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Translational

Effects of nonenzymatic glycation and fatty acids on functional properties of human albumin

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ABSTRACT

Human serum albumin nonenzymatically condenses with glucose to form stable Amadori adducts that are increased with the hyperglycemia of diabetes. The present study evaluated the influence of fatty acids, which are major endogenous ligands, on albumin glycation and of glycation on albumin conformation and exogenous ligand binding. Physiologic concentrations of palmitate, oleate, and linoleate reduced the ability of albumin to form glucose adducts, whereas glycation decreased intrinsic fluorescence, lowered the affinity for dansylsarcosine, and diminished the fatty acid–induced increase in limiting fluorescence of protein-bound warfarin that was observed with nonglycated albumin. The findings indicate that fatty acids impede the ability of albumin to undergo Amadori glucose modification and induce conformational changes affecting exogenous ligand binding, and that nonenzymatic glycation of albumin induces alterations in structural and functional properties that may have import in lipid transport and atherogenesis.

1. Introduction

Elevated plasma concentrations of albumin modified by Amadori glucose adducts associate independently with vascular complications of diabetes [1-3] and contribute to the pathogenesis of diabetic nephropathy and retinopathy through activation of cell signaling pathways and modulation of the expression of molecular mediators and structural proteins [4-13]. The role of albumin glycation in lipid transport and vascular disease, which may relate to an effect on the protein's ligand binding properties, is less clear. Albumin binds diverse endogenous and exogenous ligands including drugs and physiologic molecules such as bilirubin and hemin, and carries between 0.1 and 2 mol of fatty acid per mole of albumin [14-16]. Studies of the influence of glycation on binding of the endogenous ligands bilirubin and fatty acids, and exogenous ligands such as warfarin, dansylsarcosine, and benzodiazepine, have yielded variable results [17-23], leaving unresolved the question of whether nonenzymatic glycation modulates functional properties of the relevant ligand binding pockets. Furthermore, information is lacking on whether physiologically important fatty acids that are transported by albumin impose limitations on the ability of susceptible lysine residues to form Amadori glucose adducts, although this possibility was suggested in an early publication reporting that glycation was enhanced when fatty acids were removed from albumin before incubation with ^{14}C-glucose [24]. This issue is of interest in light of the demonstration that inhibiting the nonenzymatic glycation of albumin has therapeutic potential for preventing complications in

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patients with diabetes [25-29], in whom fatty acid levels are often elevated.

The purpose of the present experiments was to evaluate the effect of physiologically relevant fatty acids on the nonenzymatic glycation of albumin and of glycation on conformational and functional properties of the albumin protein. We assessed the influence of the physiologically relevant fatty acids palmitate, oleate, and linoleate on the ability of albumin to undergo Amadori glucose modification and examined the effects of glycation on functional and conformational properties of the protein by measuring intrinsic fluorescence, the binding of the fluorescent markers warfarin and dansylsarcosine that are specific for the site I and site II binding pockets that are, respectively, located in subdomains IIA and IIIA in the albumin molecule [30-34], and fatty acid–induced changes in limiting fluorescence. We report that fatty acids impede the ability of human serum albumin to form Amadori glucose adducts and that glycation modulates conformational and functional properties of the protein that are expressed as a marked decrease in intrinsic fluorescence, reduced binding of the ligand dansylsarcosine, and diminution of the fatty acid–induced increase in the limiting fluorescence of albumin-bound warfarin.

2. Materials and methods

Fatty acid–free albumin, warfarin, dansylsarcosine, and fatty acids (palmitate, oleate, linoleate) were obtained from Sigma-Aldrich (St Louis, MO). Glycated albumin was purified from fatty acid–free albumin by affinity chromatography on phenylboronate agarose [35,36], applying the protein in loading buffer (5 mmol/L DL-asparagine, 8.5 mmol/L L-methionine, 6 mmol/L taurine, 50 mmol/L $MgCl_2$, pH 9.0); eluting the bound (glycated) protein with 0.1 mol/L Tris HCl, pH 7.0, containing 250 mmol/L sorbitol; and desalting on NAP-5 columns (GE Healthcare, Buckinghamshire, UK) into phosphate-buffered saline, pH 7.4. Amadori glucose adducts, which complex with phenylboronate agarose under alkaline conditions and are dissociated by lowering the pH and/or with a competing polyol, represented approximately 1.5% of fatty acid–free albumin.

Glycated albumin formed after incubation of fatty acid–free albumin with glucose for 7 days at 37°C in the absence or presence of fatty acids was determined by separating the glycated from the nonglycated species by affinity chromatography on phenylboronate agarose as described above. For application to the columns, samples were defatted with activated charcoal/acetic acid (5%:10% wt/vol) and desalted on NAP-5 columns into the loading buffer. Albumin in the glycated fraction was measured by immunoassay specific for human albumin (Albuwell II; Exocell, Philadelphia, PA), using standard curves controlled for the presence of sorbitol.

Fluorescence measurements were conducted in a spectrophotofluorometer (Aminco Bowman, Silver Spring, MD). Intrinsic fluorescence due to tryptophan was measured using an excitation wavelength of 294 nm and emission wavelengths of 300 to 500 nm. The albumin preparations were dissolved in phosphate-buffered saline, pH 7.4, and analyzed at concentrations of 5-20 μmol/L. Fatty acids were completely solubilized in ethanol and were added in aliquots of 5 μL to yield final concentrations of 5 to 80 μmol/L. Measurements were controlled for any effect of 0.5% ethanol.

Warfarin and dansylsarcosine, which fluoresce upon binding to albumin, were used as probes for site I in subdomain IIA and site II in subdomain IIIA, respectively. Warfarin binding was monitored at excitation/emission wavelengths of 330/390 nm, and dansylsarcosine binding was monitored at excitation/emission wavelengths of 350/490 nm. Parallel sets of tubes were prepared containing a designated concentration of albumin (5-20 μmol/L), without or with the addition of variable amounts of fatty acids (1:1, 2:1, and 4:1 molar ratio to albumin), to which dansylsarcosine (0.25-64 μmol/L) or warfarin (0.25-128 μmol/L) was incrementally added. Albumin and fatty acids were aliquoted from stock solutions prepared as described above, controlling for any effect of ethanol as noted. Titrations to measure the limiting fluorescence of warfarin and dansylsarcosine bound to albumin in the absence or presence of fatty acids were performed as described by Sudlow et al [37], maintaining the ratio of fatty acid to albumin at 1:1, 2:1, or 4:1 while increasing the concentrations of both. Binding constants and the number of binding sites were calculated using nonlinear regression analysis (Prism by GraphPad Software, La Jolla, CA), taking into consideration the change in quantum yield of the fluorescence of the probes when bound to albumin as assessed by the limiting fluorescence value. Data under different experimental conditions were analyzed by means of analysis of variance.

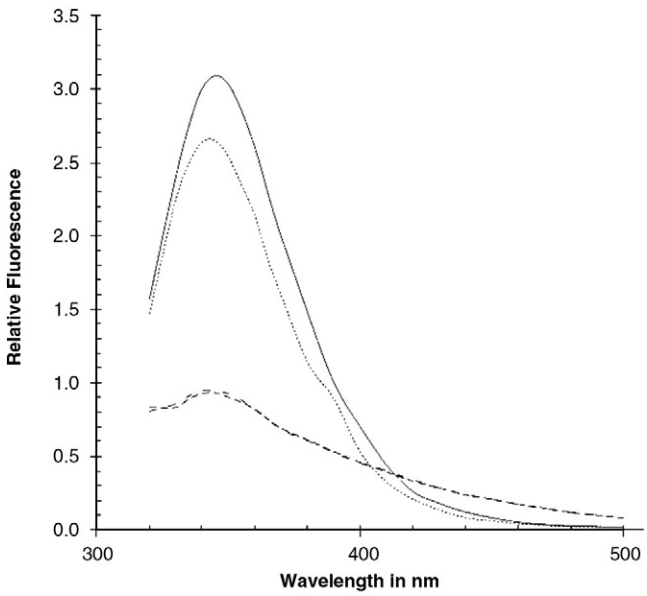

Fig. 1 – Effects of glycation and fatty acids on the fluorescence spectra of fatty acid–free albumin. The intrinsic fluorescence of 7.5-μmol/L solutions of nonglycated and glycated albumin was measured at excitation 294 nm and 300- to 500-nm emission scanning without or with a physiologic mixture of fatty acids containing 15 μmol/L each of palmitate, oleate, and linoleate. Nonglycated albumin without (_____) or with (.....) fatty acids; glycated albumin without (_ _ _ _ _ _) or with (___...___) fatty acids.

3. Results

The intrinsic fluorescence scan for fatty acid–free nonglycated albumin showed a strong symmetrical fluorescence peak at 343 nm that, in the presence of a physiologic mixture of fatty acids consisting of palmitate, oleate, and linoleate at respective molar ratios to albumin of 2:1 and in concentrations representative of those found in plasma [38], was still symmetrical but exhibited a slight leftward shift in maximal fluorescence and a modest decrease in relative fluorescence (Fig. 1). Examined individually at molar ratios to albumin of 2:1, these fatty acids did not appreciably change intrinsic fluorescence due to tryptophan of fatty acid–free albumin. Compared with the nonglycated protein, nonenzymatically glycated albumin exhibited a marked decrease in intrinsic fluorescence with loss of symmetry at the 343-nm emission peak and a modest shift to the left of the emission maximum wavelength, indicating a change in the environment of the single tryptophan 214 residue and consistent with an alteration in protein conformation, which showed little change in the presence of the fatty acid mixture (Fig. 1).

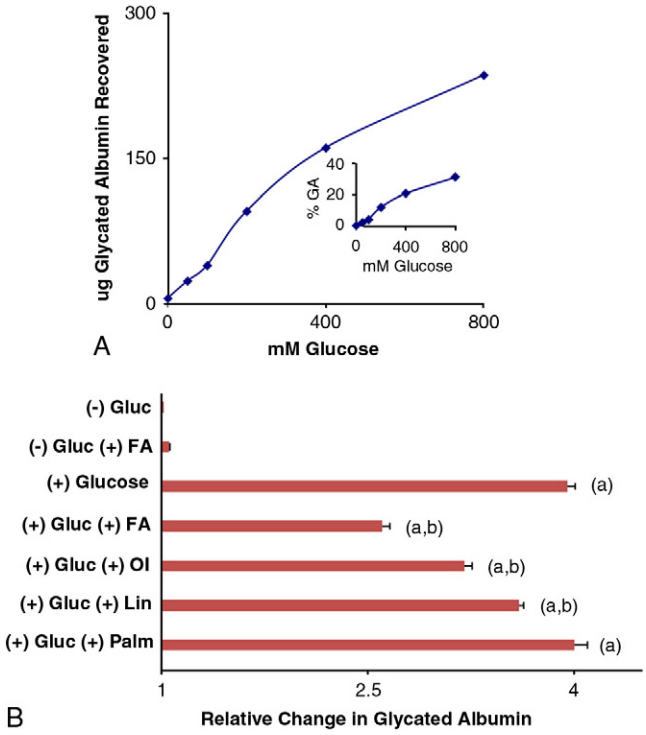

Fig. 2 – Effect of fatty acids on nonenzymatic glycation of fatty acid–free albumin. Samples containing 40 mg/mL (0.6 mmol/L) of albumin were incubated for 7 days under the indicated conditions. A, Relation between glucose concentration and formation of albumin modified by Amadori glucose adducts. B, Glycated albumin formed after incubation with 20 mmol/L glucose without or with coincubation with fatty acids as a physiologic mixture (FA) or individually (palmitate, oleate, or linoleate) at a 1:1 molar ratio to albumin. Glycated albumin formed in the absence of fatty acids assigned an arbitrary value of 1. Results are mean ± SEM, n = 3. (a) $P < .05$ compared with (–) glucose; (b) $P < .05$ compared with (+) glucose.

To assess whether physiologically relevant fatty acids modulated susceptibility of albumin to the formation of Amadori glucose adducts, fatty acid–free albumin was incubated with 20 mmol/L glucose for 7 days in the absence or presence of the 3 designated fatty acids, alone or in concert (1:1 molar ratio to albumin). Preliminary experiments validated the methodology by showing a dose-response relationship between progressively increasing glucose concentration and glycated albumin recovered from the affinity chromatography column (Fig. 2A). Coincubation of albumin with glucose in the presence of the fatty acid mixture decreased the formation of Amadori-modified glycated albumin to approximately 65% of control, whereas when added individually to incubations with albumin, these fatty acids had lesser effects on the amount of glycated albumin formed (80%, 90%, and 100% of control with oleate, linoleate, and palmitate, respectively) (Fig. 2B).

Binding of the site II probe dansylsarcosine to glycated albumin was less than to nonglycated albumin over a range of concentrations of probe tested (Fig. 3), with analysis of the data indicating decreased affinity without change in the number of specific binding sites compared with the nonglycated protein (Table 1). The limiting fluorescence value for dansylsarcosine was not affected by fatty acids, but the fluorescence of dansylsarcosine bound to fatty acid–free nonglycated and glycated albumin was, respectively, reduced approximately 30% and 15% in the presence of a mixture of the indicated fatty acids at a 2:1 molar ratio compared with the corresponding albumin preparations in the absence of the

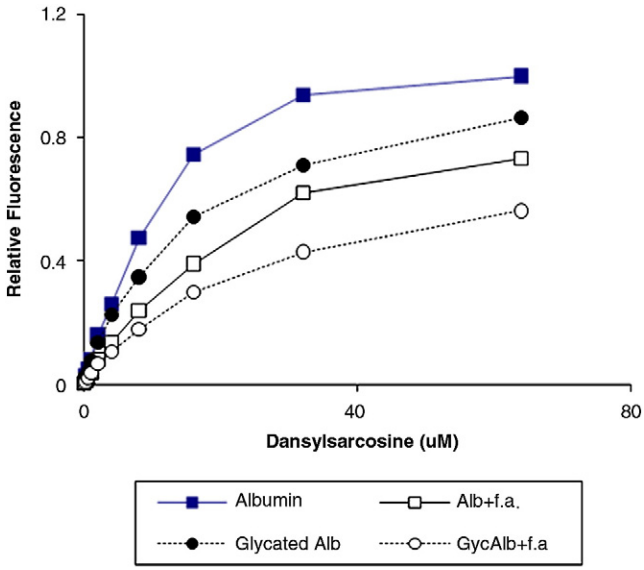

Fig. 3 – Effects of glycation and fatty acids on dansylsarcosine binding to fatty acid–free albumin. Fluorescence of increasing concentrations of dansylsarcosine at excitation/emission wavelengths of 350/490 nm added to a 10-μmol/L solution of albumin in the absence or presence of a physiologic mixture of fatty acids at 2:1 molar ratios to albumin as described in the legend to Fig. 1A. Nonglycated albumin without (▪ — ▪) and with (▫ — ▫) fatty acids; glycated albumin without (● — ●) and with (○ — ○) fatty acids. Fluorescence of fatty acid–free albumin with 64 μmol/L dansylsarcosine assigned an arbitrary value of 1 (100%).

Table 1 – Effects of glycation and fatty acids on dansylsarcosine and warfarin binding

Probe	Albumin	Addition	LFV	Kd (μmol/L)	No. sites/mol albumin
Dansylsarcosine	Nonglycated	0	1	1.7 ± 0.08	1.0
	Glycated	0	1	2.5 ± 0.02 *	0.9 ± 0.01
	Nonglycated	PM	1	3.0 ± 0.02 *	0.7 ± 0.09 *
	Glycated	PM	1	3.0 ± 0.06 †	0.6 ± 0.003 †
Warfarin	Nonglycated	0	1	3.4 ± 0.04 [a]	1.0 [a]
	Glycated	0	1	3.3 ± 0.03 [a]	0.9 ± 0.09 [a]
	Nonglycated	PM	3.26	2.0 ± 0.09 [a] *	0.6 ± 0.003 [a] *
	Glycated	PM	1.05	3.0 ± 0.10 [a]	1.1 ± 0.18 [a]

Results represent mean of 3 experiments. PM indicates physiologic mixture containing palmitate, oleate, and linoleate at 2 to 1 molar ratios to albumin; LFV, limiting fluorescence value.

[a] Corrected for quantum yield.

* $P < .05$ compared with nonglycated control (no addition).

† $P < .05$ compared with glycated control (no addition).

fatty acid mixture (Fig. 3). Analysis of these data indicated that the affinity and the number of specific binding sites in both nonglycated and glycated albumin were significantly decreased in the presence of the fatty acid mixture (Table 1). For comparative purposes, these studies were also performed with glycated albumin purified from therapeutic grade

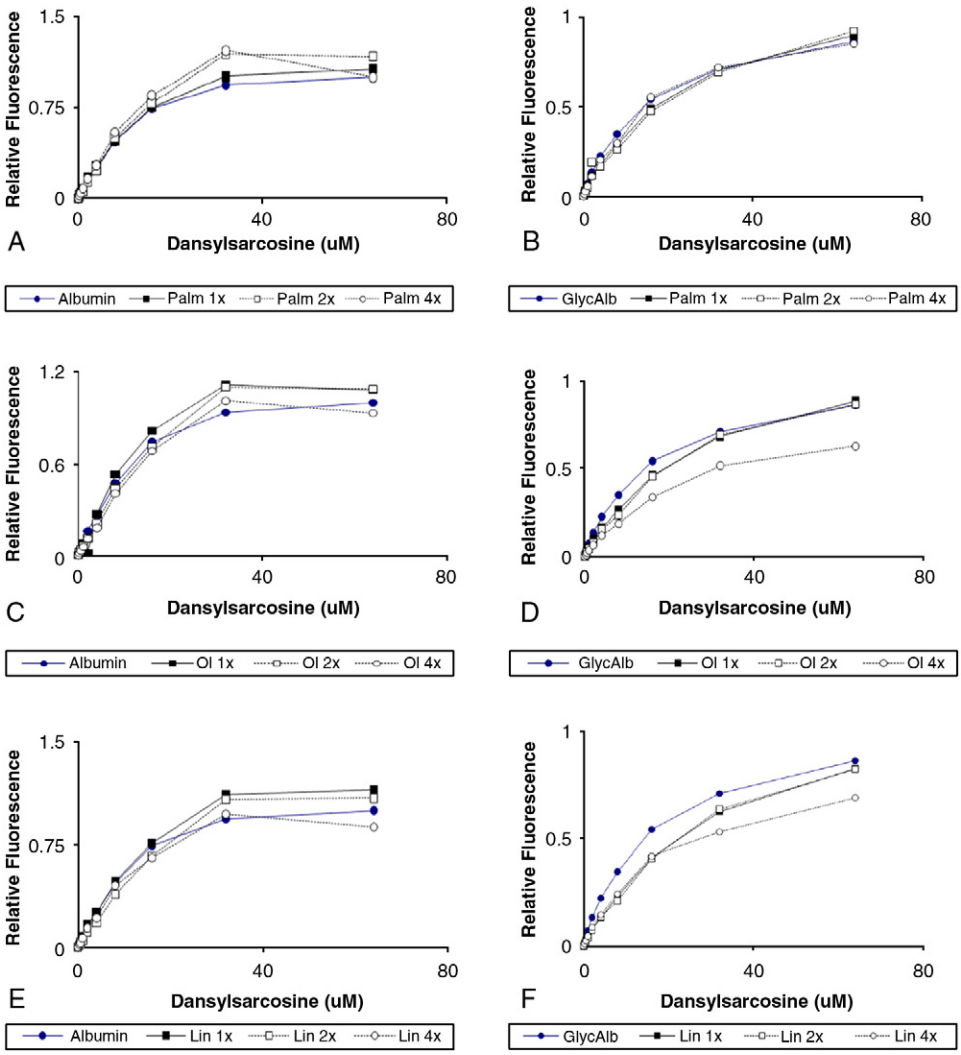

Fig. 4 – Effect of individual fatty acids on dansylsarcosine binding. Samples contained 5 μmol/L fatty acid–free nonglycated or glycated albumin and the indicated concentrations of dansylsarcosine and of palmitate (A, B), oleate (C, D), and linoleate (E, F). Fluorescence measured as described in the legend to Fig. 3. Fluorescence of fatty acid–free albumin with 64 μmol/L dansylsarcosine assigned an arbitrary value of 1.0.

albumin (Octapharma, Vienna, Austria) by affinity chromatography on phenylboronate agarose and subsequently treated with charcoal/acetic acid (5%:10% wt/vol), which removes the stabilizers (caprylic acid, acetyl tryptophan) that impair ligand binding and restores binding capacity to levels obtained with fatty acid–free albumin [39]. Dansylsarcosine binding data to this preparation of glycated albumin in the absence or presence of the fatty acid mixture yielded results very similar to those observed with glycated albumin purified from the Sigma fatty acid–free albumin (data not shown).

When these fatty acid were added individually, changes in fluorescent curves of dansylsarcosine binding were less pronounced than when added as a mixture. Palmitate added at a 1:1, 2:1, or 4:1 molar ratio to nonglycated or glycated albumin had no effect on dansylsarcosine fluorescence over the range of concentrations tested (Fig. 4A, B) or on affinity for the ligand (Table 2). Oleate added at a 1:1, 2:1, or 4:1 molar ratio did not affect dansylsarcosine fluorescence with nonglycated albumin but decreased fluorescence (Fig. 4C, D) and ligand affinity (Table 2) when added to glycated albumin. Linoleate had little effect on dansylsarcosine fluorescence (Fig. 4E, F) and variable influence on ligand affinity (Table 2) for nonglycated albumin or glycated albumin.

Binding of the site I probe warfarin to glycated albumin approximated that of nonglycated albumin over the range of concentrations of probe tested (Fig. 5), with analysis of the data indicating no change in affinity or the number of binding sites in the glycated compared with the nonglycated protein (Table 1). Warfarin fluorescence with binding to nonglycated albumin markedly increased over a range of concentrations tested in the presence of a physiologic mixture of the indicated fatty acids (Fig. 5). Most of the increased fluorescence arose from increased quantum yield according to the higher limiting fluorescence value for warfarin bound to albumin when fatty acids were added, and analysis of the data indicated increased affinity with a decrease in the number of binding sites in the presence of the fatty acid mixture (Table 1). In contrast, the fluorescence of warfarin binding to glycated albumin was minimally affected by the presence of fatty acids (Fig. 5); and the limiting fluorescence value of warfarin bound to glycated albumin was unchanged in the presence of fatty acids. Analysis of the data indicated no difference in affinity or the number of binding sites in glycated albumin in the presence of fatty acids (Table 1). Fluorescence curves and data analysis of warfarin binding data from studies with glycated albumin prepared from therapeutic grade

Table 2 – Effects of Individual fatty acids on dansylsarcosine and warfarin binding to nonglycated and glycated albumin

Probe	Albumin	Addition	LFV	Kd (μmol/L)	No. sites/mol albumin
Dansylsarcosine	Nonglycated	Palmitate 1×	1	1.8	1.1
		Palmitate 2×	1	1.9	1.2
		Palmitate 4×	1	1.8	1.0
	Glycated	Palmitate 1×	1	2.9	0.9
		Palmitate 2×	1	3.1	0.9
		Palmitate 4×	1	2.5	0.9
	Nonglycated	Oleate 1×	1	1.7	1.1
		Oleate 2×	1	2.0	1.1
		Oleate 4×	1	2.0	0.9
	Glycated	Oleate 1×	1	3.1	0.9
		Oleate 2×	1	3.0	0.9
		Oleate 4×	1	3.0	0.6
	Nonglycated	Linoleate 1×	1	1.9	1.2
		Linoleate 2×	1	2.2	1.1
		Linoleate 4×	1	1.7	0.9
	Glycated	Linoleate 1×	1	3.2	0.8
		Linoleate 2×	1	3.2	0.8
		Linoleate 4×	1	2.6	0.7
Warfarin	Nonglycated	Palmitate 1×	1.25	3.1	1.0
		Palmitate 2×	2.48	2.2	0.7
		Palmitate 4×	3.30	1.6	0.8
	Glycated	Palmitate 1×	1.05	1.8	1.1
		Palmitate 2×	1.05	0.9	0.9
		Palmitate 4×	1.05	0.7	1.0
	Nonglycated	Oleate 1×	1.68	2.8	0.8
		Oleate 2×	2.42	2.1	0.7
		Oleate 4×	3.10	1.8	0.7
	Glycated	Oleate 1×	1.05	1.7	1.2
		Oleate 2×	1.05	0.8	1.0
		Oleate 4×	1.05	1.7	1.0
	Nonglycated	Linoleate 1×	1.38	2.9	0.9
		Linoleate 2×	1.90	2.0	1.0
		Linoleate 4×	2.53	2.0	0.9
	Glycated	Linoleate 1×	1.05	1.8	1.2
		Linoleate 2×	1.05	1.3	1.0
		Linoleate 4×	1.05	1.0	1.0

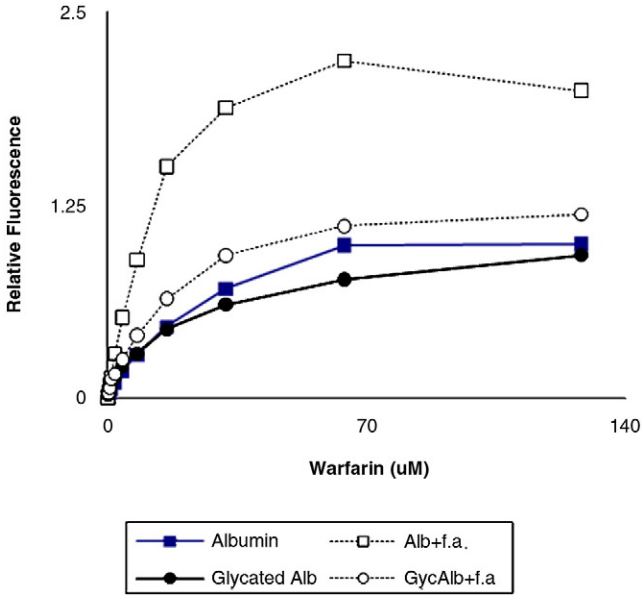

Fig. 5 – Effects of glycation and fatty acids on warfarin binding to fatty acid–free albumin. Fluorescence of increasing concentrations of warfarin at excitation/emission wavelengths of 330/390 nm added to a 10-μmol/L solution of albumin in the absence or presence of a physiologic mixture of fatty acids as described in the legend to Fig. 3. Nonglycated albumin without (▪ — ▪) and with (▫ — ▫) fatty acids; glycated albumin without (● — ●) and with (○ — ○) fatty acids. Fluorescence of fatty acid–free albumin with 128 μmol/L warfarin assigned an arbitrary value of 1 (100%).

albumin as described above and conducted in the absence or presence of the fatty acid mixture yielded results very similar to those observed with glycated albumin prepared from the Sigma fatty acid–free albumin (data not shown).

Fluorescence curves of warfarin binding were progressively higher (Fig. 6A, B) and showed increasing affinity (Table 2) with increasing molar ratios of palmitate to nonglycated or glycated albumin. Oleate or linoleate at 1:1, 2:1, and 4:1 molar ratios resulted in increased warfarin fluorescence (Fig. 6C-F) and affinity (Table 2) when added to nonglycated or glycated albumin, although the change in affinity was less at 4:1 compared with 2:1 molar ratio of oleate when added to glycated albumin (Table 2).

4. Discussion

These experiments are the first to directly demonstrate that fatty acids impose limitations on the ability of albumin to undergo Amadori glucose modification, and show that glycation markedly decreases the intrinsic fluorescence of albumin. Because the experimental conditions measured intrinsic fluorescence due to tryptophan and albumin contains a single tryptophan residue at 214, this finding is consistent with a conformational change that alters the physiochemical environment of tryptophan 214. The results of this study further indicate that nonenzymatic glycation modulates the binding of exogenous ligands for the site I and site II binding pocket in human serum albumin and diminishes the marked increase in limiting fluorescence of protein-bound warfarin that is observed when a mixture of physiologic fatty acids is added to nonglycated albumin. Because alteration in the limiting fluorescence value of albumin-bound warfarin reflects conformational change at site I consequent to fatty acid binding [33], this finding indicates that glycation renders the protein less susceptible to such change.

The demonstration that nonenzymatic glycation of albumin alters the interaction of physiologically relevant fatty acids with the albumin protein is a central and novel finding in the present study. The observed changes in binding of exogenous ligands to glycated albumin suggest that nonenzymatic glycation of albumin may have import in transport of drugs and lipids and/or in atherogenesis and are consistent with the report that glycation alters the affinity of the protein for binding of bilirubin to site I [17] and with the hypothesis that glycation of a lysine residue in domain IIIB can affect domain IIA [17,35,40]. Other studies have suggested that glycation of the lysine 525 residue located in domain IIIB, which is the principal site of nonenzymatic glycation of albumin in vivo and in vitro, may be accompanied by changes in protein conformation and altered binding of ligands at site I and/or site II [17,19,20,35]. Glycation of the lysine 199 residue, which binds acetylsalicylic acid and is located in domain IIA at the entrance to the site I binding pocket [40-42], occurs to a much lesser extent but has been reported to enhance binding of the site I probe warfarin and to decrease the affinity for bilirubin [17,20]. Whether nonenzymatic glycation of the other 2 lysines (residues 439 and 281) that may be subject to glycation in vivo [40] influences conformational or binding properties has not been directly examined; but this possibility is suggested by the proximity of the 439 residue to the mouth of the site II pocket [40], the surmised close contact of domains II and III in regions containing residues 199 and 439 [17], and the finding that recombinant albumin mutated or glycated at this residue alters tryptophan fluorescence and binding of warfarin and dansylsarcosine [20]. The glucose adducts in the glycated albumin used in the present study were assumed to be predominantly at the lysine 525 position, as reported by others [17,35,40].

The reduction in glycated albumin formation following coincubation of fatty acid–free albumin with glucose and fatty acids compared with glucose alone apparently does not derive from conformational shifts involving tryptophan 214 because fatty acids had very little effect on intrinsic fluorescence due to tryptophan, whereas nonenzymatic glycation profoundly decreased this measurement. Consideration of the main factors affecting nonenzymatic glycation, which include the pK of the amino groups, the accessibility of the lysine ε amino group, and the formation of the Amadori rearrangement [35], suggests an alternative explanation. Fatty acid–induced changes in structural features of subdomain IIA and/or IIIA may impede the ability of lysine ε amino groups to undergo the Amadori rearrangement necessary for the formation of the stable glucose adduct. This interpretation takes into account the report by Iberg and Fluckiger [40] that appropriately located positively charged amino acid residues promote local acid base catalysis, and proposes that fatty acid binding

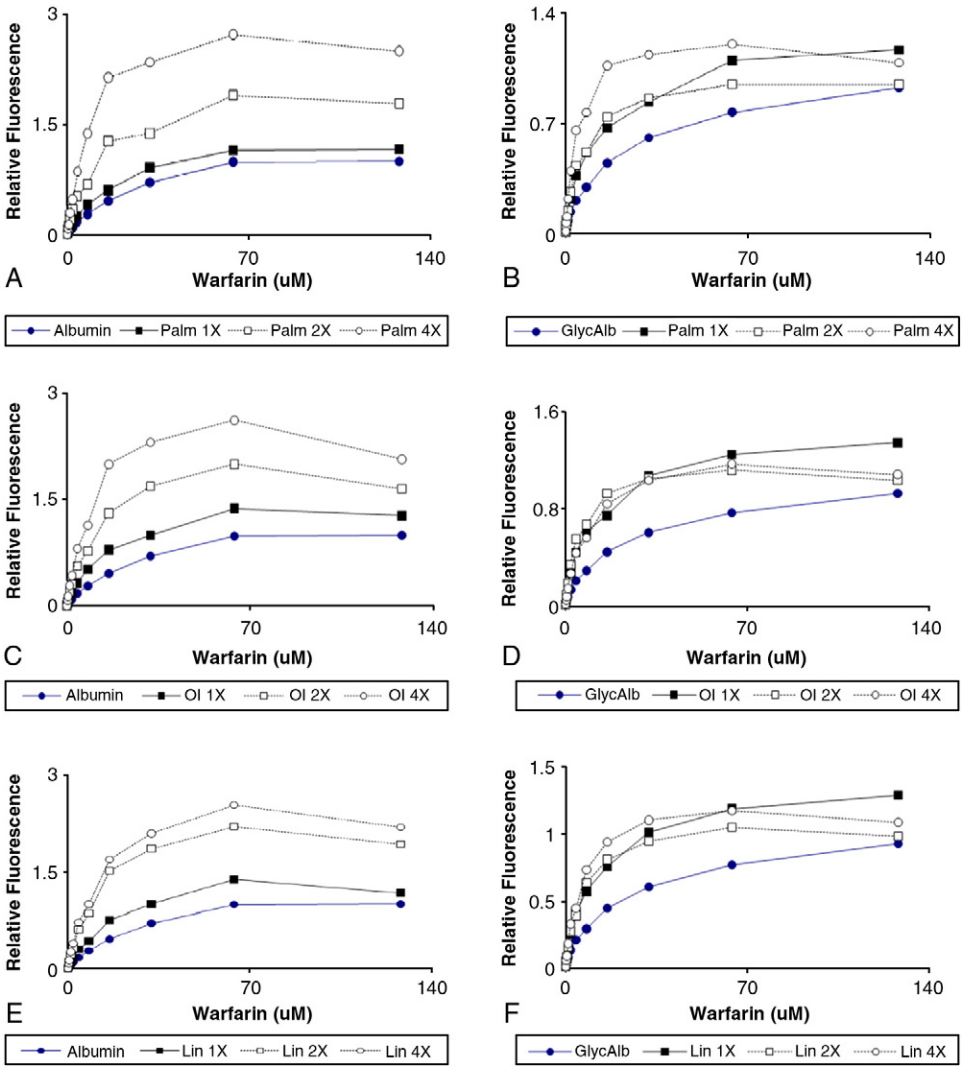

Fig. 6 – Effects of individual fatty acids on warfarin binding to albumin. Samples contained 5 μmol/L fatty acid–free nonglycated or glycated albumin and the indicated concentrations of warfarin and of palmitate (A, B), oleate (C, D), and linoleate (E, F). Fluorescence measured as described in the legend to Fig. 5. Fluorescence of fatty acid–free albumin with 128 μmol/L warfarin assigned an arbitrary value of 1.0.

at site I and/or site II may compromise local closeness of charged amino groups that act catalytically in the Amadori rearrangement at lysine residues susceptible to nonenzymatic condensation with glucose. The reduction in glycated albumin formation in the presence of fatty acids does not imply that increased levels of fatty acids are clinically beneficial, but does suggest that the influence of plasma concentrations of fatty acids on glycatability is relevant in clinical situations in which decreasing the formation of glycated albumin is desirable. The constraints imposed by fatty acids on the accessibility of potentially glycatable lysines due to coverage by a fatty acid ligand [35] or on the ability of lysine to form Amadori glucose adducts may have to be taken into account in patients with high levels of fatty acids when estimating dosages of agents directed at inhibiting the nonenzymatic glycation of albumin.

The changes in binding of exogenous ligands to nonglycated albumin in the presence of a mixture of physiologic fatty acids at 2:1 molar ratio are consistent with competition with the site II ligand and distinct from high-affinity fatty acid binding sites for longer-chain fatty acids distributed over the protein, which can affect site II binding by allosteric interference [15-17,43-46], and with the report that oleic, stearic, and myristic acids alter the conformation of the site I pocket and enhance warfarin binding at levels up to 3 to 4 mol of the fatty acid bound per mole of albumin, whereas higher concentrations may reduce the affinity of warfarin for albumin due to competition between warfarin and fatty acid for binding at domain IIA [14,45]. There is no evidence that the observed changes in binding affinity or number of sites for exogenous ligands in the presence of fatty acids affect biologic activities of the glycated protein, which are mediated by ligand-receptor interactions recognizing the fructosyllysine epitope [47].

In summary, we report that nonenzymatic glycation of albumin alters intrinsic fluorescence and binding of the exogenous ligands warfarin and dansylsarcosine, and that physiologically relevant fatty acids impede the ability of

albumin to undergo Amadori glucose modification and induce conformational changes affecting site I and site II binding properties. Although the clinical importance of these findings remains to be established, alterations in molecular conformation and in ligand binding properties of nonenzymatically glycated albumin, which is increased 2- to 3-fold in diabetes, may affect lipid transport and atherogenesis. Appreciation of the influence of fatty acids on the ability of albumin to form stable Amadori glucose adducts may bear on therapy directed at reducing the level of glycated albumin in diabetes.

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Conflict of interest statement

Disclosure statement: The authors are employed by Glycadia.

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