ABSTRACT

Objective: An elevated concentration of oxidized lipids along with the abnormal accumulation of lipids has been linked to the formation of atheromatous plaque and the development of cardiovascular diseases. This study aims to investigate if consumption of different concentrations of dietary oxidized linoleic acid alters the distribution of long chain fatty acids (LCFAs) within the liver relative to plasma in mice.

Methods: C57BL/6 male mice (n = 40) were divided into 4 groups: Standard chow as plain control (P group, n =10), Chow supplemented with linoleic acid 9 mg/mouse/day, linoleic control (C group, n=0), oxidized linoleic acid; 9 mg/mouse/day (A group, n=10) and oxidized linoleic acid 18 mg/mouse/day diet (B group, n=10). Liver and plasma samples were extracted, trans-esterified and subsequently analyzed using gas chromatography mass spectrometry (GC-MS) for LCFAs; palmitic acid, stearic acid, oleic acid, linoleic acid and arachidonic acid.

Results: LCFA methyl esters were eluted and identified based on their respective physiochemical characteristics of GCMS assay with inter assay coefficient of variation percentage (CV%, 1.81–5.28%), limits of quantification and limit of detection values (2.021 – 11.402 mg/mL and 1.016–4.430 mg/mL) respectively. Correlation analysis of liver and plasma lipids of the mice groups yielded coefficients (r=0.96, 0.6, 0.8 and 0.33) with fatty acid percentage total of (16%, 10%, 16% and 58%) for the P, C, A and B groups respectively.

Conclusion: The sustained consumption of a diet rich in oxidized linoleic acid disrupted fatty acid metabolism. The intake also resulted in elevated concentration of LCFAs that are precursors of bioactive metabolite molecule.

Keywords: Lipids; Oxidized fatty acids; Cardiovascular diseases; Atherosclerosis; Gas chromatography; Mass spectroscopy

INTRODUCTION

Oxidized linoleic acid (LA) is a bioactive metabolic derivative of LA that is synthesized by catalytic and non-catalytic oxidation of LA; one of the most abundant fatty acids in human diet, an essential fatty acid and the precursor of other omega 6 fatty acids (γ-linolenic acid [18:3ω6] and arachidonic acid [20:4ω6]). Due to LA being a substantial constituent of the human diet, oxidized LA can also be considered to be one of the most prevalent lipid oxidation products present in food with variations in its levels depending on food storage conditions.
the collection of the data or in the decision to submit the manuscript for publication. No other potential conflicts of interest relevant to this article were reported.

Author Contributions
Conceptualization: Garelnabi M; Formal analysis: Ochin CC, Wilson T; Funding acquisition: Garelnabi M; Investigation: Wilson T; Methodology: Wilson T; Project administration: Garelnabi M; Supervision: Garelnabi M; Writing - original draft: Ochin CC; Writing - review & editing: Garelnabi M, Wilson T.

and preparation conditions. The metabolic activity of oxidized LA through its metabolites 9/13-Hydroxyoctadecadienoic acid (13-HODE), 9-hydroperoxy octadecadienoic acid (9-HpODE), and 13-HpODE is extensive and could range from proinflammatory to anti-inflammatory effects. These metabolites of oxidized LA are one of the most abundant derivatives of oxidized fatty acids that can be found in both plasma and tissues as esters of phospholipids, triglycerides, cholesterol and lipoproteins as well as also components of foam cells and vascular smooth muscle in atheromatous plaques.

Long chain fatty acids (LCFAs) contain 13-22 carbon atoms and could exist as saturated, monounsaturated or polyunsaturated fatty acids. They serve in animals as components of phospholipids and other complex lipids to help maintain structural integrity of cell membranes, while as part of triglycerides they serve as strategic reserves of energy. These LCFAs are most abundant in mammals and are pertinent to their survival. Palmitic acid (saturated LCFA, 16:0) is the commonest saturated fatty acid in the human body accounting for 20-30% of its total fatty acid, stearic acid (saturated LCFA, 18:0) is more abundantly seen in animal fat than vegetable fat. Oleic acid (monounsaturated LCFA, 18:1) is synthesized from stearic acid by stearoyl-CoA 9-desaturase and is the most abundant fatty acid in adipose tissue and follows palmitic acid in its abundance in animal tissues. LA (polyunsaturated LCFA, 18:2\(\omega6\)) is the most consumed polyunsaturated fatty acid in the human diet where it serves as an essential fatty acid and is also considered an integral component of cell membrane architecture and is vital for its cell signaling functions. Arachidonic acid (polyunsaturated LCFA, 20:4\(\omega6\)) is derived from LA and is an important component of cell membrane phospholipids where it serves as a precursor of prostaglandins, leukotrienes, thromboxane, lipoxins, resolvens and exoxins. Gas chromatography-mass spectrometry (GC-MS) is an invaluable tool in lipids metabolomics profiling.

In this study, we attempted to understand if consumption of different concentrations of dietary oxidized LA alters the distribution profile of LCFAs within the liver in relation to plasma using a GC-MS method. Previous studies from our Lab clearly indicated that oxidized LA modulates plasma triglycerides levels leading to its decreased level possibly through lipolysis dependent mechanisms. These findings prompted us to further investigate the potential effects of the dietary oxidized LAs on fatty acids profile.

MATERIALS AND METHODS

1. Animals studies and sample collection
Animal use protocol was approved by the University of Massachusetts, Lowell, Institutional Animal Care and the Use Committee (IACUC). All animals were fed either standard rodent chow (Cat 8640: 22/5 rodent diet; Harlan Teklad Laboratory, Madison, WI, USA) (Table 1), oxidized LA (13-HPODE), or LA added diets for the different experiment condition groups. The oxidized LA was prepared as follows: A 50 \(\mu\)M solution of LA was made in phosphate-buffered saline, and the solution was oxidized with soybean lipoxidase (60–90 units/100 nmol, 2 hours at 37°C. The formation of HPODE was confirmed by leukomethylene blue. In brief, 40 \(\mu\)L of OxLA is added to 100 \(\mu\)L of leukomethylene blue reagent, incubated at room temperature for 10 minutes and then read in BioTek Gen5 Microplate Reader, Winooski, Vermont, USA. The amount of peroxide generated is quantitated using 13-HPODE standard purchased from Cayman, Ann Arbor, Michigan. OxLA and unoxidized LA solutions were then to Harlan Teklad Laboratory, Madison, WI, USA to incorporate in mice chow. The

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9mg/mouse/day dose was established earlier by our group from a calculated normal human consumption of linolenic acid an adjusted to mouse.\(^9\)

Forty 4-week old normal C57BL/6 male mice weighing between 18–21 g were obtained from Jackson Laboratory (Bar Harbor, ME, USA). The mice were divided into four groups, with ten mice in each group as follows: Standard chow as plain control (P group), Chow supplemented with LA 9 mg/mouse/day, linoleic control (C group), oxidized LA; 9 mg/mouse/day (A group) and oxidized LA 18mg/mouse/day diet (B group). The mice were fed the various dietary formulas for two months. All the animals were acclimatized and housed under controlled light and temperature conditions (12-hour light-dark cycle for 30 days). Water was provided \textit{ad libitum} while set amounts of diet was measured and supplied to each group weekly. At the end of the feeding period, animal chow was removed, and mice were fasted overnight. Animals were then sacrificed by using carbon dioxide. Plasma and liver samples were collected at the end of the study from each group for metabolic analysis. All other collected organ samples were quickly snap frozen in liquid nitrogen and then subsequently stored at a \(-80^\circ\text{C}\) freezer until analyzed.

### Table 1. Standard rodent chow nutrients composition

| Constituents                  | Concentration |
|------------------------------|---------------|
| Protein                      | 22.58%        |
| Fat                          | 5.23%         |
| Fiber                        | 3.94%         |
| Ash                          | 7.06%         |
| Nitrogen-free extract        | 51.19%        |
| Gross energy                 | 3.82 kcal/g   |
| Digestible energy            | 3.38 kcal/g   |
| Metabolizable energy         | 3.11 kcal/g   |
| Amino acids                  |               |
| Arginine                     | 1.55%         |
| Methionine                   | 0.37%         |
| Cysteine                     | 0.37%         |
| Histidine                    | 0.52%         |
| Isoleucine + valine          | 1.34%         |
| Leucine + tryptophan         | 1.17%         |
| Lysine + threonine           | 0.72%         |
| Phenylalanine + tyrosine     | 1.04%         |
| Minerals                     |               |
| Calcium + phosphorus         | 1.01%         |
| Sodium + potassium           | 1.40%         |
| Chlorine                     | 0.67%         |
| Magnesium                    | 0.24%         |
| Iron                         | 348.75 mg/kg  |
| Manganese                    | 104.19 mg/kg  |
| Zinc + cobalt                | 90.89 mg/kg   |
| Copper                       | 24.07 mg/kg   |
| Iodine + selenium            | 2.95 mg/kg    |
| Vitamins                     |               |
| Vitamin A + D3               | 18.93 IU/g    |
| Vitamin E                    | 109.54 IU/kg  |
| Choline                      | 2.39 mg/g     |
| Nicotinic acid               | 65.61 mg/kg   |
| Pantothenic acid + B1        | 55.13 mg/kg   |
| Vitamin B6 + vitamin B2      | 23.01 mg/kg   |
| Menadione (vitamin K3)       | 5.22 mg/kg    |
| Folic acid + biotin          | 3.61 mg/kg    |
| Vitamin B12                  | 54.60 mcg/kg  |

Standard rodent diet provided by Harlan Teklad (Cat: 8640, 22/5 rodent diet).
2. Reagents

Hexane (HPLC Grade) and Methanol (HPLC Grade), Boron Trifluoride (BF$_3$) methanol solution and fatty acid methyl ester (FAME) standards (of >99.0% purity (GC) were purchased from Sigma Aldrich (Milwaukee, WI, USA). Working standard mixed solutions were prepared freshly, diluted with hexane, and mixed thoroughly. Ultra-pure water (18.2 MΩ cm) was obtained from Millipore purification system (USA). All other chemicals and solvents were of analytical grade use without further purification, they were obtained from Fisher Scientific (Waltham, MA, USA).

3. Extraction, saponification and derivatization of liver and plasma lipids

Liver tissue was sectioned in amounts range of 0.02–0.05 grams and then spiked with 100 µL (25 ng) of Internal Standard (C17:0) FA (non-Methyl Esterified). Cold PBS (800µL) was then added to the tissue samples and subsequently homogenized. 10 µL of 10% HCL was also added to the pre-homogenized tissue matrix. Plasma in the volume of (800 µL) was spiked with 100 µL (25 ng) of Internal Standard (C17:0) FA (non-Methyl Esterified). The total lipid content in the homogenized liver and plasma samples (Neutral and Polar lipids) were then extracted by Bligh and Dyer method of lipid extraction. Glass test tubes covered with Teflon lined cap was used. The hydrophobic extract was then dried and capped under nitrogen in a chemical hood. Extraction was then followed by saponification and derivatization Boron Trifluoride-Methanol (13–15% BF$_3$) solution. Samples were dried under nitrogen in a chemical hood, covered and stored at −20°C prior to GC-MS analysis.

4. GC-MS analysis of fatty acids

A Shimadzu GCMS-QP 2010 Instrument with an Agilent, HP-5MS column, of 30m length, 0.25 mm diameter, 0.25 µm thickness, with stationary phase: 5% diphenyl, 95% dimethyl polysiloxane and tolerance temperature of 350°C was used. Helium was the carrier gas at a constant flow of 1 mL min.$^{-1}$. The temperature of the injection port was 250°C and 1 uL volume was injected in split mode with a split ratio of 50, using a 10 uL, 23-gauge glass syringe, manufactured by Hamilton, 4970 Energy Way Reno, NV 89502, USA. The effect of the temperature program on the resolution of free fatty acid isomers was investigated. The following temperature program was used; initial temperature 100°C, hold 5 min, 5°C/min ramp to 240°C. Optimum conditions were established based on the resolution of free fatty acids in the standards and then adopted towards analysis of the samples. The mass spectrometer was operated in electron ionization mode with an ionizing energy of 70 eV, ion source temperature 250°C, mass spectrum interface temperature of 250°C, electron multiplier voltage (EM Volts) 1717 V when performing selected ion monitoring scanning from m/z 50 to 500 at 3.25s per scan with solvent delay of 2.0 minutes. Analysis was performed in the full scan monitoring mode (FSM) based on the use of one target and three qualifier ions. Target and qualifier abundances were determined by autosampler injection of individual fatty acid standards under the same chromatographic conditions in full-scan mode with the mass/charge ratio ranging from m/z 50 to 500, while free fatty acids were identified according to the retention times, the target and qualifier ions, and the qualifier to target abundance ratios (Table 2, Figs. 1 and 2).

### Table 2. GCMS Quantitation of long chain fatty acid methyl esters (FAME)

| Fatty acid methyl ester (FAME) | Retention time (min) | Target ion (m/z) | Qualifier ions (m/z) | Limit of detection (µg/mL) | Limit of quantification (µg/mL) | System suitability (%CV) (n=7) |
|-------------------------------|----------------------|-----------------|---------------------|-----------------------------|-------------------------------|-----------------------------|
| Palmitic acid (C16:0)          | 6.525                | 74              | 43, 87              | 1.239                       | 2.811                         | 1.81                        |
| Heptadecanoic acid (Internal Standard) (C17:0) | 8.108          | 74              | 55, 87              | 1.016                       | 2.021                         | 1.67                        |
| Stearic acid (C18:0)           | 9.442                | 74              | 43, 143             | 1.589                       | 3.297                         | 2.96                        |
| Oleic acid (C18:1n9c)          | 9.592                | 55              | 43, 143             | 1.340                       | 3.080                         | 2.88                        |
| Linoleic acid (C18:2n6c)       | 10.092               | 67              | 81, 143             | 1.033                       | 2.289                         | 2.96                        |
| Arachidonic acid (C20:4n6)     | 13.517               | 79              | 80, 91              | 1.430                       | 3.202                         | 4.37                        |

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Quantification was based on the chromatogram peak area ratio of the target ion divided by the peak area of the internal standard.

5. Method evaluation

The performance of the method was evaluated considering different validation parameters that include the following: calibration, linearity and precision of the method, lower limit of quantification and detection (LLOQ and LLOD), biological sample stability, dilution integrity and comparative statistical data of types and components of lipids and their relative quantitative levels between the various mice groups. The qualitative analysis of mass spectrum was based on 3 ions, and the relative abundance of the ions was within 20%. The calibration curves for all of the compounds were obtained by plotting the peak area against the concentration of the corresponding calibration standards at six calibration levels. The identification of respective free fatty acids was carried out by using the retention times of the standard samples and then matched to NIST mass spectra libraries (Table 2). The limit of detection (LODs) was determined by considering a signal-to-noise ratio of 3, whereas the limits of quantification (LOQs) were determined by considering a signal-to-noise ratio of 10 (Table 2). Tests were performed in triplicate.

6. Statistical analysis

The collected data was analyzed using descriptive statistics, Student’s t-test or ANOVA. Results were expressed as mean values combined with standard deviation obtained from three experiments of triplicate repeats. Pearson correlation analysis was used to assess relationships between measured plasma and liver LCFAs. P-values < 0.05 were considered to be statistically significant.

RESULTS

1. LCFAs methyl esters

A total of five long chain free fatty acid compounds were selected for identification in plasma and liver tissue hydrophobic extracts after FAME derivatization (Table 2). The GC chromatogram sequence of elution of the selected FAMES and internal standard (IS) with
retention times were as follows; palmitic acid (6.525), heptadecanoic acid (8.108), stearic acid (9.442), oleic acid (9.592), LA (10.092) and arachidonic acid (13.517) (Fig. 1). The full scan spectra showed consistent and predominant target and qualifier ions with NIST mass spectral library matches in the range of 92-99%. The mass spectrometry parameters were optimized to maximize instrument response as indicated by their electron ionization mass spectra under the optimized method conditions for the LCFA FAMES and IS (Fig. 2). The mass spectra of the methyl ester derivatives of the LCFA analytes were identified based on the presence of their characteristic stable fragment ions such as the target ion peaks of palmitic acid, IS, stearic acid, oleic acid, LA, and arachidonic acid at m/z 74, 74, 74, 55, 67, and 79.

Fig. 2. GC-MS full scan mass spectrometric analysis of selected long chain fatty acids and internal standard methyl esters in liver and plasma samples. (A) heptadecanoic acid methyl ester (IS), (B) palmitic acid methyl ester, (C) stearic acid methyl ester, (D) oleic acid methyl ester, (E) linoleic acid methyl ester, (F) arachidonic acid methyl ester.
respectively (Table 2, Fig. 2). The molecular ion of the above LCFA FAMEs and IS analytes at m/z 270, 284, 296, 294 and 318 respectively were also noted (Fig. 2). The inter assay coefficient of variation percentage (CV%) were 1.81-5.28%, LOQ and LOD values were 2.02H1.402 µg/mL and 1.016-4.430 µg/mL respectively (Table 2). The concentration of respective fatty acids in liver and plasma samples were determined from calibration curves using FAME standards.

2. Relative quantitative distribution of LCFA in liver and plasma

Hepatic concentrations of palmitic acid, LA, oleic acid, stearic acid and arachidonic acid all showed similar distribution patterns amongst the four groups of mice (Fig. 3). The highest and lowest concentrations of these fatty acids were noted in the livers of mice in group C that were fed a diet supplemented with LA and the livers of the group B mice that were fed a diet containing a high concentration of oxidized LA respectively. The livers from mice in group A that were fed a diet containing a lower concentration of oxidized LA showed the second

![Graphs of fatty acid concentrations in liver](https://e-jla.org)

**Fig. 3.** Quantitative distribution profile of each long chain fatty acid in liver. Top to bottom is the quantitative distribution profile in liver of linoleic acid, palmitic acid, oleic acid, stearic acid, and arachidonic acid.
highest concentration of LCFA. The LCFA concentrations in the livers of the mice in the P group were in the middle between the concentrations seen in the livers of the mice in the A and B groups. This distribution was statistically significant \((p<0.001)\) for palmitic acid, LA, oleic acid and stearic acid across all groups while arachidonic acid only showed statistical significance \((p<0.001)\) in the comparison between the P and B groups. However, the distribution of the various LCFA in plasma was different for each fatty acid amongst the four groups of mice (Fig. 4). The concentration of palmitic acid was elevated in the plasma of all three groups of the experimental conditions mice when compared to the mice in the P group with the highest concentration present in plasma from mice in the A groups while the plasma in mice of C and B groups had almost similar concentrations. Plasma concentration of LA was uniformly elevated for mice in the three groups of; C, A and B, while compared to the concentration seen in the plasma of the mice in the P group. Plasma oleic acid concentration showed increased concentrations in the plasma from the mice in B, C and A groups respectively when compared to the P group. The concentration of stearic acid in the plasma

![Graphs showing quantitative distribution of LCFA in plasma](https://e-jla.org)

**Fig. 4.** Quantitative distribution profile of each long chain fatty acid in plasma. Top to bottom is the quantitative distribution profile in plasma of linoleic acid, palmitic acid, oleic acid, stearic acid, and arachidonic acid.
of mice in the experimental groups C, A, B showed plasma concentration of stearic acid were almost identical when compared to the mice in the P group. Elevated amounts of arachidonic acid were noted in the plasma of mice from both B and C groups with the plasma from the B group of mice having the greater content of these two, while the plasma concentration of arachidonic in the mice of the A group intermediate between the plasma concentrations observed in the mice from B and C groups. The was no statistical significance seen in the distribution of the LCFA in plasma, amongst the four groups of mice in (Fig. 4).

3. Comparison of LCFA profiles in liver and plasma
To compare the overall LCFA profiles of liver to plasma in mice of each respective group, a Pearson's product-moment correlation analyses were performed (Fig. 5). In the mice of the P group, plasma LCFA species had a strong positive and statistically significant relationship to their liver LCFA counterparts (r=0.96, p<0.01), the comparison in the mice of the A group revealed also a fairly strong positive correlation that was near statistical significance (r=0.8, p=0.09), while the comparison in the C group showed moderate positive correlation relationship (r=0.6) between plasma and liver LCFA. The correlation relationship between plasma and liver LCFA species in the mice of the B group had the weakest positive correlation (r=0.33).

4. Total composition ratio of LCFA in the liver and plasma
The total combined composition ratio for each LCFA across the experimental mice groups was calculated as a ratio of their concentration in plasma to liver and then followed by the summation of these ratio compositions of each LCFA to total percentages, which was then

![Graphs showing Pearson's product moment scatter plots for each group](https://e-jla.org)

**Fig. 5.** Pearson's product moment scatter plots showing correlational analysis between liver and plasma quantitative profiles of long chain fatty acids within each of the P, C, A and B groups. Correlation coefficients; P group (r=0.96), C group (r=0.6), A group (r=0.8), B group (r=0.33).

AA, arachidonic acid; LA, linoleic acid; PA, palmitic acid; SA, stearic acid; OA, oleic acid.
compared across the four mice groups in a bar chart distribution (Fig. 6). The B group had the highest composition ratio for arachidonic acid, LA, oleic acid, with stearic acid and palmitic acid having similar composition ratios. The P group had almost similar composition ratios for all the LCFA with oleic acid composition ratio being slightly lower than its counterpart ratios. The C group had the least composition ratio for all LCFA. Group A had arachidonic acid as its highest composition ratio, with stearic acid and LA having similar intermediate composition ratios while palmitic acid and oleic acids having the least composition ratios. The percentage total of the sum of LCFA composition ratios was largest in the B group at 58%, A and P groups were both at 16%, with the C group being the least at 10% (Fig. 6).

**DISCUSSION**

The GCMS assay of the hepatic and plasma samples demonstrated a great method for fatty acid analysis (Table 2, Figs. 1 and 2). GCMS system suitability for assay of these fatty acids showed great precision as revealed by the CV results for all assayed fatty acids (Table 2).

Fatty acids distribution, storage and transportation in the liver and plasma reflect the degree of exchange of these biomolecules and their constituent metabolites between the extracellular and intercellular compartments. The similar pattern of distribution of the respective fatty acids in the liver revealed that the experimental conditions had comparable...
influences on the overall fatty acid's metabolism (Fig. 3). This could be due to the LCFAs being major constituents of larger lipid molecules such as triglycerides and cholesterol esters within the liver and the blood. The fatty acids in the livers of group A mice had significantly higher concentrations than the concentration in the livers of the P group controls while the concentrations in the B group were statistically significantly less than the controls. This may be due to a dose dependent mechanism of oxidized LA associated with the biosynthesis and catabolism of fatty acids in the liver. The mechanism of the differential dose modulation of fatty acid anabolism and catabolism by oxidized LA is reinforced by the significantly increased concentration of the various fatty acids in the livers of group C mice compared to the P group and the livers of mice in A and B groups too (Fig. 3). The changes in LCFAs levels could be mediated by PPARs modulated mechanisms. PUFAs are more effective inducers of fatty acid oxidation and potent suppressers of fatty acid and triacylglycerol synthesis. It is safe to speculate that oxidized LA metabolites and their decomposed aldehydes by byproducts may exert inhibitory effects on downstream PPARs modulated genes associated with lipolysis pathways. Additionally, these oxidative byproducts may exert inhibitory effect on mitochondrial carnitine palmitoyl transporter 1 (CPT1) a mediator of fatty acids beta oxidation.

Plasma concentrations of linoleic, oleic and arachidonic fatty acids for mice in group C, A and B showed levels that were greater than the P group while palmitic and stearic acid plasma concentrations have shown a reverse pattern (Fig. 4). This could likely be attributed to the negative modulation of factors mediating metabolism of fatty acids in plasma such as plasma lipase and transport lipoproteins. The contrasting effects seen for plasma concentrations of palmitic and stearic acid could also likely be linked to their status as fatty acids that are most abundant in the viscera of animals.

The tandem relationship of concentration of the selected fatty acids between liver and plasma along with the systemic effect of the overall LCFAs profiles of both liver and plasma in each group of mice was analyzed using Pearson’s correlation (Fig. 5). The P group concentrations were consistent with the literature for the various fatty acids concentrations showing strong correlation value of (r=0.96, p<0.01) (Fig. 5); Palmitic acid concentration was higher compared to most non-essential fatty acids in animal livers, followed by LA concentration which represents the most abundant fatty acid in animals diets and then closely followed by stearic acid concentration the second most abundant fatty acid in animal tissues, arachidonic acid concentration was next in line as it is derived from LA and oleic acid concentration was least due to its high prevalence in adipose tissue than plasma or liver tissue. This pattern of fatty acids physiologic concentration was disrupted in the mice groups as indicated by their reduced positive correlation coefficients; C group (r=0.6), A group (r=0.8) and B group (r=0.33) (Fig. 5). These correlation values also point towards the dose dependent responses to oxidized LA consumption.

The total composition of each LCFAs in each of the mice groups was calculated as a ratio of plasma to liver concentration as well as the combined percentage total of these ratios into a bar chart distribution for each group of mice (Fig. 6). The B group of mice had the most elevated fatty acids compositions of linoleic, oleic and arachidonic acids. The acutely increased composition ratio of arachidonic acid in both the A and most especially the B group of mice, shows a dose dependent relationship to oxidized LA dietary intake. This outcome could positively modulate the physiologic levels of bioactive molecules such as prostaglandin, leukotrienes, thromboxane, lipoxins, resolvins and eoxins that are all derived from
arachidonic acid. These bioactive molecules are particularly important for the signaling and modulation of proinflammatory and anti-inflammatory pathways. The B group also had the highest statistically significant percentage total of LCFA compositions of over 40% when compared to the mice in the P and other groups (Fig. 6).

This indicates that increased consumption of oxidized LA could predispose to abnormalities in lipid storage with is associated with increased risk of cardiometabolic diseases such as diabetes, and cardiovascular diseases.

In conclusion, GCMS effectively separates and identifies individual LCFAs of liver and plasma. The hepatic profiles of LCFAs in mice fed oxidized LA demonstrated a dose dependent distribution pattern of liver LCFAs concentrations while in plasma the LCFA (arachidonic acid) that are precursors of bioactive messenger molecules were markedly increased in concentration. Also, cumulatively, a diet containing a high dose of oxidized LA could lead to a disrupted relationship between liver and plasma LCFAs and also facilitate disproportionate accumulation of fatty acids in the viscera. This research thus provides further insight into the influence dietary oxidized lipids in a dose dependent manner could have on fatty acid distribution and the potential effects of their downstream derivative pathways could have on lipid metabolism and its involvement in cardiovascular health.

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