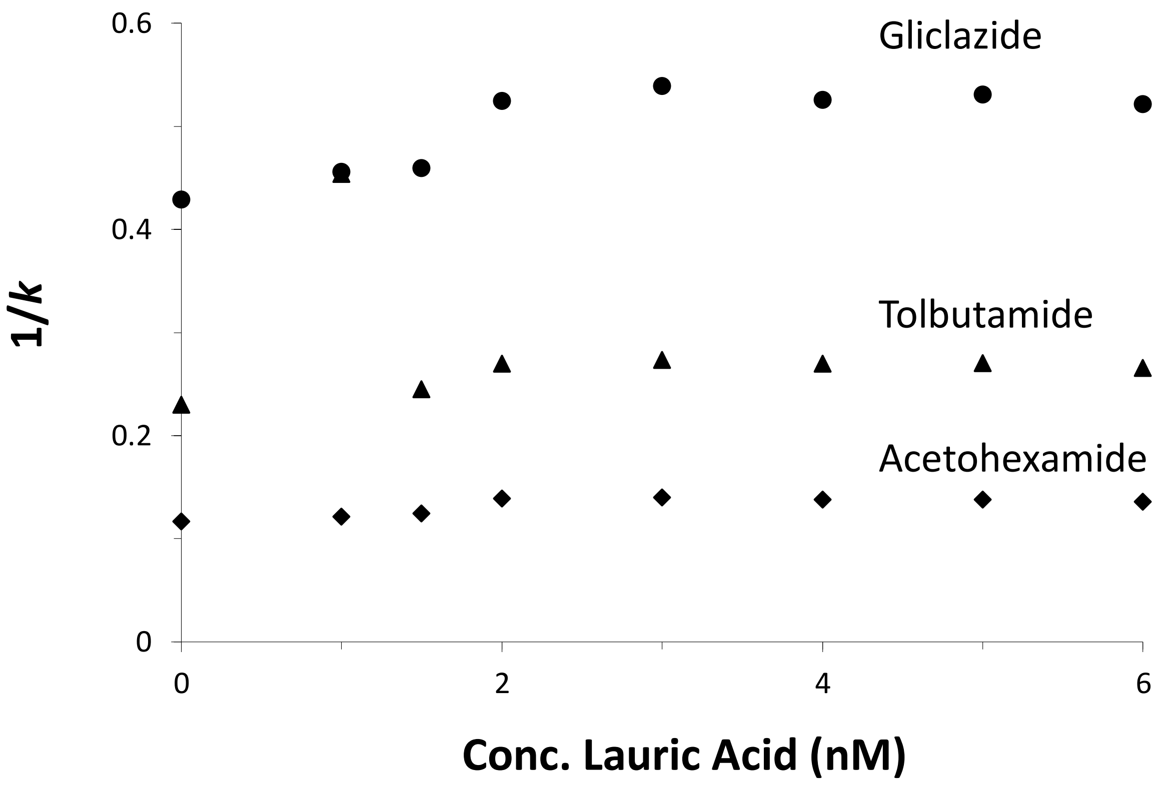
Supportive/Supplementary Material

The following pages contain additional data that were obtained when using high-performance affinity chromatography (HPAC) to examine the competition between injected drugs and applied fatty acids on columns that contained normal HSA or glycated HSA. Figure 1S gives examples of plots of $1/k$ versus fatty acid concentration (where k is the retention factor for an injected drug) in which either possible non-linear behavior was noted (e.g., see data for gliclazide or tolbutamide) or the change in $1/k$ was too small under the given conditions to conclusively determine whether or not direct competition was present (e.g., data for acetohexamide).

Tables 1S-3S provide further details on the best-fit lines that were determined for each drug and with each fatty acid in the HPAC competition studies. For drugs and fatty acids that gave a linear plot of $1/k$ versus fatty acid concentration that was significant, an estimate of the apparent association equilibrium constant for the fatty acid at the site(s) of competition is also provided, as described within the main body of the paper.

Figure Legends

Figure 1S. Change in the retention factor (*k*) for gliclazide (●), tolbutamide (▲) and acetohexamide (♦) on columns containing glycated HSA and in the presence of mobile phases containing increasing concentrations of lauric acid. Other conditions are provided in the main body of the paper.



0.6
0.4
0.2
0
1/k
Gliclazide
Tolbutamide
Acetohexamide
0
2
4
6
Conc. Lauric Acid (nM)

Figure 1S

Table 1S. Change in retention for acetohexamide on normal HSA and glycated HSA columns

Fatty Acid	Best-fit Line and Correlation Coefficient[a]	Type of Competition[b]
Normal HSA		
Lauric acid (1.5-6 nM)	$y = -2.3\ (\pm 0.6) \times 10^{7}\ x + 0.42\ (\pm 0.02)$, $r = 0.896$ ($n = 6$)	Mixed mode
Linoleic acid (100-250 nM)	$y = -2.5\ (\pm 0.5) \times 10^{5}\ x + 0.298\ (\pm 0.008)$, $r = 0.941$ ($n = 6$)	Mixed mode
Myristic acid (4-24 nM)	$y = 1.08\ (\pm 0.28) \times 10^{6}\ x + 0.206\ (\pm 0.004)$, $r = 0.887$ ($n = 6$)	Direct, $K_{aI} = 5.2\ (\pm 1.4) \times 10^{6}\ M^{-1}$
Oleic acid (125-546 nM)	$y = 5.2\ (\pm 3.1) \times 10^{4}\ x + 0.200\ (\pm 0.011)$, $r = 0.638$ ($n = 6$)[c]	Direct(?)[c], $K_{aI} = 2.6\ (\pm 1.6) \times 10^{5}\ M^{-1}$
Palmitic acid (100-400 nM)	$y = 8.4\ (\pm 1.7) \times 10^{4}\ x + 0.223\ (\pm 0.004)$, $r = 0.928$ ($n = 6$)	Direct, $K_{aI} = 3.8\ (\pm 0.8) \times 10^{5}\ M^{-1}$
Steric acid (10-80 nM)	$y = 6.6\ (\pm 1.8) \times 10^{5}\ x + 0.652\ (\pm 0.009)$, $r = 0.854$ ($n = 7$)	Direct, $K_{aI} = 1.0\ (\pm 0.3) \times 10^{6}\ M^{-1}$
Glycated HSA		
Lauric acid (1.5-6 nM)	$y = 1.3\ (\pm 1.5) \times 10^{6}\ x + 0.131\ (\pm 0.006)$, $r = 0.390$ ($n = 6$)[c]	Direct(?)[c], $K_{aI} = 9.6\ (\pm 11.4) \times 10^{6}\ M^{-1}$
Linoleic acid (100-250 nM)	$y = 1.28\ (\pm 0.12) \times 10^{5}\ x + 0.106\ (\pm 0.002)$, $r = 0.977$ ($n = 7$)	Direct, $K_{aI} = 1.2\ (\pm 0.1) \times 10^{6}\ M^{-1}$
Myristic acid (4-24 nM)	$y = 1.78\ (\pm 0.14) \times 10^{6}\ x + 0.108\ (\pm 0.002)$, $r = 0.987$ ($n = 6$)	Direct, $K_{aI} = 1.6\ (\pm 0.1) \times 10^{7}\ M^{-1}$
Oleic acid (125-546 nM)	$y = 4.0\ (\pm 3.1) \times 10^{3}\ x + 0.126\ (\pm 0.001)$, $r = 0.500$ ($n = 7$)[c]	Direct(?)[c], $K_{aI} = 3.2\ (\pm 2.4) \times 10^{4}\ M^{-1}$

Palmitic acid (50-400 nM)	$y = 1.6\ (\pm 0.2) \times 10^6\ x + 0.008\ (\pm 0.044)$, $r = 0.966$ ($n = 7$)	Direct(?)[c], $K_{aI} = 2.0\ (\pm 11.5) \times 10^8\ M^{-1}$
Steric acid (10-80 nM)	$y = 4.0\ (\pm 0.6) \times 10^5\ x + 0.121\ (\pm 0.003)$, $r = 0.946$ ($n = 8$)	Direct, $K_{aI} = 3.3\ (\pm 0.5) \times 10^6\ M^{-1}$

[a]The value of y in these plots represents the reciprocal of the retention factor, or $1/k$, and x represents the mobile phase concentration of the fatty acid. The values in parentheses represent a range of ± 1 S.D.

[b]A mixed mode interaction (e.g., direct competition plus a positive allosteric effect) was indicated by an initial decrease and then gradual increase in the retention factor for the injected drug as the concentration of fatty acids in the mobile phase was increased. A decrease in the retention factor for the drug when there was an increase in the concentration of the fatty acids could be produced by either a net negative allosteric effect or direct competition; in this study, the overall fit of the results to Eqn. (1) generally suggested that direct competition was present in most of these cases. In these latter cases, the apparent value of K_{aI} for the fatty acid, as obtained by using Eqn. (1), is also provided.

[c]The correlation coefficient obtained in this case was not significant at the 95% confidence level or, in the case of palmitic acid and glycated HSA, the resulting value of K_{aI} had a level of uncertainty that made it difficult to determine if direct competition was present in the given system. The symbol "(?)" indicates that the variation in the data was too large or the change in the retention factor was too small to obtain a reliable determination of exact type of interaction that was present or of the corresponding value of K_{aI} in the case of direct competition.

Table 2S. Change in retention for gliclazide on normal HSA and glycated HSA columns

Fatty Acid	Best-fit Line and Correlation Coefficient[a]	Type of Competition[b]
Normal HSA		
Lauric acid (1.5-6 nM)	y = -1.4 (± 0.3) × 10^8 x + 2.2 (± 0.1), r = 0.902 (n = 6)	Mixed mode
Linoleic acid (70-250 nM)	y = -1.6 (± 0.2) × 10^6 x + 1.41 (± 0.03), r = 0.961 (n = 7)	Mixed mode
Myristic acid (4-24 nM)	y = 5.4 (± 1.4) × 10^6 x + 0.84 (± 0.02), r = 0.885 (n = 6)	Direct, K_{aI} = 6.4 (± 1.7) × 10^6 M^{-1}
Oleic acid (125-546 nM)	y = -4.7 (± 1.2) × 10^4 x + 1.077 (± 0.004), r = 0.894 (n = 6)	Mixed mode
Palmitic acid (100-400 nM)	y = 5.5 (± 0.7) × 10^5 x + 0.87 (± 0.02), r = 0.965 (n = 6)	Direct, K_{aI} = 6.3 (± 0.9) × 10^5 M^{-1}
Steric acid (10-80 nM)	y = 7.4 (± 1.7) × 10^5 x + 0.993 (± 0.008), r = 0.885 (n = 7)	Direct, K_{aI} = 7.4 (± 1.8) × 10^5 M^{-1}
Glycated HSA		
Lauric acid (1.5-6 nM)	y = 8.6 (± 7.2) × 10^6 x + 0.49 (± 0.03), r = 0.513 (n = 6)[c]	Direct(?)[c], K_{aI} = 1.8 (± 1.5) × 10^7 M^{-1}
Linoleic acid (70-250 nM)	y = 6.1 (± 0.7) × 10^5 x + 0.39 (± 0.01), r = 0.972 (n = 7)	Direct, K_{aI} = 1.6 (± 0.2) × 10^6 M^{-1}
Myristic acid (4-24 nM)	y = 9.4 (± 1.3) × 10^6 x + 0.39 (± 0.02), r = 0.965 (n = 6)	Direct, K_{aI} = 2.4 (± 0.3) × 10^7 M^{-1}
Oleic acid (125-546 nM)	y = 2.9 (± 0.9) × 10^4 x + 0.472 (± 0.004), r = 0.796 (n = 7)	Direct, K_{aI} = 6.2 (± 2.1) × 10^4 M^{-1}

Palmitic acid (50-400 nM)	$y = 4.5\ (\pm 1.0) \times 10^6\ x + 0.10\ (\pm 0.22)$, $r = 0.905$ ($n = 7$)	Direct(?)[c], $K_{aI} = 4.5\ (\pm 10.2) \times 10^7\ M^{-1}$
Steric acid (10-80 nM)	$y = 1.6\ (\pm 0.1) \times 10^6\ x + 0.453\ (\pm 0.007)$, $r = 0.982$ ($n = 8$)	Direct, $K_{aI} = 3.6\ (\pm 0.3) \times 10^6\ M^{-1}$

[a]The value of y in these plots represents the reciprocal of the retention factor, or $1/k$, and x represents the mobile phase concentration of the fatty acid. The values in parentheses represent a range of ± 1 S.D.

[b]A mixed mode interaction (e.g., direct competition plus a positive allosteric effect) was indicated by an initial decrease and then gradual increase in the retention factor for the injected drug as the concentration of fatty acids in the mobile phase was increased. A decrease in the retention factor for the drug when there was an increase in the concentration of the fatty acids could be produced by either a net negative allosteric effect or direct competition; in this study, the overall fit of the results to Eqn. (1) generally suggested that direct competition was present in most of these cases. In these latter cases, the apparent value of K_{aI} for the fatty acid, as obtained by using Eqn. (1), is also provided.

[c]The correlation coefficient obtained in this case was not significant at the 95% confidence level or, in the case of palmitic acid and glycated HSA, the resulting value of K_{aI} had a level of uncertainty that made it difficult to determine if direct competition was present in the given system. The symbol "(?)" indicates that the variation in the data was too large or the change in the retention factor was too small to obtain a reliable determination of exact type of interaction that was present or of the corresponding value of K_{aI} in the case of direct competition.

Table 3S. Change in retention for tolbutamide on normal HSA and glycated HSA columns

Fatty Acid	Best-fit Line and Correlation Coefficient[a]	Type of Competition[b]
Normal HSA		
Lauric acid (1.5-6 nM)	$y = -2.7\ (\pm\ 0.7) \times 10^{7}\ x + 0.68\ (\pm\ 0.03)$, $r = 0.873$ ($n = 6$)	Mixed mode
Linoleic acid (70-250 nM)	$y = -3.8\ (\pm\ 0.5) \times 10^{5}\ x + 0.53\ (\pm\ 0.01)$, $r = 0.954$ ($n = 7$)	Mixed mode
Myristic acid (4-24 nM)	$y = 1.5\ (\pm\ 0.4) \times 10^{6}\ x + 0.38\ (\pm\ 0.01)$, $r = 0.909$ ($n = 6$)	Direct, $K_{aI} = 4.1\ (\pm\ 0.9) \times 10^{6}\ M^{-1}$
Oleic acid (125-546 nM)	$y = 1.5\ (\pm\ 0.8) \times 10^{5}\ x + 0.40\ (\pm\ 0.03)$, $r = 0.688$ ($n = 6$)[c]	Direct(?)[c], $K_{aI} = 3.8\ (\pm\ 2.1) \times 10^{5}\ M^{-1}$
Palmitic acid (100-400 nM)	$y = 1.3\ (\pm\ 0.3) \times 10^{5}\ x + 0.43\ (\pm\ 0.01)$, $r = 0.926$ ($n = 6$)	Direct, $K_{aI} = 3.1\ (\pm\ 0.6) \times 10^{5}\ M^{-1}$
Steric acid (10-80 nM)	$y = 2.8\ (\pm\ 0.6) \times 10^{5}\ x + 0.435\ (\pm\ 0.003)$, $r = 0.890$ ($n = 7$)	Direct, $K_{aI} = 6.5\ (\pm\ 1.5) \times 10^{5}\ M^{-1}$
Glycated HSA		
Lauric acid (1.5-6 nM)	$y = 2.6\ (\pm\ 2.6) \times 10^{6}\ x + 0.26\ (\pm\ 0.01)$, $r = 0.448$ ($n = 6$)[c]	Direct(?)[c], $K_{aI} = 1.0\ (\pm\ 1.0) \times 10^{7}\ M^{-1}$
Linoleic acid (70-250 nM)	$y = 2.4\ (\pm\ 0.2) \times 10^{5}\ x + 0.210\ (\pm\ 0.04)$, $r = 0.979$ ($n = 7$)	Direct, $K_{aI} = 1.1\ (\pm\ 0.1) \times 10^{6}\ M^{-1}$
Myristic acid (4-24 nM)	$y = 3.0\ (\pm\ 0.3) \times 10^{6}\ x + 0.214\ (\pm\ 0.005)$, $r = 0.982$ ($n = 6$)	Direct, $K_{aI} = 1.4\ (\pm\ 0.1) \times 10^{7}\ M^{-1}$
Oleic acid (125-546 nM)	$y = 1.7\ (\pm\ 0.4) \times 10^{4}\ x + 0.247\ (\pm\ 0.001)$, $r = 0.901$ ($n = 7$)	Direct, $K_{aI} = 7.1\ (\pm\ 1.5) \times 10^{4}\ M^{-1}$

Palmitic acid (50-300 nM)	$y = 2.1\ (\pm 0.4) \times 10^6\ x + 0.080\ (\pm 0.078)$, $r = 0.936$ ($n = 6$)	Direct(?)[c], $K_{aI} = 2.6\ (\pm 2.6) \times 10^7\ M^{-1}$
Steric acid (10-80 nM)	$y = 7.0\ (\pm 0.7) \times 10^5\ x + 0.235\ (\pm 0.003)$, $r = 0.973$ ($n = 8$)	Direct, $K_{aI} = 3.0\ (\pm 0.3) \times 10^6\ M^{-1}$

[a]The value of y in these plots represents the reciprocal of the retention factor, or $1/k$, and x represents the mobile phase concentration of the fatty acid. The values in parentheses represent a range of ± 1 S.D.

[b]A mixed mode interaction (e.g., direct competition plus a positive allosteric effect) was indicated by an initial decrease and then gradual increase in the retention factor for the injected drug as the concentration of fatty acids in the mobile phase was increased. A decrease in the retention factor for the drug when there was an increase in the concentration of the fatty acids could be produced by either a net negative allosteric effect or direct competition; in this study, the overall fit of the results to Eqn. (1) generally suggested that direct competition was present in most of these cases. In these latter cases, the apparent value of K_{aI} for the fatty acid, as obtained by using Eqn. (1), is also provided.

[c]The correlation coefficient obtained in this case was not significant at the 95% confidence level or, in the case of palmitic acid and glycated HSA, the resulting value of K_{aI} had a level of uncertainty that made it difficult to determine if direct competition was present in the given system. The symbol "(?)" indicates that the variation in the data was too large or the change in the retention factor was too small to obtain a reliable determination of exact type of interaction that was present or of the corresponding value of K_{aI} in the case of direct competition.