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Identification and quantitation of unsaturated fatty acid isomers by electrospray ionization tandem mass spectrometry: A shotgun lipidomics approach

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Abstract

Identification and quantification of unsaturated fatty acid (FA) isomers in a biological system are significant in the study of lipid metabolism and catabolism, membrane biophysics, and pathogenesis of diseases, but are challenging in lipidomics. We developed a novel approach for identification and quantitation of unsaturated FA isomers by exploiting two facts: (1) unsaturated FA anions yield fragment ion(s) from loss of CO₂ or H₂O from the anions upon collision-induced dissociation; and (2) the fragment ions yielded from discrete FA isomers have distinct profiles of the fragment ion intensity vs. collision conditions. These distinct profiles likely result from the differential interactions of the negative charge of the fragment ion with the electron clouds of the double bonds due to their different distances in discrete FA isomers. The novel approach was also extended to analyze the double bond isomers of FA chains present in phospholipids by multi-stage tandem mass spectrometry. Collectively, we developed a new approach for identification and quantification of the double bond isomers of endogenous FA species or FA chains present in intact phospholipid species. We believe that this approach should further advance the lipidomic power for identification of the biochemical mechanisms underlying metabolic diseases.

Keywords

Lipidomics; mass spectrometry; shotgun lipidomics; unsaturated fatty acid isomers

Fatty acids (FAs) play many essential roles in a biological system including providing energy sources, serving as signaling molecules, and being the major structural components in complex lipids of cellular membranes¹. FAs present in mammalian systems vary in chain length, the number of double bonds, as well as the location of these double bonds in the acyl chains. The different locations of the double bonds form the isomers of an unsaturated FA for which the chain length and the number of double bonds are identical. The composition of unsaturated FA isomers in a biological system is important in multiple folds. It reflects the dietary history and FA biosynthesis while the temporal changes of this composition display the metabolic rate of the biological system¹. Moreover, the FA signatures in phospholipids including the isomeric composition determines the membrane fluidity, which could subsequently affect the membrane protein functions^{2, 3}. Finally, the altered composition of

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

these FA isomers could also significantly influence the physiological responses and could be associated with the pathogenesis of diseases^{2, 4, 5}.

Traditionally, FA isomers are analyzed by GC and HPLC⁶. The need for derivatization, limited resolution, the time consuming, and large amount of samples are some of the caveats of those methods. Recently, mass spectrometry (MS)-based methods have been rapidly developed. For example, the method based on the fragmentation pattern analysis after high energy collision induced dissociation (CID) is one of the early developed MS methods for the direct analysis of FA isomers^{7, 8}. Hsu and Turk have explored low energy CID for identification of the double bond location in FAs^{9, 10}. However, this method requires sophisticated skills on MS analyses of lithiated FAs in positive-ion mode and interpreting the complex spectra, which prevents its general usage. Ozonolysis is the other developed method that is associated with MS¹¹⁻¹⁴. However, either a special instrumental setup or skillful data interpretation are needed in pursuing these methods. Moreover, none of these MS methods can accurately quantify the composition of FA isomers following identification. Accordingly, identification and quantification of FA isomers remain challenging in lipidomics.

The location of double bonds within a polyunsaturated FA in the majority of those found in mammals is inter-related and always interrupted between each other with a methylene group. For instance, for a FA containing 20 carbon atoms and 4 double bonds, the distribution of the double bonds is either in $\Delta^{5,6}$, $\Delta^{8,9}$, $\Delta^{11,12}$, and $\Delta^{14,15}$ positions (i.e., 5,8,11,14-eicosatetraenoic acid, n-6 20:4 FA, herein m:n was used to denote the FA or FA chain containing m of carbon atoms and n of double bonds) or in $\Delta^{8,9}$, $\Delta^{11,12}$, $\Delta^{14,15}$, and $\Delta^{17,18}$ positions (i.e., 8,11,14,17-eicosatetraenoic acid, n-3 20:4 FA). Accordingly, FA double bond isomers can be distinguished by the location of the first double bond. Although exceptions are present (e.g., conjugated linoleic acids), they only represent a minimal percentage¹⁵.

Herein, we report an alternative approach for both identification and quantification of FA isomers by using multi-dimensional (MD) MS-based shotgun lipidomics. In this method, the FA isomers was identified based on the diagnostic intensity distribution of their specific fragment ions upon varied CID conditions (e.g., collision energy or collision gas pressure, which are the variables in MDMS¹⁶). The composition of the identified FA isomers was then quantified by using calibration curves externally established using authentic FA isomers. The mechanism underlying the presence of distinct fragment ion intensity distribution over CID conditions in each FA isomer is likely due to the differential interactions between the negative charge of the fragment ion and the electron clouds of the double bonds due to the different distance between the charge site and the first double bond in each FA isomer. This novel method was applied for identification and quantitation of free FA isomers present in the mouse plasma samples. Moreover, we extended this approach for the identification and quantification of the double bond isomers of FA chains present in phospholipids of biological samples by multi-stage tandem MS (MS³). Collectively, the new approach for identification and quantification of the double bond isomers of endogenous FAs is a new addition to our MDMS-based shotgun lipidomics and should further advance the lipidomic power for identification of the underlying biochemical mechanisms of metabolic diseases.

MATERIALS AND METHODS

Materials

All FAs except 8,11,14,17-eicosatetraenoic acid (n-3 20:4 FA) were purchased from NuChek PREP, INC (Elysian, MN). The n-3 20:4 FA was purchased from Cayman Chemical (Ann Arbor, MI). All the FAs were used without further purification.

Preparation of FA Mixtures and Lipid Extracts from Biological Samples

The stock solutions of the FAs were prepared in $\text{CHCl}_3/\text{MeOH}$ (1:1, v/v). The concentration of each stock solution was quantified individually by ratiometric comparison with the internal standard $\text{d}_4\text{-16:0}$ FA by ESI/MS. The mixtures of each isomeric pair of FAs were prepared from individual FA stock solutions. The serial isomer ratios in the mixtures of each isomeric pair were obtained by fixing the concentration of one isomer while varying the other. Lipid extracts from biological samples were described in detail in the Supporting Information.

MS Analysis of Unsaturated FAs

MS analyses of FAs in the negative ion mode were performed by using a TSQ Quantum Ultra mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an automated nanospray apparatus (Advion Bioscience, Ithaca, NY) as previously described^{17, 18}. MS³ analyses of phospholipids were performed on an LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA) as previously described¹⁹.

Individual FA stock solutions were diluted to 2 pmol/ μL in $\text{CHCl}_3/\text{MeOH}/\text{isopropanol}$ (1:2:4, v/v/v). Each mixture of paired FA isomers was properly diluted to a total concentration of less than 50 pmol/ μL . Product ion scans of a diluted lipid solution were performed either by fixing collision gas pressure at 1 mT while varying collision energies from 2 to 36 eV at a step of 2 eV or by fixing collision energy while varying collision gas pressures from 0 to 3 mT at a step of 0.2 mT.

Procedures for Identification and Quantitation of FA Isomers

Detailed procedures for analysis of FA isomers were provided in Supporting Information. Briefly, a product-ion ESI mass spectrum was acquired under indicated conditions in the profile mode. Normalized absolute intensities or relative intensities of the fragment ions resulting from either the loss of CO_2 or H_2O from the FA anion were determined as following. The normalized absolute intensity of a fragment ion was determined by normalizing the absolute counts of the fragment ion in the product ion mass spectrum to the absolute counts of the molecular ion (i.e., FA anion) in the full MS scan. The relative intensity of a fragment ion was determined by normalizing the absolute counts of the fragment ion to the absolute counts of the FA anion in the product ion mass spectrum. The relative intensities were only determined from the product-ion spectra acquired under weak CID conditions where the fragment ions resulting from the loss of either CO_2 or H_2O from the FA anion were the major fragments.

RESULTS AND DISCUSSION

Product Ion MS Analysis of FAs in the Negative Ion Mode

It is well recognized that a product ion mass spectrum of a polyunsaturated FA such as 22:6 FA acquired in the negative ion mode displays a characteristic fragment ion $[\text{M}-\text{H}-44]^-$ corresponding to the facile neutral loss of CO_2 from the molecular ion^{19, 20}. When we performed a 2D MS analysis of 22:6 FA in product ion mode by varying collision energy,

we found that the intensity of this specific fragment ion varied with collision energy (Figure 1A) and displayed a Gaussian-like distribution (Figure 1B). MS analyses of other unsaturated FAs demonstrated differential Gaussian distributions that varied with chain length and double bond number of individual FAs, and in particular, varied with the location of double bonds in the FA isomers (Figure S1 in the Supporting Information).

Identification of FA Isomers

We examined essentially all the unsaturated FAs present in the mammalian FA metabolic pathway including 22:6, 22:5, 22:4, 20:5, 20:4, 20:3, 20:2, 20:1, 18:3, 18:2 (and its conjugated isomers), 18:1, and 16:1 FAs. By 2D MS analyses of these FA isomers, we found that a significant fragment ion corresponding to the neutral loss of H₂O was also present in the product-ion mass spectra of FAs. Specifically, 2D MS analyses of FAs containing three double bonds (e.g., 18:3 FA) showed not only intense fragment ions corresponding to the neutral loss of CO₂, but also abundant fragment ions corresponding to the neutral loss of H₂O from the FA anions (Figure 2). The intensity distribution of the fragment ion resulting from the loss of H₂O was Gaussian-like (data not shown), similar to that from the loss of CO₂ (Figure 1B and Figure S1). The loss of H₂O was also present in the FAs containing more than three double bonds (e.g., 22:6, 22:5 and 20:4 FAs), but much less intense than the loss of CO₂. In contrast, the FAs containing less than three double bonds (e.g., 20:2, 18:2, 18:1 and 16:1 FAs) showed a much more intense fragment ion resulting from the loss of H₂O than that from the loss of CO₂ (Figure S2). This fragment ion resulting from the loss of H₂O is useful for refining the identification and quantification obtained from the analysis of the fragment ion from the loss of CO₂ for FAs containing three or more double bonds, and particularly useful for compensating the low abundance fragment ion resulting from the loss of CO₂ for FAs containing less than three double bonds. Accordingly, the characteristic Gaussian-like distributions of intensity vs. collision energy of the specific fragment ions resulting from the loss of CO₂ or H₂O from FA anions are applicable to the identification of almost all the FA isomers in the mammalian FA metabolic pathway.

Quantification of Unsaturated FA Isomers

We then determined whether the characteristic fragment ion intensity distributions are differential in the mixtures of FA isomers at different compositions and whether a linear relationship between the fragment ion intensity distribution and FA isomeric composition is present. We employed two approaches to establish the linearity to serve as the standard curves for quantitation of FA isomers in a mixture.

In the first approach, we established the linearity by determining the normalized absolute intensities of the specific fragment ion at fixed, pre-determined collision energy for FA isomer mixtures at differential compositions (Figure 3A). This approach was simple and quick because only the product ion mass spectra of individual mixtures under fixed collision energy were acquired. However, this linearity was applicable to selected collision energies and selection of the optimal collision energy was critical. Moreover, it might be necessary to establish the standard curve every time in parallel to the analysis of real samples. This approach is therefore recommended for a quick screening to uncover significant FA isomer composition difference in a big batch of samples.

In the second approach, we established the linearity by determining the relative intensity of the specific fragment ion at varied collision energies for each mixture (Figure 3B). This linearity was only valid in the low collision energy region within which the loss of either CO₂ or H₂O is predominant. We found that the slope of this linearity was exponential to the FA isomer composition in individual mixtures (i.e., a linear relationship of the $\ln(\text{slope})$ vs.

the FA isomer composition (Figure 3C)). In contrast to the first standard curve (Figure 3A), the second standard curve (Figure 3C) could be established separately from the analysis of samples. In addition, pre-determination of the single-point, critical collision energy was not demanded. The second approach is therefore more variation-resistant than the first one and recommended for investigations desiring greater accuracy.

We found that both linear standard curves have the correlation coefficients (r^2) of better than 0.99 for a majority of the polyunsaturated FAs examined due to the presence of high abundance specific fragment ions yielded from these FAs while the correlation coefficients for the FAs containing only one or two double bonds might be relatively reduced. Moreover, the method is sufficiently sensitive for the determination of a molar composition difference of 10% between the mixtures (Figure 3), indicating that the accuracy of the method for quantification of polyunsaturated FA isomers is over 95 mol%.

Mechanisms Responsible for the Differential Fragment Intensity Distributions of FA Isomers

Since the breakage of the FA carbonyl carbon and α carbon bond is hardly affected directly by the FA chain length or unsaturation, the intensity of the yielded fragment ion during CID depends on its stability. Therefore, any factor that is able to stabilize the resultant fragment ions can enhance their intensities. To understand the mechanisms underlying the observed differential fragment intensity distributions in FA isomers, we extensively examined the effects of the location and the number of the double bonds, and the length of the acyl chain on stabilizing the fragment ions.

We found that the closer was the FA first double bond to the carbonyl carbon, the more abundant was the resultant fragment ion (Figure 4 and Figure S1). For example, the n-6 22:5 FA has its first double bond closer to the carbonyl carbon, and then had more intense fragment ion than its n-3 counterpart (Figure 4A). Other examples included 20:3 FA isomers (n-9 > n-6 > n-3) and 20:4 FA isomers (n-6 > n-3) (Figure 4B). This rule leads to establishing our approach for identification and quantification of unsaturated FA isomers. For species containing an identical position of the first double bond, we found that the more double bonds did the FA contain, the more intense was the resultant fragment ion (Figure 4). For example, the fragment ion resulting from the n-3 22:6 FA was more intense than that from the n-6 22:5 FA (both of which contain 22 carbon atoms with first double bond at the C4 position) (closed circles in Figure 4A). The same trend was also observed for the others (e.g., n-3 22:5 FA > n-6 22:4 FA (closed squares in Figure 4A); n-3 20:5 FA > n-6 20:4 FA > n-9 20:3 FA > n-15 20:1 FA (open squares in Figure 4B); etc). When the FAs contain an identical position of the first double bond and an identical number of double bonds, we found that the shorter was the FA chain, the more intense was the resultant fragment ion (Figure 4C).

These observations indicate that both the double bonds (location and number) and the acyl chain length of FAs affect the stabilization of the resultant fragment ions and subsequently their intensities. It is well known that conjugation of a charge with double bond(s) can stabilize the charge. Although direct conjugation of the double bonds with the charge in fragment ions was not present in any examined FA cases, we believe that distant interactions through spatial domain (i.e., charge-dipole interactions) between the charge and the double bond(s) (which could be called "remote conjugation") play an important role in stabilizing the fragment ions. Accordingly, if the closer is the first double bond to the charge and the more the double bonds are present in a FA chain, the stronger is the interaction between the charge and the double bonds and the more stable is the resultant fragment ion. Moreover, this interaction is more favored in a shorter FA chain due to its higher bending capability.

We also determined the effects of collision gas pressure on the fragment ion intensity distribution to further obtain mechanistic insights into the collision condition-associated intensity distribution of the fragment ions. We found that the linear relationship of the fragment ion intensity with collision gas pressure at a fixed collision energy was essentially identical to that with collision energy at a fixed collision gas pressure, for both individual FA isomers and their mixtures (Figure S3). This observation validated our approach for FA isomer identification and quantitation utilizing the established linear relationship (standard curves). These results also suggested that the fragment ion intensities essentially reflect the direct interaction between the charge of the fragment ion and the acyl chain (particularly the double bonds) under mild collision conditions where the initial fragmentation due to the loss of CO₂ or H₂O is predominant and the sequential chain fragmentation is minimal.

Applications of the Method for the Analysis of FAs in the Biological Samples

To demonstrate the application of this new approach for identification and quantification of FA isomers as a proof of concept, we determined the existence of potential FA isomers in the plasma samples of mice fed with normal chow, a high fat diet, or after 12 h fasting. The free FA profiles of these plasma samples were significantly different (Figure 5). It was found that the levels of 18:2 and 18:1 FAs increased in the plasma samples from mice fasted for 12 h in comparison to those from normal chow-fed mice. Moreover, a high fat diet feeding led to a different 18:1 and 18:2 FA profile in plasma in comparison to normal chow feeding.

Plants primarily produce the n-6 18:2 FA isomer while mammals endogenously synthesize the n-9 18:2 FA isomer. The question we asked was whether the increased content of 18:2 FA in the fasting plasma samples was from the dietary-originated 18:2 FA (e.g., 18:2 FA released from the storage depot) or was from the *de novo* synthesis or both. By determining the intensity distribution of the fragment ion resulting from the loss of H₂O from the 18:2 FA anion (Figure S4A), we found no significant difference in the fragment intensity distribution between an authentic n-6 18:2 FA and the 18:2 FAs from all the tested mouse plasma samples, which indicates that the n-6 18:2 FA isomer from the diet was the main component of the 18:2 FA in those mouse plasma samples. The significantly increased levels of the 18:2 FA in the fasting plasma samples, therefore, likely resulted from the rapid release of the stored fat at starvation.

The major isomer of the 18:1 FA in mammals is n-9 18:1 FA. Through comparison of the intensity distributions of the fragment ion resulting from the loss of H₂O from the authentic n-9 18:1 FA isomer with that from the 18:1 FA present in the plasma samples, we found that the n-9 18:1 FA was not the only 18:1 FA isomer present in mouse plasma (Figure S4B). It is known that the n-12 18:1 FA is the other 18:1 FA isomer present in biological samples while the n-7 18:1 FA is rarely occurring in nature. The maximum of the fragment ion intensity of the n-12 18:1 FA occurred at a collision energy of 20-22 eV (the same as shown in Figure S4B) and was about 25 fold higher than that of the n-9 18:1 FA. From this fact, it was estimated that approximately 6% of the n-12 18:1 FA isomer was present in the mouse plasma lipid extracts.

Extension of the Novel Approach for Identification and Quantification of FA Isomers present in the Intact Phospholipid Species

In the current study, we further examined whether the new approach could be readily extended for the analysis of unsaturated FA isomers in more complex lipids. Generally, determination of the isomers of each FA chain in an intact phospholipid species can be achieved by performing an MS³ analysis in the negative ion mode on each of the product ions from an MS/MS analysis that correspond to individual FA chains.

We noticed that, for those phospholipids containing a polyunsaturated FA chain (e.g., 22:6 FA, 22:5 FA), a sequential fragmentation occurred in an MS/MS analysis yielding a secondary fragment ion from the facile loss of CO₂ from the polyunsaturated FA product ion. Identification and quantification of the isomers of such a FA chain could therefore be achieved by an MS/MS analysis. For example, 18:0-22:6 phosphatidylethanolamine (PE) is one of the dominant PE species in mouse myocardial lipid extracts (Figure 6A)^{19, 21}. An ESI MS/MS analysis of 18:0-22:6 PE in the negative ion mode displayed abundant ions at m/z 283.3 and 327.2 corresponding to 18:0 and 22:6 carboxylates, respectively. As previously demonstrated by a high mass resolution instrument¹⁹, the peak at m/z 283.3 was isobaric and comprised of two ions, i.e., 18:0 carboxylate at m/z 283.2426 and a fragment ion at m/z 283.2637 resulting from the loss of CO₂ from 22:6 carboxylate. The intensity of the fragment ion at m/z 283.2637 varied with collision energy in the product ion analysis of 18:0-22:6 PE. A 2D MS analysis of 18:0-22:6 PE showed a similar intensity distribution of this fragment ion versus collision energy to that of the 22:6 FA (Figure 1). This example indicates that determination of some polyunsaturated FA isomers (e.g., 22:5 FA) in intact phospholipids could be performed identically as the determination of FA isomers described above.

Another dominant PE species in mouse myocardial lipid extracts is 18:0-20:4 PE (Figure 6A). A product ion analysis of 18:0-20:4 PE demonstrated the abundant product ions at m/z 283.3 and 303.2 corresponding to 18:0 and 20:4 carboxylates, respectively, as well as a low abundance fragment ion at m/z 259.2 resulting from the loss of CO₂ from the 20:4 carboxylate (Figure 6B). Unlike the facile loss of CO₂ as a secondary fragmentation from 18:0-22:6 PE molecular ion, a sequential loss of CO₂ from the 20:4 carboxylate yielded from the 18:0-20:4 PE molecular ion was not sufficient and thus, the intensity changes of this secondary fragment ion with varied collision energies were insignificant in the MS/MS analysis of the 18:0-20:4 PE ion. Accordingly, an MS³ analysis by using an LTQ-Orbitrap mass spectrometer was performed to determine the relationship of the intensity of the fragment ion yielding from the loss of CO₂ from the 20:4 carboxylate with collision energy (Figure 6C). The 2D mass spectrum of the MS³ analysis of the 20:4 carboxylate yielded from the MS/MS analysis of 18:0-20:4 PE demonstrated an intensity distribution pattern with the existence of a maximum (Figure 6C) similar to that observed for the 20:4 FA by MS/MS analysis although the collision energy values employed are different (i.e., normalized collision energy (%) for MS³ analysis by a trap instrument and collision energy (eV) for MS/MS analysis by a quadrupole instrument, respectively). This study indicates that our established approach can be extended for use in identification and quantitation of double bond isomers of the FA chains present in individual phospholipid species by MS³, which is ongoing in our laboratory.

SUMMARY

In the current study, we presented a simple and straightforward method for identification and quantitation of double bond isomers of unsaturated FAs. This method can be used to discriminate unsaturated FA isomers based on the intensity distribution of the specific fragment ion resulting from the loss of CO₂ or H₂O from FA anions with varying collision conditions. This intensity distribution can also be used to quantify the FA isomers in mixtures with a wide range of linearity. The underlying mechanism is likely due to the charge-dipole interaction between the negative charge carried by the fragment ion and the electron clouds of its double bonds, which represents the effects of remote conjugation on ion stability. Thus, the location of the first double bond, the number of double bonds, and the length of the FA chain are the major contributing factors to the stability (i.e., intensity) of the fragment ions yielded from the FA anions under mild collision conditions where the initial fragmentation from the direct loss of CO₂ or H₂O is predominant while sequential

chain fragmentation is minimal. In the study, the new method was extended to analyze the double bond isomers of FA chains present in intact phospholipids by MS³ analyses of the unsaturated FA carboxylates yielding from the MS/MS analyses of phospholipid molecular ions. Collectively, we developed an approach capable for identification and quantification of the double bond isomers of endogenous FAs and potentially of FA chains present in intact phospholipid species, thereby providing a powerful tool for the analysis of a cellular lipidome. We believe that this approach should further advance the lipidomic power for identification of the underlying biochemical mechanisms of metabolic diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Two-dimensional MS analysis of n-3 22:6 FA in the negative ion mode by varying collision energy in product ion analysis. A 2D MS analysis of 22:6 FA (Panel A) was performed by varying collision energy as indicated with a fixed collision gas pressure of 1 mT as described under “MATERIALS AND METHODS”. The distribution of the normalized absolute intensity of the fragment ion corresponding to the neutral loss of CO₂ from the FA anion vs. collision energy (Panel B) was obtained from the 2D MS analysis (Panel A).

Figure 2.

Two-dimensional MS analysis of n-6 18:3 FA in the negative ion mode by varying collision energy in the product ion analysis. A 2D MS analysis of n-6 18:3 FA was performed by varying collision energy as indicated with a fixed collision gas pressure of 1 mT as described under “MATERIALS AND METHODS” to demonstrate the differential losses of both H₂O and CO₂ from the FA anion. Other fragment ions represent the sequential fragmentation products.

Figure 3.

Examples of the determined standard curves for quantification of FA isomers. The intensity distribution of the fragment ion corresponding to the loss of CO_2 vs. collision energy of individual mixtures of 20:4 FA n-6 and n-3 isomers was obtained from the 2D MS analysis as described in Figure 1A. A linear plot of the normalized absolute intensity of this fragment ion (determined at fixed collision energy of 12 eV and collision gas pressure of 1 mT) vs. the molar composition of each mixture was obtained (Panel A). The linear relationship between the relative intensity of this fragment ion and the collision energy in the low energy region for each mixture was plotted (Panel B). The linear relationship between the natural logarithm of the slope of each linear regression in Panel B and molar composition of each mixture was displayed (Panel C). Data points represent the mean $\pm$ SD of three independent determinations.

Figure 4.

The effects of the location of the first double bond, the number of the double bonds, and the chain length of FA isomers on the intensity of the fragment ions resulting from the loss of CO₂ (Panels A and B) or from loss of CO₂ or H₂O (Panel C) from FA anions. The normalized absolute intensities of fragment ions were determined at collision energy of 12 eV and collision gas pressure of 1 mT. Panel A, the effects of the number of double bonds and the location of the first double bond on the fragment ion intensities of FAs containing 22 carbon atoms; Panel B, the effects of the number of double bonds and the location of the first double bond on the fragment ion intensities of FAs containing 20 carbon atoms; and Panel C, the effects of the FA chain length on the fragment ion intensity of FAs containing one double bond at the $\Delta^{9,10}$ position.

Figure 5.

Different fatty acid profiles of plasma samples of mice fed with normal chow, a high fat diet, or after 12-h fasting. Lipid extracts of mouse plasma samples were prepared by using a modified Bligh and Dyer procedure as previously described⁶. Mass spectra of fatty acids in the plasma samples of mice with normal chow (Panel A), a high fat diet (Panel B), and fasting for 12 h (Panel C) were acquired in the negative ion mode as described under “MATERIALS AND METHODS”. IS stands for internal standard.

Figure 6.

Determination of 20:4 fatty acyl isomers in 18:0-20:4 PE species present in mouse myocardial lipid extracts. MS analyses of mouse myocardial lipid extracts were performed (Panel A) and a representative product ion mass spectrum of 18:0-20:4 PE present in mouse myocardial lipid extracts (Panel B) was acquired in negative ion mode as described under “MATERIALS AND METHODS”. A 2D MS analysis of 20:4 carboxylate (which was yielded from the product ion analysis of 18:0-20:4 PE as shown in Panel B) was performed by varying collision energy in MS³ analysis (Panel C). IS stands for internal standard.