated albumin, and discuss the relationship between albumin glycation and diabetes complications. The

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potential application value of glycated albumin in clinical is also discussed.

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INTRODUCTION

Diabetes is a metabolic disease caused by an absolute or relative deficiency of insulin in the human body related to various pathogenic etiologies; it leads to metabolic disorders involving sugars, lipids, and proteins, with severe hyperglycemia as the main clinical manifestation[1,2]. Abnormally high glucose concentrations in patients with diabetes can cause proteins in the body to undergo non-enzymatic glycation (*i.e.*, without the involvement of glycosyltransferase), which is the initiating factor of diabetes-related complications[3,4]. Human serum albumin (HSA) is a high-abundance protein in plasma that is mainly responsible for binding and transporting various endogenous or exogenous substances (*e.g.*, fatty acids, cholesterol, and many drugs); thus, it has a profound impact on the pharmacokinetic properties and efficacy of many drugs[5,6]. In patients with diabetes, HSA has a higher probability of glycation than other proteins, so it is regarded as an indicator of glycemic control[7]. Elevated glycation levels can lead to changes in the structure and function of HSA, thus influencing the normal physiological activities of the body[8]. The distinct distributions of multiple glycation sites on the three-dimensional structure of HSA cause different degrees of glycation under a range of glucose concentrations. A non-enzymatic glycation modification at the main drug-binding site substantially affects the ability of this region to bind drugs, thereby influencing the pharmacokinetic properties and efficacies of therapeutic drugs[9]. In this paper, seven aspects of HSA and its non-enzymatic glycation are reviewed.

EXPLANATION OF NON-ENZYMATIC GLYCATION AND ITS REACTION MECHANISM

Non-enzymatic glycation (sometimes described simply as glycation) is an important post-translational modification that does not involve the catalytic activity of glycosyltransferase[10]. The reaction mainly begins with a nucleophilic addition reaction between the carbonyl group of reducing sugar and the amino group of lysine, arginine, or the N-terminus of protein[11]. Fructose and lactose are important reducing sugars in food, while glucose is the main source of energy in the human body[12]. Therefore, glucose is the primary raw material for non-enzymatic glycation in the human body. The non-enzymatic glycation process is mainly divided into three steps: (1) The carbonyl group of a reducing sugar undergoes a condensation reaction with the amino group of the protein to form a thermodynamically unstable Schiff base; (2) The unstable Schiff base is converted into a relatively stable Amadori product[13,14]; and (3) Amadori product undergoes a series of spontaneous reactions (*e.g.*, dehydration, oxidation, rearrangement, and isomerization) that can generate various carbonyl compounds, such as methylglyoxal, glyoxal, 3-deoxyglucosone, and dehydroascorbic acid[15]. These carbonyl compounds usually react more strongly than the original reducing sugars and can quickly react with proteins to form various irreversible heterostructures, which are regarded as advanced glycation end products (AGEs)[16].

GENERAL STRUCTURE AND FUNCTION OF HSA

HSA is a highly abundant protein in plasma; its concentration of approximately 35-50 g/L comprises approximately 60% of the total plasma protein content[17]. It is mainly

responsible for the regulation of plasma osmotic pressure[18] and pH, and binding various endogenous or exogenous substances (*e.g.*, fatty acids, cholesterol, and many drugs)[19]. Additionally, HSA serves as an antioxidant, mediates lipid metabolism, and sequesters toxins[17]. It is composed of 585 amino acids and 17 intramolecular disulfide bonds, with a molecular weight of 66437 kDa[8]. Crystal structure analysis has shown that HSA possesses a spherical "heart-shaped" structure comprising approximately 67% of α-helices, 23% of extended chains, and 10% of β-sheets. HSA contains three homology domains: I (amino acids 1-195), II (amino acids 196-383), and III (amino acids 384-585); each of these domains contains two subdomains (A and B). The A subdomains of both domains II and III constitute the major drug-binding regions of HSA; these are regarded as sites I (amino acids 196-292) and II (amino acids 384-489)[20].

OVERVIEW OF HSA GLYCATION

Due to the high abundance of HSA, its non-enzymatic glycation represents approximately 80% of all glycation involving circulating proteins[21]. Amadori products are the main form of glycated HSA present in the body; their amounts increase as the blood glucose concentration increases in the blood of patients with diabetes[22]. The proportion of glycated HSA in healthy people is approximately 1%-10% and can increase by 2-3-fold in patients with diabetes[8,17]. Basic amino acids on HSA, specifically, 59 lysines and 24 arginines, are regarded as potential sites of glycation.

Glucose-induced modifications strongly influence HSA functional properties and have important implications for protein activity, folding, degradation, and cell function[23,24]. Although initially harmless, these modifications can become destructive and pathogenic when they become sufficiently widespread. Figure 1 shows the mechanism of the different effects of HSA glycation on the body. First, HSA glycation change the intrinsic conformations and binding efficiencies of its major binding regions, thereby changing the drug efficacy[25]. Second, the interactions of AGEs with their receptors [receptor for AGEs (RAGE)] or other macromolecules will activate various signaling pathways such as nuclear factor κB, as well as tissue damage and metabolic complications[26]. Third, glycated HSA can also stimulate platelet activation and aggregation, thereby enhancing thrombosis and inhibiting cellular uptake of glucose[27-31]. As the main drug-binding protein in plasma, HSA strongly influences drug absorption, distribution, excretion, and efficacy characteristics[32]. Changes in HSA function caused by the pathological environment can lead to unexpected types of toxicity. Drug molecules either combine with proteins and lipids in plasma or exist in a free (*i.e.*, unbound) state in the aqueous blood environment[33]. Only free drug molecules interact with their intended targets to produce therapeutic effects[33]. In some instances, the excessive modification of HSA by non-enzymatic glycation can increase the free drug concentration, which can produce severe drug toxicity[34,35].

METHODS FOR ASSESSMENT OF GLYCATED HSA

Glycated HSA has been used as a complementary indicator to standard assays involving glycated hemoglobin (HbA1c) or real-time glucose monitoring to assess glycemic control in patients with diabetes[10]. Notably, real-time glucose monitoring only provides a single data point concerning the glycemic status of patients with diabetes, while HbA1c provides an assessment of glycemic control over 2-3 mo and may be influenced by chronic kidney disease in some patients[36,37]. In contrast, glycated HSA provides an assessment of glycemic control over 21 d and can be used as an indicator with intermediate duration (*i.e.*, between real-time glucose monitoring and assessment of HbA1c)[38]. Many methods have been developed to detect and quantify glycated HSA with the aim of predicting or preventing potential complications; these methods mainly involve the determination of total glycated HSA, as well as the qualitative and quantitative assessment of HSA glycation sites.

Methods for assessment of total glycated HSA

Immunoassays such as enzyme-linked immunosorbent assays and radioimmunoassays are often used to detect total glycated HSA[39,40]. In addition, other traditional methods for evaluation of glycated HSA include boronate affinity

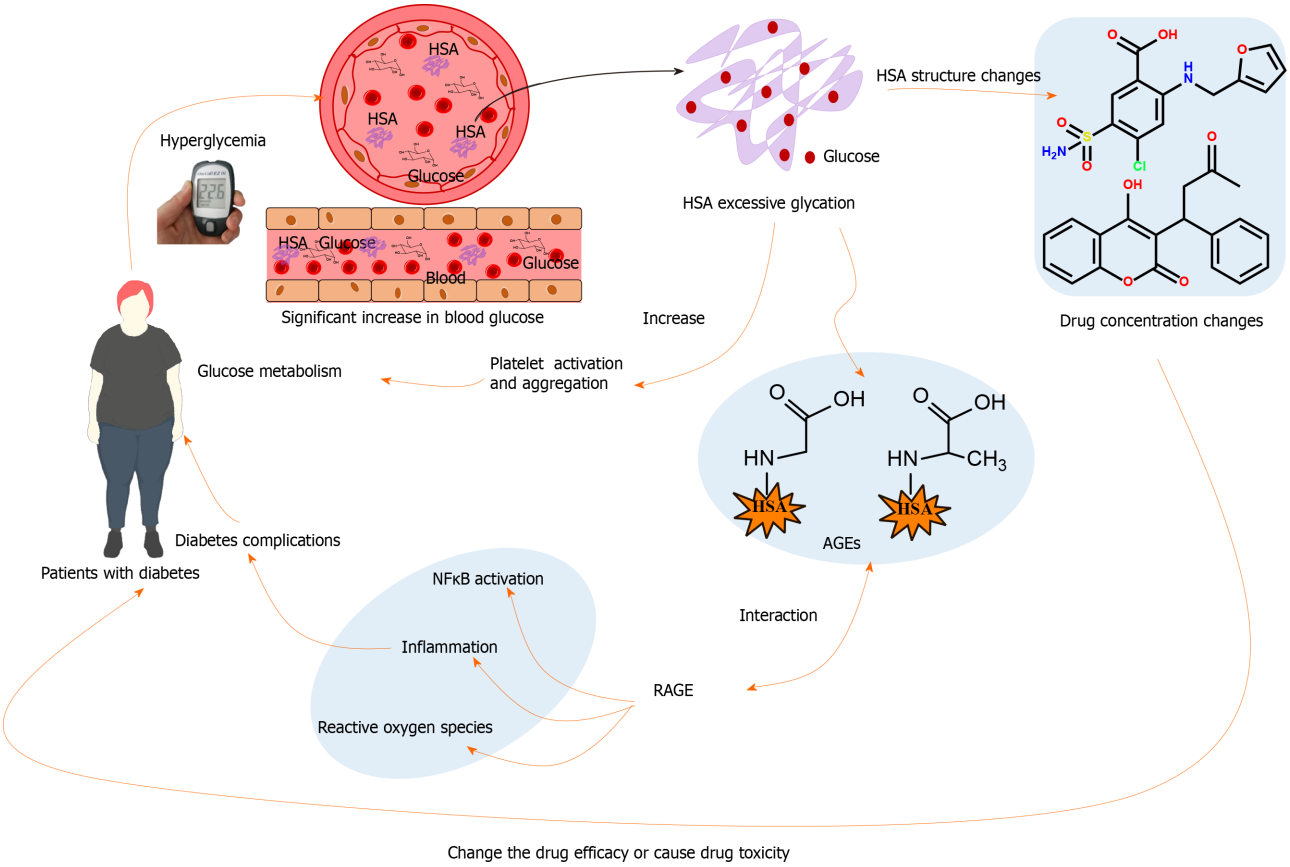

Figure 1 Mechanism of different effects of human serum albumin glycation on the human body. AGEs: Advanced glycation end products; RAGE: Receptor for advanced glycation end products; HSA: Human serum albumin.

technology; thiobarbituric acid analysis; nitro-blue tetrazolium colorimetric analysis; phenylhydrazone formation reaction; fructosamine assays; ketoamine oxidase analysis; high-performance liquid chromatography (HPLC) analysis of furosine hydrolysis by strong acid; phenylborate-containing acrylamide gel electrophoresis; and the analysis of reductive activity following alkaline solution treatment, using redox indicators[41-48]. However, the above traditional methods have their own characteristics or drawbacks. For example, colorimetric analysis methods such as nitro-blue tetrazolium and thiobarbituric acid have high unspecificity[49]; fructosamine assays provide higher specificity and reliability[50]; HPLC method has a high sensitivity[41]; phenylborate-containing acrylamide gel electrophoresis method is time-consuming and not suitable for clinical measurement[51]. In recent years, electrochemical quantitative analysis methods with high sensitivity and specificity have also been developed[52]. Intact protein analyses by high resolution mass spectrometry (MS) can also be used to determine the total glycation degree of HSA[53].

Methods for qualitative and quantitative analysis of glycation sites on HSA

HSA is rich in basic amino acids that can undergo glycation; thus, the analysis of glycation sites on HSA mainly involves the application of high-resolution MS[54]. A "Top-Down" approach combined with tandem MS is considered a standard method to accurately assess glycation sites[55-57]. In the "Top-Down" approach, HSA is first enriched and then digested with trypsin or Lys-C[7,10]. Because of glucose steric hindrance, peptides will have missed cleavage to form peptides containing glucose modifications[58]. Thus, glycated peptides exhibit a mass shift of 162 kDa in primary MS analysis, as well as a neutral loss in tandem MS analysis, and these findings can be used to locate the accurate glycation site[12]. Many types of MS with ionization modes of matrix assisted laser desorption ionization (MALDI) or electrospray ionization (*e.g.*, IT-TOF, LTQ-Orbitrap, Q-TOF, hybrid linear ion trap-Orbitrap, and MALDI-TOF MS) have been used to identify glycation sites[10,12,55,59,60]. For the quantitative analysis of glycated peptides, many approaches have been developed thus far[12,53,55,61]. Frolov *et al*[55] used the integral peak area to compare amounts of glycated peptides. In another study, isotopic labeling with ^{13}C was performed to label native proteins,

which were then digested with trypsin; the coupled ^{12}C and ^{13}C isotope peaks provided different types of quantitative information concerning the same glycated peptides[12]. Furthermore, ^{18}O- and ^{16}O-labeled H_2O has been used to hydrolyze normal and glycated HSA, respectively. The $^{16}O/^{18}O$ ratios in each digested peptide were measured to compare glycation levels[61]. Furthermore, Qiu *et al*[53] have developed an isobaric tags for relative or absolute quantitation (iTRAQ) labeling technology combined with three-stage MS (MS^3) method to compare glycation levels between healthy individuals and patients with diabetes. The iTRAQ-MS^3 method makes good use of the neutral loss of glycated peptides under collision-induced dissociation in MS/MS, and high-energy collisional dissociation in MS^3 fragmentation of the neutral loss ions were performed to precise quantification of the glycated peptides[53]. Table 1 shows the glycation sites that have been identified through qualitative and quantitative analyses. Notably, specific basic residues in HSA are involved in glycation *in vivo*[62]. Sites K525, K199, and K351 were reportedly the predominant glycation sites on HSA[62,63]. Figure 2 shows the number of reports for each potential glycation sites. Sites K12, K64, K137, K199, K233, K262, K274, K317, K378, K414, K525, K545, and K574 have been more easily identified than other sites (reported ≥ 8 times), which suggests that they are more sensitive to changes in serum glucose concentrations[7]. The underlying mechanism may be that these sites are both distributed on the HSA surface and spatially located near basic amino acids[53]. Although K199 is not completely distributed on the HSA surface, its low pKa value and spatial proximity to basic amino acids make it suitable for glycation reactions[62]. In Figure 2, we can find that some sites (*e.g.*, K20, K41, R145, R197, R209, K212, R222, R337, and K524) had never been identified in analyses of glycation modifications, indicating that they are insensitive to changes in glucose concentrations, and further explorations of the underlying mechanism are needed to determine their roles[64-71].

EFFECTS OF GLYCATION ON THE STRUCTURE AND FUNCTION OF HSA

Many functions of HSA can be attributed to its structural characteristics. The relative structural stability of HSA is mainly dependent on 17 intramolecular disulfide bonds [50]. This structural flexibility enables HSA to bind to many molecules with distinct structures[72]. The affinities of various metabolites and drugs depend on the multistage structures of binding sites, which are distributed throughout the whole HSA molecule. The major drug-binding sites of HSA are known as sites I and II[20,35,73]. Glycation contributes to various changes in HSA structure and function[74]. First, it enhances the molecular weight of HSA by attaching one or several glucose units to the basic amino acid residues of the protein. Second, glycation will change the original conformation of HSA. The intrinsic fluorescence of HSA is mainly derived from tryptophan-214 located in site I; its fluorescence is extremely sensitive to changes in the HSA environment[24,35,73]. Glycated sites located in or near Site I, such as K199, will alter the HSA structural microenvironment, thereby altering the intrinsic fluorescent characteristics of the protein. The relative fluorescence intensity of glycated HSA is reportedly reduced by 51% compared with normal HSA[75]. In addition to fluorescence chromatography, circular dichroism has also been used to study the effects of glycation on the structure of HSA[76]. Nakajou *et al*[75] used circular dichroism to compare different HSA molecules, which revealed that the secondary structure of HSA was altered after glycation with 50 mmol/L glucose. Third, the glycation of HSA will act as an oxidant and a pro-inflammatory mediator through different mechanisms[77].

Glycation-related changes in the structure of HSA can have varying effects on its abilities to bind a range of ligands. The main mechanisms that affect binding may involve steric hindrance of covalently bound glucose, the blockage of charged residues, or a combination of these two mechanisms[75]. Techniques used to study the binding affinity of glycated HSA include fluorescence spectroscopy, circular dichroism, HPLC with ultraviolet detection, and nuclear magnetic resonance[78-80]. Changes in the binding affinities of glycated HSA to various ligands are influenced by drug concentration and the degree of protein glycation[35,53,75] (see Table 2 [81-85]). Warfarin, tryptophan, and dansylsarcosine have often been used as probe compounds for HSA sites I and II in binding studies[75,76]. *In vitro* analysis has shown that HSA glycation with a range of glucose concentrations (2.5 mmol/L, 12.5 mmol/L, and 50 mmol/L) enhanced the binding of warfarin, but weakened the binding of dansylsarcosine[75]. Another study showed that both *ex vivo* (purified from the plasma of patients with diabetes) and *in vitro* glycated HSA exhibited weakened binding

Table 1 Review of the *in vivo* glycation sites of human serum albumin

Ref.	Glycation sites reported so far	Analysis tools
Iberg *et al*[63]	HSA from a diabetic patient: Lys-12, Lys-199, Lys-233, Lys-281, Lys-317, Lys-351, Lys-439, Lys-525, Lys-534	Amino acid analysis after hydrolysis in HCl
Garlick *et al*[64]	Freshly purified human serum albumin: Lys-525	Cation exchange chromatography
Frolov *et al*[55]	HSA from five T2DM patients: Lys-12, Lys-51, Lys-64, Lys-162, Lys-174, Lys-181, Lys-233, Lys-262, Lys-276, Lys-351, Lys-359, Lys-378, Lys-414, Lys-475, Lys-525, Lys-545	Q-TOF-MS
Kisugi *et al*[56]	HSA from a female diabetic patients: Lys-64/Lys-73, Lys-199, Lys-136/ Lys-137, Lys-233, Lys-274/Lys-276, Lys-317, Lys-389, Lys-439, Lys-534, Lys-525	QSTAR Pulsar-i mass spectrometer
Frolov *et al*[57]	HSA from 5 T2DM patients and 4 healthy subjects: Lys-12, Lys-51[1], Lys-64[1], Lys-73, Lys-93, Lys-137, Lys-162, Lys-174[1], Lys-181[1], Lys-205, Lys-233[1], Lys-262[1], Lys-274, Lys-351, Lys-359[1], Lys-378[1], Lys-414, Lys-475, Lys-525, Lys-545[1], Lys-557[1] (detected only in diabetic samples), Lys-574	Nano-ESI-LTQ Orbitrap XL MS with ETD
Bai *et al*[10]	HSA from a healthy subject and a diabetic patient: Lys-64, Lys-93, Lys-190, Lys-199, Lys-205, Lys-225, Lys-233, Lys-240, Lys-262, Lys-274, Lys-281, Lys-317, Lys-323, Lys-351, Lys-372, Lys-378, Lys-413, Lys-432, Lys-475, Lys-525, Lys-545, Lys-557, Lys-557/ Lys-560/ Lys-564, Lys-564, Lys-573/ Lys-574	IT-TOF-MS/MS
Zhang *et al*[7]	HSA from clinical T2DM, IGT, NGT and 389 volunteers: Lys-12/ Lys-20[1], Arg-144, Arg-186/ Lys-190[1], Arg-222/ Lys-225, Lys-240, Arg-336, Lys-372, Lys-414/ Arg-428[1]. (8 glucose sensitive sites)	Agilent MSD trap
Anguizola *et al*[59]	HSA from individual clinical plasma samples: Arg-10, Lys-12, Arg-10/Lys-12[1], Arg-98[1], Arg-160, Lys-162, Lys-190, Lys-199, Lys-276, Lys-281, Lys-276/Lys-281[1], Lys-286[1], Lys-313, Lys-317, Lys-372, Lys-428, Lys-432, Arg-484, Arg-485, Arg-484/ Arg-485[1], Lys-545, Lys-557, Lys-560, Lys-564[1], Lys-573/ Lys-574[1]	MALDI-TOF-MS
Priego-Capote *et al*[12]	HSA from human Plasma: Lys-64, Lys-73, Lys-93, Lys-106, Lys-136, Lys-137, Lys-159, Lys-174, Lys-181, Lys-195, Arg-218, Lys-233, Lys -240, Lys-262, Lys-274, Lys-323, Lys-359, Lys-372, Lys-378, Lys-389, Lys -402, Lys-413, Lys-432, Lys-436, Lys-439, Lys-444, Lys-466, Arg-472, Lys-475, Lys-500, Lys-519, Lys -525, Lys-573	Hybrid linear ion trap-Orbitrap MS
Korwar *et al*[65]	HSA from clinical plasma samples: Lys-12, Lys -64[1], Lys -136, Lys-137, Lys-159[1], Lys-402[1], Lys-414[1], Lys-466[1], Lys-525[1]	Hybrid quadruple Q-Exactive Orbitrap MS
Zhang *et al*[60]	HSA from 12 NGT, 11 IGT and 8 T2DM: Lys-4[1], Lys-12, Lys-51, Lys-64[1], Lys-73, Lys-136, Lys-137, Lys-159, Lys-162, Lys-181[1], Lys-190[1], Lys-195, Lys-199[1], Lys-205, Lys-225, Lys-233[1], Lys-262, Lys-274, Lys-276, Lys-317[1], Lys-351, Lys-378, Lys-414, Lys-432[1], Lys-436[1], Lys-475, Lys-525, Lys-538, Lys-545, Lys-562[1], Lys-573, Lys-574	Ion Trap LC-MS
Miyamoto *et al*[66]	HSA from 8 diabetic patients: Lys-51, Lys-64/ Lys-73, Lys-136/ Lys-137, Lys-159/ Lys-162, Lys-190/ Lys-195/ Lys-199/ Lys-205, Lys-233, Lys-262, Lys-274/ Lys-276, Lys-313/ Lys-317, Lys-351, Lys-378/ Lys-389, Lys-432/ Lys-436/ Lys-439, Lys-525, Lys-534/ Lys -536/ Lys-538/ Lys-541, Lys -545, Lys-573/ Lys-574	QSTAR Pulsar-i MS
Brede *et al*[67]	HSA from plasma: Lys-12, Lys-137, Lys-414, Lys-525[1]	Q-TOF MS
Spiller *et al*[68]	HSA from 48 T2DM patients and 48 non-diabetic: Lys-64, Lys-73, Lys-93, Lys-174, Lys-181, Lys-233, Lys-262, Lys-359, Lys-378, Lys-414, Lys-525, Lys-545, Lys-574	QTRAP 4000
Spiller *et al*[69]	HSA from 5 T2DM patients and 5 non-diabetic individuals: Lys-64[1], Lys-73[1], Lys-181[1], Lys-262[1], Lys-378[1], Lys-574[1]	ESI-QqLIT-MS (4000
Takátsy *et al*[70]	HSA from diabetic patients and healthy individuals: Arg-81, Lys-93, Arg-98, Lys-106, Arg-114, Lys-190, Lys-199, Arg-218, Arg-257, Lys-276, Lys-317, Arg -348, Lys-372, Lys-378, Lys-389, Lys-413, Lys-436, Lys-439, Lys-444, Lys-466, Arg-484, Arg-485, Lys-500, Lys-519, Arg-521, Lys-564, Lys-536, Lys-538, Arg-445, Lys-541, Lys-560, Lys-573	MALDI TOF MS
Greifenhagen *et al*[71]	HSA from 5 diabetic patients: Lys-12, Lys-64, Lys-137, Lys-190, Lys-199, Lys-274, Lys-276, Lys-525	ESI-Orbitrap-MS
Qiu *et al*[53]	HSA from 4 diabetic patients and 4 healthy subjects: Lys-4, Lys-12, Lys-51[1], Lys-64[1], Lys-73, Arg-81, Lys-93[1], Arg-98, Arg-117, Lys-136, Lys-137, Lys-162[1], Lys-174, Lys-181, Arg-186, Lys-199[1], Lys-205, Lys-233[1], Lys-240, Arg-257, Lys-262[1], Lys-274, Lys-276, Lys-281, Lys-286, Lys-313[1], Lys-317, Lys-323[1], Lys-351, Lys-359, Lys-372, Lys -378[1], Lys-389, Lys-402[1], Lys-410, Lys-414[1], Lys-436, Lys-439, Lys-466[1], Lys-475[1], Lys-519, Lys-525[1], Lys-538, Lys-541, Lys-545[1], Lys-557[1], Lys-564[1], Lys-573, Lys-574[1]	LTQ Orbitrap Velos Pro MS

[1]Represents glycation sites detected at higher quantities in diabetic patients than in healthy individuals. HSA: Human serum albumin; ESI: Electrospray ionization; NGT: Normal glucose tolerance; T2DM: Type 2 diabetes mellitus; MS: Mass spectrometry.

interactions with warfarin[35]. Joseph *et al*[76] proved that the binding of L-tryptophan was enhanced by 4.7-5.8 fold under glycation conditions similar to those in patients with diabetes, although the binding of warfarin remained unchanged. Notably, the above contradictory results concerning warfarin were obtained under relatively nonphysiological conditions *in vitro*. Qiu *et al*[53] found that the affinity of warfarin for HSA was greater in plasma from patients with diabetes. The level of free warfarin was also reduced in subsequent pharmacokinetic experiments[53]. Furthermore, a retrospective clinical study revealed that the anticoagulant effect of warfarin was

Table 2 Effects of glycation on the binding of human serum albumin to various ligands

Ref.	Ligands	*In vivo/ vitro/ex vivo*	Glycation level of HSA	Binding affinity
Nakajou *et al*[75]	Warfarin	*In vitro*	HSA glycated with 2.5 mmol/L, 12.5 mmol/L, and 50 mmol/L glucose	↑
Baraka-Vidot *et al*[35]	Warfarin	*In vitro* and *Ex vivo*	HSA purified from blood and HSA glycated with 25 mmol/L or 100 mmol/L glucose	↓
Joseph *et al*[76]	Warfarin	*In vitro*	HSA glycated with 0.5 mol/L glucose	→
Qiu *et al*[53]	Warfarin	*In vivo*	HSA from diabetic patients	↑
Joseph *et al*[76]	Tryptophan	*In vitro*	HSA glycated with 0.5 mol/L glucose	↑4.7-5.8-fold
Nakajou *et al*[75]	Dansylsarcosine	*In vitro*	HSA glycated with 2.5 mmol/L, 12.5 mmol/L, and 50 mmol/L glucose	↓
Qiu *et al*[53]	Heparin	*In vitro* and *in vivo*	HSA from diabetic patients	→
Guerin-Dubourg *et al*[81]	Copper	*In vivo*	HSA purified from diabetic patients and control individuals	↓16%
Koizumi *et al*[82]	Furosemide	*In vitro*	Prepared from HSA, and commercial HSA	↓
Okabe *et al*[83]	Phenylbutazone	*In vitro*	Each mole of HSA contains 1.94 moles of glucose	↓
Yamazaki *et al*[84]	Fatty acids	*In vitro*	HSA glycated with 100 mmol/L glucose	↓
Karp *et al*[85]	Diazepam	*In vitro*	HSA glycated with 140 mmol/L glucose	→
Karp *et al*[85]	Bilirubin	*In vitro*	HSA glycated with 140 mmol/L glucose	↓30%
Okabe *et al*[83]	Ibuprofen	*In vitro*	Each mole of HSA contains 1.94 moles of glucose	↓20
Okabe *et al*[83]	Dansylproline	*In vitro*	Each mole of HSA contains 1.94 moles of glucose	↓25%
Okabe *et al*[83]	Flufenamic acid	*In vitro*	Each mole of HSA contains 1.94 moles of glucose	↓
Koizumi *et al*[82]	Naproxen	*In vitro*	Prepared from HSA, and commercial HSA	→

"→": No change; "↑": Increase; "↓": Decrease; HSA: Human serum albumin.

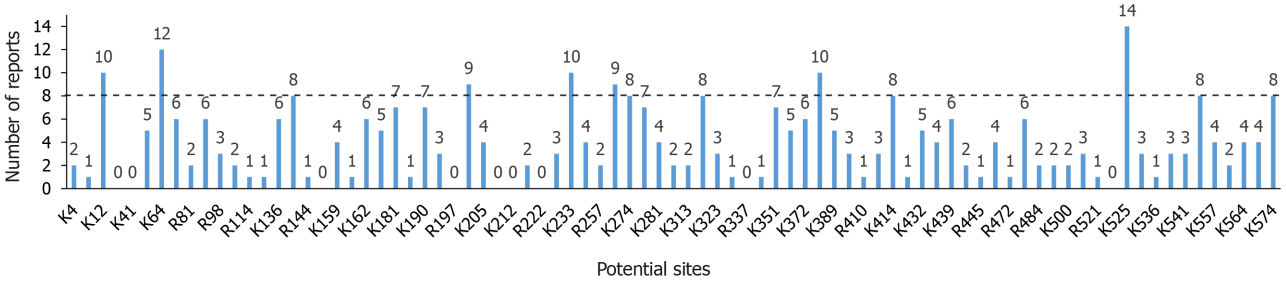

Figure 2 Number of reports for each potential glycation site. Dotted line represents that the number of reports reaches 8 times.

reduced in patients with diabetes[53]. These *in vivo* findings may provide better reference data with respect to warfarin binding.

HSA GLYCATION AND COMPLICATIONS

Chronic hyperglycemia is the primary condition associated with complications of diabetes. Hyperglycemia leads to excessive irreversible accumulation of AGEs on long-lived proteins, such as HSA and HbA1c. The degrees and durations of protein exposure to abnormally high levels of glucose are closely related to the degrees and rates of progression of nephropathy, stroke, neuropathy, retinopathy, and cardiovascular disease[86]. There remain questions concerning how the accumulation of AGEs promotes the development of these lesions. There are three main consequences of the formation of AGEs: (1) Cross-linking of various extracellular proteins[87]; (2) Changes in cell–matrix interactions[88,89]; and (3) Changes in DNA

structure and function[90]. HSA is the main protein in blood circulation; patients with diabetes exhibit significantly greater levels of the HSA-related AGEs[91]. Interactions between AGEs and RAGEs alter cellular signals and gene expression, thereby enhancing the secretion of pro-inflammatory molecules and leading to oxidative stress reactions in patients with diabetes[92].

HSA GLYCATION AND CLINICAL APPLICATIONS

Glycation is a continuous process in the human body. Elevated levels of glycated proteins are associated with elevated levels of blood glucose in patients with diabetes. Thus, there is considerable interest in measuring the glycation levels in patients with diabetes; these data can be used for diagnosis, treatment, and prognosis[93,94]. For many years, HbA1c has been used for the clinical monitoring of long-term blood glucose control[95]. However, HbA1c monitoring has some limitations. Because the lifespan of HbA1c is approximately 3 mo, rapid changes in serum glucose status (*e.g.*, treatment response) are not clearly reflected in HbA1c measurements[96,97]. In some individuals, an abnormally elevated HbA1c value may be recorded, such as patients with hemoglobin variants[96,98], patients with rapid changes in glucose control, patients with iron-deficiency anemia, patients with HIV, or pregnant patients[99-102]. In patients with reduced erythrocyte lifespan, such as those with liver cirrhosis[103], hemolytic anemia[104], chronic kidney disease, and/or hemorrhage, the recorded values of HbA1c will decrease[105,106]. HSA glycation has been suggested as an alternative clinical indicator to circumvent many limitations of HbA1c assessment. The level of HSA glycation is not affected by hemoglobin genetic variations or changes in erythrocyte lifespan[107]. Compared with HbA1c, glycated HSA has a much shorter half-life and is therefore more sensitive to changes in glycemic status. The levels of glycated HSA reflect the average plasma glucose level over a 2-wk interval[94,108]. Therefore, glycated HSA is a more dynamic indicator of glycemic control, which can be used to evaluate the drug treatment efficacy and short-term changes in glucose control. In patients with pre-diabetes, the total degree of HSA glycation does not provide all possible information regarding short-term fluctuations in plasma glucose concentrations because of the high number of possible glycation sites. Therefore, the comparison of the glycation degree of specific HSA sites sensitive to glucose (*e.g.*, K525 and K199) can be used as clinical biomarkers for the occurrence and early diagnosis of diabetes[53,65]. However, it is noteworthy that glycated HSA levels are also influenced by hypoalbuminemic conditions such as malnutrition, nephrotic syndrome, liver cirrhosis, or other liver and renal disease[109]. Further verification is needed to determine whether and how glycated albumin can be used as an indicator of hyperglycemia under these conditions.

CONCLUSION

Hyperglycemia leads to enhanced HSA glycation in patients with diabetes; this highly non-enzymatic glycation at multiple sites can impact the function of HSA as a drug carrier. In this review, we have presented a detailed summary of non-enzymatic glycation sites identified thus far *in vivo*; we have also discussed the impacts of non-enzymatic glycation on the three-dimensional structure and biological functions of HSA. It would be useful to determine how modifications in HSA glycation affect drug treatments for a range of diseases. Glycated HSA may serve as a new clinical indicator for assessment of glycemic control, potentially as an alternative for the long-term indicator HbA1c. Additional *in vivo* studies are needed to determine the effects of glycated HSA on combinations and efficacies of various drugs, thereby providing reference data to aid in the guidance of clinical treatment for patients with diabetes.

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