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Benzo[a]pyrene Uptake by Serum Lipids: Correlation with Triglyceride Concentration[1] (41969)

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Abstract. An *in vitro* study of the relationship between benzo[*a*]pyrene (BaP) association with serum lipoproteins (LP) and LP composition was conducted using human subjects. BaP partitioning into different serum LP ranged from 53 to 71% of available BaP. Efficiency of BaP partitioning was examined for the relationship with lipid components of different sera. The data indicate that triglyceride (TG) concentrations were more directly correlated with BaP uptake than were concentrations of other LP components. Adjusting sera to a uniform TG concentration (96.5 mg/dl) resulted in the same BaP uptake for each serum type, while adjusting sera to contain a uniform cholesteryl ester concentration (104.6 mg/dl) did not result in similar BaP uptake among serum types. Analysis of serum LP composition suggested that marked differences in both BaP uptake and serum TG concentrations among the subjects were due mainly to differences in serum very low density lipoprotein (VLDL) concentrations. A correlation study using 14 human subjects showed that serum TG concentration was the best predictor ($r = 0.973$, $P < 0.001$) for BaP uptake by serum, followed by phospholipid ($r = 0.658$, $P < 0.01$) and total cholesterol ($r = 0.514$, $P < 0.05$) concentrations. The results indicate that serum TG concentration (typically VLDL–TG) may be the primary factor affecting BaP uptake by serum LP, and suggest that a small change in serum TG concentration could cause a significant increase in BaP uptake by serum LP, contributing to an increased level of circulating carcinogen. © 1984 Society for Experimental Biology and Medicine.

The uptake of lipophilic xenobiotic compounds by plasma lipoproteins (LP) has been extensively studied. Among these lipophilic compounds are carcinogens such as methylcholanthrene (1), *p*-dimethylaminoazobenzene (2), and benzo[*a*]pyrene (BaP) (3–6). Shu and Nichols (3) investigated the uptake of BaP by human plasma LP *in vitro* and reported that BaP associates mainly with very low density lipoproteins (VLDL) and low density lipoproteins (LDL), and that BaP can transfer among LP classes. Busbee *et al.* (5, 7) reported BaP uptake to be correlated with total LP cholesterol concentration in serum and lymph, while Shu and Nichols (3) demonstrated that BaP uptake by LP is correlated with the total lipid volume of each LP class. In studies of the uptake of BaP and its hydroxylated metabolites by plasma LP, Shu and Nichols (4, 6) found that the distribution of these compounds into the albumin-rich serum fraction increased while their uptake by VLDL and LDL decreased with increased hydroxylation of the compounds.

Busbee *et al.* (5) recently reported a role for human serum LP in the intracellular transport of BaP, suggesting that VLDL and LDL may function to internalize BaP sequestered in LP by adsorptive endocytosis of the LP, and that this phenomenon may be measured when BaP is present in serum at concentrations too low to detect direct partitioning into cells. Since BaP is metabolized by microsomal oxygenase enzymes to mutagenic and carcinogenic derivatives in genetically responsive cells (8–10), this finding indicates the potential importance of BaP association with LP *in vivo*.

Chen *et al.* (2) suggested the extremely apolar lipid core of VLDL and LDL to be a possible binding site for *p*-dimethylaminoazobenzene, a lipophilic carcinogen. How-

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ever, Maliwal and Guthrie (11) showed that association of lipophilic compounds with LP is not a form of binding; rather, it is based on partitioning of the compounds from an aqueous phase into the LP lipid core. Since partitioning is apparently the mechanism by which BaP is taken up by LP, concentrations of triglycerides (TG) and esterified cholesterols (CE) in the apolar lipid core should be of greater importance in BaP uptake by serum LP than either free cholesterol or phospholipids (PL) (11, 12).

In this paper we present a study of the relationship between BaP uptake by, and the composition of, serum LP. We propose that the TG concentration of LP is more directly associated with the degree of BaP uptake and the transport by serum than are the concentrations of other LP components.

Materials and Methods. Four male subjects, 34 to 40 years old, were employed in the initial phase of this study. Three of the subjects were normal and healthy. One subject (subject 3) expressed genetic hypertriglyceridemia (hyperlipoproteinemia type IV) but was in apparent good health. A total of 14 male subjects, ages 30 to 45, were used in the last BaP uptake correlation phase of the study. Fasting blood was drawn by venipuncture, and serum was separated from cell fractions. The serum was preserved with 0.02% NaN_3.

Separation of LP using high-performance liquid chromatography (HPLC). Separation of serum LP was completed using the HPLC procedures of Busbee *et al.* (13), which employed modified silica gel size exclusion columns (a Spherogel TSK-4000 SW column with a TSK-125 guard column). Serum was eluted through the HPLC system at 1 ml/min using 0.01 *M* sodium phosphate buffer (pH 6.8) containing 0.02% NaN_3. The column effluent was monitored by spectral analysis at 254 nm, and 0.25-ml elution fractions were collected. The fractions containing VLDL, LDL, or high density lipoproteins (HDL) were pooled separately.

[^3H]BaP association with serum LP. Partitioning of BaP into serum LP was determined using the procedures of Busbee *et al.* (5). In 13 × 100-mm glass tubes, 500 μl of serum was mixed with 690 μl of phosphate-buffered saline (pH 7.4) containing human serum albumin (0.435 mg HSA/ml PBS). To this mixture was added 10 μl of an ethanol solution (96%) containing [^3H]BaP ([G-^3H]benzo(*a*)pyrene, 2×10^7 dpm/mole, 0.375 μmole/liter; New England Nuclear, Boston, Mass.; radiochemical purity of [^3H]BaP was determined, by radiometric assessment of the eluate of an Ultrasphere ODS HPLC analysis, to be greater than 99%.), making a total volume of 1.2 ml. The preparations were vortex-mixed, and incubated for 30 min in the dark at 37°C in a shaking water bath. After incubation, the tubes were placed in an ice bath, and 200 μl of the incubation mixture was removed from each tube to radiometrically determine the [^3H]BaP concentration (total [^3H]BaP available in the mixture). To the remaining preparations, we added 200 μl of charcoal/dextran suspension (6% activated, washed NORIT A and 0.06% dextran, mol wt = 70,000, in PBS). The preparation was vortex-mixed, placed in an ice bath for 30 min, and centrifuged at 3000*g* for 10 min. After centrifugation, 200 μl of the supernatant (equivalent to 167 μl of the original incubation mixture) was removed, and the [^3H]BaP concentration ([^3H]BaP partitioned into serum LP) was determined. The amount of [^3H]BaP partitioned into serum LP was expressed as the percentage of total [^3H]BaP available in the mixture. Background levels of [^3H]BaP were obtained by performing the assay without serum (1.190 ml of HSA/PBS + 0.010 ml [^3H]BaP) for control experiments, and subtracted from the value determined for each sample.

Determination of LP composition. Concentrations of proteins, PL, and free and esterified cholesterols were determined by the procedures of Bradford (14), Naito (15), and Allain *et al.* (16), respectively. TG concentration was determined using a commercially available TG Enzymatic Reagent kit (Cat No. 335UV; Sigma Co., St. Louis, Mo.).

Results. Table I presents data for the composition of serum lipids from four human subjects, and for BaP uptake by the different sera. The BaP uptake was quite different among the subjects, showing that subject 3 had the highest value (71%) followed by subject 2 (62%), and subjects 1 and 4 (53–56%). Comparison of these values with the serum lipid composition of the subjects

TABLE I. BaP Uptake by Human Sera: A Comparison with Serum Lipid Concentrations[a]

Subject	BaP uptake[b] %	BaP uptake[b] μg/ml serum	Lipid (mg/dl) PL[c]	Cholesterol Free	Cholesterol Ester	Cholesterol Total	TG	Total
1	53.4	1.40	148.9	43.8	104.6	148.4	96.5	393.8
2	61.9	1.62	161.5	49.5	118.7	168.2	196.0	525.7
3	70.7	1.86	227.0	50.2	133.2	183.4	349.5	759.9
4	55.9	1.46	242.0	53.0	120.2	173.2	102.0	517.2

[a] Each value represents an average of triplicate determinations.
[b] 500 μl of serum was incubated with 5.2 pmole (1.31 μg) of [^{3}H]BaP (total available) in 1.2 ml of incubation mixture. Values are expressed as either the percentages of the total available BaP or μg BaP/ml serum.
[c] PL, phospholipids; TG, triglycerides.

showed that TG concentrations appeared to be best correlated with BaP uptake ($r = 0.991$, $P < 0.001$).

Serum samples were adjusted to contain the same concentrations of TG (96.5 mg/dl), or CE (104.6 mg/dl). Data for BaP uptake by the adjusted sera are presented in Table II. BaP uptake was equivalent in sera with the same TG concentrations, while adjusting CE concentrations to the same level resulted in different BaP uptake for each serum type.

Data for the composition analysis of isolated LP from the four serum samples are presented in Table III. Serum VLDL concentration of subject 3 was 0.23 μ*M*, followed by subject 2 (0.11 μ*M*), and subjects 1 and 4 (0.05 μ*M*). These results suggest that differences in serum TG concentrations among the subjects are reflected mainly by VLDL concentrations. Subject 3 is a type IV hyperlipoproteinemic. Due to the extremely high VLDL level, serum concentrations of VLDL components, including TG and total lipids, for subject 3 were distinctly different from those of the other subjects. This was not observed by analysis of intact sera as shown in Table I. Although subject 3 exhibited the highest LDL–TG concentration among the four subjects, the LDL concentration differences among the subjects were not as distinct as were the differences in VLDL concentrations.

In a subsequent study of correlation between BaP uptake and lipid composition, sera from 14 normal human subjects, 30 to 45 years, were examined. There was very little difference in the ratios of total cholesterols (TC) to CE concentrations among the initial four subjects, therefore, data for TC concentration were used rather than values for BaP uptake correlated to free cholesterol vs CE. Data shown in Fig. 1–3 indicate that BaP uptake was correlated bestwith serum TG concentration ($r = 0.973$, $P < 0.001$), followed by PL ($r = 0.658$, $P < 0.01$), and total cholesterol (TC) ($r = 0.514$, $P < 0.05$) concentrations. Data showing relationships between variables (Table IV) indicate that the correlations of BaP uptake with LP components were TG > total lipids (TL) > PL

TABLE II. A Comparison of BaP Uptake by Human Sera Adjusted to Contain the Same Concentrations of Either Triglycerides or Cholesteryl Esters[a]

Subject	BaP uptake[b] by TG[c] (96.5 mg TG/dl serum) %	BaP uptake[b] by TG[c] (96.5 mg TG/dl serum) μg BaP/mg TG	BaP uptake by CE (104.6 mg CE/dl serum) %	BaP uptake by CE (104.6 mg CE/dl serum) μg BaP/mg CE
1	52.0	1.41	52.6	1.34
2	51.7	1.41	58.7	1.50
3	50.0	1.40	64.6	1.65
4	51.9	1.41	54.5	1.38

[a] Each value represents an average of triplicate determinations.
[b] Adjustment to contain the same serum concentrations of either TG or CE was achieved by diluting intact serum with phosphate-buffered saline (pH 7.4) containing human serum albumin (0.435 mg HSA/ml PBS), and 500 μl of adjusted serum was incubated with 5.2 pmole (1.31 μg) of [^{3}H]BaP (total available) in 1.2 ml of incubation mixture. Values are expressed as either the percentages of the total available BaP or μg per mg TG (or CE).
[c] TG, triglycerides; CE, cholesteryl esters.

TABLE III. THE CHEMICAL COMPOSITION OF HUMAN SERUM LP[a]

			Lipid [mg/dl (%)]						
				Cholesterol					
LP	Subject	Protein [mg/dl (%)[d]]	PL[b]	Free	Ester	Total	TG	Total	LP concentration[c] (μM)
VLDL	1	11.0 (12.0)	13.3 (14.6)	4.3 (4.7)	9.7 (10.6)	14.0 (15.3)	53.0 (58.1)	80.3 (88.0)	0.05
VLDL	2	14.0 (7.2)	14.2 (7.3)	9.4 (4.8)	11.5 (5.9)	20.9 (10.7)	145.3 (74.8)	180.4 (92.8)	0.11
VLDL	3	30.0 (7.3)	50.2 (12.1)	17.6 (4.3)	33.0 (8.0)	50.6 (12.3)	282.2 (68.3)	383.0 (92.7)	0.23
VLDL	4	7.0 (7.6)	14.3 (15.6)	6.1 (6.7)	8.0 (8.7)	14.1 (15.4)	56.2 (61.4)	84.6 (92.4)	0.05
LDL	1	41.8 (21.0)	48.0 (24.1)	26.2 (13.2)	59.4 (29.9)	85.6 (43.1)	23.5 (11.8)	157.1 (79.0)	0.86
LDL	2	58.8 (21.8)	66.1 (24.5)	23.7 (8.8)	80.3 (29.8)	104.0 (38.6)	40.8 (15.1)	210.9 (78.2)	1.17
LDL	3	70.9 (22.9)	77.3 (25.0)	21.6 (7.0)	78.4 (25.3)	100.0 (32.3)	61.4 (19.8)	238.7 (77.1)	1.35
LDL	4	51.2 (21.0)	71.6 (29.4)	24.0 (9.8)	72.0 (29.5)	96.0 (39.3)	25.0 (10.3)	192.6 (79.0)	1.06
HDL	1	174.5[e] (52.7)	96.6 (29.1)	12.6 (3.8)	35.0 (10.6)	47.6 (14.4)	12.6 (3.8)	156.8 (47.3)	14.40
HDL	2	168.2 (52.7)	93.4 (29.2)	12.7 (4.0)	31.3 (9.8)	44.0 (13.8)	13.8 (4.3)	151.2 (47.3)	13.89
HDL	3	177.6 (52.7)	106.2 (31.9)	11.4 (3.4)	15.8 (5.6)	27.2 (9.0)	21.2 (6.4)	154.6 (47.3)	14.44
HDL	4	239.0 (52.7)	145.6 (32.1)	20.4 (4.5)	39.6 (8.7)	60.0 (13.2)	9.2 (2.0)	214.8 (47.3)	19.73

[a] Each value represents an average of triplicate determinations.
[b] PL, phospholipids; TG, triglycerides.
[c] LP concentrations were derived from the chemical composition data and published values for average molecular weights of VLDL, LDL, and HDL (12).
[d] (%) Calculated as follows: individual component/(protein + total lipid) × 100.
[e] Protein concentrations of HDL were determined (see Materials and Methods) and correlated for accuracy with published compositional analysis data (12).

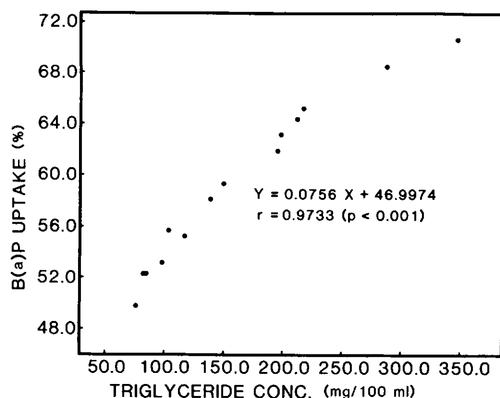

FIG. 1. Correlation between BaP uptake by serum and serum triglyceride (TG) concentration (n = 14).

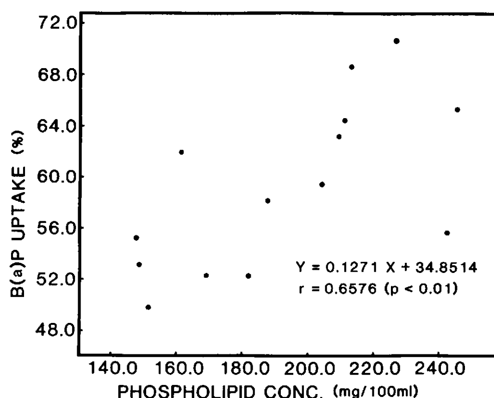

FIG. 3. Correlation between BaP uptake by serum and serum phospholipid (PL) concentration (n = 14).

> TC, and that the high correlation between BaP uptake and TL (r = 0.961, $P < 0.001$) resulted from the high correlation between TG and TL (r = 0.924, $P < 0.001$).

Discussion. Busbee *et al.* (5) demonstrated that the efficiency of available BaP uptake (concentrations between 3.08 pmole and 1.5 nmole/assay) by serum is constant if the serum LP concentration is constant. They suggested the LP concentration to be a major parameter affecting BaP uptake by serum. In the present study significant differences were observed in BaP uptake by different sera. These marked differences in BaP uptake by the different sera employed seem to be due to the compositional differences of serum LP. Chen *et al.* (2) reported that the apolar lipid core contents of VLDL and LDL dictate uptake of the lipophilic carcinogen, *p*-dimethylaminoazobenzene. This reasoning was carried further by Maliwal and Guthrie (11) who demonstrated that LP uptake of lipophilic compounds is based on partitioning of the compounds from an aqueous phase into the nonaqueous LP core. They suggested that concentrations of TG and CE in the lipid core of LP may affect the partitioning of lipophilic compounds into serum LP. Although other studies (3–7) have shown that BaP, a lipophilic carcinogen, partitions into LP, preferentially into VLDL and LDL, these reports did not address the relationship between uptake of BaP by serum and the concentrations of apolar LP components such as TG and CE. In this study, we examined the relationship between BaP uptake and serum LP components.

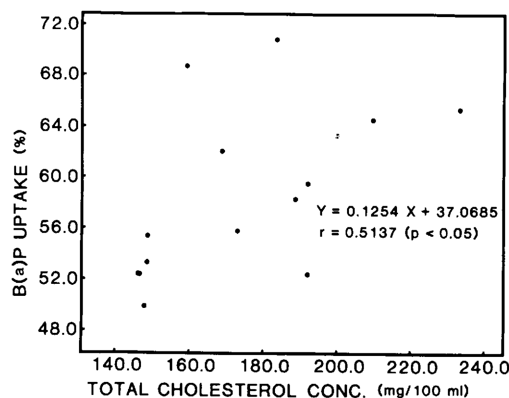

FIG. 2. Correlation between BaP uptake by serum and serum total cholesterol (TC) concentration (n = 14).

Intact serum samples were adjusted to contain the same TG concentration (96.5 mg/dl) among the subjects, resulting in marked differences of the other lipid components. Under these conditions, BaP uptake by the TG-adjusted sera was equivalent, suggesting a direct relationship between TG concentration and BaP uptake. Since CE, the other component of the apolar lipid core of LP, has the potential to affect partitioning of BaP (2, 11), intact serum samples were adjusted to contain the same CE concentration (104.6 mg/dl) among four subjects. Even though CE concentrations of the subject sera were the same, the CE-adjusted sera retained differences in TG concentration and exhibited distinct differences in BaP uptake.

TABLE IV. CORRELATION BETWEEN HUMAN SERUM LEVELS OF TRIGLYCERIDES, TOTAL CHOLESTEROLS, PHOSPHOLIPIDS, AND TOTAL LIPIDS, AND BaP UPTAKE BY SERUM

	BaP uptake	TG[a]	TC	PL	TL
BaP uptake	1.000[b]	0.973 ($P < 0.001$)	0.514 ($P < 0.05$)	0.658 ($P < 0.01$)	0.961 ($P < 0.001$)
TG	0.973 ($P < 0.001$)	1.000	0.375 ($P < 0.10$)	0.565 ($P < 0.05$)	0.924 ($P < 0.001$)
TC	0.514 ($P < 0.05$)	0.375 ($P < 0.10$)	1.000	0.660 ($P < 0.01$)	0.658 ($P < 0.001$)
PL	0.658 ($P < 0.01$)	0.565 ($P < 0.05$)	0.660 ($P < 0.01$)	1.000	0.808 ($P < 0.001$)
TL	0.961 ($P < 0.001$)	0.924 ($P < 0.001$)	0.658 ($P < 0.01$)	0.808 ($P < 0.001$)	1.000

[a] TG, triglycerides; TC, total cholesterols; PL, phospholipids; TL, total lipids.
[b] Pearson correlation coefficients ($n = 14$).

Subject 3 has been diagnosed as having familial hyperlipoproteinemia type IV, which is characterized by high levels of serum VLDL and TG. The increased VLDL level noted in the analysis of LP composition of this person's serum was seen to be associated with an elevated serum TG concentration and with elevated efficiency of BaP uptake.

We have conducted a study using 14 subjects to investigate the correlation between BaP uptake and serum lipid composition. Although serum concentrations of all LP components showed different degrees of correlation with BaP uptake, the TG concentration was shown to be the best predictor for BaP uptake by serum. The correlation between BaP uptake and TL ($r = 0.961$, $P < 0.001$) was not substantially different from the slightly higher correlation between BaP uptake and TG alone ($r = 0.973$, $P < 0.001$). A high correlation between TG concentration and TL concentration ($r = 0.924$, $P < 0.001$) was observed, which, in part, contributed to the high correlation between BaP uptake and total lipid concentration. The results from this study suggest that serum TG concentration is the best single predictor for BaP uptake. This, in turn, suggests that a small change in serum VLDL concentration, which greatly affects serum TG concentration, may cause a dramatic increase in BaP uptake by serum LP, contributing to an increased serum level of circulating carcinogen.

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