Dietary Fats Affect Rat Plasma Lipoprotein Secondary Structure as Assessed by Fourier Transform Infrared Spectroscopy

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ABSTRACT This investigation was undertaken to determine by Fourier transform infrared spectroscopy the effects of diets enriched with fish, sunflower or olive oils on the secondary structure of plasma HDL and LDL from rats, as well as the effects on lipid unsaturation and acyl chain lengths. Controls were fed a commercial diet. In HDL, random coil conformation was relatively high in rats fed the fish diet, probably due to the irregular geometry of polyunsaturated fatty acids interacting with apoproteins. Parallel structural behaviors were observed for rats fed control and olive oil diets. The lowest lipid unsaturation level was found in HDL of rats fed olive oil, and acyl chain lengths were slightly increased by the three fats. Rats fed olive oil had the lowest percentage of LDL β-sheets and these were more abundant in rats fed the fish oil diet. The least lipid unsaturation in LDL was in rats fed the olive oil diet. No significant differences in acyl chain lengths were observed. Certain protein conformational changes and/or apoprotein composition differences due to dietary fat may affect the binding between lipoproteins and their receptors in cells. J. Nutr. 131: 1898–1902, 2001.

KEY WORDS: • dietary fat • lipoproteins • rats • secondary structure
• Fourier transform infrared spectroscopy

Plasma lipoproteins are particles that carry cholesterol to different tissues. Cardiovascular disease has been related to diet and lipoprotein composition. Numerous epidemiological studies have demonstrated that high levels of plasma cholesterol, especially that transported in LDL, and/or low concentrations of cholesterol transported in HDL are associated with an increase in the risk of atherosclerosis and cardiovascular disease. Fatty acids are the most important dietary component that determines the concentrations of plasma lipids. The association between diet, plasma lipid concentrations and atherosclerosis has been well documented and reviewed (1). Studies carried out by Parthasarathy et al. (2) have shown the relationship between oleic and linoleic acid-rich diets and the lipoprotein composition associated with atherogenic risk, as well as the different composition of the dietary fat and the LDL oxidation susceptibility. This susceptibility is composition-dependent, depending mainly on the fatty acids of the phospholipids in the outer layer of the lipoprotein particles. Although lipoproteins in normal plasma present different LDL subclasses that vary in terms of size, lipid composition, apoprotein content and metabolic properties (3–5), we have studied the particles without previous separation into subclasses. In the present study, we used rats as an animal model for evaluating four dietary fats effects on apoprotein structure by infrared spectroscopy.

The objective of the present study was to establish the secondary structure of plasma apolipoproteins in relation to the dietary oil, as assessed by Fourier transform infrared, showing the possible structural changes that the fatty composition of the diet induces in the secondary structure of the apolipoproteins in LDL and HDL particles. Structural changes in LDL have been shown to alter their metabolism and atherogenic potential. We have used animal and not human models because it is very difficult to feed an appropriate number of persons particular oil diets and, moreover, human commercial food consists usually of heterogeneous fat components.

The infrared amide I band arises primarily from in-plane C=O stretching vibrations, and partly from in-plane N-H bending vibrations (6). The exact location of the amide I band in the infrared spectrum depends on hydrogen bonding and the conformation of the protein backbone (6). In heteropolypeptides and in real-world proteins, there exist a variety of domains containing polypeptide fragments in different conformations. Thus, the observed amide I band contours of proteins are usually complex composites that consist of a number of overlapping component bands, representing helices, β-sheets, turns and unordered structures. The currently available techniques of resolution enhancement, such as Fourier deconvolution and derivative spectroscopy, usually allow the identification of these otherwise hidden component bands. Moreover, band-fitting procedures allow for quantitative evaluation of
the various components of protein secondary structure, such as α-helices, β-structures and turns, on the basis of measuring the fractional areas (integrated intensities) of the fitted component bands. These areas are directly related to the relative populations of the conformational structures represented by these components.

MATERIALS AND METHODS

Subjects. Forty male Wistar rats from an in-house colony were subdivided into four dietary groups (10 animals per group) weighing between 70 and 90 g for 2.5 mo until their killing. One group control and three experimental groups were fed synthetic diets with 5 g/100 g in fat prepared in our laboratory (7,8). The three diets differed primarily in their fatty acid composition: one with 42% (n-3) PUFA (fish oil) (9); another with 60% (n-6) PUFA (sunflower oil) and the third with 75% monounsatuated fatty acid (olive oil) (9). RMN commercial rat diet (in pellet form) provided by Harlam Ibérica (Barcelona, Spain) was used as the commercial control with 2.2 g/100 g fat. The rats were allowed free access to food and water. The above animals were killed by decapitation, and the corresponding blood samples were immediately centrifuged to separate the plasma.

Lipoproteins. IDL + LDL (d 1.006–1.063 kg/L) and HDL (d 1.063–1.21 kg/L) were isolated from 9 mL of plasma obtained at each time point by sequential ultracentrifugation in a Beckman TL100 ultracentrifuge using a NVTi rotor (Beckman Coulter, Fullerton, CA). These lipoproteins were then purified by dialysis against saline solution (pH 7.0) for 12 h.

Infrared spectroscopy. Infrared spectra were recorded at room temperature with a Perkin-Elmer 2000 instrument (Norwalk, CT) using a high sensitivity deuterated triglycerine sulfate detector and a spectral resolution of 2 cm⁻¹. The spectra of LDL and HDL were recorded using the film procedure. This was followed by placing ∼30 µL of lipoprotein aqueous suspension on an infrared window and evaporating under vacuum. The spectra were transferred to a personal computer, where small amounts of water vapor were subtracted when needed. Quantitative information on protein structure was obtained through decomposition of the amide I band into its constituents. The spectral resolution enhancement was performed as described previously (10), and the amide I band decomposition was performed using the curvefit routine running under SpectraCalc (Galactic, Salem, NH). The amide I band components were fitted with weighted sums of Lorentz and Gaussian functions. The choice of the starting parameters was assisted by Fourier self-deconvolution and second derivative spectroscopy. The initial heights were set at ∼90% of those in the original spectrum for the bands in the wings and for the most intense component, and at ∼70% of the original intensity for the other bands. The mathematical solution of the decomposition may not be unique, but if restrictions are imposed such as the maintenance of the initial band positions in an interval of ± 1 cm⁻¹, the preservation of the bandwidth within the expected limits, or the agreement with theoretical boundaries or predictions, the result becomes, in practice, unique.

Data analysis and statistics. The area percentages of amide I band components are reported as the mean for each group. Student’s t tests were used to determine differences between experimental groups and controls.

RESULTS AND DISCUSSION

Protein secondary structure and lipid/protein ratio

HDL. The spectra of the HDL are shown in Figure 1. These show three bands corresponding to the C=O stretching motions (14C=O) of lipid carbonyl groups (band centered near 1735 cm⁻¹), carbonyl groups of protein backbone (band centered near 1655 cm⁻¹, which is the so-called amide I band) and N-H groups (6N-H band centered at 1550 cm⁻¹, so-called amide II). Although the height of the 1735 cm⁻¹ band relative to that located at 1655 cm⁻¹ was variable when controls were compared with those fed the experimental fats, the lipid carbonyl band area was smaller than that of the amide I band in each sample, as expected from the composition of the HDL. In fact, these usually contain ∼50% apoprotein, 20% phospholipids, 10% cholesterol esters and 20% unesterified cholesterol (4). We estimated average absorbivitics for carbonyl and amide I bands of carbonyl lipid fraction and protein fraction, respectively, which showed that the absorptivity of the 1735 cm⁻¹ lipid band relative to that of the amide I band was ∼2. Because of this result and the above lipoprotein composition, it was expected that the area of the amide I band in each sample would be higher than that of the corresponding 1735 cm⁻¹ lipid band.

The relative intensities of ν(C=O) lipid and amide I bands were smaller in the rats fed the fish oil or sunflower oil diets than in controls or in the rats fed the olive oil diet. In contrast, the spectral profiles of the amide I and amide II bands differed in the experimental groups compared with controls, which means that these diets affected protein secondary structure and/or apoprotein composition.

With the aim of quantifying the changes observed in the spectral profiles, we have done the curve fitting of the amide I band generated by the protein backbone (Fig. 2). The assignment of the amide I component bands to conformational structures (Table 1) seems at first difficult because the number of bands is larger than the number of expected protein secondary structures, and because natural proteins do not always exhibit the same behavior as model molecules and homopolypeptides that are thought to reflect these secondary structures (10). Still, some of the bands could be unambiguously assigned (Table 1), whereas for others, reasonable approximations could be made in comparison with data from other techniques. Thus, the band ∼1655 cm⁻¹ corresponds to α-helix plus nonstructured peptide, but only α-helix in N-deuterated polypeptide backbone. β-Turns are located between 1660 and 1685 cm⁻¹, whereas the extended structures (β-strands) give rise to signals in the region 1610–1640 cm⁻¹. Two different groups of bands are seen in this region, one in the 1625–1640 cm⁻¹ range and the other group in the 1610–1625 cm⁻¹ range. The first group of bands arise from intramolecular C = O vibrations of α-helix, the latter is found in denatured proteins (11,12) but it is not so common in native proteins. In these, the bands falling in the 1610–1625 cm⁻¹ range were first found in concanavalin A (13) and were assigned to peptides in an extended configuration, with a hydrogen bonding pattern formed by peptide residues not taking part in intramolecular β-sheet but rather hydrogen...
bonded to other molecular structures, e.g., forming intermolecular hydrogen bonding in monomer-monomer interaction. They were also found in triosephosphate isomerase (14), and even in human LDL (15). These low frequency bands were called "β-edge" bands, typical of the outer strands of β-sheets (13,14). This pattern also implies intermolecular hydrogen bonding, as postulated for the low-frequency bands in irreversibly aggregated proteins (10), or in monomer-monomer contacts, as in concanavalin A (13). Aromatic ring vibration of tyrosine residue can also generate a band usually appearing near 1615 cm⁻¹ (16).

Figure 3 shows, as a characteristic feature, similar area percentages for all the amide I band components in HDL proteins from olive oil and control diets. In contrast, the spectral behavior of HDL from rats fed fish oil diet tended to differ from that of controls, where this secondary structure was less abundant. Only the fish oil diet generated a visible band at 1674 cm⁻¹, and the most prominent bar of the 1650 amide I band component corresponded to HDL from rats fed this diet (Fig. 3). By contrast, its area percentage at 1681 cm⁻¹ was the lowest one. The sunflower oil diet resulted in the most intense band components at 1622 and 1615 cm⁻¹.

**LDL.** Figure 4 includes the infrared spectra of LDL in the 1800–1500 cm⁻¹ region. Apart from the fact that the lowest nC=O intensity relative to that of amide I band was present in control samples, there seemed to be some differences within the amide I spectral region. This was particularly true for LDL from rats fed olive oil diet, which produced the lowest proportion of β-structure compared with the rest of the samples. Thus, this diet generated 8.91% of the 1623 cm⁻¹ band component, whereas the corresponding percentages in the LDL of rats fed fish oil and sunflower oil diets were 15%, 14% and 11%, respectively (Fig. 5). A similar situation could be described for the other β-structure band component at 1615 cm⁻¹. The fish oil diet generated weaker intensities at 1651 cm⁻¹ relative to controls. This band component can be attributable to α-helices and random coil polypeptide backbone. In contrast, it is obvious that

### Table 1

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1 In some lipoprotein band components the frequencies may be noncoincident, ~1–2 cm⁻¹ apart.
2 In undeuterated proteins, the band components near this frequency may include contributions of unordered structure.
random coil formation involves the presence of turns, which usually appear in the 1660–1675 cm$^{-1}$ range. Because the band components at 1661 and 1671 cm$^{-1}$ of LDL from rats fed the fish oil diet did not significantly differ from controls, we can tentatively assign the 1651 cm$^{-1}$ intensity decrease in LDL of rats fed the fish oil diet to a concomitant decrease of α-helices. This can be easily explained by considering the lipid composition of this diet. In fact, fish fats are rich in PUFA, which contain cis C=C conformation (turns) in their hydrocarbon chains (16). In general, fatty acid composition in serum and plasma lipoproteins reflects, to a large extent, the fatty acid composition of the diet (17). Consequently, the lipids surrounding the apolipoproteins of rats fed fish and sunflower oil diets may adapt preferentially to random or β-polypeptide conformation. In fact, we have found (unpublished results) that PUFA induce turns and β-sheet structures in proteins.

The above diet-dependent protein conformational changes in LDL and HDL are not parallel. This can be explained by considering that apoprotein and lipid compositions in HDL and LDL particles are different, thus leading to particular lipid-protein interactions and subsequent protein structures adopted by each type of lipoprotein particles.

Unsaturation and acyl chains

With the aim of knowing the relationship between type of dietary fat and its effect on the amount of lipid unsaturation in lipoprotein particles, we have reported the intensity of the 3015 cm$^{-1}$ band relative to that of 1745 cm$^{-1}$ band (Fig. 6), which are generated by the stretching motions of C-H groups attached to C=C bonds and by the lipid nC = O vibration, respectively. The most important differences in LDL were in those of rats fed the olive oil diet, which produced the least amount of unsaturation. This result is consistent with the lipid composition of the olive oil diet, which is less unsaturated than the others. By contrast, no differences were observed in HDL.

In contrast, Figure 7 shows the relative intensities of the 2954 and 2926 cm$^{-1}$ bands, which depend on the acyl chain length and/or chain branching. The former is produced by the asymmetrical stretching vibration, $\nu_{\text{as}CH_3}$, and the second is generated by the $\nu_{\text{as}CH_2}$ mode with contribution from $\nu_{\text{s}CH_3}$ (18). Consequently, the 2954/2926 cm$^{-1}$ band ratio can be considered an indirect measurement of the amount of CH$_3$ groups relative to CH$_2$ groups; that is, an indirect measurement of the lipid acyl chain length or chain branching. In LDL, there were no marked differences among diet groups. In HDL,
controls contained hydrocarbon chains shorter than those of rats fed the experimental fats.

In conclusion, the effects of dietary fats (fish, sunflower and olive oils) on the secondary structures of HDL and LDL as well as on some structural properties of their lipids were studied by infrared spectroscopy. Concerning HDL structure, the most distinct spectroscopic behaviors were associated with the fish oil diet, which produced relatively high contents of random coil, probably due to the presence of the irregular geometries of PUFA. Other spectral features revealed similar structural behaviors in apoproteins from controls and rats fed the olive oil diet. Interestingly, this olive oil diet resulted in the lowest unsaturation level and acyl chains of HDL in rats fed the experimental diets seemed to be slightly longer than controls.

Regarding the protein secondary structure of LDL particles, olive oil produced the lowest proportion of \( \beta \)-structure with this being more abundant in LDL from rats fed the fish oil diet. In contrast, no differences were observed in acyl chain lengths or branching, and as in HDL, the lowest level of unsaturation was in rats fed the olive oil.

These data suggest that certain protein conformational changes and/or apoprotein composition differences produced by some diets may be the primary factors that determine the magnitude of binding between these lipoproteins and their corresponding receptors in cells.

LITERATURE CITED