A COMPARATIVE ANALYSIS OF THE BINDING OF DIFFERENT LONG CHAIN FREE FATTY ACIDS BY HUMAN SERUM ALBUMIN

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1. Introduction

Free fatty acids (FFA) are bound by human serum albumin (HSA) as multiple complexes of FFA–HSA which are characterized by stepwise equilibrium constants [1], leaving only minute amounts in the unbound form for uptake by tissues [2,3]. The bound FFA dissociates thus replenishing the supply of unbound FFA [3]. The distribution of FFA between the free form and fatty acid bound by HSA as multiple complexes of FFA–HSA can be computed [4] using stepwise equilibrium constants [5] and a computer program [6]. The purpose of the present report is to provide such values for six long fatty acids at different molar ratios of FFA/HSA. The implications of this theoretical analysis with respect to the delivery of FFA by HSA to tissues are also considered.

2. Materials and methods

The distribution of different FFA (12:0, 14:0, 16:0, 18:0, 18:1 and 18:2) between the unbound form, the bound form and the amount bound as complexes with HSA was computed on an IBM 370/168 computer [6] using stepwise equilibrium constants for the FFA [5]. The values used are in fig.2. The protein concentration was 580 μM (4 g/dl), based upon a molecular weight of 69 000.

3. Results and discussion

3.1. *Computed concentrations of unbound fatty acids and free protein*

As shown in fig.1, the computed level of unbound FFA increased rapidly in a non-linear fashion, with quantitative differences due to the structures, as the concentration of FFA and the molar ratio of FFA to albumin increased. In all cases the unbound form amounted to only a small fraction of the total (less than 0.03%), which is in accord with experimental studies [2,3]. Figure 1 also shows the rapid decrease in free or uncomplexed protein as the concentration of FFA increased.

3.2. *Distribution of fatty acids among the complexes with HSA*

Graphic plots of the concentrations of the complexes as a function of the concentration of FFA and the molar ratios of FFA/HSA are presented in fig.2. At low molar ratios of FFA/HSA, the complex of one FFA per HSA would be the most abundant. However, as the total concentration of FFA increased there would be a progressive shift to complexes with greater numbers of FFA per HSA. Note the differences in the plots with differences in the structure of the fatty acid.

3.3. *A comparison of the contribution of the complexes in the delivery of different FFA to tissues*

Experimental studies of the delivery of FFA to tissues showed that about one-fourth of the circulating FFA was removed in transit through an organ such as the liver [7–9]. In this study an analysis was made of the removal of one-fourth of the FFA at molar ratios of FFA/HSA of 0.5, 1.0, 2.0, 3.0, and 4.0. For conciseness, table 1 only shows the contributions of the complexes when the molar ratio upon delivery to the tissue was 0.5 and 2.0 releasing 72.5 and 290 μmol FFA, respectively. At a molar ratio of

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0.5 (table 1,A), the complexes with one and two moles of FFA would contribute most of the FFA, while at a molar ratio of 2 (table 1,B) the higher complexes would provide greater portions of the FFA. At a molar ratio of 4, the complexes with 4 and 5 moles of FFA would be the greatest contributors. The contributions of the complexes in the delivery of FFA are again influenced by the structure of the fatty acid.

The amounts of FFA contributed were in great excess of the amounts which were unbound. This relationship can be expressed as the ratio of the amount of FFA contributed divided by the amount not bound, e.g., at a total FFA concentration of 1160 μM this ratio for each FFA were: 230 (12:0), 1277 (14:0), 4677 (16:0), 9235 (18:0), 17 500 (18:1) and 2377 (18:2). This implies that the rate of dissociation of

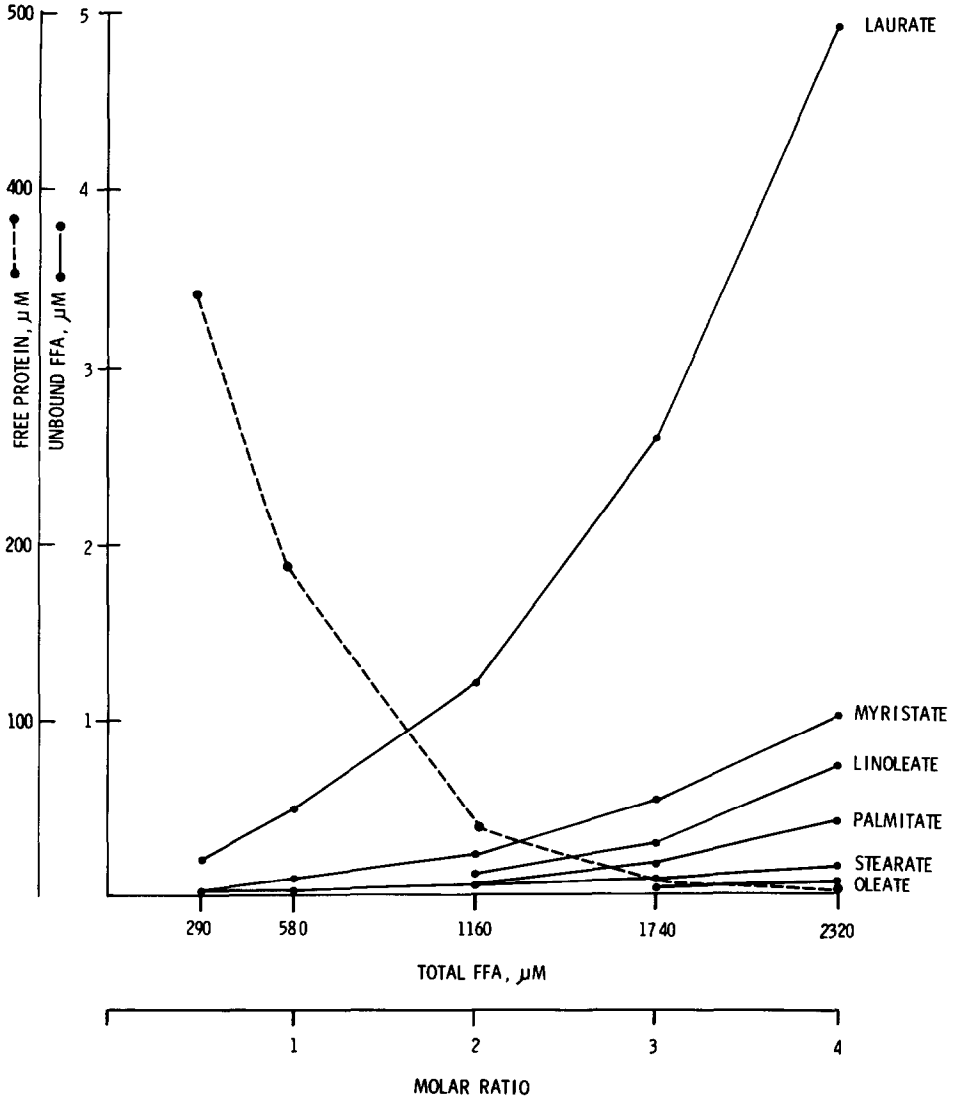

Fig.1. Concentration of unbound FFA and free protein as a function of FFA/HSA molar ratio. The concentration of unbound fatty acid was computed using the association constants presented in fig.2 for a concentration of HSA of 580 μM. The curve for free protein is based upon calculations using the constants for palmitate; the others were similar but are not included for the sake of simplicity.

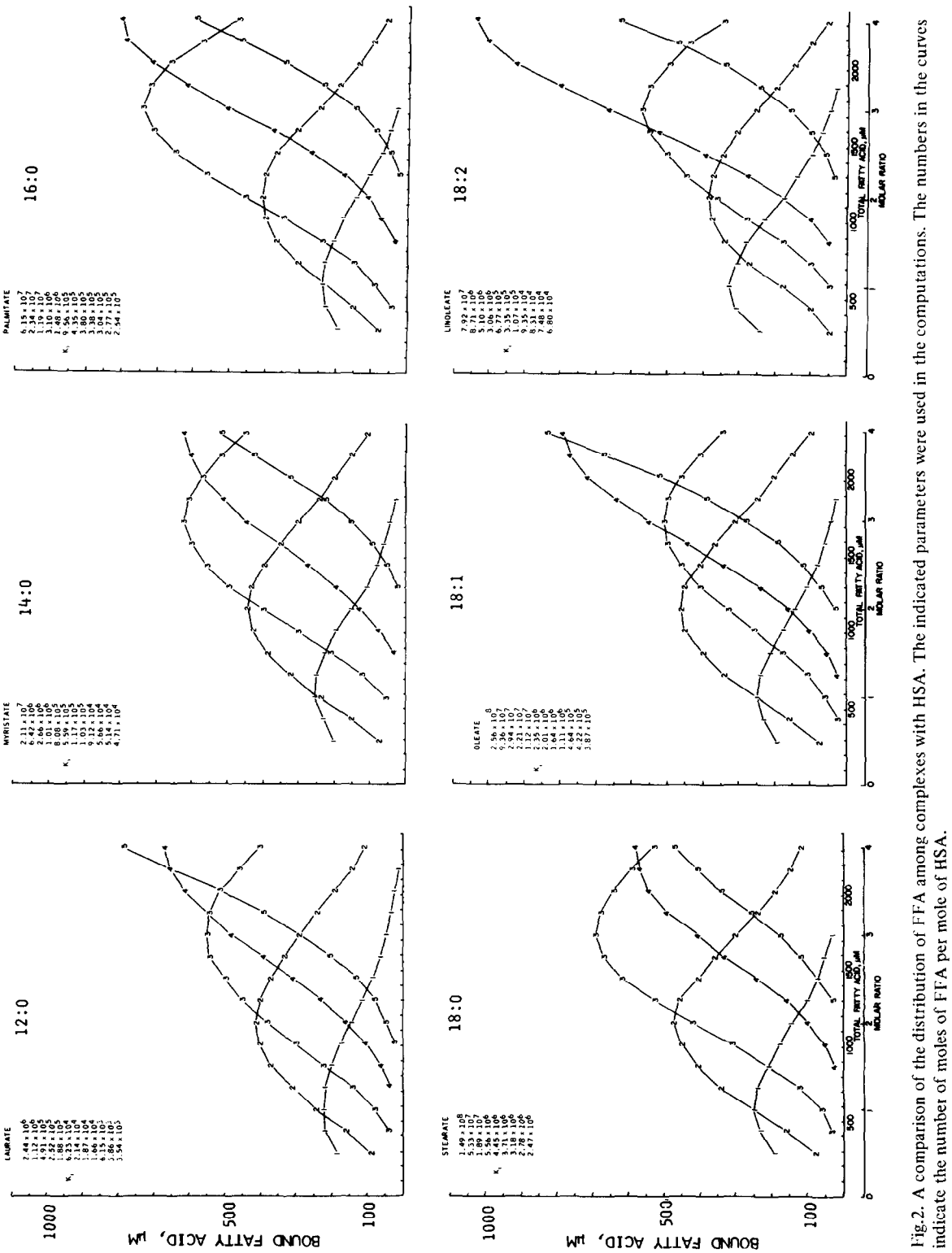

Fig.2. A comparison of the distribution of FFA among complexes with HSA. The indicated parameters were used in the computations. The numbers in the curves indicate the number of moles of FFA per mole of HSA.

Table 1
The amount of free fatty acid (μmoles/l) contributed by each complex as one-fourth of the FFA is removed at FFA/HSA molar ratios of 0.5 and 2

Complex	Laurate (12:0)	Myristate (14:0)	Palmitate (16:0)	Stearate (18:0)	Oleate (18:1)	Linoleate (18:2)
A. Molar ratio 0.5						
1	29.4 (41)	35.7 (49)	31.9 (44)	33.6 (46)	33.0 (46)	50.4 (70)
2	34.4 (47)	31.4 (43)	32.7 (45)	33.4 (46)	33.0 (46)	19.9 (14)
3	7.9 (11)	5.1 (7)	7.6 (10)	5.3 (7)	4.8 (7)	2.1 (3)
4	0.7 (1)	0.2 (0)	0.4 (1)	0.2 (0)	0.4 (1)	0.1 (0)
B. Molar ratio 2						
1	−55.0 (−19)	−72.1 (−25)	−65.5 (−23)	−73.3 (−25)	−66.3 (−23)	−92.6 (−32)
2	43.5 (15)	55.6 (19)	49.7 (17)	52.8 (22)	55.5 (19)	52.8 (18)
3	160.2 (55)	193.8 (67)	214.6 (74)	213.0 (73)	157.0 (54)	183.6 (63)
4	100.8 (35)	85.4 (29)	79.5 (27)	70.5 (24)	111.3 (38)	129.7 (45)
5	36.2 (12)	23.1 (8)	10.8 (4)	14.4 (5)	30.7 (11)	15.6 (5)
6	3.8 (1)	3.8 (1)	0.8 (0)	2.2 (1)	1.6 (1)	0.8 (0)
7				0.2 (0)		

The values in the parentheses are the percent contribution of that site; the minus signs designate opposite changes resulting from redistribution. Changes less than 0.01 μmol/l are not included. The complex indicates the number of moles of FFA per mole of HSA.

the FFA from the complexes with HSA could be a limiting factor in the clearance of FFA. If this is the case, the rate of blood flow through the organs would affect the clearance of the different FFA from the plasma. Such clearance would depend upon the relative contributions of the complexes with the high and intermediate number of molecules of FFA providing FFA to the cells at different rates.

Since, according to the present analysis, the relative amounts of FFA removed from each complex changes as a function of both the molar ratio of FFA to HSA and the structure of the FFA, it seems possible that the removal of different FFA from the circulation is modulated by various factors: the molar ratio of FFA to albumin, the structure of the FFA, and the rate of blood flow through the organ.* Differences in these factors could account for some of the contradictory results noted in the experimental studies concerning the influence of the structure of the fatty acid on its relative rate of uptake [10]. It is also conceivable from the present data that such variations could influence the results obtained using an individual radioactive fatty acid as a tracer to measure the rates of clearance of the exchangeable pool of FFA (11). A related observation [10] is that under specific experimental conditions the uptake of FFA by the liver can be measured accurately with either palmitic or oleic acid as a tracer, although differences were observed in the fractional uptake of individual FFA.

* The relative rates of uptake of different FFA can be complicated in vivo because of the presence of variable amounts of other fatty acids competing for binding sites and other factors [4].

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