

Stearidonic-Enriched Soybean Oil Modulates Obesity, Glucose Metabolism, and Fatty Acid Profiles Independently of *Akkermansia muciniphila*

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Scope: Previous studies have suggested that diets rich in omega-3 and low in omega-6 long-chain polyunsaturated fatty acids (PUFAs) can limit the development of metabolic syndrome (MetS). Transgenic soybeans yielding oils enriched for omega-3 PUFAs represent a new and readily-available option for incorporating omega-3 PUFAs into diets to provide health benefits.

Methods and Results: Transgenic soybean oils, enriched for either stearidonic acid (SDA) or eicosapentaenoic acid (EPA), are incorporated into diets to test their effects on limiting the development of MetS in a mouse model of diet-induced obesity. Supplementation with SDA- but not EPA-enriched oils improved features of MetS compared to feeding a control wild-type oil.

Because previous studies have linked the gut microorganism *Akkermansia muciniphila* to the metabolic effects of feeding omega-3 PUFAs, the causal contribution of *A. muciniphila* to mediating the metabolic benefits provided by SDA-enriched diets is investigated. Although *A. muciniphila* is not required for SDA-induced metabolic improvements, this microorganism does modulate levels of saturated and mono-unsaturated fatty acids in host adipose tissues.

Conclusion: Together, these findings support the utilization of SDA-enriched diets to modulate weight gain, glucose metabolism, and fatty acid profiles of liver and adipose tissue.

1. Introduction

The prevalence of obesity and obesity-related comorbidities, including metabolic syndrome (MetS), continues to increase world-wide.^[1] Although nutritional interventions are routinely recommended to promote weight loss and control blood glucose levels, some individuals experience benefits from these therapeutic approaches while others do not.^[2,3,4] Consequently, there is no consensus on which interventions are most effective. However, diets rich in long-chain polyunsaturated fatty acids (PUFAs) with unsaturation at the omega-3 carbon (n-3) have been associated with a low incidence of MetS in certain populations.^[5-7,8] The mechanisms underlying the metabolic benefits provided by n-3 PUFAs are not well understood but may include decreases in adipose tissue mass and inflammation^[9] as well as modulation of the gut microbiome.^[10,11] For example, feeding mice a high fat (HF) diet rich in n-3 PUFAs from fish

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oil limited the development of MetS compared to feeding a HF diet containing lard and also increased the abundance of *Akkermansia muciniphila*, a gut microorganism linked to improved metabolic health.^[11] Altogether, these findings support the development of novel foods and dietary interventions with higher n-3 and lower omega-6 (n-6) PUFA levels to prevent or treat MetS.

Fish oil is currently the major source of n-3 PUFAs incorporated into foods, but meeting future global demand using wild fisheries will be challenging.^[12,13] Terrestrial-based sources hold great potential to meet the future demand for oils rich in n-3 PUFAs in a sustainable fashion. For example, soybeans (*Glycine max*) are widely grown around the world, provide the majority of vegetable oil for food applications and can be genetically enhanced to produce oils high in n-3 PUFAs.^[14] Indeed, multiple transgenic soybean events have already been developed that yield oils with greater n-3 and lower n-6 PUFA levels compared to oils from wild-type (WT) soybeans.^[15–19] However, studies conducted to date on n-3 enriched soybean oils have shown inconsistent effects on host metabolism, perhaps because the levels of n-3 and n-6 PUFAs are insufficient to provide the desired benefits.^[15–19] Research efforts have also led to the development of soybeans with higher levels of the n-3 PUFAs alpha-linoleic acid (ALA), stearidonic (SDA), and eicosapentaenoic (EPA) acid and lower levels of the n-6 PUFA linoleic acid (LA) compared to WT or previously described transgenic soybean oils.^[14,20] These new soybean oils have been used as a feedstock for aquaculture and have successfully increased n-3 PUFA content in fish flesh compared to feeding a WT commodity soybean oil.^[20] Considering that foods containing SDA may provide metabolic benefits by increasing EPA levels in host tissue,^[21] we hypothesized that novel soybean oils enriched in SDA or EPA could limit the development of MetS.

In this study, two transgenic soybean oils were fed to mice to test their ability to limit the development of MetS in a mouse model of diet-induced obesity. Both oils contained lower levels of n-6 PUFAs (14.2%) and higher levels of ALA (11.0%) compared to the WT oil. However, one transgenic oil contained higher levels of SDA (21.7%) compared to the WT oil (and is referred to as “SDA-enriched oil”). The other transgenic soybean oil tested contained higher SDA levels (16.3%) plus EPA (5.43%) compared to the WT oil (and is referred to as “EPA-enriched oil”). HF diets supplemented with SDA- but not EPA-enriched soybean oil improved features of MetS compared to feeding a HF diet supplemented with a WT control soybean oil. Because previous studies have linked *A. muciniphila* to the metabolic effects of feeding n-3 PUFAs, we further investigated the causal contribution of *A. muciniphila* to mediating the metabolic benefits provided by feeding an SDA-enriched HF diet.

2. Results

2.1. Supplementation with Soybean Oil Enriched in SDA but not EPA Reduced Body Weight Gain and Fat Mass

Because studies have suggested that foods containing SDA or EPA may improve features of MetS,^[22,23] we investigated the physiological and metabolic effects of soybean oils enriched in these n-3 fatty acids in mice fed HF diets. Feeding a HF diet con-

taining 1.3% (w/w) n-3 PUFAs from SDA-enriched soybean oil to mice for eight weeks limited body weight gain and fat mass deposition compared to a HF diet supplemented with the WT soybean oil (Figure 1). In contrast, a HF diet containing 1.3% (w/w) n-3 PUFAs from soybean oil enriched in both SDA plus EPA did not affect body weight gain or fat mass deposition. Importantly, no differences in food consumption were observed among any of the test diets (Figure S3D–E, Supporting Information).

Hierarchical interactions in mice based on body weight have been shown to influence their food consumption and growth.^[24–26] We therefore utilized body weight differences between animals in a cage as a metric to stratify individuals into dominant or subordinated mice (see Section 4 for details). Stratification of the data revealed decreases in body weight gain and adipose tissue deposition upon feeding an SDA-enriched HF diet and were primarily observed in dominant but not subordinated mice (Figure 1). Feeding diets containing 1.3% n-3 PUFAs from SDA- but not EPA-enriched soybean oil decreased fasting glucose (Figure S4A–C, Supporting Information). However, feeding these oils had no significant effect on plasma concentrations of insulin or leptin (Figure S4D–I, Supporting Information). Together, these data demonstrate that feeding HF diets supplemented with a soybean oil enriched in SDA but not EPA can limit body weight gain and fat mass accretion.

2.2. Consumption of SDA- and EPA-containing HF Diets did not Alter Gut Microbiome Composition

The inclusion of n-3 PUFAs in the diet may provide metabolic benefits via modulation of the gut microbiota.^[27] Here, we investigated whether the observed effects on body weight and fat mass deposition during feeding of SDA- or EPA-enriched diets were accompanied by changes in global or specific features of the gut microbiome. Microbiome analysis after eight weeks of feeding the test diets revealed that neither a HF diet supplemented with SDA- or EPA-enriched oils induced notable changes in the microbiome composition compared to feeding a HF control diet (Figure S5, Supporting Information). These results indicate that the effects of feeding an SDA-enriched HF diet on body weight gain and fat mass are not related to changes in gut microbiome composition.

2.3. Metabolic Benefits from Feeding Diets Containing an SDA-Enriched Soybean Oil Occurred Independently of *A. muciniphila*

Caesar et. al. previously showed that feeding a diet high in n-3 PUFA content to mice prevented the development of MetS and increased the abundance of *A. muciniphila*.^[11] In our first feeding study, all mice were ordered from a commercial vendor and confirmed by both 16S rRNA gene sequencing and species-specific qPCR to not harbor *A. muciniphila* (Figures S5A, Supporting Information). These results therefore suggested that the effects of an SDA-enriched HF diet on body and fat mass arose independently of *A. muciniphila*. Considering the work by Caesar et. al. as an example for potential synergistic metabolic interactions between a gut microorganism such as *A. muciniphila* and

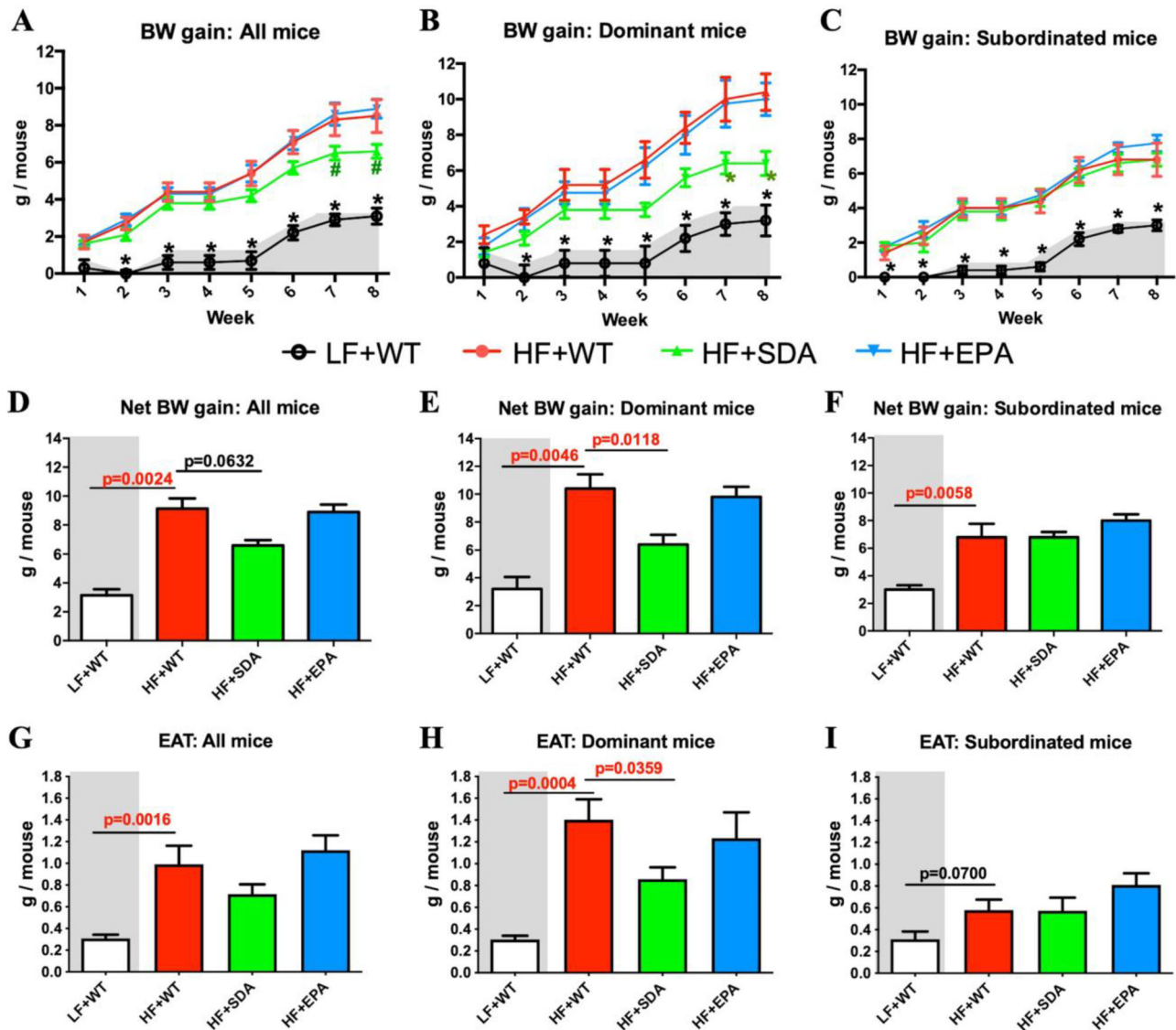


Figure 1. Feeding an SDA-enriched but not an EPA-enriched high fat diet limited body weight gain and fat mass deposition. Body weight (BW) gained during the study for A) all mice, B) dominant mice, and C) subordinated mice. Black * indicates significant differences between low fat (LF) versus high fat (HF) treatments. Green * indicates significant differences between HF+WT versus HF+SDA; # represents significance of SDA X Time. Shaded area corresponds to LF diet control. Net body weight gain of mice after eight weeks of HF diet feeding in D) all mice, E) dominant mice, and F) subordinated mice. Epididymal adipose tissue (EAT) mass in G) all mice, H) dominant mice, and I) subordinated mice. For bar graphs, *p*-values are shown for specific pairwise comparisons. Significant *p*-values (<0.05) are shown in red. Marginal *p*-values (0.05–0.07) are shown in black.

diets high in n-3 PUFAs such as our SDA-enriched HF diet, we conventionalized germ-free C57BL/6 mice with an *Akkermansia*-free complex mouse microbiome previously described by Martinez et. al.^[28] Mice were then either colonized with or without *A. muciniphila* and fed HF diets supplemented with either WT or SDA-enriched soybean oil (Figure S6A, Supporting Information). The dietary n-3 PUFAs from SDA-enriched soybean oil were increased from 1.3% to 3.2% (w/w) to increase the resolution of SDA-mediated metabolic effects in the second feeding study.

Consistent with our first feeding study, mice fed a 3.2% SDA-enriched HF diet for 12 weeks gained less body weight and deposited less fat compared to mice fed a HF diet containing WT

soybean oil (Figure 2). These physiological improvements from feeding an SDA-enriched diet were primarily present among dominant mice. No changes in food consumption were observed among treatments (Figure S6B–C, Supporting Information). Also, plasma leptin levels (but not other satiety hormones) were reduced in mice colonized with *A. muciniphila* and fed an SDA-enriched diet compared to all other HF diet fed mice (Figure S7, Supporting Information).

A. muciniphila colonization of mice receiving the HF diet supplemented with WT soybean oil reduced blood glucose levels compared to control mice (Figure 3A–C, Supporting Information), a finding consistent with other reports in the literature.^[29] Feeding a 3.2% SDA-enriched HF diet reduced glycemia levels

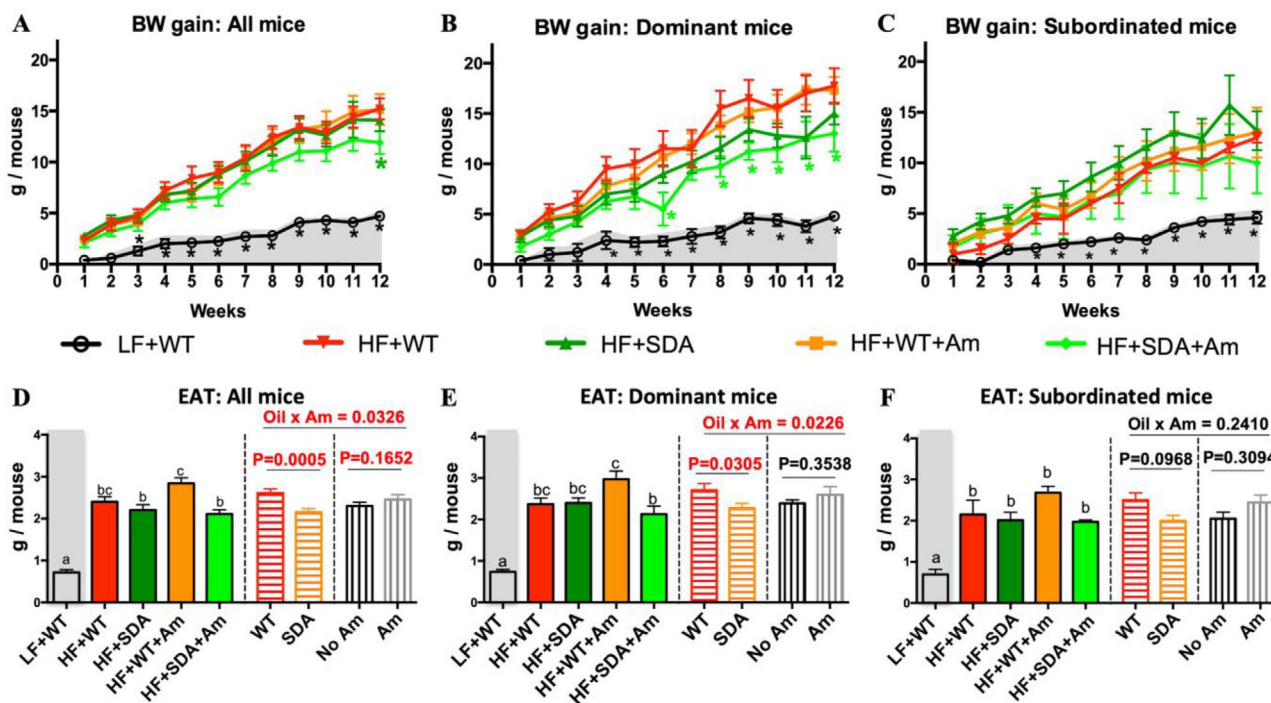


Figure 2. An SDA-enriched diet and *A. muciniphila* colonization limited body weight gain and fat mass deposition. Body weight (BW) gained during the study for A) all mice, B) dominant mice, and C) subordinated mice. Black * indicates significant differences between low fat (LF) versus high fat (HF) treatments. Green * indicates significant differences between HF+WT versus HF+SDA. Shaded area corresponds to LF diet control. Epididymal adipose tissue (EAT) mass in D) all mice, E) dominant mice, and F) subordinated mice. Treatments with different letters are significantly different from one another by Tukey Test. Factorial analysis was performed only with HF treatments by Two-Way ANOVA. *p*-values from this analysis are presented for main effects and interactions (Oil X Am). Significant *p*-values (<0.05) are shown in red. Non-significant *p*-values are shown in black.

in all mice regardless of whether they were colonized with or without *A. muciniphila*, indicating that SDA supplementation did not provide additional metabolic benefits in the presence of *A. muciniphila*. All mice fed an SDA-enriched diet regardless of *A. muciniphila* colonization status also had lower plasma levels of insulin-associated hormones, but these effects were primarily present in dominant mice (Figure 3D–I, Supporting Information). Together, these data indicate that SDA-mediated improvements in glucose and insulin metabolism can occur independently of *A. muciniphila*.

Mice fed an SDA-enriched HF diet also had lower plasma levels of the pro-inflammatory cytokine TNF- α and adipose tissue expression of the chemokine CCL2 (Figure 4). These effects were mainly present in dominant mice (Figure 4B,E,H). A significant main effect of *A. muciniphila* colonization on plasma resistin levels was also observed (Figure 4I). However, contrary to a previous report,^[11] no significant correlations between *A. muciniphila* abundance and plasma levels of TNF- α or adipose tissue expression of CCL2 were found (Figure S8, Supporting Information). Moreover, microbiome composition was not notably altered by SDA or *A. muciniphila* presence (Figure S9, Supporting Information). Altogether, these results suggest that the presence of *A. muciniphila* is not required for an SDA-enriched HF diet to limit the development of metabolic inflammation.

Early feeding trials conducted with high oleic acid soybean oil (Plenish) described improved features of MetS in mice that were associated with liver dysfunction.^[18] We therefore measured hepatic gluconeogenesis and fatty acid oxidation in all

treated mice. No alterations in hepatic functions of gluconeogenesis or fatty acid oxidation were observed in mice fed SDA- or EPA-enriched soybean oil as determined by gene expression of glucose-6-phosphatase (G6Pase; Figure S10A–C, Supporting Information) and carnitine palmitoyltransferase I alpha (CPT1 α ; Figure S10D–F, Supporting Information), respectively. These results suggest that feeding an SDA-enriched HF diet to mice did not alter hepatic gluconeogenesis and fatty acid beta-oxidation.

2.4. Mice fed an SDA-Enriched Diet Incorporated More n-3 and Less n-6 PUFAs into Adipose Tissue and Liver

Metabolic improvements following SDA consumption have been linked to changes in fatty acid profiles in host tissues.^[21] We therefore determined whether the metabolic benefits derived from an SDA-enriched diet were associated with changes in the fatty acid profile of adipose tissue and liver. All mice fed an SDA-enriched HF diet showed distinct fatty acid profiles in epididymal adipose tissue (EAT) featuring increased levels of SDA and ALA as well as decreased amounts of LA (Figure 5). Incorporation of n-3 PUFAs in mice fed an SDA-enriched diet was consistent for all mice and found to be independent of dominance effects or *A. muciniphila* presence (Figure S11, Supporting Information). Similarly, specific increases in EPA and docosahexaenoic acid as well as reduced levels of LA and arachidonic acid were observed in livers of mice fed an SDA-enriched HF diet (Figure S12, Supporting Information). Altogether, these findings indicate that feeding

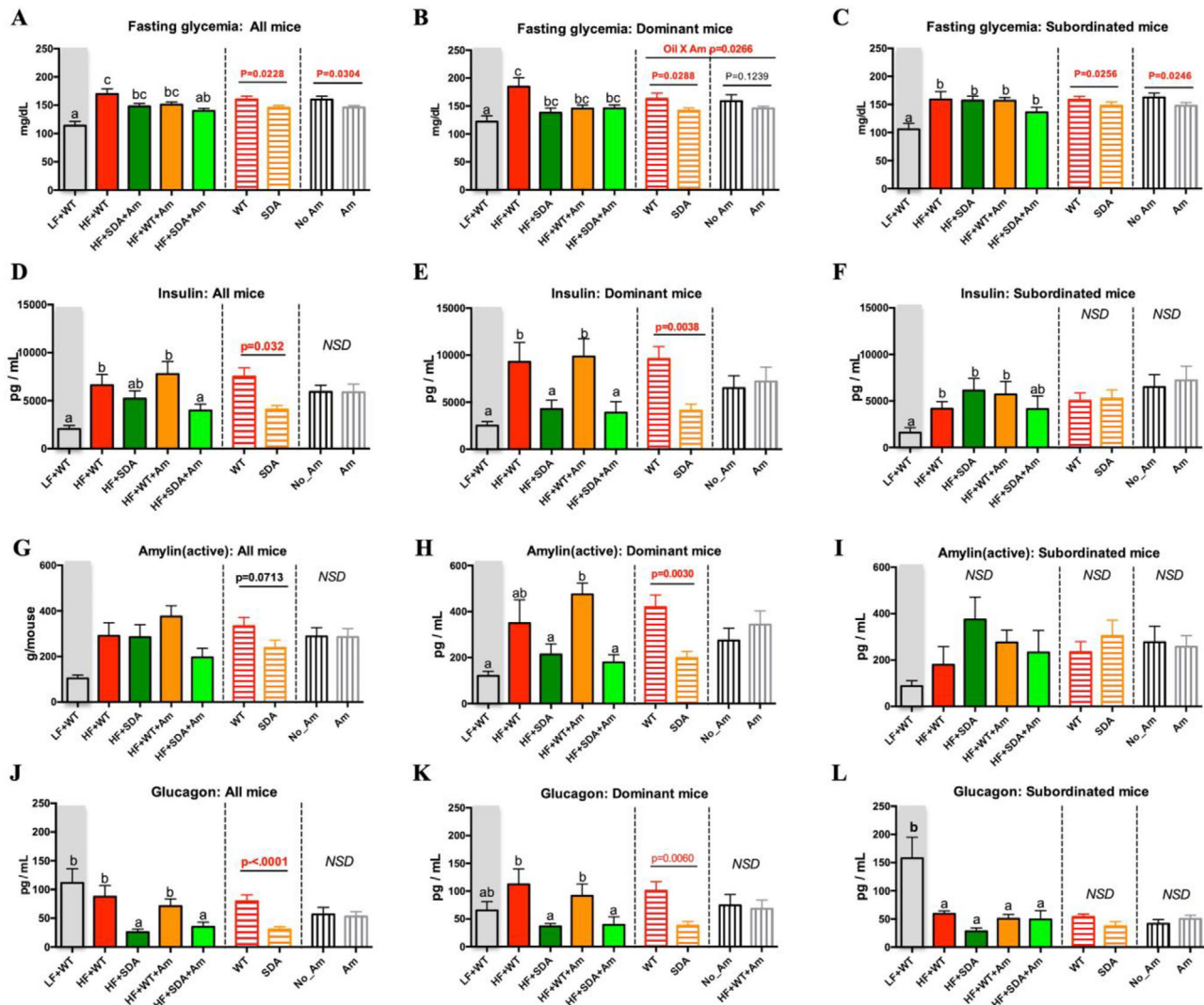


Figure 3. Metabolic benefits from feeding diets containing an SDA-enriched soybean oil occurred independently of *A. muciniphila*. Fasting plasma glycemia measured at week 11 in A) all mice, B) dominant mice, and C) subordinated mice. Fasting plasma insulin measured at necropsy in D) all mice, E) dominant mice, and F) subordinated mice. Fasting plasma active amylin measured at necropsy in G) all mice, H) dominant mice, and I) subordinated mice. Fasting plasma glucagon measured at necropsy in J) all mice, K) dominant mice, and L) subordinated mice. Treatments with different letters are significantly different from one another by Tukey Test. Shaded area corresponds to low fat (LF) diet control. Factorial analysis was only performed with high fat (HF) diet-fed treatments by Two-Way ANOVA. p -values from this analysis are presented for main effects and interactions (Oil X Am). Only significant interactions are shown. Significant p -values (<0.05) are shown in red. Marginal p -values (0.05–0.07) are shown in black. NSD means no significant main effects were observed.

an SDA-enriched HF diet increased incorporation of n-3 PUFAs into host adipose tissue and liver independently of *A. muciniphila* colonization.

2.5. *A. muciniphila* and an SDA-Enriched Diet Influenced Levels of SFAs and Monounsaturated Fatty Acids in Adipose Tissue

Saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) in tissues have been linked to metabolic benefits;^[30] we therefore determined MUFA levels in the adipose tissue and liver of all mice. EAT levels of palmitoleic (C16:1n7) and oleic (C18:1n9) acid were significantly lower in mice colonized with *A.*

muciniphila (Figure 6). Moreover, percentages of palmitic (16:0) but not stearic (C18:0) acid in the EAT were higher in mice colonized with *A. muciniphila* and fed an SDA-enriched HF diet compared to all other treatments (Figure S13A–C, Supporting Information). However, levels of stearic acid in the EAT were significantly influenced by the interaction between oil type and *A. muciniphila* presence (Figure S13D–F, Supporting Information). Hence, mice harboring *A. muciniphila* experienced changes in adipose tissue profiles of SFAs and MUFAs during SDA feeding.

Because SFA and MUFA profiles may be influenced by desaturation and elongation,^[16] we next assessed such parameters in adipose tissue and liver of all treated mice. We observed a reduction in the C16:1/C16:0 ratio in mice harboring

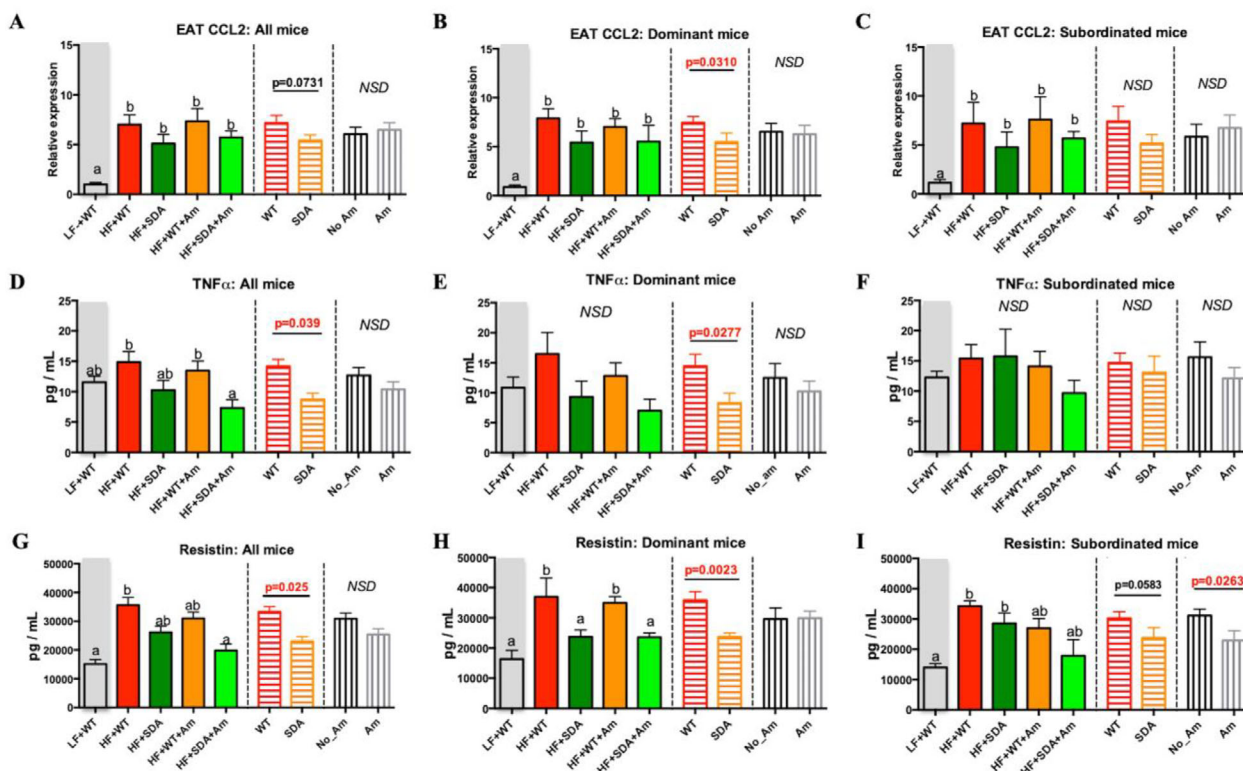


Figure 4. Feeding an SDA-enriched diet reduced plasma TNF- α levels and CCL2 gene expression levels in adipose tissue. Relative expression of CCL2 in epididymal adipose tissue (EAT) from A) all mice, B) dominant mice, and C) subordinated mice. Fasting plasma TNF- α levels measured at necropsy in D) all mice, E) dominant mice, and F) subordinated mice. Fasting plasma resistin levels measured at necropsy in G) all mice, H) dominant mice, and I) subordinated mice. Treatments with different letters are significantly different from one another by Tukey Test. Shaded area corresponds to low fat (LF) diet control. Factorial analysis was only performed with high fat (HF) diet-fed treatments by Two-Way ANOVA. p -values from this analysis are presented for main effects and interactions (Oil X Am). Only significant interactions are shown. Significant p -values (<0.05) are shown in red. Marginal p -values (0.05–0.07) are shown in black. NSD means no significant main effects were observed.

A. muciniphila compared to mice without this gut microorganism (Figure S13G–I, Supporting Information), which may indicate lower desaturation by the enzyme stearoyl-CoA desaturase-1 (SCD-1).^[16] However, colonization with *A. muciniphila* and feeding an SDA-enriched diet increased SCD-1 expression (Figure S14A–C, Supporting Information). Moreover, no significant correlations between C16 and the longer C18 fatty acids were found (Figure S14D–E, Supporting Information). Hence, the different profiles of SFAs and MUFAs in the EAT of mice with and without *A. muciniphila* were not related to desaturation or elongation processes.

In contrast to our observation in the EAT, *A. muciniphila* presence did not significantly affect levels of SFAs and MUFAs in the liver (Figure S15, Supporting Information). Nonetheless, mice fed an SDA-enriched HF diet did experience increased hepatic levels of palmitic acid compared to mice fed a HF diet with WT soybean oil (Figure S15G–I, Supporting Information). This observation is consistent with the lower hepatic expression of SCD-1 in the liver of mice fed SDA-enriched diet (Figure S10G–I, Supporting Information). Altogether, these results show that feeding an SDA-enriched HF diet to mice can influence hepatic levels of SFAs, MUFAs and their desaturation by SCD-1.

3. Discussion

Previous studies have suggested that diets rich in n-3 and low in n-6 PUFAs can prevent the development of MetS.^[7,8] Soybeans that yield oils enriched in n-3 PUFA and low in n-6 PUFA levels represent a readily-available option for incorporating n-3 PUFAs into the diet to provide health benefits. However, conflicting reports make it unclear whether transgenic soybean oils with such fatty acid profiles can mitigate MetS.^[15–17] In this study, we incorporated soybean oils enriched in n-3 PUFAs into test diets to systematically assess their ability to improve features of MetS in mice. We found that feeding HF diets with 1.3% n-3 PUFAs from SDA- but not from EPA-enriched soybean oil improved body weight and fat mass compared to mice fed a HF diet supplemented with WT soybean oil. Moreover, feeding a HF diet supplemented with 3.2% n-3 PUFAs from SDA-enriched soybean oil improved glucose metabolism, inflammation and PUFA profiles in adipose tissue and liver, and such changes did not require the presence of the gut microorganism *A. muciniphila*. However, *A. muciniphila* did modulate SFA and MUFA levels in host adipose tissues. Altogether, these observations suggest that SDA-enriched soybean oil can be utilized in dietary strategies to limit obesity and its metabolic comorbidities.

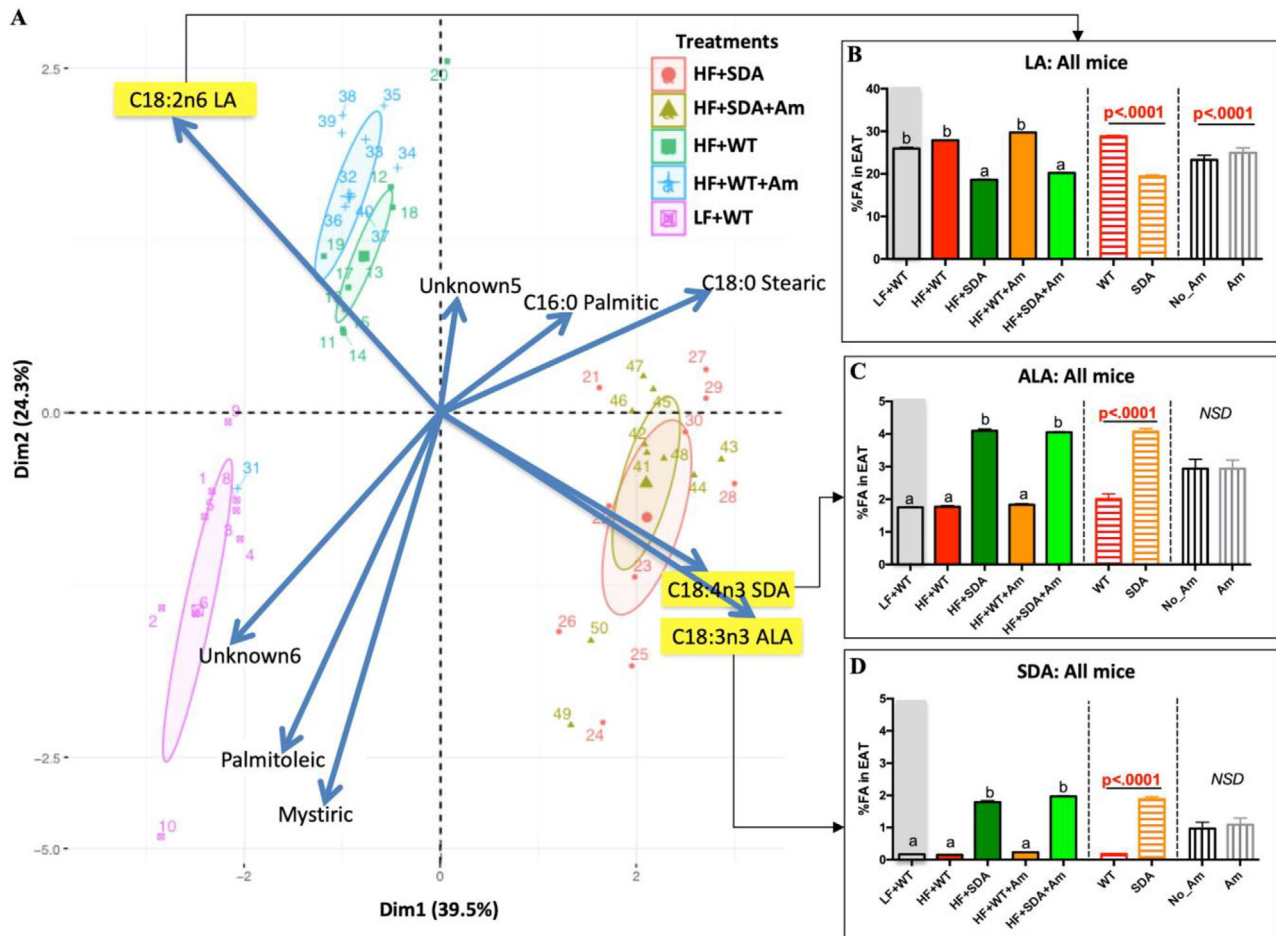


Figure 5. Mice fed an SDA-enriched diet incorporated more n-3 and less n-6 PUFAs into adipose tissue. A) principal component analysis of fatty acid profiles. Arrows are eigenvectors depicting direction towards which a specific fatty acid drives differences among treatments. Ovals represent the 95% confidence interval for data in each treatment. Specific levels of B) linoleic, C) alpha-linolenic, and D) stearidonic acid in epididymal adipose tissue (EAT). Treatments with different letters are significantly different from one another by Tukey Test. Shaded area corresponds to low fat (LF) diet control. Factorial analysis was only performed with high fat (HF) diet-fed treatments by Two-Way ANOVA. p -values from this analysis are presented for main effects and interactions (Oil X Am). Only significant interactions are shown. Significant p -values (<0.05) are shown in red. Marginal p -values (0.05 – 0.07) are shown in black. NSD means no significant main effects were observed.

Traditionally, dietary formulations aimed at attenuating MetS have focused on maintaining low n-3:n-6 PUFA ratios between 1:1 to 1:4.^[31,32] However, Enos et. al. demonstrated that creating those ratios by increasing ALA in a HF diet was not sufficient to attenuate MetS in mice.^[33] Here, we demonstrated that SDA- but not EPA-enriched soybean oil improved body weight and fat mass despite the fact that both oils contained similar levels of ALA that were greater than those in the WT oil. These findings support the idea that changing the dietary n-3:n-6 PUFA ratio or increasing ALA alone is insufficient to limit MetS, and that the source of the PUFA must also be considered. One potential explanation for this observation may be that host conversion of ALA into the longer n-3 PUFAs SDA and EPA is highly inefficient compared to the conversion of SDA into EPA.^[34] Although the EPA-enriched soybean oil also contained SDA, the levels were lower than those present in the SDA-enriched soybean oil and may not have been sufficient to affect weight gain and metabolic parameters. Another possible explanation for why SDA- but not EPA-enriched oil limited the development of MetS is the higher unsaturation

of EPA compared to SDA, which may make EPA more easily oxidized prior to intestinal absorption.^[35,36] Although the exact reason for these differential responses is not yet clear, our findings do indicate that SDA- but not EPA-enriched soybean oil may be incorporated into diets to limit the development of MetS.

We also observed that feeding mice a HF diet supplemented with SDA-enriched soybean oil provided benefits to metabolism, inflammation and PUFA profiles in adipose tissue and liver independently of changes in the fecal microbiome or the presence of *A. muciniphila*. Our observations differ from those of Caesar et. al. who reported that protection against MetS when mice were fed a HF diet containing fish oil was associated with increased *A. muciniphila* levels and major changes in fecal microbiome composition.^[11] Differences in microbiome effects between these two studies may be due to the amounts of n-3 PUFAs in the diets. In Caesar et. al., total replacement of lard in a HF diet by fish oil likely created higher levels and different types of n-3 PUFAs compared to our lard-based HF diet supplemented with a genetically enhanced soybean oil. Although most dietary

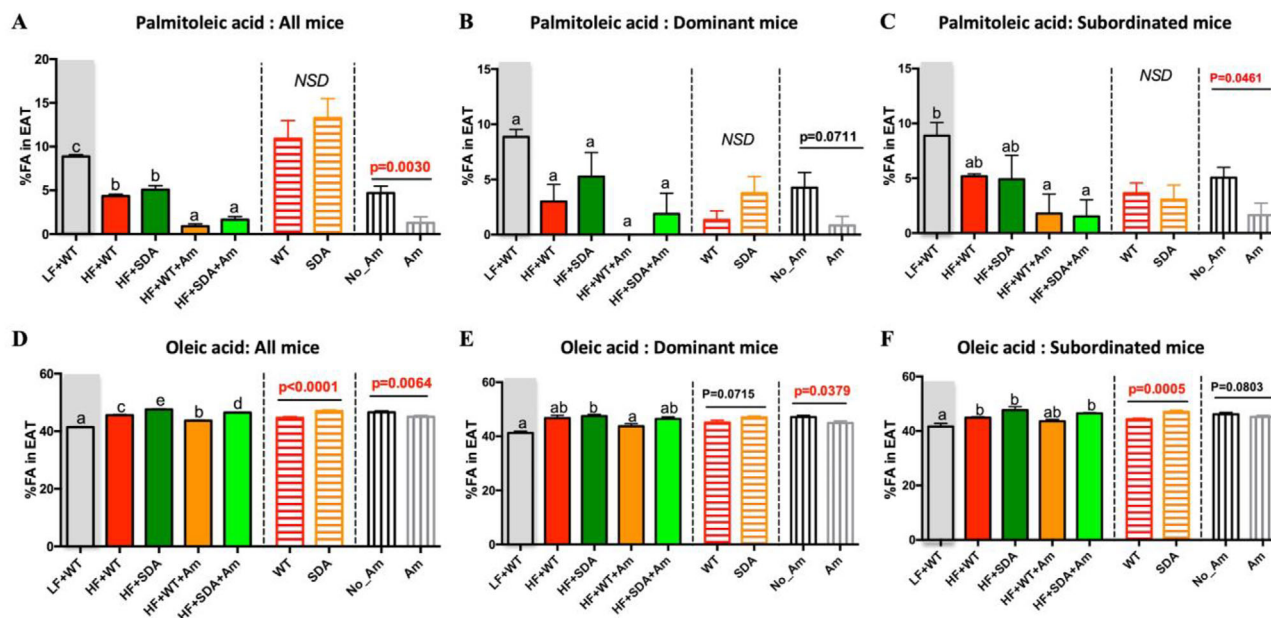


Figure 6. A. *muciniphila* reduced levels of MUFAs in adipose tissue. Palmitic acid in epididymal adipose tissue (EAT) from A) all mice, B) dominant mice, and C) subordinated mice. Oleic acid in EAT from D) all mice, E) dominant mice, and F) subordinated mice. Treatments with different letters are significantly different from one another by Tukey Test. Shaded area corresponds to low fat (LF) diet control. Factorial analysis was only performed with high fat (HF) diet-fed treatments by Two-Way ANOVA. *p*-values from this analysis are presented for main effects and interactions (Oil X Am). Only significant interactions are shown. Significant *p*-values (<0.05) are shown in red. Marginal *p*-values (0.05–0.07) are shown in black. NSD means no significant main effects were observed. All values are presented as percentages of all fatty acids.

fatty acids are primarily absorbed in the small intestine,^[37] the diet formulation in Caesar et. al. may have been sufficient to allow some n-3 PUFAs to pass through to the large intestine and subsequently influence the fecal microbiome. Further studies are clearly needed to identify the exact effects of specific PUFAs and their quantities on the microbiome.

A notable finding from our present study is that supplementation with a genetically enhanced soybean oil limited the development of MetS without negatively affecting liver functions. This result stands in contrast to a previous report in which liver functions were impaired in mice fed for 23 weeks with a HF diet supplemented with either olive oil or soybean event Plenish, both of which are high in oleic acid.^[18] It is possible that we did not observe liver dysfunction because our feeding study was only 12 weeks in duration. Another explanation may involve differences in oil composition. For example, feeding olive oil has generated conflicting results concerning alterations in liver metabolism,^[38] whereas studies feeding extra virgin olive oil consistently report protection against liver disease.^[39] Although the exact mechanisms are currently unknown, results from our study suggest that an SDA-enriched vegetable oil, exemplified by the soybean oil described in this study, represents a promising alternative to limit the development of MetS while preserving hepatic functions.

Importantly, we observed that mice colonized with *A. muciniphila* and fed an SDA-enriched HF diet experienced increased deposition of SFA and decreased accumulation of MUFA in adipose tissue compared to control mice. *A. muciniphila* has been shown to provide multiple metabolic benefits in mouse models of diet-induced obesity via mechanisms that are yet to be defined.^[29] One possible mechanism underlying these observed

metabolic benefits is that *A. muciniphila* directly produces SFAs that are then incorporated into adipose tissue. Previous reports have described that *Bifidobacterium* species and other gut bacteria are capable of generating conjugated LA and SFAs.^[37,40,41] The observed reduction in the levels of the MUFAs oleic and palmitoleic acid in the adipose tissue of mice harboring *A. muciniphila* did not correlate with changes in desaturation or elongation processes, suggesting that *A. muciniphila* decreased MUFA levels by an alternative pathway. Because the presence of *A. muciniphila* reduced fasting glucose in this and other studies,^[42,30] it is possible that *A. muciniphila* improves glucose metabolism via a mechanism that includes modulation of SFA and MUFA levels in adipose tissue.

In conclusion, this study demonstrates that dietary supplementation with genetically enhanced soybean oils enriched in SDA can prevent the development of MetS without negatively affecting hepatic function. These benefits occurred independently of changes in the microbiome and did not require the presence of the gut microbe *A. muciniphila*. However, colonization with *A. muciniphila* did alter adipose tissue levels of SFA and MUFA. Altogether, these findings support the use of SDA-enriched diets to limit the development of MetS and to modulate SFA and MUFA profiles in adipose tissue.

4. Experimental Section

Animals, Diets, and Bacteria: All “Thorne” (genetic background of the transgenic events)^[43] wild-type and genetically enhanced lineages of soybeans (*Glycine max* (L.) Merr) were created and grown as part of the University of Nebraska’s soybean biotechnology program. The fatty acid

profiles of the selected transgenic events utilized in this study are shown in Table S1, Supporting Information. Oils were expelled from seeds (REF. 645.7737, AgOilPress) at the UNL Food Processing Center and shipped to Research Diets Inc. (New Brunswick, NJ) for incorporation into rodent diets. D12450K (10% Kcal Fat) was fed as a low-fat control diet modified to contain the wild type soybean oil (LF-WT). The soybean oil in diet D12451 (45% Kcal Fat) was replaced by either wild type (HF-WT), stearidonic-enriched (HF+SDA), or eicosapentaenoic acid-enriched (HF+EPA) soybean oils.

For the first feeding study, eight-week-old male C57BL/6J mice were purchased from The Jackson Laboratories (Bar Harbor, ME) and acclimated for three days on a chow diet (LabDiet 5K67, Purina Foods, St. Louis, MO) prior to the introduction of test diets, which were fed for eight weeks. For the second feeding study, six-to-eight-week-old germ-free male C57BL/6 mice were raised at the University of Nebraska-Lincoln in gnotobiotic isolators as previously described.^[44] Germ-free mice were transferred from isolators to individually-ventilated cages, housed two to three mice per cage, fed a chow diet (LabDiet 5K67) and conventionalized via oral gavage with 100 μ L each of a conventional mouse microbiota diluted at 1:10 (w/v; grams of ceca per milliliters of PBS plus 10% glycerol) as previously described.^[44] This conventional mouse microbiome was described previously by Martinez et. al.,^[45] and was tested by qPCR for the absence of *A. muciniphila* as previously described.^[28] One week later, mice were orally gavaged with either 1×10^8 CFU *A. muciniphila* BAA-835 or PBS in a 100 μ L volume. One week after *A. muciniphila* was administered, mice were introduced to the test diets and fed for 12 weeks. The Institutional Animal Care and Use Committee at UNL approved all procedures involving animals (protocols 817 and 1215).

Dosage Information: Compositions of the soybean oils tested in this study differed in SDA and EPA content but not in the abundance of other fatty acids (Table S1, Supporting Information). The recommended amount of n-3 PUFAs for human clinical trials is at least 1% (w/w).^[42] In the first study, a purified HF diet (with lard as the main source of fat) was modified to contain 1.3% (w/w) of n-3 PUFAs from either transgenic or WT soybean oil (Tables S2–S3, Supporting Information). Specific fatty acid profiles of the formulated diets are shown in Table S3, Supporting Information. To increase the resolution of SDA-mediated metabolic effects in the second study, HF diets were supplemented with 3.2% (w/w) n-3 PUFAs from soybean oil (Tables S4–S6, Supporting Information). This amount corresponded to two times the caloric content of soybean oil used in the first study. In all feeding studies, 200 ppm each of citric acid and tert-butylhydroquinone were added to the oils to prevent oxidation prior to their incorporation into diets. Test diets were replaced two times per week during all feeding studies.

Determining Dominance in Mice: Previous studies have suggested that hierarchical interactions in mice are based on body weight and can influence their development and food consumption.^[24–26] Consequently, body weight differences between animals in a cage were used as a metric to determine patterns of dominance and subordination. The heaviest mouse in a cage was identified at 5 time points, over the first 7 weeks of feeding, for each trial and designated as the dominant mouse (Figure S1, Supporting Information). Employing this approach failed to clearly identify dominance in three out of the 53 cages; mice in these three cages were therefore not included when stratifying results by dominance. Previous studies have utilized plasma levels of testosterone, progesterone and cortisol for defining dominance in mice.^[24,25] In the studies, these hormones were not significantly correlated with the body weight of the mice ($p < 0.05$, Figure S2, Supporting Information). In fact, these hormones failed to identify prominent differences between mouse weights (e.g., cages 6 and 7, Figure S1, Supporting Information). This observation is consistent with recent studies showing that hormone levels are influenced by factors other than dominance (e.g., despotic social interactions).^[46–48] In addition, no significant differences were observed in the initial body weight when mice were classified as either dominant or subordinated (Figure S3A–C, Supporting Information). Together, these data indicate that body weight can be used to represent dominance of mice in cages. Hence, all data was stratified and analyses conducted using dominance-based body weights; results are presented in

three formats: for all mice together (ten mice per treatment), for the mice classified as dominant (4–5 per treatment), and for the mice classified as subordinated (5–6 mice per treatment).

Blood Analyses: Mice were fasted for 6 h at the start of the daily light cycle one week prior to necropsy. Glucose sampling was performed from tail blood using a glucometer (Aviva Accu-check, Roche Diagnostics, Indianapolis, IN), and tail blood samples were collected for use in an insulin ELISA assay kit (Mercodia, Uppsala, Sweden) according to manufacturer specifications. On necropsy day, cardiac blood from 6 h fasted mice was collected and later analyzed for gut hormones and gut peptides using a mouse metabolic magnetic bead panel (Milliplex, Millipore, Billerica, MA).

RNA Expression, Microbiome, and Fatty Acid Analyses: Experimental procedures and analyses for RNA isolation, RT-PCR, bacterial 16S rRNA gene sequencing, and fatty acid profiles are described in Supporting Information.

Statistical Analyses: In the first feeding study, p -values are shown for specific pairwise comparisons. Significant p -values (<0.05) are shown in red. Marginal p -values (0.05–0.07) are shown in black. In the second feeding study, factorial analysis was performed only with HF treatments by Two-Way ANOVA. The factors analyzed included oil type (WT versus SDA) and *A. muciniphila* presence/absence (Am versus No Am). p -values from this analysis are presented for main effects of each factor individually and for interactions (Oil X Am). Significant p -values (<0.05) are shown in red. Non-significant p -values are shown in black. In all experiments, data were assessed for outliers using a Grubbs test and are presented as mean $\pm$ SEM. Treatments with different letters are significantly different from one another by Tukey Test. Weight gain was analyzed by two-way ANOVA repeated measures.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

Akkermansia muciniphila, metabolic syndrome, polyunsaturated fatty acids, soybean oil

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