LA and ALA prevent glucose intolerance in obese male rats without reducing reactive lipid content, but cause tissue-specific changes in fatty acid composition

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Matravadia S, Zabielski P, Chabowski A, Mutch DM, Holloway GP. LA and ALA prevent glucose intolerance in obese male rats without reducing reactive lipid content, but cause tissue-specific changes in fatty acid composition. Am J Physiol Regul Integr Comp Physiol 310: R619–R630, 2016. First published January 13, 2016; doi:10.1152/ajpregu.00297.2015.—While the cause of Type 2 diabetes remains poorly defined, the accumulation of reactive lipids within white adipose tissue, skeletal muscle, and liver have been repeatedly implicated as underlying mechanisms. The ability of polyunsaturated fatty acids (PUFAs) to prevent the development of insulin resistance has gained considerable interest in recent years; however, the mechanisms-of-action remain poorly described. Therefore, we determined the efficacy of diets supplemented with either linoleic acid (LA) or α-linolenic acid (ALA) in preventing insulin resistance and reactive lipid accumulation in key metabolic tissues of the obese Zucker rat. Obese Zucker rats displayed impaired glucose homeostasis and reduced n–3 and n–6 PUFA content in the liver and epididymal white adipose tissue (EWAT). After the 12-wk feeding intervention, both LA- and ALA-supplemented diets prevented whole body glucose and insulin intolerance; however, ALA had a more pronounced effect. These changes occurred in association with n–3 and n–6 accumulation in all tissues studied, albeit to different extents (EWAT > liver > muscle). Triacylglycerol (TAG), diacylglycerol (DAG), ceramide, and sphingolipid accumulation were not attenuated in obese animals supplemented with either LA or ALA, suggesting that preservation of glucose homeostasis occurred independent of changes in reactive lipid content. However, PUFA-supplemented diets differentially altered the fatty acid composition of TAGs, DAGs, and PLs in a tissue-specific manner, suggesting essential fatty acid metabolism differs between tissues. Together, our results indicate that remodeling of the fatty acid composition of various lipid fractions may contribute to the improved glucose tolerance observed in obese rats fed PUFA-supplemented diets.

Specifically, diacylglycerol (DAG) accumulation (8, 21, 23, 41, 42, 46) has been proposed to antagonize insulin signaling by mediating protein kinase C (PKC) serine phosphorylation of the insulin receptor substrate (IRS), thereby inhibiting the insulin signaling cascade (16). In addition to DAGs, ceramides have also been associated with the induction of insulin resistance in a variety of tissues (1, 6, 40, 43, 46), as ceramide accumulation reduces the activation of Akt (35) to attenuate GLUT4-mediated glucose uptake (22). Further, reactive lipids have been linked to inflammation, including facilitating the effects of TNFα (38) and inducing JNK signaling (6), which is thought to result in serine phosphorylation-mediated inhibition of IRS. In addition, activation of inflammation has been linked to IL-1β mediated reductions in IRS protein, further attenuating the biological effects of insulin (17). Collectively, these studies suggest a causal link between reactive lipid accumulation and insulin resistance in peripheral tissues. Therefore, treatment strategies that reduce reactive lipid species in these tissues may be beneficial in managing and/or preventing insulin resistance.

Diets supplemented with omega–3 (n–3) polyunsaturated fatty acids (PUFAs) have been shown to improve glucose tolerance (20, 24, 30, 39); however, the majority of these past studies have examined eicosapentaenoic acid (EPA, 20:5n–3) and docosahexaenoic acid (DHA, 22:6n–3). Very little is known regarding the effects of α-linolenic acid (ALA, 18:3n–3); however, we have previously shown that a diet supplemented with flaxseed (i.e., rich in ALA) prevents obesity-induced insulin resistance (24). The effect of ALA on reactive lipids in diverse tissues is unknown; however, carnitine palmitoyltransferase (CPTI) has a high sensitivity for ALA-CoA moieties (9), and PUFAs in general, and ALA in particular has a high rate of oxidation within skeletal muscle (11). It is therefore possible that preferential oxidation of ALA within mitochondria may reduce the accumulation of reactive lipid species in diverse tissues.

In contrast to the recognized benefits of n–3 PUFAs, diets enriched with omega–6 (n–6) PUFAs have traditionally been viewed as detrimental primarily because they are precursors for pro-inflammatory eicosanoids (13). However, evidence suggests that this notion is overly simplified (18). Additionally, we recently reported that a diet supplemented with safflower [i.e., rich in linoleic acid (LA, 18:2n−6)] protected against obesity-induced insulin resistance (24). The mechanism-of-action involved in LA-mediated improvements in insulin sensitivity, and the potential alterations in reactive lipid profiles in key insulin sensitizing tissues following LA consumption, remain unknown. However, similar to ALA, CPTI—a key rate-limit-
ing enzyme for mitochondrial fatty acid oxidation—has a high sensitivity to LA (9), and this PUFA is oxidized to a greater degree in muscle than saturated fatty acids (e.g., stearate) (11). Therefore, in the current study we aimed to determine if supplementing diets with ALA and LA could improve the reactive lipid profiles of skeletal muscle, liver, and epididymal WAT (EWAT) of obese Zucker rats. It was hypothesized that both PUFA-supplemented diets would reduce reactive lipids in all tissues studied.

MATERIALS AND METHODS

Animals. Five-week-old male lean (n = 8) and obese (n = 24) Zucker rats were purchased from Charles River (St. Constant, Quebec, Canada). Animals were housed in a temperature-regulated room on a 12:12 h light-dark cycle with water available ad libitum. At 6 wk of age animals commenced a 12-wk dietary intervention (n = 8 per group). Lean and obese control animals were given unrestricted access to control diet, while obese animals fed PUFA-supplemented diets were pair-fed to match for caloric intake of obese controls (see below for details). Thereafter, animals were anesthetized with an injection of pentobarbital sodium (60 mg/kg), and the red tibialis anterior muscle, EWAT, and liver were excised and immediately placed in liquid nitrogen. The University of Guelph Animal Care Committee approved all procedures.

Diets and feeding. All diets used in the present study were purchased through Research Diets (New Brunswick, NJ). Daily food consumption of lean and obese rats fed the control diet (No. AIN-93G; 20% protein, 64% carbohydrate, and 16% fat; 4.00 kcal/g) was recorded by weight to pair-feed rats given LA (No. AIN-93G + 10% safflower oil; 20% protein, 54% carbohydrate, and 26% fat; 4.24 kcal/g) and ALA (No. AIN-93G + 10% flaxseed oil; 20% protein, 64% carbohydrate, and 16% fat; 4.00 kcal/g) supplemented diets to ensure caloric consumption was similar across diets. Fatty acid composition of the diets was confirmed by gas chromatography (Table 1).

Whole body glucose tolerance. Four-hour-fasted animals underwent an intraperitoneal glucose (IPGT, 2 g/kg) and insulin (IPITT, 0.1 U/kg) tolerance test separated by 48 h. Blood was collected from a tail vein at various time points, and blood glucose was determined with a glucometer (Freestyle Lite, Abbott Laboratories, St. Laurent, QC, Canada). To determine the area under the curve (AUC) during the insulin tolerance test, the baseline values were adjusted to examine the “response” of the insulin injected. Specifically, the AUC baseline was adjusted to the final glucose value during the ITT to negate any starting differences in fasting blood glucose concentrations.

Analysis of tissue lipid content. Lipids were extracted by a modified Folch method (12). Frozen samples were cleaned, freeze-dried, and powdered in an aluminum mortar with a stainless steel pestle, pre-cooled in liquid nitrogen. Powdered samples were transferred into glass tubes containing 2 ml of methanol containing an antioxidant (0.01% BHT) at −20°C, followed by a 4 ml addition of chloroform and 1.5 ml of water. To correct for the extraction and assay losses, 100 µl of internal standard mixture composed of triheptadecanoyl (C17:0 TAG), diheptadecanoyl (C17:0 DAG), and diheptadecanoyl phosphatidylcholine (C17:0 PC) was added. Lipids within the chloroform layer were fractionated via thin-layer chromatography (silica plate 60, 0.25 mm, Merck) by using heptane, isopropyl ether, and acetic acid (60:40:3 ratio vol/vol/vol) for TAG, DAG, and PL separation. Dried plates were sprayed with 0.2% solution of 2’7’-dichlorofluorescin in methanol and briefly exposed to ammonia vapor, while bands were visualized under UV light. Lipid bands were identified according to the standards (Sigma) and scraped off the plates. Separated lipids were methylated in 14% boron trifluoride-methanol (28), while fatty acid methyl esters were extracted with pentane (44). Samples were dissolved in hexane and analyzed with a Hewlett-Packard 5890 Series II gas chromatograph, an Agilent J&W CP-Sil 88 capillary column (50 m × 0.25 mm id), and a flame-ionization detector. The oven temperature was programmed from 130 to 220°C at 5°C/min and held at 220°C for 32 min. Subsequently, retention times of standards and individual fatty acids were quantified. Total amount of each fraction for TAG, DAG, and PL were counted as a sum of identified long chain fatty acids. The following fatty acid species were identified and quantified: myristic (14:0), palmitic (16:0), palmitoleic (16:1n–7), stearic (18:0), oleic (18:1n–9), linoleic (18:2n–6), α-linolenic (18:3n–3), arachidic (20:0), arachidonate (20:4n–6), eicosapentaenoic (20:5n–3), behenic (22:0), docosahexaenoic (22:6n–3), and nervonic (24:1n–9) acids.

Ceramide and sphingolipids were measured by HPLC, as previously described (15). Briefly, a small volume (50 µl) of the chloroform phase containing lipids was extracted, as described above, and was transferred to a fresh tube containing 40 pmol of N-palmitoyl-D-erythro-sphingosine (C17 base) as an internal standard. The samples were evaporated under a nitrogen stream, dissolved in 1.2 ml of 1 M KOH in 90% methanol, and heated at 90°C for 60 min to convert ceramide into sphingosine. This digestion procedure does not convert complex sphingolipids, such as sphingomyelin, galactosylceramide, or glucosylceramide, into free sphingoid bases (25). Samples were then partitioned by the addition of chloroform and water. The upper phase was discarded and the lower phase was evaporated under nitrogen. The content of free sphingosine liberated from ceramide was then analyzed by HPLC as described above. The calibration curve was prepared with N-palmitoylsphingosine (Avanti Polar Lipids) as a standard. The chloroform extract used for the analysis of ceramide

<table>
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<th>Table 1. Fatty acid composition of diets</th>
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<td>Relative % of Total Lipid Detected</td>
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contains small amounts of free sphingoid bases. Therefore, the concentration of ceramide was corrected for the level of free sphingosine measured in the same sample.

**Western blotting.** Whole muscle homogenate (10 μg) was separated by electrophoresis by SDS-PAGE and transferred to polyvinylidene difluoride membranes. The following commercially available antibodies were used: total and phosphorylated JNK1/2 (Cell Signaling) and total IRS (Millipore). Bands were visualized by enhanced chemiluminescence (Western Lightning Plus-ECL, PerkinElmer) and a FluorChem HD2 Alpha Innotech imager, and quantified by using the software provided. Multiple proteins were detected from the same Western blot by cutting gels before transferring onto a single membrane to minimize variability. As a result, Ponceau staining was used to confirm consistent loading.

**Statistics.** All statistical analyses were conducted with Prism software (GraphPad Software, La Jolla, CA). A one-way analysis of variance (ANOVA) was used to determine the effects of LA and ALA supplementation, followed by a Tukey post hoc test when appropriate. A P < 0.05 was considered statistically significant. For fatty acid analyses in each lipid fraction within a tissue, we also accounted for multiple comparisons by using a Bonferroni correction. In these instances significance was accepted as P < 0.0035 (i.e., 0.05/14 fatty acids = 0.0035). Values are reported as means ± SE throughout the manuscript.

**RESULTS**

**Effects of LA and ALA on whole body glucose homeostasis.** Obese animals displayed elevated body weight; however, differences within obese animals were not observed as a result of pair feeding (Fig. 1A). Despite a lack of difference in body weight, ALA and LA supplementation attenuated the elevation in fasting blood glucose observed in obese animals (lean: 5.7 ± 0.9 mM; obese: 14.2 ± 1.0 mM; obese ALA: 9.4 ± 1.1 mM; obese LA: 10.0 ± 1.3 mM; P < 0.05). In addition, while obese control animals displayed impaired glucose and insulin tolerance (Fig. 1, B–E), the AUCs during glucose and insulin tolerance tests indicated that obese animals supplemented with either ALA or LA were not significantly different from lean animals (Fig. 1, B–E). However, only ALA-supplemented animals displayed decreased AUC relative to obese controls (Fig. 1, B–E), suggesting ALA is more efficient in preventing the obese Zucker phenotype.

**Effects of LA and ALA on global tissue lipid profiles.** The observed differences in whole body glucose and insulin tolerance between obese animals were not associated with reductions in fasting serum free fatty acid levels (lean: 259 ± 22 μM; obese: 847 ± 170 μM; obese ALA: 957 ± 174 μM; obese LA: 700 ± 65 μM; P < 0.05, all obese higher than lean). We therefore next aimed to determine if muscle, liver, and EWAT, three tissues known to influence whole body glucose homeostasis, displayed deficiencies in either total n–3 or n–6 PUFAs that could be prevented with ALA or LA supplementation. To accomplish this, we summed the n–3 (ALA, EPA and DHA) and n–6 (LA and AA) of the various TAG, DAG, and PL lipid fractions within each tissue. This approach revealed that the skeletal muscle of obese control animals exhibited increased n–3 and n–6 PUFAs compared with lean animals (Fig. 2, A and B). In obese animals supplemented with either ALA or LA, muscle tissue levels of n–3 and n–6 PUFAs were further increased, respectively, compared with the obese control. In contrast to muscle, the liver and EWAT of obese animals showed significant depletions in both n–3 and n–6 PUFAs compared with lean animals (Fig. 2, A and B) that were recovered following consumption of the respective diets. Therefore, ALA and LA diets increased n–3 and n–6 PUFAs in all three tissues; however, only liver and EWAT originally showed reductions in these fatty acids in obese animals.

Given the observation that ALA and LA supplementation prevented glucose intolerance, albeit to different extents, and affected lipid composition within peripheral tissues, we next examined the tissue-specific effects on individual lipid fractions. In general, while the obese phenotype increased TAG, DAG, and ceramides, neither ALA nor LA altered these responses in the muscle, liver, or EWAT (Fig. 3, A–E). The exceptions were that ALA increased TAG within EWAT (Fig. 3A), increased ceramide within muscle (Fig. 3D), and decreased sphingolipids within the liver (Fig. 3E). While total PL content was increased in muscle of obese animals, and decreased in liver and EWAT, neither ALA nor LA altered the total PL content in any of the tissues (Fig. 3C). Altogether, these data suggest that ALA and LA do not dramatically alter the total tissue levels for TAGs, DAGs, PLs, ceramides, or sphingolipids in association with improvements in glucose homeostasis. Therefore, we next examined the fatty acid composition of these lipid fractions within each tissue.

**Changes in TAG composition.** Within all tissues, consumption of the ALA diet enriched ALA and EPA n–3 PUFAs and concomitantly decreased n–6 PUFAs (LA and AA; Fig. 4, A–C). The opposite pattern of enrichment was observed following LA supplementation, where n–6 PUFAs were generally increased relative to control animals at the expense of n–3 PUFAs (Fig. 4, A–C). However, there were tissue-specific differences in various fatty acids, which are highlighted with boxes in Fig. 4. Specifically, the ALA diet increased TAG-DHA within the liver (Fig. 4B) and did not reduce TAG-LA within EWAT, compared with the obese control (Fig. 4C). The LA diet systematically reduced TAG-ALA and TAG-DHA in all three tissues, while TAG-EPA was reduced in both liver and EWAT, but not muscle. In addition to the dietary effects on PUFA profiles, ALA and LA also altered the levels of specific monounsaturated (MUFA) and saturated (SFA) fatty acids in the TAG fraction. This is potentially important, as palmitoleic, palmitic, and stearic acids have been associated with insulin resistance (15, 42). In the current study, neither palmitic acid nor stearic acid in the TAG fraction were altered in any tissue in response to the dietary interventions (Fig. 4, A–C), while across all tissues ALA supplementation decreased TAG behenic acid. In contrast to these consistent tissue responses, LA and ALA decreased TAG palmitoleic acid in the liver only (Fig. 4, A–C). Finally, LA increased TAG nervonic acid only within EWAT (Fig. 4C). Collectively, these results highlight the relatively consistent responses in TAG composition following LA and ALA supplementation between muscle, liver, and EWAT.

**Changes in DAG composition.** While the response of DAG-ALA in the three tissues was relatively consistent between the two diets (Fig. 5, A–C), the responses of LA, AA, EPA, and DHA were not (highlighted with boxes). Specifically, DAG-AA was only increased in EWAT following LA consumption compared with the obese control. ALA consumption had no effect on DAG-LA levels in the liver, but did reduce DAG-LA in the muscle and EWAT. Furthermore, ALA consumption increased DAG-EPA in only the liver, and increased
DAG-DHA within EWAT (Fig. 5, A–C). Overall, the PUFA diets had little effect on the SFA and MUFA composition of DAG; however, both diets reduced DAG myristic and stearic acids in muscle (Fig. 5A) and increased DAG oleic acid in the liver. Collectively, these results highlight the ability of ALA and LA to decrease the SFA content of DAGs within muscle.

Changes in total PL composition. In contrast to the liver-specific increases observed for DAG-EPA following ALA consumption (Fig. 5B), all three tissues showed strong increases in PL-EPA with this diet (Fig. 6, A–C). Overall, the changes in PL-LA, PL-ALA, and PL-EPA were relatively consistent across tissues. However, in contrast, PL-AA was only increased in muscle following ALA supplementation (Fig. 6A). Collectively, these results highlight the ability of muscle to incorporate AA and DHA into PLs, as well as the ability of EPA to be enriched into the PL fraction in all three tissues.

Changes in markers of tissue inflammation. Given the known link between reactive lipids and inflammation, we next examined JNK phosphorylation, a common marker of inflammation. Only the liver displayed an increase in JNK phosphorylation with obesity, but neither ALA nor LA altered this...
response (Fig. 7, A–F). In addition, activation of inflammation has been shown to reduce IRS1 protein content (17). In the current study, obesity was associated with reductions in IRS1 protein in the muscle (Fig. 7G) and liver (Fig. 7H), but not in EWAT (Fig. 7I). Neither ALA nor LA altered these responses. Combined, these data further substantiate the lack of improvements in reactive lipids observed with ALA and LA supplementation.

**DISCUSSION**

The current study has investigated the effects of diets supplemented with either ALA or LA on the lipid profiles of key metabolic tissues influencing whole body glucose homeostasis in a rodent model of obesity. Our data show that both ALA and LA prevent glucose intolerance, although ALA is more efficient. However, an attenuation of reactive lipid accumulation (i.e., DAGs and ceramides) is not required for the prevention of whole body glucose intolerance seen with increased consumption of either essential PUFA. Furthermore, the PUFA-supplemented diets differentially altered the fatty acid composition of TAGs, DAGs, and PLs in a tissue-specific manner. Together, our results suggest that remodeling the fatty acid composition of various lipid fractions may contribute to the improved glucose homeostasis observed in obese rats fed PUFA-supplemented diets.

While TAG is now widely considered an inert storage depot (21, 34), reactive lipids have gained considerable interest as a mechanism to cause insulin resistance in diverse tissues (1, 6, 8, 21, 23, 40–43, 46). However, in the current study, the prevention in whole body glucose and insulin intolerance following PUFA supplementation was not associated with reductions in DAG or ceramide content in any tissue studied, with the exception of ALA reducing hepatic sphingolipids, a lipid fraction strongly associated with WAT insulin resistance (5, 42). The controversy surrounding which lipid species, if any, independently mediate the induction of insulin resistance extends beyond the current observations. Indeed, increases in DAG content can occur without the expected induction of insulin resistance within both muscle (2, 36) and liver (26), suggesting individual lipid species may have a more important role. For example, DAGs containing stearic acid have been linked to liver and muscle insulin resistance (15, 42). While neither ALA nor LA had any effect on DAG-stearic acid levels within the liver, both diets reduced muscle DAG-stearic acid to levels equivalent to lean controls, which may contribute to the improved glucose homeostasis.

In the current study, despite similar glucose/insulin tolerance, the ceramide content of muscle in ALA-supplemented animals was higher than lean controls. Since the ALA diet does not contain higher palmitate concentrations, these data may

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**Fig. 2.** The effect of ALA and LA diets on total n–3 (A) and n–6 (B) polyunsaturated fatty acids. Values are reported in muscle, liver, and epididymal white adipose tissue (EWAT) and are expressed as means ± SE; n = 8 for each measure. Similar letters indicate a lack of statistical difference between groups.
Fig. 3. The effect of ALA and LA diets on total triacylglycerol (TAG) (A), diacylglycerol (DAG) (B), phospholipids (C), ceramides (D), and sphingolipids (E). Values are reported in muscle, liver, and EWAT and are expressed as means ± SE; n = 8 for each measure. Similar letters indicate a lack of statistical difference between groups.
suggest ALA supplementation altered the metabolism of the liver or EWAT in a manner that increased the delivery of saturated fatty acids to muscle, although this remains speculative. Regardless, the ceramide content of obese animals, in all tissues studied, cannot explain the prevention of glucose intolerance following ALA and LA supplementation.

The prevention in whole body glucose intolerance in the current study may also extend from rectifying the apparent derangements in essential n–3 and n–6 PUFA content in obese liver and EWAT. The accumulation of PUFAs within the tissues studied may indirectly, through alterations in gene transcription and/or metabolism, affect lipid-sensitive signaling pathways associated with insulin sensitivity. This latter point is particularly noteworthy given that we observed increases in EPA, DHA, and AA levels in the three tissues. Both rodents and humans have the ability to convert ALA into EPA and DHA, as well as LA into AA, through a series of desaturation and elongation steps (known as the FADS pathway) (3, 4, 7). Interestingly, a relationship between reduced FADS pathway activity and impaired insulin action and increased adiposity has been previously reported (32, 45). The FADS pathway is highly active in the liver (29) and we have recently reported that this pathway is also functional in adipocytes (33). Our fatty acid data clearly shows that interconversion of ALA and LA into their longer chain counterparts occurred; however, we our unable to ascertain in which tissue(s) this interconver-

Fig. 4. The effect of ALA and LA diets on TAG fatty acid composition. Values for saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids are reported in muscle (A), liver (B) and EWAT (C). Values represent means ± SE, expressed as a percentage of lean control (dotted line); n = 8 for each measure. Fatty acids were compared across the four groups by one-way ANOVA and Tukey post hoc tests. Multiple testing was accounted for by using a Bonferroni correction (i.e., P < 0.05/14 fatty acids). Similar letters indicate a lack of statistical difference between groups. Where letters are absent, significance with an ANOVA and Tukey post hoc tests was not attained.
sion occurred. Therefore, it is not possible to definitively conclude whether the improvements in glucose homeostasis seen in obese rats supplemented with flaxseed stem from ALA or EPA/DHA or a combination of all three fatty acids. This is pertinent given that Lanza et al. (20) recently reported that whole body glucose tolerance was improved in mice fed a high-fat diet supplemented with menhaden oil, thereby demonstrating that EPA and DHA are capable of improving metabolic health independent of ALA. Future studies in which the FADS pathway is inhibited are required to uncouple the interconversion of ALA to EPA/DHA, and better delineate the relationship of specific PUFAs and insulin sensitivity.

**Perspectives and Significance**

The present data highlights the ability of both LA and ALA to prevent glucose intolerance in a rodent model of obesity, although the effect is greater following ALA supplementation. The current dietary approach did not replace saturated fatty acids with PUFAs, but rather increased the total fat composition of the diet, suggesting that supplementation of essential PUFAs in the diet prevents glucose intolerance in the absence of reductions in either caloric intake or total ingestion of fat. While caution is warranted in extrapolating rodent data to humans, the genetic mutation within the obese Zucker rat
(leptin deficiency) results in hyperphagia-induced obesity and insulin resistance, and therefore follows a similar disease progression normally described in humans. While the current results support the idea that PUFA supplementation can improve glucose homeostasis, the underlying mechanism(s) remain debatable. A major working model for diet-induced insulin resistance is focused on reactive lipids; however, neither ALA nor LA reduced total tissue levels of DAGs or ceramides, thereby challenging this long-standing hypothesis. While specific reactive lipid subspecies have been suggested to be more important in the induction of insulin resistance, there was no consistency between tissues. These data either challenge the necessity of increasing reactive lipids in mediating insulin resistance, or suggest tissue specificity exists with respect to which reactive lipids attenuate insulin responsiveness. With respect to this latter point, accumulation of the downstream products of ALA and LA metabolism (i.e., EPA, DHA, and AA) within tissues appears to be unique, and future research should focus on elucidating the mechanisms-of-action for this observation.

**Fig. 6.** The effect of ALA and LA diets on phospholipid fatty acid composition. Values for SFA, MUFA, and PUFA are reported in muscle (A), liver (B), and EWAT (C). Values represent means ± SE, expressed as a percentage of lean control (dotted line); n = 8 for each measure. Fatty acids were compared across the four groups by one-way ANOVA and Tukey post hoc tests. Multiple testing was accounted for by using a Bonferroni correction (i.e., P < 0.05/14 fatty acids). Similar letters indicate a lack of statistical difference between groups. Where letters are absent, significance with an ANOVA and Tukey post hoc tests was not attained.
Unfortunately, the current study does not delineate which tissue(s) contribute to the observed improvement in whole body glucose homeostasis. However, we have previously reported in these same animals that skeletal muscle insulin-stimulated Akt phosphorylation is improved following both ALA- and LA-supplemented diets, and therefore muscle is likely contributing to the observed responses (24). However, DAG was not reduced in muscle, and ALA actually increased ceramide content. Therefore, it is clear that a reduction in total DAG and ceramide content is not required for improvements in skeletal muscle insulin signaling. In addition, given the more robust accumulation of PUFAs in WAT and liver, as well as the known large contribution of the liver to postprandial glucose homeostasis (27), it is likely that these tissues also display an improved response to insulin, although this remains to be determined. Future research utilizing hyperinsulinemic euglycemic clamps with tracers are required to unravel the tissue-specific alterations in insulin sensitivity following ALA and LA supplementation to better understand the observations of the current study. Regardless of these gaps in our knowledge, the present data highlights the ability of LA and ALA to prevent the induction of glucose intolerance, and challenges the importance of reactive lipids in mediating these responses.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


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**Fig. 7.** The effect of ALA and LA diets on JNK1 (A–C) and JNK2 (D–F) phosphorylation and insulin receptor substrate 1 (IRS1) content (G–I). Values represent means ± SE, expressed as a percentage of lean control (dotted line); n = 8 for each measure. Similar letters indicate a lack of statistical difference between groups. Where letters are absent, significance with an ANOVA was not attained.
REFERENCES


