Apolipoprotein C-III$_0$ lacks carbohydrate residues: use of mass spectrometry to study apolipoprotein structure

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Abstract Apolipoprotein C-III (apo C-III) is a 79 amino acid glycoprotein. The sugar moiety of apoC-III is attached to amino acid residue 74 and is thought to consist of 1 mole of galactose, 1 mole of N-acetyl-galactosamine, and either 0, 1, or 2 moles of sialic acid. This results in three isoproteins called C-III$_0$, C-III$_1$, and C-III$_2$ designated by the number of sialic acid residues. It has been assumed, although not experimentally tested, that apoC-III$_0$ lacks sialic acid residues but possesses the D-galactosyl-(1-3)-N-acetyl-D-galactosamine sugar backbone. To verify the structure of the three apoC-III isoproteins, we applied the method of ^{252}Cf plasma desorption mass spectrometry to measure the exact molecular weight (M_r) of each of the isoproteins. Our data confirmed the proposed structure of apoC-III$_1$ and apoC-III$_2$. However, the difference in mass between apoC-III$_1$ (9420.6, 9420.0, 9422.2 daltons) and apoC-III$_0$ (8763.9, 8764.9, 8765.5 daltons, respectively, in three subjects) suggests that the latter is missing not just sialic acid but the entire sugar moiety. This finding may have important implications for the metabolism of apoC-III. The accuracy and reproducibility of M_r measurements described in this paper suggest that this technique holds promise for the detection of apolipoprotein amino acid substitutions or modifications undetected by conventional techniques such as isoelectric focusing. —**Ito, Y., J. L. Breslow, and B. T. Chait.** Apolipoprotein C-III$_0$ lacks carbohydrate residues: use of mass spectrometry to study apolipoprotein structure. *J. Lipid Res.* 1989. **30:** 1781-1787.

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Apolipoprotein C-III (apoC-III) is the most abundant of the three apoC proteins and accounts for 50% of VLDL and 2% of HDL protein (1). The function of apoC-III is uncertain; however, in vitro apoC-III inhibits the activation of lipoprotein lipase (LPL) by apoC-II (2, 3) and reduces the uptake of intestinal lymph chylomicrons by the perfused liver (4, 5). Thus, apoC-III may play a regulatory role in the catabolism of triglyceride-rich lipoproteins.

ApoC-III is a 79 amino acid glycoprotein (6-10). The site of sugar attachment is the threonine at residue 74. The sugar moiety of apoC-III has been found to be 1 mol of galactose, 1 mol of N-acetyl-galactosamine, and 0, 1 or 2 mol of sialic acid (6, 11, 12). This results in three apoC-III isoproteins that are separable by isoelectric focusing. The isoproteins are designated by the number of sialic acid residues and are called C-III$_0$, C-III$_1$, and C-III$_2$, and comprise approximately 14%, 59%, and 27% of plasma apoC-III, respectively (13). Previously analysis of the trisaccharide structure of apoC-III$_1$ has revealed that one sialic acid is attached to the C3 position of galactose in the D-galactosyl-(1-3)-N-acetyl-D-galactosamine sugar moiety. In the tetrasaccharide structure of apoC-III$_2$, the additional sialic acid is attached to the C6 position of the N-acetyl-D-galactosamine (**Fig. 1**) (14). It has been assumed that apoC-III$_0$ simply lacks both of these sialic acids, but possesses the D-galactosyl-(1-3)-N-acetyl-D-galactosamine sugar backbone (6). However, to our knowledge, this has not been tested experimentally.

To verify the structures of the three apoC-III isoproteins, we applied the method of ^{252}Cf plasma desorption mass spectrometry (15) to measure the exact molecular weight (M_r) of each of the isoproteins. This technique has been used to measure the masses of proteins with an accuracy of 1 part in 5,000 to 10,000 daltons (16) or 2 parts in 1,000 to 35,000 daltons (17, 18). Our data confirmed the proposed structure of apoC-III$_1$ and apoC-III$_2$. However, the difference in mass between apoC-III$_1$ and apoC-III$_0$ suggests that the latter is missing not just sialic acid but the entire sugar moiety. We conclude that ^{252}Cf plasma desorption mass spectrometry is useful in identifying post-translational changes in proteins that would be difficult to detect by conventional analyses.

Abbreviations: apo, apolipoprotein; M_r, molecular weight; VLDL, very low density lipoprotein.

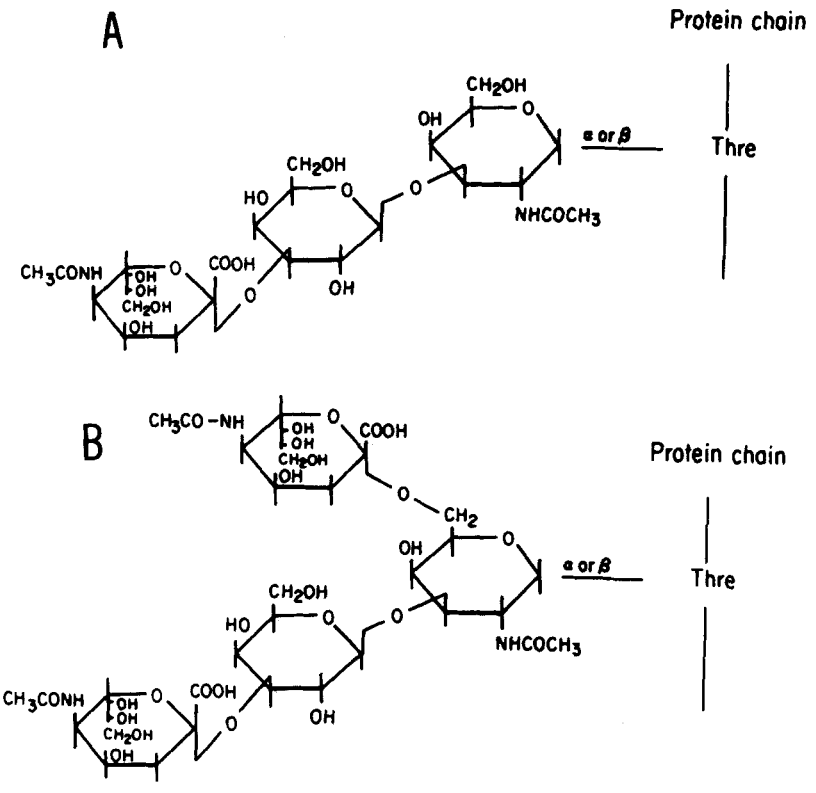

Fig. 1. Proposed structure of the sugar moiety of (A) apoC-III$_1$ and (B) apoC-III$_2$. Structure (B) reproduced from reference 14 with permission.

METHODS

The three apoC-III apolipoproteins were isolated using a slight modification of the method of Weisweiler, Friedel, and Schwandt (19). To this end, approximately 10–15 ml of plasma was obtained from each of three fasting hypertriglyceridemic subjects. At the time of blood sampling, these patients had triglyceride levels of 1091, 5967, and 356 mg/dl, and were designated subjects 1, 2, and 3, respectively. Chylomicron and VLDL fractions ($d < 1.006$ g/ml) were separated by preparative ultracentrifugation and delipidated with methanol–ether 1:3 (v/v) (20). ApoVLDL was dissolved in 10 mM Tris/base in 6 M urea buffer (pH 8.6) and spun in a Beckman Airfuge (100,000 *g*) (Beckman, Palo Alto, CA) for 10 min to remove insoluble proteins. Subsequently, 0.2 ml of this solution, containing up to 1.5 mg of apoVLDL protein, was applied to a Superose 12 gel filtration column (Pharmacia, Piscataway, NJ) equilibrated with the same buffer and run at a flow rate of 0.5 ml/min.

The largest peak, accounting for approximately 75% of total proteins, was applied to a Mono Q ion-exchange column (Pharmacia) and fractions were eluted with a linear gradient of 0–0.14 M NaCl in 10 mM Tris/base in 6 M urea buffer (pH 8.6), at a flow rat of 1.0 ml/min over 30 ml (**Fig. 2**). The fractions containing the apoC-III isoproteins were dialyzed extensively in 5 mM NH_4HCO_3 buffer and lyophilized.

Purity was examined by one-dimensional isoelectric focusing (Fig. 2). The gel was prepared in 6 M urea buffer containing 7.5% acrylamide, 0.2% N,N'-methylene-bis-acrylamide (BIS), and 2% ampholines (pH 4–6.5). It was prefocused for 1 h at 4°C at 110 V, and then approximately 50 μg of protein was applied. Focusing was carried out for 17 h at 4°C at 250 V. After focusing, the gel was fixed in trichloroacetic acid–sulfosalycilic acid, stained with Coomassie Blue (0.1%) in methanol–water–acetic acid 4.5:4.5:1.0, and then destained.

The mass spectrometric measurements were performed on the ^{252}Cf plasma desorption time-of-flight mass spectrometer constructed at the Rockfeller University and previously described (21, 22). Samples were prepared by adsorption on a thin nitrocellulose film of 1–2 nmol of protein from a solution of 0.1 nmol protein/μl of 0.1% TFA, 0.2 mM EDTA, 2 mM 18-Crown-6, and 2 mM reduced glutathione (23). After adsorption, the nitrocellulose film was washed thoroughly with 0.1% trifluoroacetic acid to remove residual low molecular weight impurities and then was placed into the vacuum lock of the mass spectrometer to be thoroughly dried by evacuation. The resulting layer of protein molecules bound to the nitrocellulose film was then inserted into

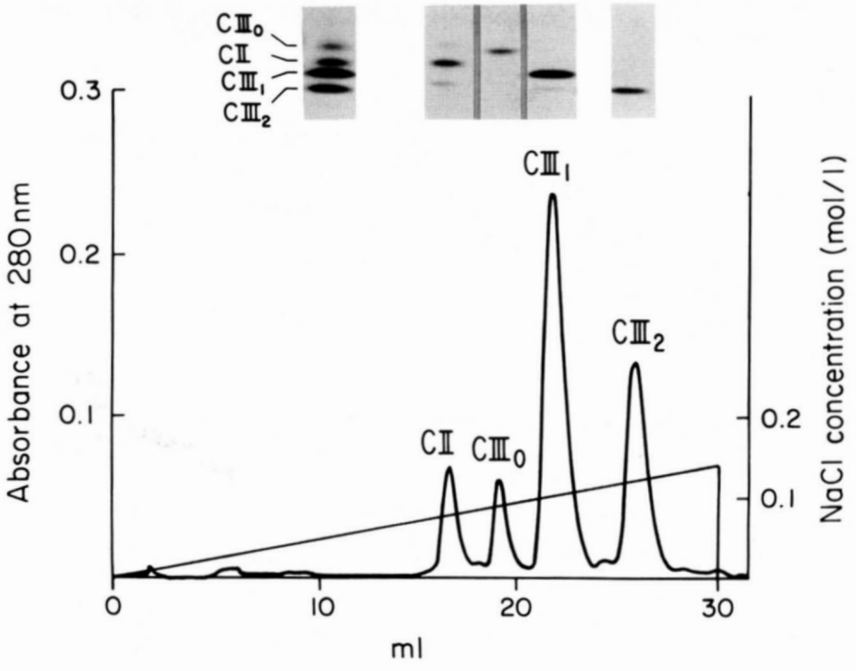

Fig. 2. Ion exchange chromatogram of apoC proteins and isoelectric focusing patterns before and after separation.

the mass spectrometer for analysis.

The spectra obtained from the apoC-III samples exhibited both singly and doubly protonated molecule ion peaks. These are designated $(M_S + H)^+$ and $(M_S + 2H)^{2+}$, respectively. The subscript s denotes the number of sialic acid residues on the protein. Data accumulation times ranged between 3 and 16 h. The accuracy of the mass determinations was, in general, better than 200 ppm (0.02%) for those samples that yielded a clearly discernable, sharp, doubly protonated molecule ion peak. The accuracy of the masses determined from the singly protonated molecule ion peaks was considerably lower (0.2%) since they were weaker and less well-defined. Thus the masses were determined using the doubly protonated molecule ion peaks.

RESULTS

The elution profile of the VLDL apoC proteins from the Mono Q ion-exchange column is shown in Fig. 2. The isoelectric focusing pattern of the proteins before separation and from each peak are also shown. The apoC-III isoproteins separated in this manner were used for M_r measurements by ^{252}Cf plasma desorption mass spectrometry. **Fig. 3** shows the mass spectra obtained with each of the three apoC-III isoproteins from subject 1. The mass spectrum of apoC-III_2 (Fig. 3A) yields a broad peak in the $(M_2 + 2H)^{2+}$ region. There is no well defined sharp component indicating that the ionized molecule has undergone extensive unimolecular fragmentation during the mass analysis (24, 25). Since virtually none of the $(M_2 + 2H)^{2+}$ ions appear to survive the mass analysis intact, these data can only be used to estimate a lower limit for the M_r of apoC-III_2. This lower limit corresponds to the mass of the highest minor peak (indicated by the arrow in Fig. 3A), and is 9700.2 daltons. This is 11.9 daltons smaller than the calculated M_r of 9712.1 daltons, based upon the reported apoC-III primary amino acid sequence plus one molecule each of galactose, and N-acetyl-galactosamine and two molecules of sialic acid (**Table 1**).

The mass spectrum of apoC-III_1 (Fig. 3B) yields a more defined peak in the $(M_1 + 2H)^{2+}$ region. This corresponds to an M_r of 9420.6 daltons. This measurement is only 0.2 daltons smaller than the calculated M_r of apoC-III_1 of 9420.8 daltons. The broad shoulder observed on the low mass side of the sharp $(M_1 + 2H)^{2+}$ peak is consistent with the loss of the sialic acid moiety from ionized apoC-III_1 during mass analysis.

The mass spectrum of apoC-III_1 (Fig. 3C) yields a sharp peak in the $(M_0 + 2H)^{2+}$ region. This corresponds to an M_r of 8763.9 daltons. This is 656.7 mass units smaller than the measured M_r of apoC-III_1. This difference in M_r is virtually identical to the 656.6 daltons that would correspond to the replacement of the entire carbohydrate moiety of apoC-III_1, galactose, N-acetyl galactosamine, and sialic acid, by hydrogen. This finding strongly suggests that apoC-III_0 lacks any carbohydrate. In fact, the measured M_r of apoC-III_0 is also very close to the calculated M_r of 8764.2 daltons based upon 79 amino acids alone with no additional sugar molecules. The measurements of apoC-III_0 and apoC-

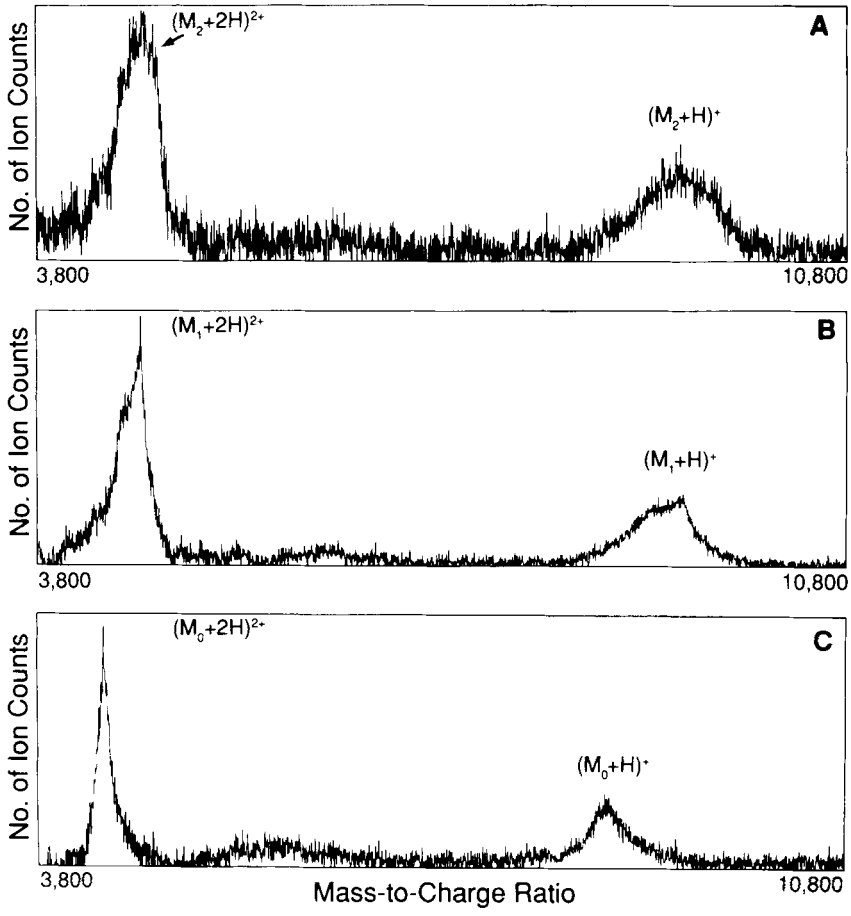

Fig. 3. Partial ^{252}Cf plasma desorption mass spectra of (A) apoC-III_2, (B) apoC-III_1, and (C) apoC-III_0. The singly and doubly protonated intact apoC-III molecules are designated $(M_S + H)^+$ and $(M_S + 2H)^{2+}$, respectively, where the subscript s denotes the number of sialic acid residues. The molecular weights of the apoC-III proteins obtained from these spectra are given in Table 1.

III_1 in two other subjects confirmed this finding (Table 1). To evaluate the reproducibility of the measurement, the same specimen of apoC-III_0 from subject 1 was prepared independently for mass spectrometry and two more runs gave M_rs of 8769.0 (4.8 mass units larger than the calculated M_r) and 8765.4 (1.2 mass units larger than the calculated M_r).

An independent measure of the M_rs was obtained using the singly protonated molecule ion peaks. The measured molecular weights for C-III_0 from subjects 1, 2, and 3 were

TABLE 1. Calculated and measured molecular weight of apoC-III (daltons)

	Measured M_r[b]		
Calculated M_r[a]	Subject 1	Subject 2	Subject 3
ApoC-III_0 8764.2	8763.9 (- 0.3)	8764.9 (+ 0.7)	8765.5 (+ 1.3)
ApoC-III_1 9420.8	9420.6 (- 0.2)	9420.0 (- 0.8)	9422.2 (+ 1.4)
ApoC-III_2 9712.1	≥ 9700.2 (- 11.9)		

[a]Average M_r was calculated using the eight most abundant isotopic components. The M_r of apoC-III_0 was calculated based upon the reported 79 amino acids without carbohydrate residues, apoC-III_1 with a galactose-N-acetyl galactosamine and one sialic acid, and apoC-III_2 with a galactose-N-acetyl galactosamine and two sialic acids. The measurement of apoC-III_0 in subject 1 was repeated two more times independently, showing M_rs of 8769.0 (+ 4.8) and 8765.4 (+ 1.2) daltons, respectively. The apoC-III_2 peak was broad, resulting from extensive fragmentation of the carbohydrate residues (see Fig. 3A). Therefore, the measurements were not done on subjects 2 and 3.

[b]The difference between the measured and calculated M_r is in parentheses.

respectively 8756 (− 8), 8764 (0), and 8773 (+ 9). The differences between the measured and calculated M_rs are given in parentheses and are within the large experimental errors (± 20) expected from the weak, poorly defined singly protonated peaks.

DISCUSSION

By measuring the M_r of the apoC-III isoprotein, with ^{252}Cf plasma desorption mass spectrometry, we have shown that apoC-III_0 lacks not only any sialic acid residue but also the D-galactose-N-acetyl-D-galactosamine sugar backbone, which is present in apoC-III_1 and apoC-III_2. We doubt that our finding is an artifact of the preparation procedure or mass spectrometry, since apoC-III_1 and C-III_2 were treated identically to apoC-III_0 and retain the O-glycosidic bond.

The principal site of apoC-III synthesis is the liver (26–28). It had been assumed that this apolipoprotein was secreted as apoC-III_2 and neuraminidase activities in plasma produced apoC-III_1 and apoC-III_0. Now this view must be modified. If apoC-III_2 and apoC-III_1 are the precursors of apoC-III_0, then it would require both neuraminidase as well as another type of glycosidase to entirely remove the carbohydrate moiety and produce apoC-III_0. Alternatively, apoC-III_2 might be the precursor of apoC-III_1, whereas apoC-III_0 may be secreted as such from the liver. Against the latter hypothesis is a recent study that showed that human apoC-III expressed in the mouse fibroblast cell line C127 is secreted exclusively in the disialated form (29).

Our findings might also have implications for the mechanism whereby apoC-III is cleared from plasma. It is postulated that prior to removal sialoglycoproteins are desialated revealing a penultimate galactose (30). This is then recognized by the abundant asialoglycoprotein receptor on the surface of hepatocytes and receptor-mediated endocytosis occurs. In the case of apoC-III, it appears that in none of the three isoforms is the penultimate galactose revealed. This implies that another clearance mechanism may exist for apoC-III independent of the asialoglycoprotein receptor. It is of interest that apoE also exists in plasma in sialated and nonsialated forms (31, 32), and the latter also lacks the entire sugar backbone (33). Therefore, this phenomenon appears to have similar implications for apoE secretion, processing, and removal as noted above for apoC-III.

It is not at all certain that the carbohydrate moiety of apoC-III has any physiological function. In one study, apoC-III_1 and apoC-III_0 were equal in their ability to inhibit hepatic uptake of triglyceride-rich lipoproteins (5). In another study, members of one Japanese family were found to have a point mutation of $74_{Thr \rightarrow Ala}$ (34, 35). This prevented the formation of the O-glycosidic linkage and caused all plasma apoC-III to be in the apoC-III_0 isoprotein form. This reportedly did not affect serum lipoprotein profiles or atherosclerosis susceptibility.

There is increasing interest in the detection of structural variations in apolipoproteins that might cause lipoprotein abnormalities. Thus far the principal method has been isoelectric focusing. Several amino acid substitutions, especially in apoA-I and apoE, have been found using this approach (36). Unfortunately, isoelectric focusing only detects mutations affecting charge. Other mutations not changing the net charge of the protein are left undetected. In selected cases, direct amino acid or nucleic acid sequencing has revealed significant mutations (36), but this approach is labor-intensive, time-consuming, and not practical as a screening technique.

Mass spectrometric analysis may overcome some problems of the conventional approaches. In 1986, Jabs et al. (37) identified a possible structural variation in apoA-I by isoelectric focusing and reported the use of time-of-flight secondary ion mass spectrometry to identify the specific amino acid substitution. In their study, they digested purified apoA-I with trypsin and separated the resultant peptides by HPLC. The aberrant peptide was subjected to mass spectrometry. This technique allows determination of peptide masses up to 3000 daltons, and the peptide in question was within this size range. In the current study, we used ^{252}Cf plasma desorption mass spectrometry. This differs from the method of Jabs et al. (37) in that the present technique allows for the direct examination of a higher M_r range. In fact, we show satisfactory accuracy and reproducibility of measurements with M_rs up to 9400 daltons. In our study of the apoC-III isoproteins, we have shown that our method can be used to study post-translational protein modifications, not easily studied by conventional techniques. Although not now reported, the method can also be used to detect most amino acid substitutions.

There are limitations to the present technique. The protein must be purified prior to analysis, and it takes 12 h in a typical run to derive the mass spectrum of a single specimen. Finally, at present the upper limit of the M_r of proteins that can be studied with acceptable accuracy appears to be 10,000 to 20,000 daltons. Larger molecules require enzymatic digestion or chemical cleavage and peptide purification prior to mass spectrometry. The approach described here does not replace conventional techniques, but is expected to play an important complementary role in the detection of post-translational modifications and primary sequence variations of proteins.

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REFERENCES

1. Herbert, P. N., G. Assmann, A. M. Gotto, Jr., and D. S. Fredrickson. 1982. Familial lipoprotein deficiency: abetalipoproteinemia, hypobetalipoproteinemia, and Tangier disease. *In* The Metabolic Basis of Inherited Disease. J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, J. L. Goldstein, and M. S. Brown, editors. McGraw-Hill, New York. 589- 651.
2. Brown, V. W., and M. L. Baginsky. 1972. Inhibition of lipoprotein lipase by an apoprotein of human very low density lipoprotein. *Biochem. Biophys. Res. Commun.* **46:** 375-382.
3. Krauss, R. M., P. M. Herbert, R. I. Levy, and D. S. Fredrickson. 1973. Further observations on the activation and inhibition of lipoprotein lipase by apolipoproteins. *Circ. Res.* **33:** 403-411.
4. Shelburne, F., J. Hanks, W. Meyers, and S. Quarfordt. 1980. Effect of apoproteins on hepatic uptake of triglyceride emulsions in the rat. *J. Clin. Invest.* **65:** 652-658.
5. Windler, E., Y-S. Chao, and R. J. Havel. 1980. Regulation of the hepatic uptake of triglyceride-rich lipoproteins in the rat: opposing effects of homologous apolipoprotein E and individual C apoproteins. *J. Biol. Chem.* **255:** 8303-8307.
6. Brewer, H. B., Jr., R. Shulman, P. Herbert, R. Ronan, and K. Wehrly. 1974. The complete amino acid sequence of alanine apolipoprotein (apoC-III), an apolipoprotein from human plasma very low density lipoprotein. *J. Biol. Chem.* **249:** 4975-4984.
7. Hospattankar, A. V., H. B. Brewer, Jr., R. Ronan, and T. Fairwell. 1986. Amino acid sequence of human plasma apolipoprotein C-III from normolipidemic subjects. *FEBS Lett.* **197:** 67-73.
8. Sharpe, C. R., A. Sidoli, C. S. Shelley, M. A. Lucero, C. C. Shoulders, and F. E. Baralle. 1984. Human apolipoproteins AI, AII, CII and CIII. cDNA sequences and mRNA abundance. *Nucleic Acids Res.* **12:** 3917-3932.
9. Protter, A. A., B. Levy-Wilson, J. Miller, G. Bencen, T. White, and J. J. Seilhamer. 1984. Isolation and sequence analysis of the human apolipoprotein CIII gene and the intergenic region between the apoAI and apoCIII genes. *DNA.* **3:** 449-456.
10. Karathanasis, S. K., V. I. Zannis, and J. L. Breslow. 1985. Isolation and characterization of cDNA clones corresponding to two different human apoC-III alleles. *J. Lipid Res.* **26:** 451-456.
11. Brown, W. V., R. I. Levy, and D. S. Fredrickson. 1970. Further characterization of apolipoproteins from the human plasma very low density lipoproteins. *J. Biol. Chem.* **245:** 6588-6594.
12. Albers, J. J., and A. M. Scanu. 1971. Isoelectric fractionation and characterization of polypeptides from human serum very low density lipoproteins. *Biochim. Biophys. Acta.* **236:** 29-37.
13. Kashyap, M. L., L. S. Srivastava, B. A. Hynd, and P. S. Gartside, and G. Perisutti. 1981. Quantitation of human apolipoprotein C-III and its subspecies by radioimmunoassay and analytical isoelectric focusing: abnormal plasma triglyceride-rich lipoprotein apolipoprotein C-III subspecie concentrations in hypertriglyceridemia. *J. Lipid Res.* **22:** 800-810.
14. Vaith, P., G. Assmann, and G. Uhlenbruck. 1978. Characterization of the oligosaccharide side chain of apolipoprotein C-III from human plasma very low density lipoproteins. *Biochim. Biophys. Acta.* **541:** 234-240.
15. MacFarlane, R. D., and D. F. Torgerson. 1976. Californium-252 plasma desorption mass spectrometry. *Science.* **191:** 920-925.
16. Grace, L. I., B. T. Chait, and F. H. Field. 1987. A system for collecting high-resolution time-of-flight mass spectrometric data. *Biomed. Environ. Mass Spectrom.* **14:** 295-299
17. Sunquist, B., I. Kamensky, P. Hakansson, J. Kjellberg, M. Salehpour, S. Widdiyasekera, J. Fohlman, P. A. Peterson, and P. Roepstorff. 1984. Californium-252 plasma desorption time-of-flight mass spectrometry of proteins. *Biomed. Environ. Mass Spectrom.* **11:** 242-257.
18. Craigh, A. G., A. Eugstrom, H. Bennich, and I. Kamensky. 1987. Enhancement of ion yields in plasma desorption mass spectrometry. Presented at the 35th ASMS Conference on Mass Spectrometer and Allied Topics, May 24-29, Denver Colorado. Proceedings: 528-529.
19. Weisweiler, P., C. Friedel, and P. Schwandt. 1986. Isolation and quantification of apolipoproteins C-I, C-II, and C-III_{0-2} in very low-density lipoproteins by "high-performance" anion-exchange chromatography. *Clin. Chem.* **32:** 992-994.
20. Herbert, P. N., L. L. Bausserman, L. O. Henderson, R. J. Heinen, M. J. LaPiana, E. C. Church, and R. S. Shulman. 1978. Apolipoprotein quantitation. *In* The Lipoprotein Molecule. H. Peeters, editor. Plenum Publishing Corporation, New York. 35-56.
21. Chait, B. T., W. C. Agosta, and F. H. Field. 1981. Fission fragment ionization (^{252}Cf) mass spectrometry: positive and negative spectra and decomposition mechanisms for seven compounds. *Int. J. Mass Spectrom./Ion Proc.* **39:** 339-366.
22. Chait, B. T., B. F. Gisin, and F. H. Field. 1982. Fission fragment ionization mass spectrometry of alamethicin I. *J. Am. Chem. Soc.* **104:** 5157-5162.
23. Chait, B. T., and F. H. Field. 1986. A rapid, sensitive mass spectrometric method for investigating microscale chemical reactions of surface adsorbed peptides and proteins. *Biochem. Biophys. Res. Commun.* **134:** 420-426.
24. Chait, B. T., and F. H. Field. 1981. Fission fragment ionization mass spectrometry: metastable decompositions. *Int. J. Mass Spectrom./Ion Proc.* **41:** 17-29.
25. Chait, B. T. 1983. Prompt and metastable decompositions in ^{252}Cf fission fragment ionization mass spectrometry. *Int. J. Mass Spectrom./Ion Proc.* **53:** 227-242.
26. Zannis, V. I., S. F. Cole, C. Jackson, D. M. Kurnit, and S. K. Karathanasis. 1985. Distribution of apolipoprotein A-I, C-II, C-III, and E mRNA in fetal human tissues. Time-dependent induction of apolipoprotein E mRNA by cultures of human monocyte-macrophages. *Biochemistry.* **24:** 4450-4455.
27. Haddad, I. A., J. M. Ordovas, T. Fitzpatrick, and S. K. Karathanasis. 1986. Linkage, evolution, and expression of the rat apolipoprotein A-I, C-III, and A-IV genes. *J. Biol. Chem.* **261:** 13268-13277.
28. Wu, A. L., and H. G. Windmueller. 1979. Relative contributions by liver and intestine to individual plasma apolipoproteins in the rat. *J. Biol. Chem.* **254:** 7316-7322.
29. Roghani, A., and V. I. Zannis. 1988. Mutagenesis of the glycosylation site of human apoCIII: O-linked glycosylation is not required for apoCIII secretion and lipid binding. *J. Biol. Chem.* **263:** 17925-17932.
30. Ashwell, G., and J. Hartford. 1982. Carbohydrate-specific receptors of the liver. *Annu. Rev. Biochem.* **51:** 531-554.
31. Jain, R. S., and S. H. Quarfordt. 1979. The carbohydrate

content of apolipoprotein E from human very low density lipoproteins. *Life Sci.* **25:** 1315–1323.
32. Zannis, V. I., and J. L. Breslow. 1981. Human very low density lipoprotein apolipoprotein E isoprotein polymorphism is explained by genetic variation and posttranslational modification. *Biochemistry.* **20:** 1033–1041.
33. Rall, S. C., Jr., M-E. Wernett-Hammond, S. J. Lauer, A. Corsini, D. Walker, and J. M. Taylor. 1989. Glycosylation of human apolipoprotein E: the carbohydrate attachment site is threonine 194. *J. Biol. Chem.* **264:** 9094–9101.
34. Maeda, H., H. Uzawa, and R. Kamei. 1981. Unusual familial lipoprotein C-III associated with apolipoprotein C-III-O preponderance. *Biochim. Biophys. Acta.* **665:** 578–585.
35. Maeda, H., R. Hashimoto, T. Ogura, S. Hiraga, and H. Uzawa. 1987. Molecular cloning of a human apoC-III variant: Thr 74→Ala 74 mutation prevents O-glycosylation. *J. Lipid Res.* **28:** 1405–1409.
36. Breslow, J. L. 1988. Apolipoprotein genetic variation and human disease. *Physiol. Rev.* **68:** 85–132.
37. Jabs, H. U., G. Assman, D. Greifendorf, and A. Benninghoven. 1986. High performance liquid chromatography and time-of-flight secondary ion mass spectrometry: a new dimension in structural analysis of apolipoproteins. *J. Lipid Res.* **27:** 613–621.