Oleic Acid Allows More Apoprotein A-1 to Bind with Higher Affinity to Large Emulsion Particles Saturated with Cholesterol*

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Apoprotein (apo) A-1 binding to large triolein-rich emulsion particles saturated with cholesterol has been examined as a function of the oleic acid content. Six emulsion systems were formed containing 0.3–1.0% (by weight) oleic acid, 82.9–86.3% triolein, 10.6–7.2% egg yolk phosphatidylcholine, and 6.7–5.5% cholesterol. The average emulsion particle diameters calculated from these lipid compositions ranged between 84 and 116 nm. Negative stain electron microscopy of an emulsion containing 1% oleic acid showed a polydisperse population of only large spherical particles with a mean diameter of 116 ± 54 nm. The calculated cholesterol concentrations of the particle surface and core for the six emulsions were 43.3 ± 1.1 and 5.6 ± 0.2 mol%, respectively, and were rather constant. Therefore, when the surface oleic acid concentrations increased from 2.6 to 10.1 mol%, the phospholipid concentration decreased from 55.1 to 45.9 mol%. In the core, oleic acid increased at the expense of triolein. In the range studied a nearly 4-fold increase in the surface oleic acid content produces a similar increase in the binding capacity (N) and reduces the dissociation constant (Kₐ). The changes in the Kₐ and N values were linearly dependent on the surface oleic acid concentration. These data show that oleic acid allows more apoA-1 to bind with higher affinity to large emulsion particles saturated with cholesterol.

In the extracellular compartment, free fatty acids are transported primarily by albumin (1), with small portions either carried by plasma lipoproteins (2) or dissolved in the lipid bilayer of plasma membranes (3). Fatty acids readily distribute from albumin to bilayers (4, 5) or from lipoproteins to cells (6), and the stoichiometry of this process is under study in this laboratory (7). In the plasma membrane amphiphatic lipids like fatty acids move rapidly in the plane of the membrane (laterally) (8) or traverse the membrane (9) to become available to a myriad of enzymatic conversions in the interior of the cell.

In circulation, long chain triacylglycerols in large plasma lipoproteins (d < 1.006) are hydrolyzed by lipases (10) to generate protonated fatty acids (11) which are poorly soluble in water (12, 13) and could transiently accumulate in the particle, if transfer to albumin and cell membranes is rate limiting. In whole plasma, the molar ratio of free fatty acids to albumin ordinarily varies between 0.14 and 1.9, with a basal value of about 0.6, and may reach 4 after strenuous exercise (14). We have shown the upper limit of fatty acid binding of saturated long chain fatty acids (15, 16) and oleic acids (17) is about 7–9 mol of fatty acid/albumin. Plasma obtained from large blood vessels probably does not exceed this maximal ratio, but in the capillary at the site of hydrolysis more fatty acids may be released than can be bound to albumin and significant amounts of fatty acid may accumulate in lipoprotein interfaces. The current work examines the effect of oleic acid on the binding of the exchangeable plasma apoprotein (apoA-1) to triglyceride-rich, remnant-like emulsions.

Recently, the ionization and distribution of oleic acid in chylomicrons between surface and core was shown to mimic the behavior of this fatty acid in chylomicron-like emulsion particles with identical morphology and similar lipid composition (7). The partitioning of oleic acid between the surface and core of large emulsion particles at pH 7.4 was independent of the particle cholesterol content (18). An equilibrium binding study showed that the affinity of large emulsion particles for the exchangeable apoproteins apoA-1 and apoA-3 was independent of particle cholesterol content, but the protein binding capacity declined when the surface cholesterol exceeded 34 mol% (19). In this paper, equilibrium binding of apoA-1 to a series of large triolein particles saturated with cholesterol was studied as a function of the oleic acid concentration in the particle surface. Oleic acid was varied between 2 and 10 mol% in the particle surface. We found that under these conditions an increase in emulsion oleic acid content increases the affinity and the capacity for the binding of apoA-1.

**EXPERIMENTAL PROCEDURES**

**Materials**

Triolein (TO) and cholesterol (CH) were obtained from Nu-Chek Prep (Elysian, MN) and oleic acid from Sigma. Egg phosphatidylcholine (PC) was obtained from Lipid Products (Nutfield Ridge, Great Britain). Lipids were certified >99% pure by the supplier. [1H]Triolein, [14C]cholesterol, [14C]oleic acid, and [14C]phosphatidylcholine were all obtained from Amersham Corp. The radiocuhemical purity of [14C]-lipids was >99% and of [1H]tri olein was >98%. The purities of these lipids were verified by analytical thin layer chromatography.

**Methods**

Preparation of Emulsions—Emulsions of six different compositions were prepared. Appropriate amounts of lipids ([1H]TO, [14C]CH and [14C]PC) were pipetted from chloroform stock solutions into 20-ml glass vials. The specific activities of the lipids were: 526 cpm/μg for [1H]TO, 2360 cpm/μg for [14C]oleic acid, 465 cpm/μg for [14C]CH, and 165 cpm/μg for [14C]PC. The total amount of lipids in the starting mixtures was either 50 or 40 mg, with 80% by weight as TO and 8%

*The abbreviations used are: apoA-1, apoprotein A-1; TO, triolein; CH, cholesterol; PC, phosphatidylcholine.
by weight as CH. The amounts of the other lipids were varied as described in Tables I and II.

The samples were dried under N₂ on a water bath (25 °C) and vacuum-dessicated at 4 °C overnight. The dried lipids were suspended in 10 ml of 10 mM phosphate buffer (pH 7.4) containing 150 mM NaCl, 0.01% Tween 20, and 0.02% NaN₃. The samples were then emulsified by a 10-min sonication under N₂ on an ice-water bath using a Branson sonifier model W-350 set at 90 watts continuous power.

After sonication the dispersions were transferred to polyallomer centrifuge tubes, overlayed with 1 ml of water, and centrifuged at 23,000 × g for 10 min in a Beckman SW 41 rotor at 25 °C. The centrifuge was operated without breaking. The top 0.6 ml were isolated with a tube slicer and transferred with a 3-ml plastic syringe into a 15-ml plastic conical screw cap tube. Transfers of the creamy layer were completed with a 200-μl rinse of buffer. Final volumes were measured and recorded, and 20 μl was taken for lipid analysis. The volume of the remaining infranatant was also measured and recorded, and 150 μl was taken for lipid analysis.

**Lipid Analysis**—Aliquots of the emulsion were taken with a Hamilton syringe and spotted under N₂ as short bands on Silica Gel G precoated thin layer chromatography plates (Analytech, Inc., Newark, Del.). TLC programs were developed with hexane:ether:18 N acetic acid (55:45:1) as the mobile phase (20), which gave a good separation of the four lipid classes used. The lipid spots were located by a brief iodine vapor treatment. The iodine was allowed to sublime and the lipid (TO, CH, and PC) spots were scraped from the plates into scintillation vials, or fatty acids into vials equipped with screw caps. The mass of TO, CH, and PC was determined by liquid scintillation counting in a 1215 Rack Beta counter (LKB-Wallac, Turku, Finland) using Liquiscint (National Diagnostics, Mansfield, MA) as scintillant fluid. Since we observed that small amounts of free fatty acids (≤0.15 mg) were generated during the preparation of emulsions due to hydrolysis of TO and PC, the mass of fatty acid was not determined by scintillation counting but by gas-liquid chromatography according to the method of Derksen and Cohen (21) with heptadecanoic acid as internal standard.

**Isolation of ApoA-1**—Human delipidated high density lipoprotein (HDL) was purchased from New England Nuclear (Boston, MA) in liquid form at a concentration of 1 mg/ml. The protein concentration was measured spectrophotometrically at 280 nm and as compared to the absorbance of a 0.01% solution of bovine serum albumin as a standard (24) and by measuring the absorbance at 280 nm in a Perkin-Elmer Lambda 5 spectrophotometer using a molar extinction coefficient of 92,000 for apoA-1. Isolated apoA-1 was stored in the cold room and used within 7 days.

**Assay of the Binding ApoA-1 to Emulsion Particles**—Binding assays were performed in duplicate at room temperature. Six "polyallomer centrifuge tubes" (5 × 20 mm, Beckman) each containing 175 μl of assay mixture were incubated for 20 min in an A-100/18 rotor followed by centrifugation at 20,000 × g for 10 min in a Beckman Airfuge. After centrifugation, the bottoms of the tubes were punctured with a 27-gauge needle and 100 μl were collected with a gas-tight Hamilton syringe. This aliquot (bottom fraction) and the tube with the left over (top fraction) were transferred separately to glass tubes. γ counting was performed in an LKB 1276 Minigamma spectrophotometer with a sample elevator setting of 0.5 cm. In all experiments, the total amount of protein added to each assay mixture was accounted for by the sum of the bottom and top fractions. In each assay mixture the buffer and emulsion content were kept constant while the protein content was increased to 10, 20, 40, 60, or 80 μg/ml.

Two rotors were employed during the execution of the binding study protocols comparing emulsions with different oleic acid compositions. Twelve tubes could be processed per h and protocols were completed within 6 h after the isolation of the emulsion fractions.

**Analysis and Expression of Binding Data**—For each assay mixture the following concentrations were measured or derived: [PC] = lecithin concentration; [P₀] = free protein concentration = (bottom fraction); [Pₕ] = total protein concentration = bottom + top fractions; [P₀/ₕ] = bound/free protein concentration.

Using these values, the data were analyzed, assuming a single isothermal equilibrium and a finite number of discrete, equivalent, and non-interacting binding sites on the surface of the lipid particles. This equilibrium has been described mathematically (25) according to Equation 1.

\[ K_d = \frac{[P]_0 - [P]_h}{[P]_h} \]  

in which the \( K_d \) value is the dissociation constant and the term \( \frac{[P]_0 - [P]_h}{[P]_h} \) represents the concentration of unoccupied binding sites at protein levels below the saturation level.

Equation 1 transforms to \( P = \frac{[P]_0[P]_h}{[P]_h} - K_d \) and yields the parameters \( K_d \) and \( N \) (the number of binding sites 'n relation to phospholipid).

**Calculation of Particle Diameter**—The method for the calculation of particle diameter from chemical compositions has been previously described (26, 27). The method takes into account that triolein, cholesterol and oleic acid, distribute between the emulsion surface and core phases. For larger particles (diameter > 100 nm), as used in this study, the oil phase contains a significant amount of cholesterol and oleic acid. Therefore, the ratio of oil to surface masses for the particle is increased and the diameter becomes about 10% larger than the value estimated assuming all the cholesterol and oleic acid is at the surface (26).

**Electron Microscopy**—The isolated emulsion fractions were diluted with pH 7.4 phosphate buffer and were fixed for 30 min with OsO₄. Final lipid and osmium concentrations are 2-4 mg/ml and 0.1-0.8%, respectively. A 5-μl droplet of fixed emulsion were applied for 10 s to a Formvar carbon-coated 300-mesh copper grid. All film surfaces were made hydrophilic by glow discharge and used within 2 weeks. Excess emulsion was removed by Pasteur pipette and filter paper and replaced by a 5-μl droplet of 1% sodium phosphotungstate; after a few seconds the excess was removed and the stained residue on the grid was air-dried. Each grid quadrant (field) was photographed with a Hitachi HU-11C electron microscope at an acceleration voltage of 75 kV. The instrument was calibrated with a carbon grating replica (Ernest F. Fullam, Inc.) and magnification was 15,000. Each field contained 100-200 particles. Particles were measured systemically using a Peak X × magnifier with a graticule 1-20 mm in 0.1 mm divisions. Only discrete spherical particles greater than 260 Å were tabulated, and about 400 particles were accumulated in a histogram.

**RESULTS**

**Fig. 1** shows the indigenous fatty acid content of a starting liquid mixture (data point on the left) as well as the typical
Oleic Acid Enhances ApoA-1 Binding to Model Lipoproteins

Effects of hydration (second data point from the left) and sonication of the lipid mixture for various lengths of time (other data points). 50 mg of "pure" total lipid contained 60 \( \mu \)g of oleic acid. Purity checks of the purchased lipid revealed no detectable other lipids in the cholesterol and egg lecithin, but the triolein contained 0.15% (w/w) oleic acid and 0.35% (w/w) diolein. Upon hydration of the dried lipid film, the amount of free fatty acid doubled while a 10 min sonication released another 30 \( \mu \)g of fatty acids. Gas-liquid chromatographic profiles further revealed that most of the fatty acid was oleic acid (>90%) with minor amounts of palmitate, stearate and linoleate. Thus, the procedure for making emulsions results in the hydrolysis of 0.6% triolein and egg lecithin into diolein, lysolecithin, and free fatty acids. It follows that the isolated large emulsion particles always contain certain amounts of fatty acids (0.3% (w/w) of total lipid), lysolecithin (0.4% (w/w) of lecithin), and diolein (0.8% (w/w) of triolein) (see also top of Table 1).

Table 1 illustrates the preparation of two sonicated emulsions from lipid mixtures containing 0 and 0.92% by weight of oleic acid added at the expense of egg lecithin. When oleic acid is added to the starting mix the yields of cholesterol, egg lecithin, and oleic acid are reduced in the floated emulsion fraction, elevated in the bottom fraction, while no change occurs in the overall recovery of these components (compare the top and bottom parts of the last three columns in Table 1). Thus, the addition of oleic acid decreases the amounts of cholesterol and egg lecithin and increases the amount of triolein (relative to the starting mix) recovered in the isolated emulsion particles.

Amount of Free Oleate in the Surface and the Average Size of Large Emulsion Particles Saturated with the Cholesterol Can Be Calculated from the Composition Data—The mean particle diameter of these two emulsion fractions along with those of four similarly composed and isolated emulsion fractions, but ranging stepwise in oleic acid content from 0.3 to 1.0 are listed in Table II. Increasing the oleic acid content from 0.3 to 1.0% by weight in these emulsions increases the triolein content from 82.9 to 86.3% and decreases the egg lecithin content from 10.6 to 7.2%, while the sum of the cholesterol and oleic acid content remains rather constant (6.5 ± 0.2%). The stepwise increase in oleic acid content 0.3, 0.5, 0.7, and 1.0% increases the calculated mean particle size from 84 to 88 to 100 to 114 ± 2 nm, respectively. Fig. 2A shows an electron micrograph of emulsion 6 listed in Table II. The emulsion contains only large, round (presumably spherical) particles, and no lamellar structures were seen. The size frequency distribution of the particles diameters in Fig. 2A is displayed as a histogram in Fig. 2B. The particle diameters range between 45 and 320 nm (116 ± 54 was the mean ± S.D. of 360 particles measured in Fig. 2A). In addition to the size, the calculated average composition of the particle surface and core of these emulsions is described in Table III. The calculated molar concentrations of cholesterol in both the surface and the core are 43.3 ± 1.1 and 5.6 ± 0.2 mol%, respectively, and they are rather constant. The increase of oleic acid in the surface occurs primarily at the expense of egg lecithin, while in the core this increase is offset by triolein. The interaction of apolipoprotein apoA-1 to these particles is considered below.

Binding of ApoA-1 to Large Emulsion Particles Containing Increasing Oleic Acid and Constant High Cholesterol Concentrations in Surface and Core—The six emulsions described in Tables II and III were used in binding studies with apoA-1 (Fig. 3). Without added oleic acid the binding of apoA-1 to large triglyceride-rich particles was reproducible, saturable, and the low amounts were very similar to those found in previous studies using emulsions saturated with cholesterol (19) (bottom two curves, Fig. 3). With emulsions containing added oleic acid, more protein binds to the particles when more oleic acid was present in the surface at each of the protein concentrations. For every emulsion as the protein concentration in the incubations was increased larger amounts of apoA-1 bound to the surface of the particles without reaching saturation at the highest protein levels (top curves, Fig. 3). The results from Fig. 3 transform into linear plots by graphing the phosphatidylcholine \times free protein/bound protein \((P_F/P_B)\) against the free protein \(P_F\) concentration (25, 29). In Fig. 4 a dissociation constant \(K_d\) is represented as the ordinate intercept and the \(N\) value, the saturation level of the protein binding onto the surface of the emulsion particles is obtained as the slope.

Fig. 4 shows that both the slope and the intercept changes when the particle oleic acid content increases. Table IV lists the \(K_d\) and \(N\) values of the six emulsions. A 3-4-fold increase in surface oleic acid content produces a similar increase in \(N\) value and divides the \(K_d\) value almost in half. When the \(K_d\) and \(N\) values are plotted separately as a function of the surface

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**Table I**

<table>
<thead>
<tr>
<th>Starting compound</th>
<th>Mix</th>
<th>Sonicated</th>
<th>Emulsion*</th>
<th>Floated emulsion composition</th>
<th>Bottom fraction composition</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
<td>mg</td>
<td>%</td>
<td>mg</td>
<td>mg</td>
<td>%</td>
</tr>
<tr>
<td>Triolein</td>
<td>40.0</td>
<td>39.86</td>
<td>7.7</td>
<td>25.50</td>
<td>82.9</td>
<td>65</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>4.0</td>
<td>4.00</td>
<td>8.0</td>
<td>2.09</td>
<td>6.7</td>
<td>52</td>
</tr>
<tr>
<td>Egg lecithin</td>
<td>6.0</td>
<td>5.99</td>
<td>12.0</td>
<td>3.15</td>
<td>10.1</td>
<td>52</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>0.0</td>
<td>0.15</td>
<td>0.3</td>
<td>0.09</td>
<td>0.3</td>
<td>60</td>
</tr>
<tr>
<td>Triolein</td>
<td>40.0</td>
<td>39.86</td>
<td>7.7</td>
<td>26.50</td>
<td>86.3</td>
<td>66</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>4.0</td>
<td>4.00</td>
<td>8.0</td>
<td>1.65</td>
<td>5.5</td>
<td>42</td>
</tr>
<tr>
<td>Egg lecithin</td>
<td>5.54</td>
<td>5.53</td>
<td>11.1</td>
<td>2.18</td>
<td>7.2</td>
<td>39</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>0.46</td>
<td>0.61</td>
<td>1.2</td>
<td>0.29</td>
<td>1.0</td>
<td>47</td>
</tr>
</tbody>
</table>

* After sonication the lipid composition is corrected according to the data in Fig. 1. The triolein contains 0.8% (w/w) diolein and the lecithin contains 0.4% lysolecithin.

* Emulsion fraction isolated after centrifugation (\(q^2 = 475 \times 10^7 \text{ rad } s^{-1}\)). Lipid analysis is performed as described under "Methods." The value for triolein does not include diolein (0.8%); however, the value for lecithin does include 0.4% (w/w) lysolecithin.

* This fatty acid was present in the starting material and also generated during hydration and sonication. 90% of this fatty acid is oleic acid (see Fig. 1).
Oleic Acid Enhances ApoA-1 Binding to Model Lipoproteins

**TABLE II**

<table>
<thead>
<tr>
<th>Preparations</th>
<th>Emulsion lipid composition</th>
<th>Particle diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% by weight</td>
<td>Lipid composition</td>
</tr>
<tr>
<td>1</td>
<td>82.9</td>
<td>6.2 10.6 0.3</td>
</tr>
<tr>
<td>2</td>
<td>82.9</td>
<td>6.7 10.1 0.3</td>
</tr>
<tr>
<td>3</td>
<td>82.9</td>
<td>6.5 9.8 0.5</td>
</tr>
<tr>
<td>4</td>
<td>84.8</td>
<td>6.1 8.4 0.7</td>
</tr>
<tr>
<td>5</td>
<td>85.3</td>
<td>6.0 7.2 1.0</td>
</tr>
<tr>
<td>6</td>
<td>86.3</td>
<td>5.5 7.2 1.0</td>
</tr>
</tbody>
</table>

*a* Preparations 2 and 6 are described in Table I. Other preparations were started with 40 mg of total lipid comprised of 32 mg of TO, 3.2 mg of cholesterol, and a combined total of 4.8 mg of egg lecithin (PC) with added oleic acid (OA). Preparations 1, 3, 4, and 5 contained 0.18, 0.36, and 0.72 mg of added oleic acid respectively.

The lipid composition values are the average of two determinations. For radiochemical determinations the coefficient of variations (C.V.) for TO values in the percent lipid composition of 0.1%, the C.V. for PC values is 1%, and for CH values the C.V. is 4%. The C.V. for oleic acid determinations by GLC has been reported (21).

The particle diameter was calculated from the lipid composition data with the aid of a computer program (27), assuming spherical emulsion structure and the appropriate partition of TO, CH, and oleic acid into surface and core. The values for the surface to core distribution ratio of CH and oleic acid are very similar and equal approximately 11 (18). Also listed are the mean diameters ± S.D. of the particles in emulsions 1 (19) and 6 as measured by negative stain electron microscopy (Fig. 2).

oleic acid concentration in Figs. 5 and 6, an apparent linear relationship exists between the decrease in $K_d$ ($r = -0.834$) and the increase in $N$ ($r = 0.908$) since the solid lines represent the least squares fit to the data.

**DISCUSSION**

Separating free from lipid-bound proteins quickly in an Airfuge (29) has provided a technique to measure the interaction of exchangeable apoproteins to triolein emulsions as a function of the cholesterol content (19, 30). Because large amounts of fatty acids are generated during lipolysis of triglyceride-rich lipoproteins, a major aim for the present work was to measure the effect of fatty acid on the binding of apoA-1 to defined emulsion systems. Thus, oleic acid was increased and egg lecithin decreased in the different emulsions. Indeed, the isolated particles had surface monolayers in which oleic acid and egg lecithin concentrations changes reciprocally (Table III). Other surface-active compounds capable of interacting with apoA-1 such as diolein (31) and lysolecithin (32) were minimal and constant (Table I). These two compounds together with endogenous oleic acid were shown to come from three separate origins during the formation of emulsions. With respect to fatty acids, the first 40% was present in the starting lipid, another 40% came from lipid hydrolysis upon hydration of the dry lipid film, and the last 20% was liberated during sonication (Fig. 1). Although egg lecithin and cholesterol were found to be 99.9% pure, triolein was only 99.5% pure, containing enough oleic acid to account for the first 40% of the endogenous fatty acid. Hydration of the dry lipid film on a glass surface may produce fatty acids via alkaline hydrolysis. When 10–15 ml of water distilled at pH 6.8 was added to the disposable glassware used, the pH increased to 8.5, indicating the alkaline nature of the glass surface. Hydrolysis during sonication was linear with time, but the amount generated in 10 min was small (Fig. 1). Together, these findings imply that sonicated emulsions will always have a small but constant amount of lyssolecithin, diolein, and fatty acids, primarily oleic acid. This observation prompted us to select and study the effect of oleic acid on the association of apoA-1 with triolein-rich emulsions that were saturated with cholesterol in order to standardize the cholesterol concentration. Note that the core and surface concentrations of cholesterol remain constant as oleic acid increases (Table III).

Addition of oleic acid at the expense of egg lecithin did not change the yield of the total lipids floated in the isolated large

**FIG. 2.** Negative staining electron micrographs of large particles from emulsion 6 (Table II). A, emulsions were diluted and fixed with osmium, plated on Formvar grids, and stained with 1% sodium phosphotungstate, pH 7.2. Grids were observed and photographed with a Hitachi-11C electron microscope. The bar is equivalent to 258 nm and magnification is ×38,783. B, histogram of the particle size-frequency distribution from emulsion 6. See “Methods” for the measurements of particle sizes.

**TABLE III**

<table>
<thead>
<tr>
<th>Emulsion</th>
<th>Surface composition</th>
<th>CH/PC</th>
<th>Core composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TO CH PC OA</td>
<td></td>
<td>TO CH OA</td>
</tr>
<tr>
<td>1</td>
<td>0.30 41.9 55.1 2.69</td>
<td>0.76</td>
<td>94.4 5.27 0.34</td>
</tr>
<tr>
<td>2</td>
<td>0.21 44.8 52.3 2.66</td>
<td>0.85</td>
<td>93.9 5.73 0.34</td>
</tr>
<tr>
<td>3</td>
<td>0.21 43.8 51.5 4.48</td>
<td>0.88</td>
<td>93.8 5.63 0.57</td>
</tr>
<tr>
<td>4</td>
<td>0.16 43.6 49.6 6.45</td>
<td>0.86</td>
<td>93.4 5.69 0.87</td>
</tr>
<tr>
<td>5</td>
<td>0.09 44.2 45.9 9.77</td>
<td>0.96</td>
<td>92.8 5.91 1.31</td>
</tr>
<tr>
<td>6</td>
<td>0.15 41.8 48.0 10.07</td>
<td>0.87</td>
<td>93.1 5.52 1.33</td>
</tr>
</tbody>
</table>

*a* The surface and core composition was calculated by the phase analysis method of Miller and Small (26) using the computer program and assumptions as described in Table II.

*b* The CH/PC mole ratio was used to calculate the TO solubility in the surface as described by Spooner and Small (28).
emulsions particles (compare top and bottom, Table I). Systematic increases in the oleic acid content of the isolated particles were accompanied with stepwise higher triolein contents and concomitant lower lecithin and cholesterol contents. These systematic and opposing changes in triolein and phospholipid content of necessity increased the calculated average particle diameter (Table II). The large average diameter of emulsion particles containing 1% oleic acid was verified by electron microscopy (Fig. 2).

As was the case with cholesterol (30) confirming the change in the average calculated particle diameter as a function of oleic acid content is difficult to obtain by negative stain electron microscopy. Small differences in the particle size frequency distribution can be noted (Fig. 2) but the polydispersity (large S.D.) (Table II) of these particles populations does not allow simple statistical significance between average particle diameters compared over the entire range. However, the polydispersity and ranges of the particle diameters of the isolated emulsions are very similar. Furthermore, the surface and core composition of the emulsion particles listed in Table

![Graph](image)

**FIG. 3.** Binding profiles of apoA-1 to large particles in emulsions 1 through 6 (in ascending order) with increasing oleic acid content in the surface (Table III). For each profile the PC content of the assay mixtures was constant but varied slightly from one emulsion to another in between 360 and 428 \( \mu \)M. apoA-1 was increased from 0.4 to 2.8 \( \mu \)M. Free protein was separated from bound with an Airfuge as described under “Methods.” Two separate experiments are shown for emulsions containing 0.3 and 1.0% by weight of oleic acid (open versus closed symbols).

![Graph](image)

**FIG. 4.** Linearized plots of the data from Fig. 3 according to equation \( P_1 =\frac{N[PC][P]}{[P]} - K_d \) (20, 24). The solid lines represent the least squares fit to the data. \( K_d \) and \( N \) calculated from the six lines are given in Table IV.

**TABLE IV**

Parameters for the binding of apoA-1 to the emulsion particles characterized in Tables II and III.

<table>
<thead>
<tr>
<th>Emulsion</th>
<th>( K_d ) (g of apoA-1/g PC)</th>
<th>apoA-1 molecules/particle (mean)</th>
<th>mol of apoA-1/1000 mol PC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g of apoA-1/g PC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.98 ( \times ) 10(^{-2} )</td>
<td>2.73 ( \times ) 10(^{-2} )</td>
<td>16.8 (84)</td>
</tr>
<tr>
<td>2</td>
<td>2.21 ( \times ) 10(^{-2} )</td>
<td>3.44 ( \times ) 10(^{-2} )</td>
<td>20.5 (84)</td>
</tr>
<tr>
<td>3</td>
<td>1.97 ( \times ) 10(^{-2} )</td>
<td>6.42 ( \times ) 10(^{-2} )</td>
<td>41.8 (88)</td>
</tr>
<tr>
<td>4</td>
<td>1.39 ( \times ) 10(^{-2} )</td>
<td>7.36 ( \times ) 10(^{-2} )</td>
<td>60.9 (100)</td>
</tr>
<tr>
<td>5</td>
<td>0.93 ( \times ) 10(^{-2} )</td>
<td>8.81 ( \times ) 10(^{-2} )</td>
<td>87.5 (112)</td>
</tr>
<tr>
<td>6</td>
<td>1.31 ( \times ) 10(^{-2} )</td>
<td>14.50 ( \times ) 10(^{-2} )</td>
<td>158.7 (116)</td>
</tr>
</tbody>
</table>

**FIG. 5.** \( K_d \) (Table IV) is plotted as a function of the oleic acid content in the surface of the emulsion particles (see Table III).

**FIG. 6.** \( N \) (Table IV) is plotted as a function of the oleic acid content in the surface of the emulsion particles (see Table III).

III are invariant with respect to cholesterol molar percentage. These emulsion particle qualities allowed the evaluation of the equilibrium binding of apoA-1 with emulsions containing increasing amounts of oleic acid (Fig. 3).

The dissociation constant \( (K_d) \) and binding capacity \( (N) \) (Fig. 4 and Table IV) of apoA-1 to large emulsion particles saturated with cholesterol changed linearly and in opposite ways as a function of increasing surface oleic acid content (Figs. 5 and 6). Thus, both the affinity and the capacity for protein binding increased. The effects of surface oleic acid are different from cholesterol, where an increase in surface cholesterol content did not change the affinity and started to decrease the capacity only when the surface concentration exceeded 34 mol% (30). These results suggest that if one could construct an emulsion particle that was saturated with cholesterol but with no fatty acid or lysolecithin present the protein binding capacity would be zero. The line in Fig. 6 goes virtually through zero. In the absence of fatty acids cholesterol
largely blocks the penetration of the protein between the acyl chains of the phospholipids and thus eliminates most of the binding sites. However, cholesterol is unable to do this in the presence of free fatty acids. This interpretation is compatible with our observation that cholesterol does not affect the distribution of fatty acids into surface phospholipid domains. When fatty acids are present in the emulsion apoA-1 can penetrate into the surface and probably interact with the acyl chains.

The mechanism by which fatty acids induce apoA-1 to bind to emulsion particles is not understood. However, the slope of the curve in Fig. 6 suggests that about 70 fatty acids are added to each particle for each additional molecule of apoA-1 bound. Since the fatty acids partition largely into the surface (at pH 7.4, the apparent distribution coefficient/surface/core $K_{ac} = 12$) (18), and since about one-half of the fatty acid (at pH 7.4) is ionized (17, 33) about 30–33 negatively charged carboxyls are added to the surface for each additional apoA-1 bound. Interestingly, apoA-1 contains 35 potentially positively charged (34) arginines and lysines. Is it possible that some charge interaction between the ionized fatty acid and apoA-1 lysines and arginines mediates the binding? Negatively charged ionized fatty acids incorporated into the surface might also fluidize the cholesterol-rich surface to some extent. This might allow an increased free volume in the interfacial region for apoA-1 to bind. Furthermore, the 70 fatty acids bound to the surface would probably occupy an area of about 30–35 $\text{Å}^2$/fatty acid (35); thus, the surface area covered by 70 fatty acids would be on the order of roughly 2100–2500 $\text{Å}^2$. The interfacial area of apoA-1 spread at an interface at 20–25 mMN/m is about 10 $\text{Å}^2$/amino acid (36) or about 2400 $\text{Å}^2$/apoA-1 molecule. Thus, the surface area occupied by the fatty acids and the surface area of apoA-1 capable of binding to the interface are about the same. This does not necessarily imply that the apoA-1 binds directly to the fatty acids but the concordance of areas is provocative.

The fact that the affinity appears to go up as the amount of apoA-1 bound increases may have to do with minor conformational changes occurring in apoA-1 as the result of some specific fatty acid binding. It could also be related to a difference in surface to which the apoA-1 is bound, i.e. more or less fatty acid. Neither of these possibilities can be addressed by the present studies.

The physiological importance of the high affinity between apoA-1 and fatty acids in emulsions is as yet unknown. It is possible that the function of apoA-1 on a chylomicron particle is different from its function on a high density lipoprotein particle (25, 32). On high density lipoprotein apoA-1 is a cofactor for lecithin-cholesterol-acyltransferase. On a chylo- micron, apoA-1 may aid the lipoprotein lipase reaction by being an acceptor for protonated fatty acids and moving this product away from the enzyme center. Subsequently apoA-1 may keep the proton for $\alpha$-helix stabilization (37) and transfer the deprotonated fatty acid to water, making it more likely that the ionized and hydrated fatty acid stays in the surface.

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