SUPPLEMENTARY DATA

Supplementary Table 1. Demographic and clinical characteristics of human subjects

	Warfarin		Heparin (Low Molecular)		
	Diabetes	Non-diabetes	Diabetes	Non-diabetes	P value
Number	13	14	13	13	
Sex (M/F)	6/7	6/8	6/7	7/6	ns
Age (Y)	63.9 ± 3.2	64.6 ± 3	66.6 ± 4.6	66.5 ± 3.3	ns
BMI (kg/m^2)	28.6 ± 3.3	28.3 ± 2.9	27.5 ± 3.1	28.1 ± 2.6	ns
FPG (mmol/L)	8.3 ± 1.1	5.2 ± 1.3	9.1 ± 1.6	5.51 ± 1.2	****
DD (Y)	19 ± 5	Non	21 ± 6	Non	
SBP (mmHg)	140.3 ± 28.9	139.5 ± 26.1	138.5 ± 24.4	136.7 ± 23.8	ns
DBP (mmHg)	80.2 ± 16	82.5 ± 17	79.3 ± 15	81.6 ± 18	ns
TC (mmol/L)	4.7 ± 0.85	5.1 ± 0.91	4.9 ± 0.94	5.2 ± 0.93	ns
TG (mmol/L)	2.1 ± 1.3	1.9 ± 1.4	2.2 ± 1.2	1.8 ± 1.3	ns
ALT (U/L)	25.3 ± 19.8	26.5 ± 20.2	24.9 ± 21.3	23.1 ± 18.9	ns
CREA (μmol/L)	88.2 ± 13.5	89.5 ± 12.7	90.6 ± 11.2	91.3 ± 13.7	ns
BT-INR	1.19 ± 0.09	1.25 ± 0.1	1.21 ± 0.11	1.30 ± 0.15	ns
AT-INR	2.67 ± 0.11	3.43 ± 0.14	2.51 ± 0.12	2.63 ± 0.16	*

Notes:

BMI: Body mass index; FPG: Fasting plasma glucose; DD: Diabetes duration; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TC: Total cholesterol; TG: Triglycerides; ALT: Alanine transaminase; CREA: Creatinine; BT-INR: INR of before treatment; AT-INR: INR of after treatment. The dose of warfarin is generally determined according to INR, it is targeted to an INR of range 2.0 to 3.0 (1,2). In general, the doses of low molecular weight heparin was 5,000 unit intravenous bolus followed by 32,000 unit per 24 hours, and the dose was adjusted to maintain the APTT at 6 hours within the therapeutic range of 1.5 to 2.5 times control (3).

Literatures 1-3:

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Supplementary Table 2. Summary of HSA glycation sites identified or quantified in previous *in vivo* studies

Potential sites[1]	1[2]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
N-TERM																
K4										√√*						
R10							√									
K12	√		√√			√√	√		√√*	√	√√	√√				√
K20																
K41								√√								
K51			√√			√√*		√√		√					√	
K64			√√	√	√	√√*		√√		√√*	√√*		√√*	√√*	√	√
K73						√√*		√√		√			√√*	√√*		
R81																
K93					√	√√		√√					√√*			
R98							√									
K106								√√								
R114																
R117																
K136				√				√√		√	√√					
K137						√√		√√		√	√√	√√			√	√
R144									√√							
R145																
K159								√√		√	√√					
R160							√									
K162			√√			√√	√			√					√	
K174			√√			√√*	√	√√					√√*			
K181			√√			√√*		√√		√√*			√√*	√√*		
R186																
K190					√		√		√√*	√√*						√
K195								√√		√						
R197							√									
K199	√			√	√		√			√√*					√	√
K205					√	√√				√						
R209																
K212																
R218								√√								
R222																
K225					√				√√	√						
K233	√		√√	√	√	√√*		√√		√√*			√√*		√	
K240					√			√√	√ √							
R257																
K262			√√		√	√√*		√√		√			√√*	√√*	√	

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K274					√	√√		√√		√					√	√
K276			√√	√			√			√						√
K281	√				√		√									
K286							√									
K313																
K317	√			√	√		√			√√*					√	
K323					√			√√								
R336									√√							
R337																
R348																
K351	√		√√		√	√√				√	√√*				√	
K359			√√			√√*		√√					√√*			
K372					√		√	√√	√√*							
K378			√√		√	√√*		√√		√			√√*	√√*	√	
K389				√				√√								
K402								√√								
R410																
K413					√			√√								
K414			√√			√√			√√	√	√√*	√√	√√*			
R428							√									
K432					√		√	√√		√√*					√	
K436								√√		√						
K439	√			√				√√								
K444								√√								
R445																
K466								√√			√√*					
R472								√√								
K475			√√		√	√√		√√		√						
R484							√									
R485							√									
K500								√√								
K519								√√								
R521																
K524																
K525	√	√	√√	√	√	√√		√√		√	√√*	√√	√√*		√	√
K534	√			√											√	
K536																
K538										√√*						
K541																
K545			√√		√	√√	√			√			√√*		√	
K557					√	√√*	√									
K560							√			√						
K564					√		√									
K573								√√		√						
K574					√	√√	√			√			√√*	√√*	√	
Total glycation sites	9	1	16	10	24	22	23	35	8	32	9	4	13	6	16	8

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√ - Glycation sites that were identified in literatures
√√ - Glycation sites that were quantified in literatures
√√* - Glycation sites that were quantified with significant differences between healthy and diabetic subjects

Notes:

[1]There are 84 potential glycation sites on HSA.

[2]The serial numbers 1-16 represent 16 literatures of previous *in vivo* study. The tick mark represents that the site was identified in corresponding literatures; double tick mark represents that the site was quantified; asterisk represents that the site was significantly different between healthy subjects and diabetic patients.

Literatures 1-16:

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Ahmad F, Saboury AA, Ganjali MR, Norouzi P. Spectroscopic studies of the effects of glycation of human serum albumin on L-Trp binding. Protein Pept Lett 2007;14:13-8.
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Supplementary Table 3. The contribution of each site glycation on warfarin binding

Sites	*Kd* (μM)	Fold change in affinity after glycation	Fold change in glycation during diabetes	Multiply the fold of affinity change after glycation by fold of glycation change during diabetes
K199	1.72	2.03	1.77	3.59
K233	2.43	1.43	1.25	1.79
K414	3.27	1.06	2.00	2.12
K262	2.79	1.25	1.45	1.81
K475	2.78	1.25	1.46	1.83
K402	3.61	-1.03	1.33	-1.37
K162	4.47	-1.28	1.54	-1.97
K436	3.91	-1.12	1.51	-1.69
K439	3.93	-1.13	1.52	-1.72
Net effect				4.39
Net effect excluding K199				0.79

Notes:
The *Kd* values were calculated from the modeling simulation.

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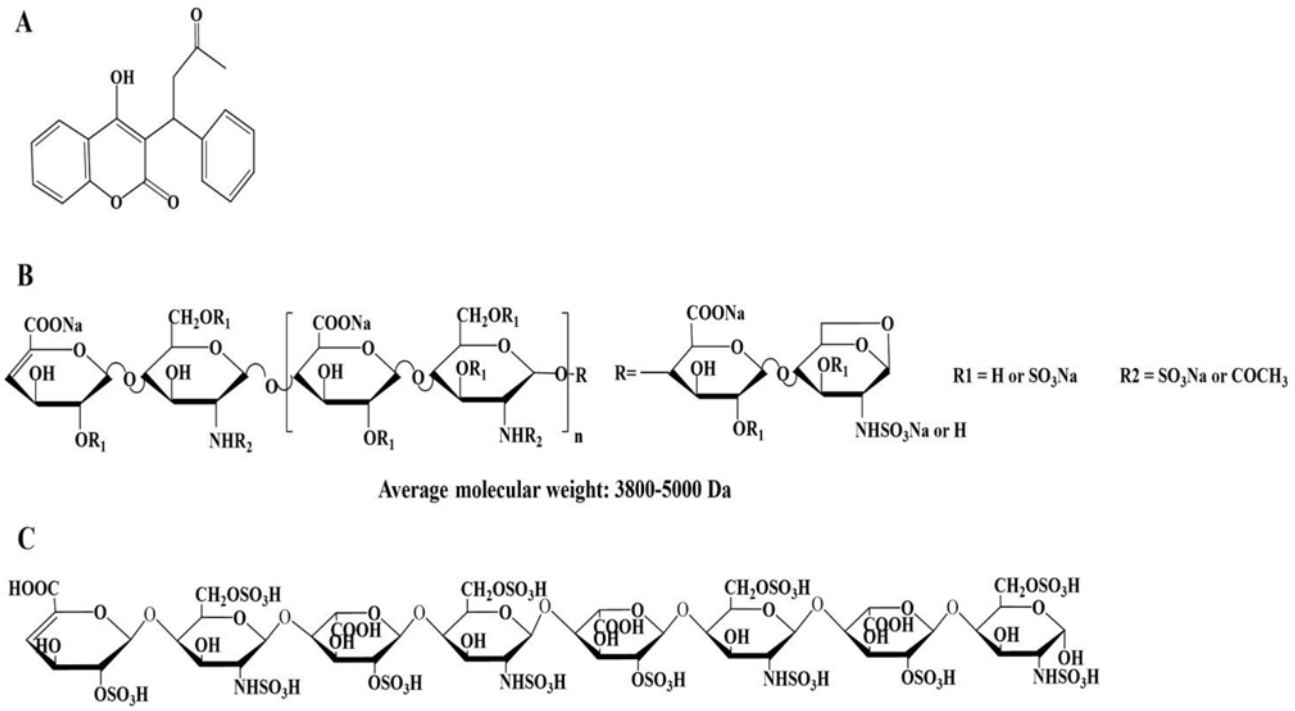
Supplementary Figure 1. Structure of warfarin, enoxaparin and a heparin octasaccharide. (A) Structure of warfarin. (B) Structure of enoxaparin sodium. (C) Structure of a heparin octasaccharide.



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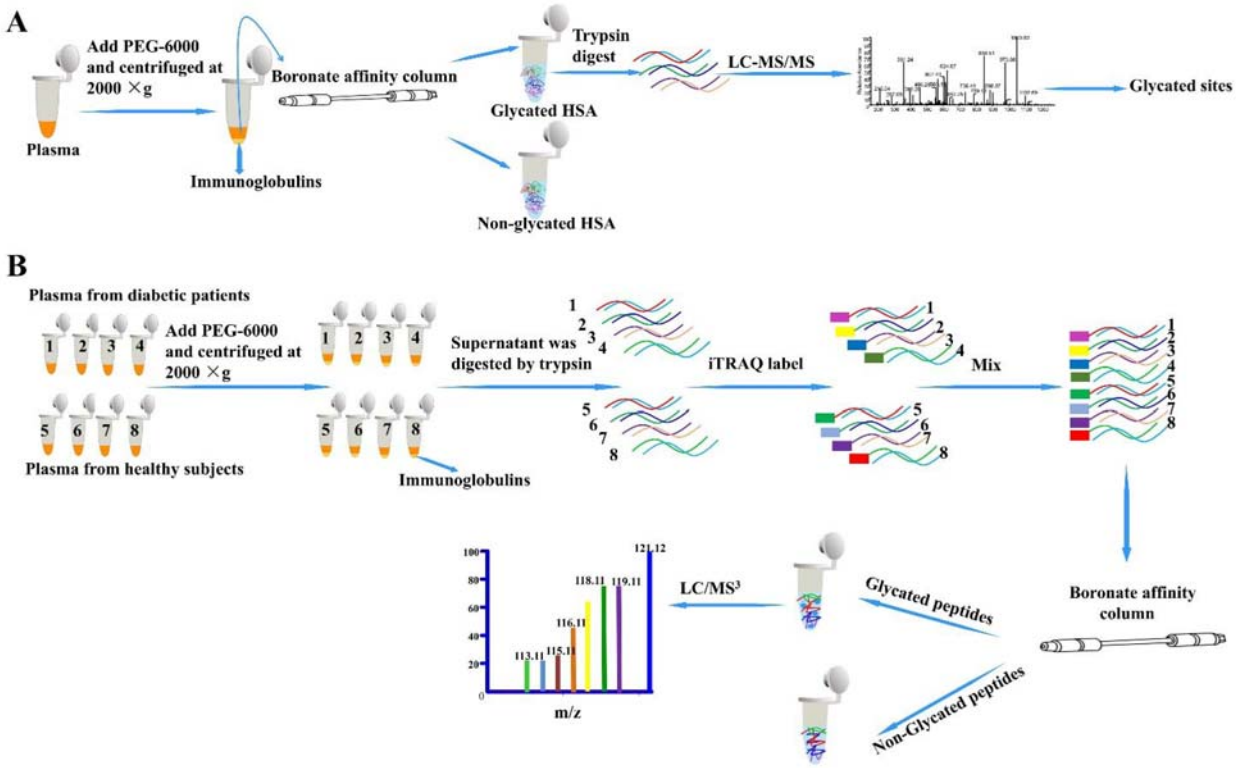
Supplementary Figure 2. The workflow of qualitative and quantitative analysis of HSA glycation sites. (A) Workflow for the identification of glycation sites in diabetic patients and healthy subjects. (B) Workflow for the quantitative comparison of glycation sites between diabetic patients and healthy subjects.



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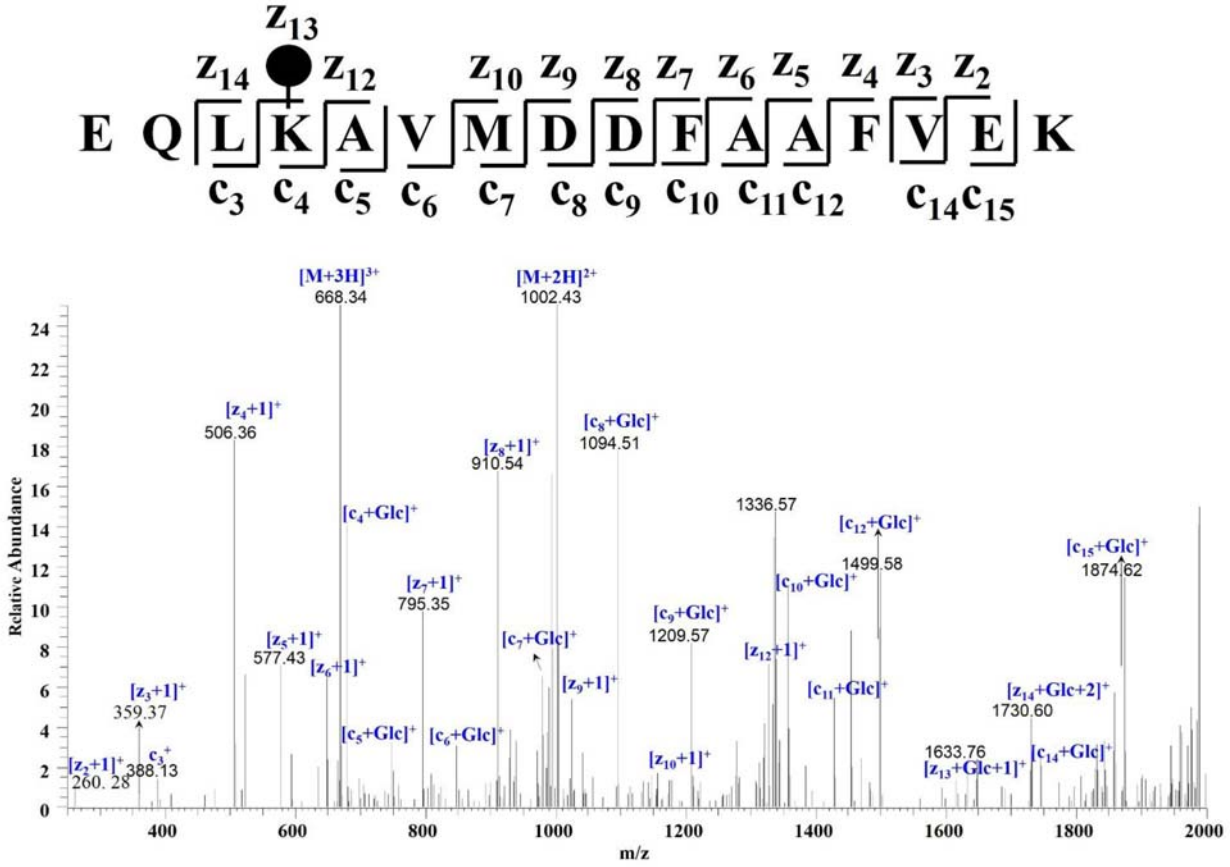
Supplementary Figure 3. Confirmation of glycation site K545 using ETD-MS/MS. ETD fragmentation of the parent-ion of peptide $E_{542}QLK_{Glc}AVMDDFAAFVEK_{557}$ (*m/z* 668.33, +3 charge) resulted in peptide bonds breakage, which generated a series of fragment ions with the glucose moiety attached. Two pairs of fragment ions, c_3 and c_4 + Glc, z_{12} + 1 and z_{13} + Glc + 1, confirmed that the glucose was attached at K545.



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Supplementary Figure 4. Distribution of identified glycation sites on the tertiary structure of HSA. HSA was visualized in the views of "surface" in figure A-F and "ribbon structure" in figure G-L. In figure A-F, domain I, II, and III of HSA were colored by magenta, green and cyan, the major binding regions subdomain IIA and IIIA were colored by palecyan and limon, and glycation sites identified in current study were colored by salmon. In figure G-L, the HSA backbone was colored by cyan and glycation sites identified in current study were shown by sticks, the residue K199 was colored by blue. (A) The front side of HSA in the view of surface. (B) The top side of HSA in the view of surface. (C) The back side of HSA in the view of surface. (D) The left side of HSA in the view of surface. (E) The bottom side of HSA in the view of surface. (F) The right side of HSA in the view of surface. (G) The front side of HSA in ribbon structure. (H) The top side of HSA in ribbon structure. (I) The back side of HSA in ribbon structure. (J) The left side of HSA in ribbon structure. (K) The bottom side of HSA in ribbon structure. (L) The right side of HSA in ribbon structure.

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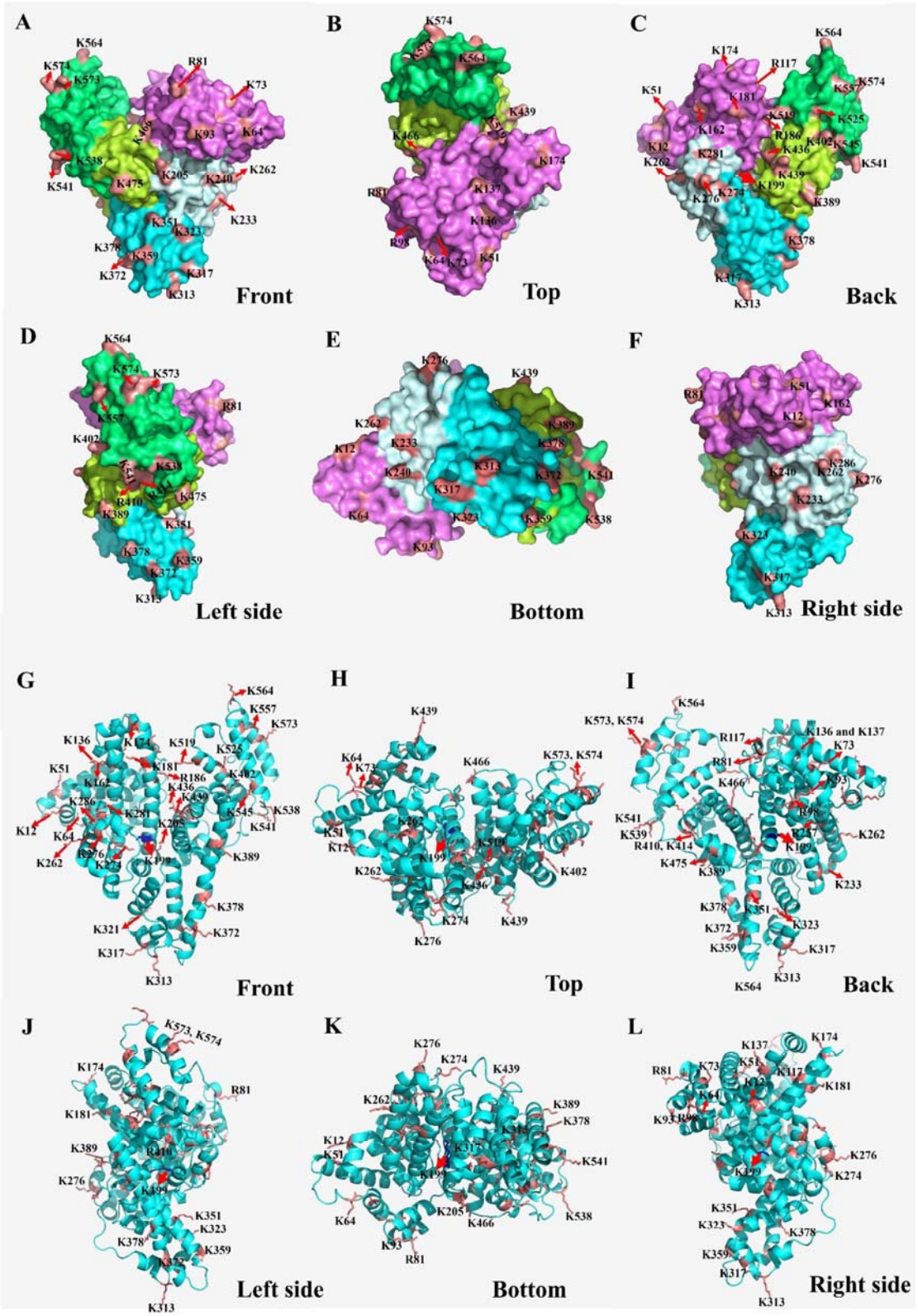

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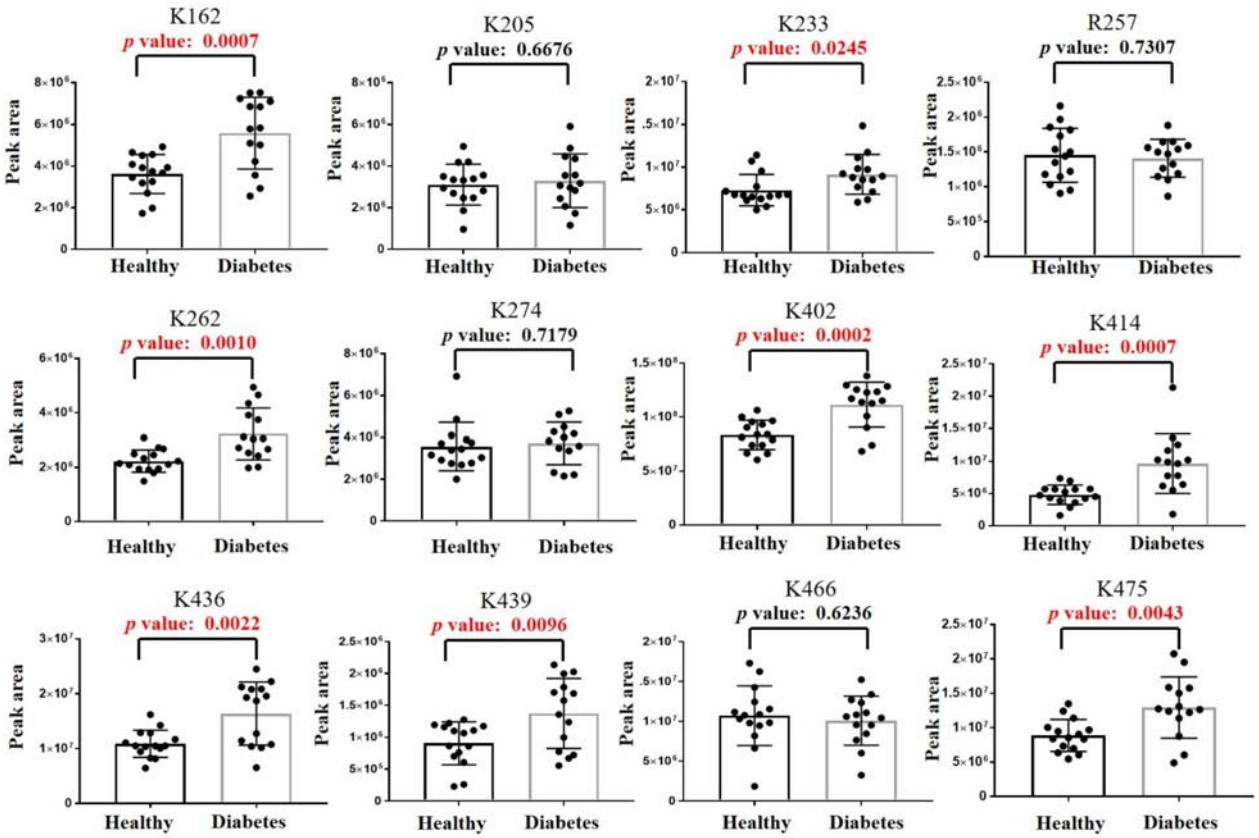
Supplementary Figure 5. nLC-MS/MS-MRM analysis of 12 glycation sites in plasma from diabetic patients and healthy subjects.

The amount of glycation at each glycation site was analyzed for healthy subjects (n = 15) and diabetic patients (n = 14), the statistical differences were analyzed using an unpaired t-test with a 95% confidence interval.



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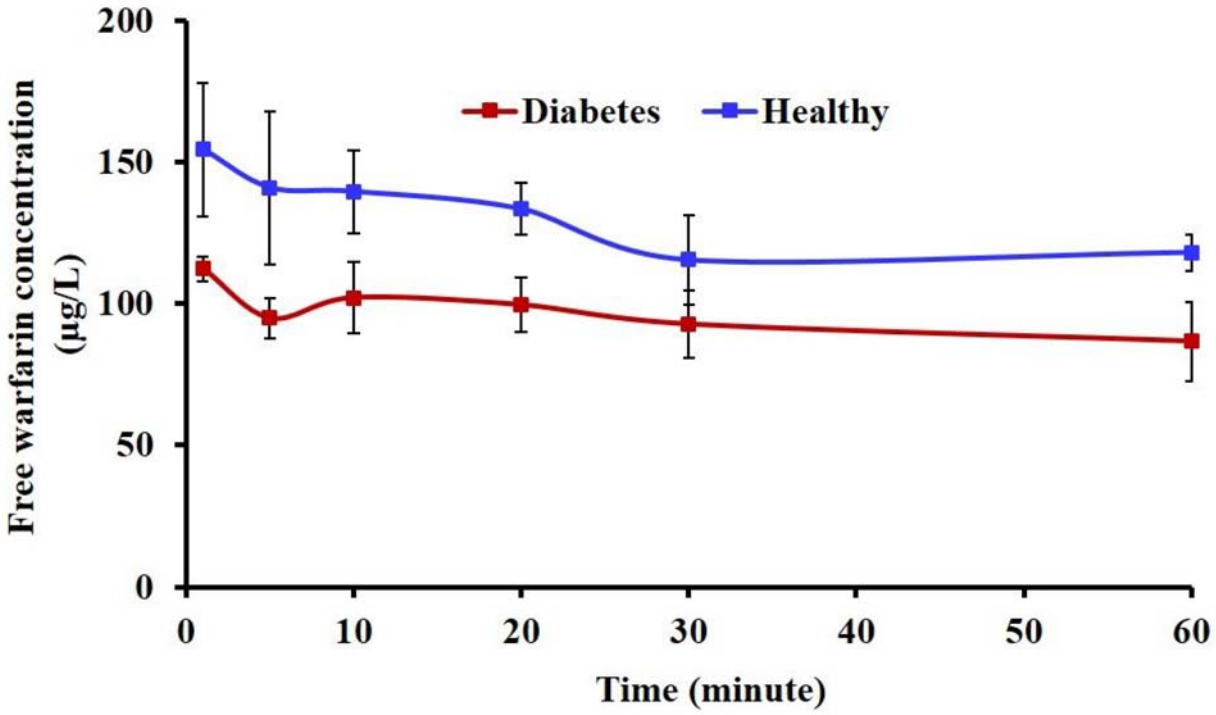
Supplementary Figure 6. Saturating binding study for warfarin

Free drug levels of warfarin after incubation for 1 min, 5 min, 10 min, 20 min, 30 min and 60 min with freshly collected plasma from diabetic patients (n = 5) and healthy subjects (n = 5). Data were presented as mean ± SD.



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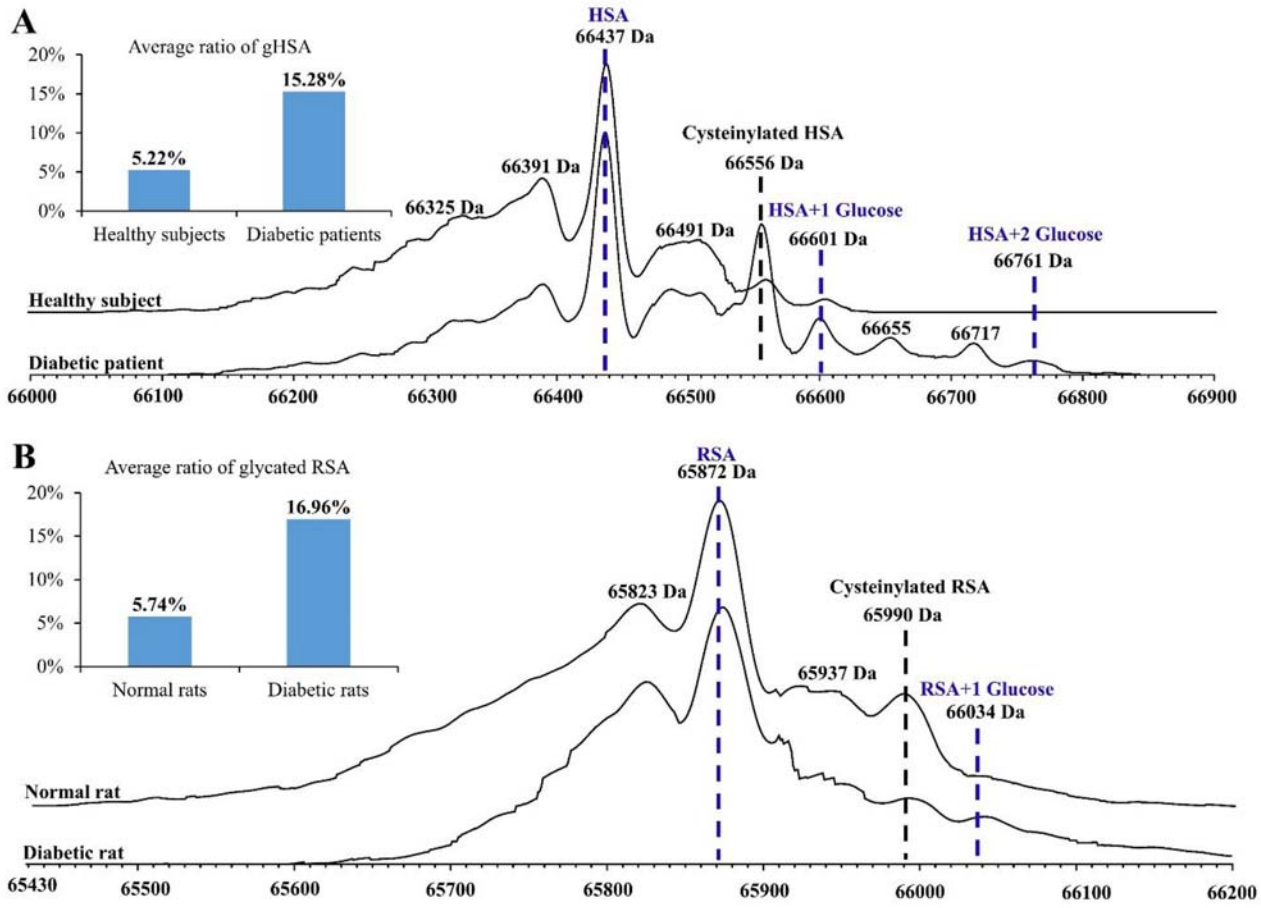
Supplementary Figure 7. Profiles of intact human and rat serum albumins and their glycated forms. (A) The deconvoluted MS spectra of HSA from healthy and diabetic subjects. The molecular weight of HSA was about 66,437 Da, while gHSA were approximately 66,601 Da (attaching one glucose molecule) or 66,761 Da (attaching two glucose molecules). The peak at 66,556 Da represents the cysteinyl adducted HSA. The percentage of gHSA was calculated from the intensity ratio of gHSA to total HSA. gHSA accounted for 15.28% in diabetic patients while it was 5.22% in healthy subjects. (B) The deconvoluted MS spectra of albumin from normal and diabetic rats. The molecular weight of rat serum albumin (RSA) was approximately 65,872 Da, while its glycated form with one glucose molecule was 66,034 Da. The peak 65,990 Da represents the cysteinyl adducted RSA. The glycated RSA accounted for 16.96% in diabetic rats while it was 5.74% in normal rats.



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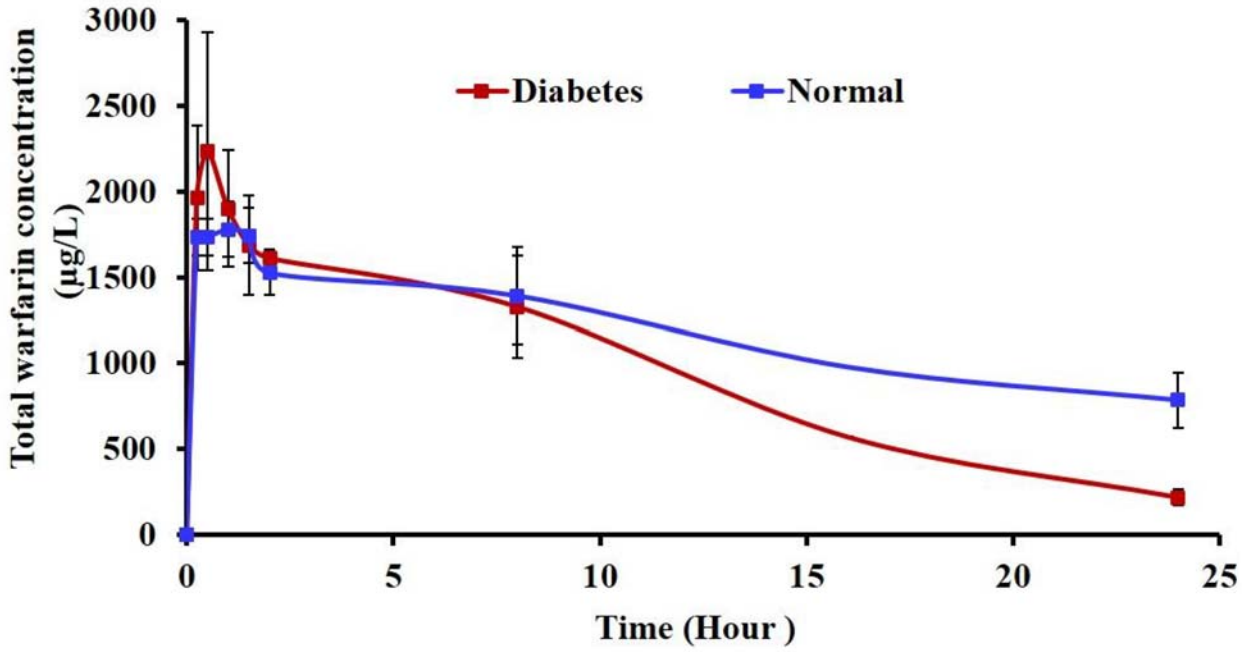
SUPPLEMENTARY DATA

Supplementary Figure 8. Total warfarin in plasma from diabetic and healthy rats

Total concentration of warfarin at different time points were compared between diabetic rats (n = 3) and normal rats (n = 3). Data were presented as mean ± SD.



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