Dioxin Alters the Human Low-Density and Very Low-Density Lipoprotein Structure with Evidence for Specific Quenching of Trp-48 in Apolipoprotein C-II

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ABSTRACT: The intercellular transport of cholesterol and triglycerides via lipoproteins interacting with their receptors is a critical component in human lipid metabolism. The delivery of cholesterol to cells is accomplished primarily through low-density lipoproteins (LDLs), while the transport of fatty acids to adipose and muscle tissue is accomplished primarily through the actions of very low-density lipoproteins (VLDLs). Disruption of lipoprotein structure leading to impaired binding between these lipoproteins and their obligate receptors is a known risk factor for cardiovascular disease. Because of recent investigations linking 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure in humans with coronary artery disease, investigations have been carried out by fluorescence and circular dichroism to evaluate conformational changes in LDL and VLDL structure upon binding of TCDD. These studies demonstrate that, at a molar ratio of three TCDD molecules to one lipoprotein molecule, TCDD binds and disrupts the secondary and tertiary lipoprotein structure. Circular dichroism studies show that residues within the inner core of apoC-II, which compose a four-α-helix bundle when this apolipoprotein is associated with VLDL, are directly affected upon binding TCDD. Fluorescence also indicates the specific interaction of Trp-48 within apoC-II upon TCDD binding. We found that the TCDD/apoC-II complex suffers a 5-fold reduction in its ability to bind lipoprotein lipase compared to untreated apoC-II. The interaction of TCDD with LDL markedly altered the secondary structure of apoB reducing its α-helical content. These cumulative responses in lipoprotein structure may impair the LDL and VLDL cellular uptake leading to a buildup of serum lipoproteins and fats thus hastening the development of coronary artery disease.

The intercellular transport of cholesterol, cholesteryl esters, and triacylglycerols is accomplished through interactions between lipoproteins, their receptors, and certain accessory enzymes. Low-density lipoproteins (LDLs)[1] deliver cholesterol esters to cells via the low-density lipoprotein receptor (LDLR) for use in membranes and steroid hormone synthesis. Very low-density lipoproteins (VLDLs) primarily transport triacylglycerols to skeletal muscle and adipose tissue. Also integral to VLDL uptake (predominately adipocytes) is the interaction between VLDL and lipoprotein lipase (LPL) (*1*). LPL hydrolyses VLDL and chylomicron triglycerides facilitating cellular uptake and has been postulated to assist in anchoring the lipoprotein prior to endocytosis (*2*). Apolipoprotein C-II is obligatory for LPL hydrolysis of VLDL, but apoB-100 as well as apoC-III and several of the apoE apolipoproteins have been shown to play a role in receptor binding of in vitro systems (*3*). A disruption of these lipoproteins and apolipoproteins, particularly in the case of apoC-II and apoB-100, could impair VLDL and LDL cellular uptake.

Recent investigations link 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure in humans to cardiovascular heart disease (*4*). Consequently, environmental exposure to toxic compounds, which alter the structure of low-density lipoproteins (LDLs) and very low-density lipoproteins (VLDLs) with the result of decreased cellular uptake, may contribute to the incidence of coronary artery disease in these individuals. LDL and VLDL are taken up by cells through receptor-mediated endocytosis (*5*). Here, we examine the interactions of TCDD with various lipoproteins in an effort to evaluate the ability of TCDD to alter lipoprotein structure sufficiently enough to impair cellular uptake.

Because of the nonpolar nature of dioxin, it is reasonable to expect these compounds to have a high affinity for fats and fat-carrying compounds, such as lipoproteins in the blood. Importantly, TCDD interaction with VLDL and LDL may disrupt the receptor pathway and add to the observation that cardiovascular disease is found in large cohorts exposed to this contaminant (*6*). Animal studies have found that a

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[1] Abbreviations: LDLs, low-density lipoproteins; VLDLs, very low-density lipoproteins; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; LDLR, low-density lipoprotein receptor; LPL, lipoprotein lipase; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; CD, circular dichroism; K_{eq}, binding constant.

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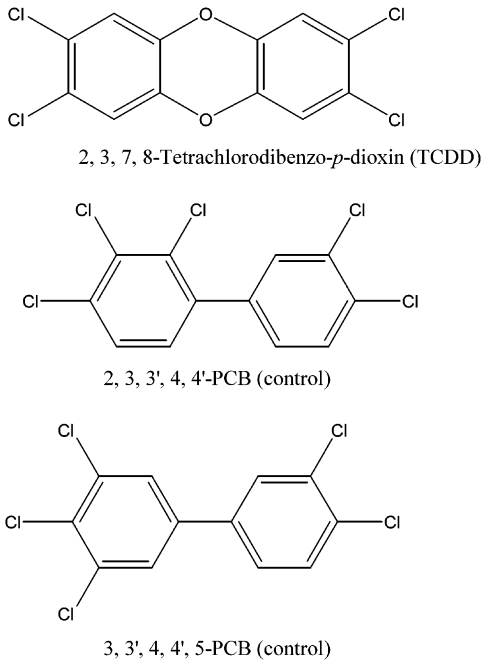

FIGURE 1: Structures of TCDD and PCB controls. TCDD is a coplanar molecule. 2,3,3′,4,4′-PCB and 3,3′,4,4′,5-PCB are also coplanar and described as "dioxin-like" because of the limited rotation about the two rings due to their chlorination pattern.

single dose of TCDD alters serum lipid composition, causing a nineteen-fold increase in VLDLs and a 4-fold increase in LDLs. A marked increase in serum cholesterol, triglycerides, and phospholipids is also observed 7 days after exposure (*7*). The source of this increase in both serum lipids and lipoproteins is as yet unknown.

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) coexist in the environment and are linked to numerous toxic responses in humans (*8*, *9*). The most toxic of the 75 congeners of dioxin is TCDD and is the subject of this investigation. These compounds may be released to the environment during the manufacturing of chlorinated organic solvents such as hexachlorophene and the herbicide 2,4,5-T and in the production of certain paper products. TCDD is a planar molecule with eight potential chlorination sites, where the two aromatic rings are bound by two oxygen atoms (Figure 1). The controls chosen for this study were 2,3,3′,4,4′-PCB and 3,3′,4,4′,5-PCB because both are planar molecules with similar molecular mass compared to TCDD (Figure 1). Importantly, PCBs have not been reported to alter serum lipid levels and are not presently associated with cardiovascular disease.

Fluorescence spectroscopy was employed to determine the binding affinity between TCDD and PCB controls with human LDL, VLDL, and apoC-II, using the intrinsic fluorescence of tryptophan and tyrosine residues. This allowed us to probe the effect of binding without mutating the proteins of interest. Only three amino acids reliably fluoresce: phenylalanine, tyrosine, and tryptophan. The emission wavelength of tryptophan is extremely sensitive to neighboring side-chain interactions, solvent interactions, and the polarity of its surroundings. This complexity is attributed to the two long wavelength absorption bands of tryptophan and their overlapping transitions to the ^1L_a and ^1L_b excited states. Consequently, each state's dipole moment differs such that ^1L_a and ^1L_b respond differently, where lower energy emission attributed to the ^1L_a excited state is seen with increasing solvent polarity (*10*). For this reason, tryptophan offers many advantages as an internal probe.

Circular dichroism (CD) spectroscopy was employed to elucidate changes in the secondary structure of both human LDL and apoC-II. Binding data from fluorescence have shown that TCDD alters the electronic environment of tryptophan and tyrosine residues (*11*, *12*). These data also suggest a general unfolding of the proteins when complexed with TCDD. Therefore, CD may be used to augment previous work and provide conformationally relevant data for the interaction of TCDD with these critical constituents of lipid metabolism.

CD spectroscopy shows that at low concentrations of TCDD (3:1 molar ratio of TCDD/lipoprotein), TCDD binds and diminishes the secondary structure of LDL apoB-100, reducing α helix and β sheet. CD studies show that residues comprising the inner core of the apoC-II, which form a four-α-helix bundle when complexed with VLDL, are affected upon binding TCDD. We found the TCDD/apoC-II complex suffers a 5-fold reduction in its ability to bind LPL compared to untreated apoC-II. Together, these interactions may lead to the inactivation of LDL and VLDL cellular uptake contributing to a buildup of serum lipoproteins and fats thus hastening the development of atherosclerosis.

MATERIALS AND METHODS

Human LDL and VLDL samples were purchased from Calbiochem and put into solution using phosphate-buffered saline (PBS) and deionized/distilled water. Samples were stored at 10 °C and used within 3 days of preparation. The sample was dialyzed to remove salts. Purity was found to be >95% by gel electrophoresis. Normal human LDL contains a single monomeric glycoprotein, apoB100. Molarity of LDL was calculated using the estimated molecular mass of 550 kDa of the protein (*13*). The sample was added to deionized/distilled H_2O and PBS purchased from Fisher Scientific and diluted to 1.212×10^{-13} M.

ApoC-II was also purchased from Calbiochem and put into solution using PBS and deionized/distilled water. Samples were refrigerated at 10 °C. The molecular mass of apoC-II was estimated at 8800 Da (*3*) and diluted to 1.893×10^{-13} M. Purity was assessed by SDS−PAGE and found to be >95%.

Fluorescence measurements were performed using a SPF-500C spectrofluorometer from SLM Instruments Inc. equipped with a xenon lamp. Lamp intensity was set between 550 and 650 V. Emission spectra were corrected for lamp output and photon multiplier tube deviation using rhodamine B as a quantum counter as described by Lakowicz (*14*). Samples were placed in sealed cuvettes under nitrogen gas to avoid quenching from atmospheric oxygen (*15*). TCDD and PCB samples were purchased from Ultra Scientific. Samples were placed into solution using deionized/distilled water and 2-propanol. Three scans were taken for each data point, and the average was taken. All experiments were performed six separate times. Excitation and emission slit widths were set at 10 nm, and gain was set at 100 for all experiments. Data were collected at 1-nm increments. The sample was sonicated and then allowed 6 min to reach equilibrium before performing an emission scan from 300 to 500 nm. The time required

to reach equilibrium was determined by scanning emission every minute until the emission spectrum stabilized.

Fluorescence steady-state emission data were obtained by taking the area of the emission spectra between 300 and 500 nm. Binding constants were assessed using the Stern−Volmer equation (*15*).

$$F_0/F = 1 + K[Q] \quad (1)$$

Here, F_0 is the initial intensity of emission, F is the intensity at a specific concentration of the quencher in units of molarity, and K is the binding constant (K_{eq}).

To quantify the degree to which submerged fluorophores gain access to the solvent as a consequence of TCDD binding, a modified Stern−Volmer equation was applied for the VLDL data (*15*). The fluorescence intensity of the protein prior to the addition of TCDD is given by eq 2. Here, F_0 is

$$F_0 = F_{0a} + F_{0b} \quad (2)$$

the initial emission intensity, F_{0a} is the initial intensity of fluorophores accessible to the solvent, and F_{0b} is the initial intensity of those buried within the protein.

The solvent accessibility of fluorophores and their quenching constant may be determined by use of the modified Stern−Volmer equation.

$$F_0/\Delta F = 1/(f_a K_a[Q]) + 1/f_a \quad (3)$$

Here, f_a is representative of the portion of initial fluorescence because of the fluorophores that are accessible to the solvent, where $(f_a K_a)^{-1}$ is the slope, f_a^{-1} is the y intercept, and K_a is the Stern−Volmer quenching constant.

Data for the change in the proportion of fluorophores that are solvent accessible upon VLDL binding TCDD was determined by taking the ratio of the value for f_a over the two different concentration ranges for TCDD.

$$f_{a\alpha}/f_{a\beta} = \Delta f_a \quad (4)$$

Here, $f_{a\alpha}$ is the degree of solvent-accessible fluorophores for the data collected from 620 fM to 3.11 pM TCDD and $f_{a\beta}$ is the degree of solvent-accessible fluorophores at 0−620 fM TCDD for VLDL data.

For CD experiments, the samples were maintained at a low molarity (3.64×10^{-7} M for LDL; 5.68×10^{-7} M for apolipoprotein C-II) to reduce the possibility of protein aggregation in solution. Each protein sample was run at four concentration ratios of TCDD to the protein (0:1, 3:1, 6:1, and 9:1). All samples were run at 25 °C. CD studies were performed on an Aviv 62DS CD spectropolarimeter (Aviv Biomedical, Inc., Lakewood, NJ) equipped with a thermoelectric temperature controller. Samples were run a total of four times, and the average was taken with a sweep time of 10 s per data point. Human LDL was scanned from 250 to 200 nm with a bandwidth of 1 nm in a 1-mm path length cell. Human apoC-II was scanned from 250 to 195 nm with a bandwidth of 0.5 nm in a 2-mm path length cell. Multiple spectra were recorded, averaged, corrected for buffer baseline, and normalized to molar ellipticity, [θ]. Molar ellipticity values, [θ], were calculated according to the equation: [θ] (deg cm^2/decimol) = $\theta \times$ MRW/10 $\times$ l $\times$ c, where θ = experimental ellipticity in millidegrees, MRW = mean residue weight of the amino acids, l = path length of the cell in centimeters, and c = concentration of apoC-II or LDL protein in grams per milliliter. After the calculation of the molar ellipticity, the percentage of α helix was calculated (*11*, *12*).

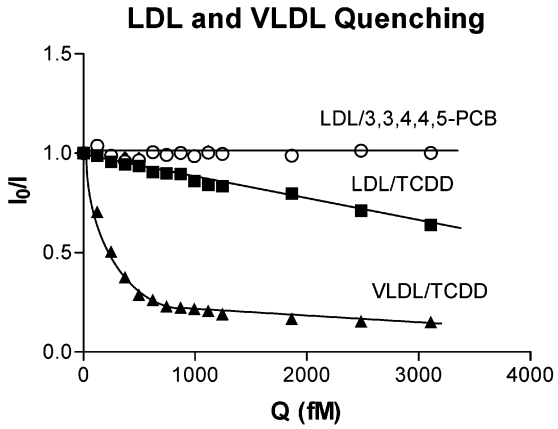

FIGURE 2: Fluorescence data for the binding of TCDD with human LDL and VLDL at 285 nm excitation using Stern−Volmer equation, where $p < 0.0001$ for both LDL and VLDL data calculated using GraphPrism.

For the binding assay between apoC-II and LPL, apoC-II was incubated with TCDD overnight at a molar ratio of 6:1 TCDD to apolipoprotein. TCDD was added to apoC-II as previously described, sonicated, allowed to sit at 24 °C for 30 min, and then refrigerated at 4 °C. Samples were scanned three separate times, and the average was taken. Experiments were performed independently on six separate occasions.

RESULTS

Fluorescence Spectroscopy. Fluorescence quenching experiments were used to determine the binding constant between TCDD; 2,3,3′,4,4′-PCB; and 3,3′,4,4′,5-PCB with LDL, VLDL, and apoC-II and to assess any structural changes initiated by TCDD. Polychlorinated biphenyls were used as controls.

TCDD bound human LDL, VLDL, and apoC-II significantly over the controls. We found the TCDD/LDL complex to have a $K_{eq} = 1.16 \times 10^{11}$ M^{-1} with an R^2 value of 0.9789 (Figure 2). The slope of these data is negative and therefore indicates that increasing amounts of TCDD result in greater fluorescence from the lipoprotein. This behavior has been seen in other systems where the addition of a denaturing agent increases an emission of the protein because of the movement of buried fluorophores to the surface of the protein (*10*, *16*). It is important to note that, at higher concentrations of TCDD, the emission from LDL was so great that it was at the detection limit of the spectrofluorometer. These data also show a red shift in the emission spectra and are indicative of tryptophan residues moving into a more polar environment such as that encountered with greater solvent accessibility. Neither PCB controls demonstrated binding activity with LDL.

Identical experiments were performed using TCDD and PCB controls with human VLDL to determine binding and to assess structural changes resulting from such interactions (Figure 2). Again, a strong response occurs when TCDD is reacted with VLDL, but no activity is observed between VLDL and PCB controls. We then assessed the binding over

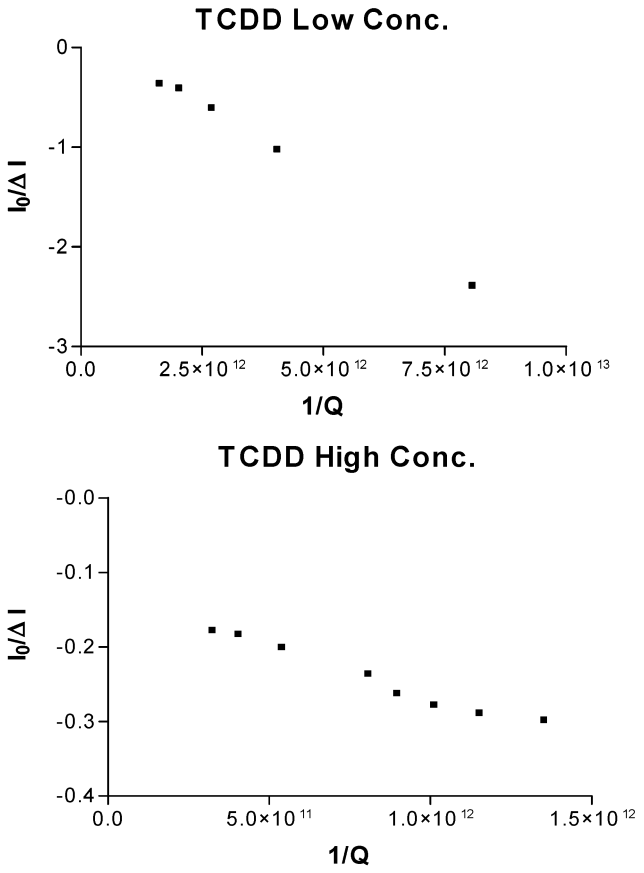

FIGURE 3: (a) Results of modified Stern−Volmer equation applied to the VLDL data set taken from a concentration range of 0−6.2 $\times$ 10^{-13} M. This graph gives the value of f_a^{-1} as the *y* intercept, which is an indication of the amount of accessible fluorophores. These data give an R^2 value of 0.997. (b) Results of modified Stern−Volmer equation applied to the VLDL data set taken from a concentration range from 7.43 $\times$ 10^{-13} to 3.11 $\times$ 10^{-11} M. $p <$ 0.0001 for both data sets calculated using GraphPrism.

two concentration ranges. Data taken for TCDD at 0 concentration going to 620 fM gave $K_{eq} = 1.69 \times 10^{12}$ M^{-1} with an R^2 value of 0.843. Data taken for TCDD with VLDL from 620 fM to 3.11 pM gave $K_{eq} = 2.54 \times 10^{10}$ M^{-1} with an R^2 value of 0.897. Importantly, binding data for both LDL and VLDL gave a negative slope. This is indicative of an increase in the emission of the protein with each successive titration of TCDD.

To quantify the degree to which submerged fluorophores gain access to the solvent, a modified Stern−Volmer equation was applied for the VLDL data (*14*). Parts a and b of Figure 3 show the plot of $F_0/\Delta F$ versus $1/[Q]$ for VLDL binding TCDD, where $(f_aK_a)^{-1}$ is the slope and f_a^{-1} is the *y* intercept. These data show the presence of two separate trends when the modified Stern−Volmer equation was employed for the entire VLDL data set (data not shown). In both graphs, the *y* intercept is f_a^{-1}, where f_a indicates the value of initially accessible fluorophores. In Figure 3a, f_a is equal to 4.35, and in Figure 3b, f_a *is* equal to 7.46. Dividing the value for f_a at high concentrations versus the value of f_a at lower concentrations gives a ratio for the change in accessible fluorophores of 1.71. This comes from the use of eq 4. Δf_a is the value of accessible fluorophores and nearly doubles over the course of the experiment. Values of K_a between the two data sets decrease as the protein unfolds. The data within the range of 0−620 fM TCDD give a $K_a = 1.19 \times 10^{12}$ M^{-1} compared

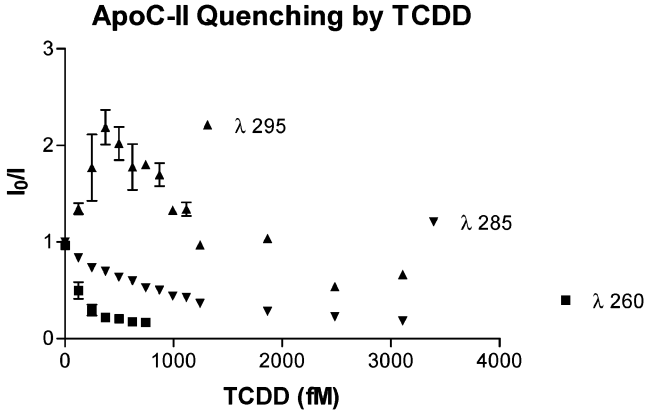

FIGURE 4: Fluorescence data for the unfolding of human apoC-II with quenching of Trp residues using Stern−Volmer equation. Excitation was performed at 260 (▲), 285 (■), and 295 nm (◆). Excitation at 295 nm shows quenching of the lone tryptophan residue in apoC-II at approximately 1:1 ratio of TCDD/apoC-II. Excitation at 260 and 285 nm showed the general unfolding pattern.

to the K_a for the data taken from 620 fM to 3.11 pM TCDD, where $K_a = 2.54 \times 10^{10}$ M^{-1}. These data demonstrate a biphasic response to TCDD binding, with two distinct equilibrium expressions for the two concentration ranges. We see from the data plotted using the modified Stern−Volmer equation that the percent of solvent-accessible fluorophores increases by approximately 2-fold over these two concentration ranges and that the equilibrium expression for the data taken between 0 and 620 fM TCDD is greater than the expression at higher concentrations.

Because of the critical role apoC-II plays in cellular uptake, we performed steady-state experiments to measure binding between this apolipoprotein and TCDD (Figure 4). This was done to determine the value of K_a and to assess any structural changes apparent in either blue or red shifts in the emission spectra. Binding was assessed using three different excitation wavelengths (260, 285, and 295 nm) to quantify the effect of binding on both tryptophan and tyrosine fluorophores. Excitation wavelengths of 260 and 285 nm produce a progressive increase in tryptophan/tyrosine emission intensity and a slight red shift in the emission spectra from 331 to 336 nm as the TCDD concentration is increased (Figure 4). Excitation at 260 and 285 nm results in a nonlinear plot similar to that seen in the earlier VLDL data. Again, controls showed no response (data not shown). The 260 nm excitation resulted in a dramatic increase in emission intensity, which overcame the light intensity limits of the instrument. However, when an excitation wavelength of 295 nm was employed, the emission intensity of apoC-II decreased as the TCDD concentration rose to 384 fM. ApoC-II and TCDD reached a molar ratio of 1:1 at 189 fM. Data collected over this range also show a blue shift from 353 to 331 nm.

To test if these perturbations in protein structure are sufficient to impede or destroy the ability of apoC-II to bind LPL, a binding assay using LPL and apoC-II both treated and untreated with TCDD was performed. Fluorescence spectroscopy steady-state measurements were employed in which LPL was held at a constant concentration with increasing amounts of C-II. The molar ratio of the apoC-II/TCDD complex was maintained for all samples at a 1:6 molar ratio of apoC-II/TCDD. Figure 5 shows statistically significant evidence for the quenching of LPL by apoC-II

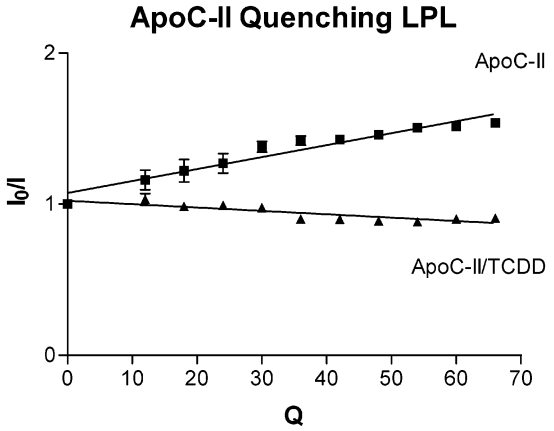

FIGURE 5: Stern−Volmer plot of apolipoprotein C-II quenching of LPL at an excitation wavelength of 285 nm. Emission was scanned from 300 to 500 nm with the integral of the quantum yield used to calculate the change in fluorescence. Concentration of C-II or C-II/TCDD is given in nanomolars. The ratio of TCDD/C-II was maintained at 6:1 for all concentration ranges, $p < 0.001$, calculated using GraphPrism.

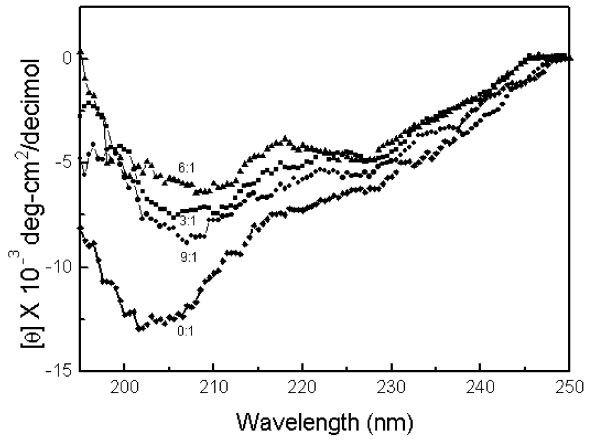

FIGURE 6: CD spectra of human apolipoprotein C-II in the presence of TCDD. Spectra were recorded from 250 to 195 nm over a concentration range of 0.0 TCDD to a molar ratio of 9:1 TCDD/C-II.

that has not been incubated with TCDD compared to C-II that has been complexed with TCDD.

CD Spectroscopy. CD was used to further characterize lipoprotein and apolipoprotein conformation in the presence of TCDD. CD spectra of ApoC-II and LDL were acquired in the absence of TCDD to examine their conformation prior to TCDD binding. Each sample was run at three different concentrations of TCDD starting at a molar ratio of 3:1 TCDD/protein and concluded at a molar ratio of 9:1 TCDD/protein. Both apoC-II and LDL apoB exhibited significant alterations in their secondary structure in response to TCDD, as described in detail below and in Figures 6 and 7.

Using CD, we identified changes in the conformation of apoC-II, which resulted in a loss of the α-helix structure upon addition of TCDD as seen in Figure 6. The α-helical content of apoC-II calculated, on the basis of $[\theta]_{222\ nm}$, the wavelength associated with the $n-\pi^*$ transition in a right-handed α helix, at 0:1, is 25% and is reduced to 19, 19, and 21% upon successive additions of TCDD (3:1, 6:1, and 9:1, respectively). The CD spectrum of native human LDL apoB is characterized by a broad minimum, characteristic of a protein that has a large amount of secondary structure, ~40% α helix and 20% β sheet (*11*, *12*). Figure 7 presents CD spectra of LDL and TCDD at ratios from 0:1 (no TCDD) to 9:1. In the absence of TCDD, the spectrum of LDL is as described above and observed previously. Addition of TCDD to LDL (3:1, 6:1, and 9:1) progressively alters the CD spectrum of LDL. In Figure 7, a progressive loss of both the α-helical and β-sheet content is observed with increasing TCDD concentrations. Changes in α-helical content may be estimated based on the value of the molar ellipticity measured at 222 nm (*11*, *12*). This is clearly evident in Figure 7, where a progressive loss of signal at 222 nm is observed with increasing amounts of TCDD. The α-helical content of LDL calculated, on the basis of $[\theta]_{222\ nm}$, at 0:1 is 38.6% and decreases to 30.8, 26.0, and 23.4% upon successive additions of TCDD (3:1, 6:1, and 9:1, respectively). Similar observations are made at 217 nm, a wavelength associated with the β-sheet content of proteins, where a progressive loss of signal is observed upon addition of TCDD (*11*, *12*). Thus, even at low ratios of TCDD, the conformation of LDL apoB is altered significantly.

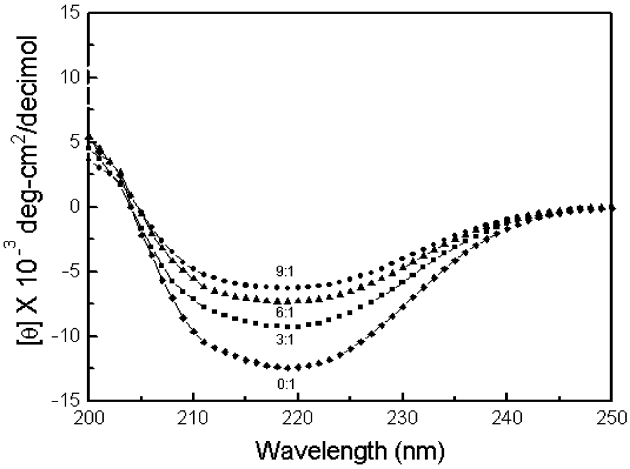

FIGURE 7: CD spectra of human LDL treated with an increasing concentration of TCDD. Molar ratio of TCDD/LDL apoB-100 goes from an initial ratio of 0:1 to a maximum of 9:1 at a 3:1 ratio increase at every addition.

DISCUSSION

Because recent investigations link exposure to TCDD in humans with cardiovascular disease, we examined the interactions of TCDD with various lipoproteins in an effort to evaluate the ability of TCDD to alter the lipoprotein structure sufficiently enough to impair cellular uptake thereby contributing to serum lipid levels. This study shows that at low molar ratios of 3:1 TCDD/lipoproteins, both the structure and biological activity of apoC-II, LDL, and VLDL are affected. LDL and VLDL have multiple tyrosine and tryptophan residues of varying degrees of solvent accessibility. Figure 2 demonstrates the selectivity of TCDD for protein elements within LDL and VLDL. The increase in emission intensity of both LDL and VLDL when complexed with TCDD compared to the controls is in agreement with a system that undergoes a dramatic change in conformation such that a large proportion of buried fluorophores are moved to the surface. It is our view that, as LDL or VLDL binds TCDD, a conformational change occurs rendering some fluorophores more accessible to the solvent and likely more surface-located.

To quantify the degree to which submerged fluorophores within VLDL gain solvent access as a result of the lipoprotein binding TCDD, we analyzed the data over two concentration ranges using a modified Stern−Volmer equation. The values for f_a show that at the lower concentrations there are fewer accessible fluorophores compared to higher concentrations. This supports the theory that, as greater amounts of TCDD bind VLDL, a change in protein structure occurs whereby more fluorophores gain access to the solvent. This is seen in the fluorescence spectra where greater concentrations of TCDD increase the quantity of the emission spectra. These calculations quantify what may be understood intuitively as the increase in solvent-accessible fluorophores as greater amounts of TCDD bind VLDL.

Human apolipoprotein C-II is found on VLDL and chylomicrons and is needed for proper binding between the triglyceride-rich lipoproteins and LPL. LPL is also involved in lipid hydrolysis and plays a role in facilitating the interaction of lipoproteins with their cellular receptors (*2*). To quantify the binding of TCDD to both tryptophan and tyrosine residues within apoC-II, excitation wavelengths of 260, 285, and 295 nm were employed. Tryptophan absorption occurs from approximately 240 to 305 nm with an absorption maximum near 285 nm. Tyrosine absorption occurs in the range from 240 to approximately 290 nm, so that excitation at 295 nm will select for tryptophan over tyrosine, while excitation at shorter wavelengths will excite both fluorophores (*18*). Therefore, exciting the protein at 260 and 285 nm will result in emission from both tyrosine and tryptophan. Excitation at 260 nm will produce greater emission from tyrosine because of the increase in tyrosine absorbance at this wavelength. Excitation at 295 nm will excite only tryptophan, and so the emission spectra obtained from exciting the molecule at 295 nm will include only tryptophan emission. Figure 4 shows that at excitation wavelengths of 260 and 285 nm apoC-II exhibits greater emission at higher concentrations of TCDD, which is consistent with the behavior of LDL and VLDL at those wavelengths. However, excitation at 295 nm shows maximum quenching of apoC-II by TCDD at a molar ratio of approximately 2:1 TCDD/apoC-II. Importantly, there is only one tryptophan in all of apoC-II, Trp-48, which is located in the lipid-binding region of the protein. This indicates either TCDD binds Trp-48 moving the residue into a less polar environment (*18*) or that, as TCDD binds apoC-II, a neighboring amino acid side chain quenches Trp-48. This paper provides specific binding data not previously reported.

We chose to study the binding of LPL to apoC-II to quantify any potential loss in biological activity for these molecules. The structures of apoC-II involved in lipid interactions have been shown to reside at the N terminus (residues 1−51) (*19*). The C-terminal (residues 52−79) is needed for the activation of LPL, which is thought to involve protein/protein interactions between C-II and LPL (*20*). Earlier work predicted two or three amphipathic helical regions, which serve to coordinate C-II lipid and water interfaces (*21*). Recent work using apoC-II complexed with micelles shows that helix mobility within apoC-II is involved in the activation of LPL (*22*).

Figure 5 shows quenching of LPL by apoC-II using the Stern−Volmer equation. Little quenching of LPL is observed when the TCDD/apoC-II complex is titrated. A negative slope is seen in this data set and may be explained by the observed increase in apoC-II emission following incubation with TCDD. This supports the observation that the negative slope seen in the C-II/TCDD data is the result of increased unwinding of the apolipoprotein. These results are consistent with our theory that TCDD binding human apoC-II induces a significant structural change in the protein such that it may no longer function as untreated apoC-II. Importantly, such defects in apoC-II on VLDL may alter the ability of LPL to perform its essential function of lipid hydrolysis leading to hypertriglyceridemia, a major risk factor for heart disease. The data for LPL binding both untreated and TCDD complexed apoC-II provide insight into possible mechanisms by which TCDD may affect the metabolism of lipoproteins in living cells. While these data are the result of in vitro studies and do not constitute a biological response within a living entity, the observed binding trends do shed light on the ability of TCDD to alter the structure of apoC-II such that it no longer recognizes this essential enzyme in lipid metabolism.

CD studies (Figures 6 and 7) corroborate previous data obtained using fluorescence spectroscopy techniques that indicate a loss of structure when the proteins are complexed with TCDD. A significant alteration in the secondary structure of both proteins studied was observed at relatively low levels of TCDD. Even at a low molar ratio of 3:1 TCDD/protein, there was a significant alteration in the secondary structure of both LDL apoB and apoC-II.

The CD spectrum of apoC-II, similar to that reported previously, is characterized by a deep minimum at 202 nm and a broad shoulder centered at 222 nm (*23*−*25*). Such a spectrum is typical of a highly unordered, loosely folded, or random coil, protein that also contains some α helix. In its native lipid-bound state or in detergent micelles, apolipoprotein C-II has a highly ordered secondary structure, with a four-α-helix bundle at its core with the remainder of the protein composed of loops. Zdunek et al. (*22*) propose that, in its native lipid environment on VLDL, the four-α-helix bundle of apoC-II undergoes protein/protein interactions within the bundle and with the loop regions and that these interactions are essential to the proper function of the protein. The effect of TCDD on the secondary structure of apoC-II at low molar ratios is compelling. Significant changes in the ellipticity of apoC-II at 222 nm suggest that the α-helical content is altered, accompanied by substantial alteration in the overall structure of the protein.

LDL apoB-100, the sole protein component of normal human LDL, has a molecular weight of 550 000 Da. Scans of LDL were performed from 250 to 200 nm, a wavelength range over which the protein component of LDL, apoB, alone exhibits a CD signal. At wavelengths below 200 nm, some of the lipid components of LDL also exhibit CD signals, contributing to the observed CD spectrum and complicating the interpretation of the spectrum.

It is interesting to note that, while the loss of structure in LDL is progressive with each addition of TCDD, we see a significant loss of structure in apolipoprotein C-II with the first addition of TCDD. Subsequent additions of TCDD to apoC-II further increase the loss of the signal at 222 nm, but this disturbance is not of the same magnitude seen after the first addition. This may well be due to the increased α-helical and β-sheet content in LDL because of apolipo-

protein B-100, as well as its association with the lipid. As TCDD is added, an increased loss of the signal from both the 222 and 217 nm regions of the spectra are observed, but the total α-helical and β-sheet content is not abolished. This observation correlates to the respective molecular weights of apoB-100 compared to apoC-II (550 000 and 8800 Da, respectively) and supports the theory that, while TCDD may disturb LDL at low concentrations, similar molar ratios of TCDD to apoC-II result in a more substantial destruction of the overall secondary structure of the protein. CD spectroscopy correlates earlier findings using fluorescence spectroscopy that the addition of TCDD to both LDL and apoC-II results in the loss of the secondary structure within these proteins. It is important to note that these effects are seen in both these proteins at relatively low molar ratios of TCDD. In both CD studies, we found that the addition of TCDD at a molar ratio of 3:1 TCDD/protein significantly altered the protein structure.

We have determined that in the case of VLDL the number of solvent-accessible fluorophores increases by nearly 2-fold upon treatment of TCDD. CD spectroscopy has shown that apoC-II and LDL undergo significant conformational changes resulting in a substantial loss of the secondary structure upon binding TCDD. TCDD at a 9:1 molar ratio resulted in substantial reduction in α-helical content in apoC-II and also in LDL apoB. This is in keeping with earlier fluorescence binding data that demonstrates a general unwinding of these proteins upon binding TCDD.

Binding experiments performed with apoC-II and LPL demonstrated TCDD complexation altered this biologically important interaction. Untreated apoC-II effectively bound LPL, while apoC-II that had been incubated with TCDD prior to the experiment lost its ability to bind its obligate lipase. This indicates that TCDD binding apoC-II affects one of the biologically significant properties of this apolipoprotein. The observed pathologies because of TCDD exposure are numerous. It is more than likely that a variety of pathways may be affected by TCDD and would be relevant to the observation of the increased risk of coronary artery disease in humans exposed to this environmental contaminant. This paper demonstrates the deleterious effect of TCDD on human LDL, VLDL, and the apolipoprotein C-II, which may in part account for the increased risk of heart disease following toxic exposure.

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