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# Effect of Trans Fatty Acid on Insulin Responsiveness and Fatty Acid Composition of Lipid Species of 3T3-L1 Adipocytes

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Kenichi Ishibashi, Yoshihiro Takeda and Gen-ichi Atsumi

Additional information is available at the end of the chapter

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## Abstract

Trans fatty acids (TFAs) have at least one non-conjugate double bond in trans and TFAs are divided into two groups such as naturally or industry-occurring groups. Recent studies reveal that intake of industrial TFA is linked to increased risk of cardiovascular disease. Moreover, several studies suggest that intake of industrial TFA increases risk of diabetes, although other studies show that intake of industrial TFA is not associated with diabetes. Therefore, we used adipocytes which play important roles in glucose metabolism and development of diabetes, and our previous study showed that persistent exposure to elaidate, a major industrial TFA, impairs insulin-dependent glucose uptake of adipocytes. Since phospholipid acts as a scaffold for molecules of insulin signaling, we analyzed intracellular distribution of elaidate and fatty acid composition of lipid species. Incorporated elaidate is esterified into triglyceride and phospholipid. Moreover, elaidate-esterified phospholipids are distributed in various organelles. Intriguingly, persistent exposure to elaidate reduces the amount of oleate in phospholipid of mitochondria and plasma membrane and disturbs the equilibrium between bent and linear-shaped chain fatty acid. Therefore, disturbed equilibrium of fatty acid composition of phospholipid should be considered to elucidate the mechanism for impaired insulin responsiveness of adipocytes exposed to elaidate.

**Keywords:** elaidate, trans fat, insulin responsiveness, phospholipids

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## 1. Introduction

### 1.1. Intake of trans fatty acids from daily diet

Trans fatty acids (TFAs) are defined as unsaturated fatty acids which have at least one non-conjugated double bond in the trans form [1] and are divided into naturally or industry-occurring group. A major naturally occurring TFA is vaccenic acid (*t*11-18:1), and it is produced by gastric bacteria of ruminants, for example, cow and goat [2]. Since meat and milk of ruminants contain vaccenic acid [3], we intake vaccenic acid from daily diet and vaccenic acid is contained in human blood [4]. On the other hand, a major industry occurring TFA is elaidate (*t*9-18:1), which is an artificially produced cis form of unsaturated fatty acids during hydrogenation of vegetable oil and food frying [5]. Therefore, we intake elaidate from shortening, margarine and fried food such as fried potato, snack food and instant noodles [6, 7]. It is reported that elaidate as well as vaccenic acid is contained in our blood at approximately 10–30  $\mu\text{M}$  [4, 8].

### 1.2. Relationship between intake of industrial trans fatty acids and risk of cardiovascular disease and governmental regulation of dietary intake of trans fatty acids

It has been revealed that intake of TFAs-rich diet reduces HDL cholesterol level and raises LDL cholesterol level in healthy subjects [9]. Moreover, retrospective cohort study shows that intake of TFAs-rich diet is associated with higher risks of cardiovascular disease (CVD) [10]. These findings are supported by systematic review and meta-analysis [11, 12]. Furthermore, de Souza et al. also reveal that mortality of CVD is associated with intake of industrial TFAs, but not ruminant TFAs [11]. Consideration of those adverse effects of industrial TFAs on health, US Food and Drug Administration decided to eliminate industrial TFAs from food supply until 2018. Moreover, Denmark, Switzerland, Austria, Canada and Singapore have policy to limit or diminish industrial TFAs in food. Some other countries in Asia such as Korea and China obligate to labeling of content of TFAs. These governmental regulations reduce intake of industrial TFAs from daily diet, although industrial TFAs are contained in food in many countries [13, 14].

### 1.3. Is intake of trans fatty acids a risk factor for various diseases?

Emerging evidences suggest that intake of TFAs is associated with the development of various diseases. Systematic review by Barnard et al. indicates that intake of TFAs is associated with risk of cognitive disorders [15]. Moreover, Golomb et al. reports that dietary TFAs intake is negatively related to memory of the word in younger adults [16]. In addition, intake of TFAs is inversely related to sperm count in young healthy men [17]. However, since these studies do not distinguish between industrial TFAs and ruminant TFAs, it cannot be concluded that the adverse effects are caused by intake of industrial TFAs.

A systematic review by de Souza et al. also analyzes the effects of intake of TFAs on diabetes [11]. Intake of TFAs has no association with the risk of diabetes. However, intriguingly, when TFAs are divided into industrial and ruminant TFAs, intake of ruminant TFAs reduces the risk of diabetes. Although there is no data about industrial TFAs in the study, these results suggest

that intake of industrial TFAs elevates the risk of diabetes. This hypothesis is supported by in vitro study using rat fed with industrial TFAs-rich diet [18]. However, other groups report that industrial TFAs do not alter insulin responsiveness in epidemiological study [19], in vivo [20] and in vitro study [21]. Hence, the roles of industrial TFAs in development of insulin resistance and diabetes remain unclear.

#### **1.4. Elaidate, an industrial trans fatty acid, impairs insulin responsiveness of adipocytes**

What causes the differences in the effects of industrial TFAs on insulin resistance and diabetes? Osso et al. analyzed the effects of industrial TFAs for about 8 weeks [18]. On the other hand, Louheranta et al. and Lovejoy et al. analyzed for 2–4 weeks, respectively [19, 20]. Moreover, Granados et al. cultured 3T3-L1 adipocytes for 24 h in the presence of 100  $\mu$ M elaidate [21]. We focused on the exposure period to industrial TFAs. To examine the effects of persistent exposure to industrial TFAs, 3T3-L1 preadipocytes were cultured and differentiated into adipocytes in the presence of 10  $\mu$ M elaidate which is close to physiological concentration in human plasma [4, 8]. Persistent exposure to elaidate before and during differentiation impaired insulin-dependent glucose uptake and translocation of glucose transporter 4 (GLUT4) to the plasma membrane [22]. On the other hand, culture of 3T3-L1 adipocytes with 10  $\mu$ M elaidate for 24 h did not alter insulin-dependent glucose uptake. Thus, our findings reveal new factor “period” for consideration of adverse effects of TFAs and emphasize the risk of persistent intake of industrial TFAs for development of diabetes.

#### **1.5. How does elaidate impair insulin responsiveness of adipocytes?**

Stimulation with insulin activates insulin-signaling cascades [23]. In brief, insulin binding to insulin receptor activates its kinase domain, and insulin receptor phosphorylates itself and insulin receptor substrates followed by activation of phosphoinositide 3-kinase (PI3K). PI3K converts phosphatidylinositol (3,4)-bisphosphate ( $\text{PIP}_2$ ) to phosphatidylinositol (3,4,5)-trisphosphate ( $\text{PIP}_3$ ).  $\text{PIP}_3$  acts as a scaffold of Akt at the plasma membrane, and it enables mTORC2 and PDK to phosphorylate and activate Akt. Activated Akt accelerates translocation of GLUT4 storage vesicles (GSVs) to the plasma membrane [24], and GLUT4 transports extracellular glucose into the cells. Our unpublished data indicate that insulin-dependent phosphorylation of Akt, but not insulin receptor, is suppressed in adipocytes persistently exposed to elaidate. Therefore, suppressive effects of elaidate on insulin signaling are partial but not overall. One possibility is that expression or activation of PI3K, mTORC2 or PDK1 may be repressed in adipocytes persistently exposed to elaidate. Another possibility is that alteration in fatty acid composition of phospholipids affects activation of Akt. It is reported that saturated fatty acid-contained phosphatidylcholine prevents full activation of Akt in vitro [25]. Moreover, oleate-contained  $\text{PIP}_3$  enhances activation of Akt in vitro [26]. Thus, we should carefully analyze the effects of elaidate on insulin signaling around Akt.

Our previous data also indicate that incorporated elaidate may affect insulin responsiveness of adipocytes. Elaidate acts as a ligand and can activate G protein-coupled receptor 120 at several hours [27], although culture of adipocytes with 10  $\mu$ M elaidate for 20 min or 24 h did not

alter insulin-dependent glucose uptake [22]. Therefore, ligand property of elaidate will not be involved in an impairment of insulin responsiveness. Another possibility is that incorporated elaidate impairs insulin responsiveness. Extracellular fatty acids are incorporated through FATP and CD36 [28, 29], and then fatty acids are converted into acyl-CoA derivatives by acyl-CoA synthetase [30]. Acyl-CoA derivatives are degraded by  $\beta$ -oxidation or esterified into lipid species such as triglyceride and phospholipid. It is reported that elaidate is esterified into triglyceride and phospholipid of heart of rats fed with elaidate-contained diets [31]. Moreover, elaidate is incorporated into adipose tissue of rabbits fed with elaidate-contained diets [32], although intracellular distribution of elaidate in lipid species of adipocytes is not fully understood. Therefore, we provide new data about intracellular lipid distribution of elaidate in adipocytes.

## 2. Incorporation of extracellular elaidate and its distribution in lipid species of adipocytes

### 2.1. Materials and methods

#### 2.1.1. Cell culture, differentiation and addition of fatty acids

3T3-L1 pre-adipocytes were purchased from Japanese Collection of Research Bioresources (Osaka, Japan). Cells were cultured in Dulbecco's modified Eagles medium containing 4.5 mg/L D-glucose (DMEM-high Glucose; Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS, S1820; Biowest, Nuaille, France), 50 units/ml penicillin and 50  $\mu$ g/ml streptomycin (P7081; Sigma-Aldrich, St. Louis, MO). Before differentiation into adipocytes, cells were cultured for 10 days in the presence of 10  $\mu$ M of bovine serum albumin (BSA)-conjugated elaidate (E4637; Sigma-Aldrich), stearate-d35 (BX-245; Olbracht Serdary Research Laboratories, Toronto, Canada), oleate-d17 (9000432; Cayman Chemical, Ann Arbor, MI), elaidate plus stearate (S4751; Sigma-Aldrich), elaidate plus oleate (O1382; Sigma-Aldrich) or BSA alone (vehicle), which was prepared as described [22]. Then, cultured 3T3-L1 pre-adipocytes were differentiated into adipocytes using 3-isobutyl-1-methylxanthine (I7018; Sigma-Aldrich), dexamethasone (D4902; Sigma-Aldrich) and insulin (I5500; Sigma-Aldrich) for 8 days in the presence of BSA-conjugated fatty acids as described [22].

#### 2.1.2. Subcellular fractionation

Subcellular fractionation was performed as described by Rangel et al. [33]. In brief, cells were washed twice with cold phosphate buffered saline (PBS) and once with lysis buffer (10 mM Tris-HCl, pH 7.4/250 mM sucrose/1 mM EDTA). Then, scraped cells were homogenized in lysis buffer with 7 mL-scale Dounce homogenizer (Kontes Glass Co, Vineland, NJ), and the homogenates were centrifugated at  $1000 \times g$  for 5 min with MX-107 (TOMY, Tokyo, Japan). The pellet was collected as nuclear fraction. The supernatant was further centrifugated with  $20,000 \times g$  for 20 min, and the pellet was collected as mitochondrial fraction. Then, the supernatant was ultracentrifugated at  $100,000 \times g$  for 60 min with Himac CS 150GX (Hitachi, Tokyo, Japan). The supernatant was collected as cytosolic fraction which will contain light vesicles,



and the pellet was collected as fraction of the plasma membrane. Each pellet was resuspended in PBS and sonicated for 10 s using a ultrasonic homogenizer (UR-21P; TOMY).

### *2.1.3. Analysis of fatty acids composition of triglyceride, free fatty acid and phospholipid*

Total lipids were extracted from each fraction using a modified Bligh & Dyer method [34]. Triglyceride, free fatty acid and phospholipid in total lipids were separated with thin-layer chromatography (Silica gel 60, no. 1.05721.0001; Merck, Darmstadt, Germany) using development solvent (petroleum ether:diethyl ether:acetic acid = 82:18:1, v/v/v). Isolated triglyceride, free fatty acid and phospholipid was hydrolyzed and methylated using 10% toluene (no. 209-06791; Wako, Osaka, Japan) and hydrogen chloride-methanol reagent (5–10%) (X0038; Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) for 1 h at 100°C. Then, amount of fatty acids in each lipid species was analyzed by GC-MS-QP2010 Ultra (SHIMADZU, Kyoto, Japan) as described [22]. Efficiency of extraction, isolation and methylation was normalized with triheicosanoic acid (B-422; Olbracht Serdary Research Laboratories) for triglyceride, nonadecanoic acid (N5252-1G; Sigma-Aldrich) for free fatty acid and 1,2-diheptadecanoyl-sn-glycero-3-phosphorylcholine (no. 1400; Matreya LLC Inc., Pleasant Gap, PA) for phospholipid. The amount of fatty acids was also normalized with cell number.

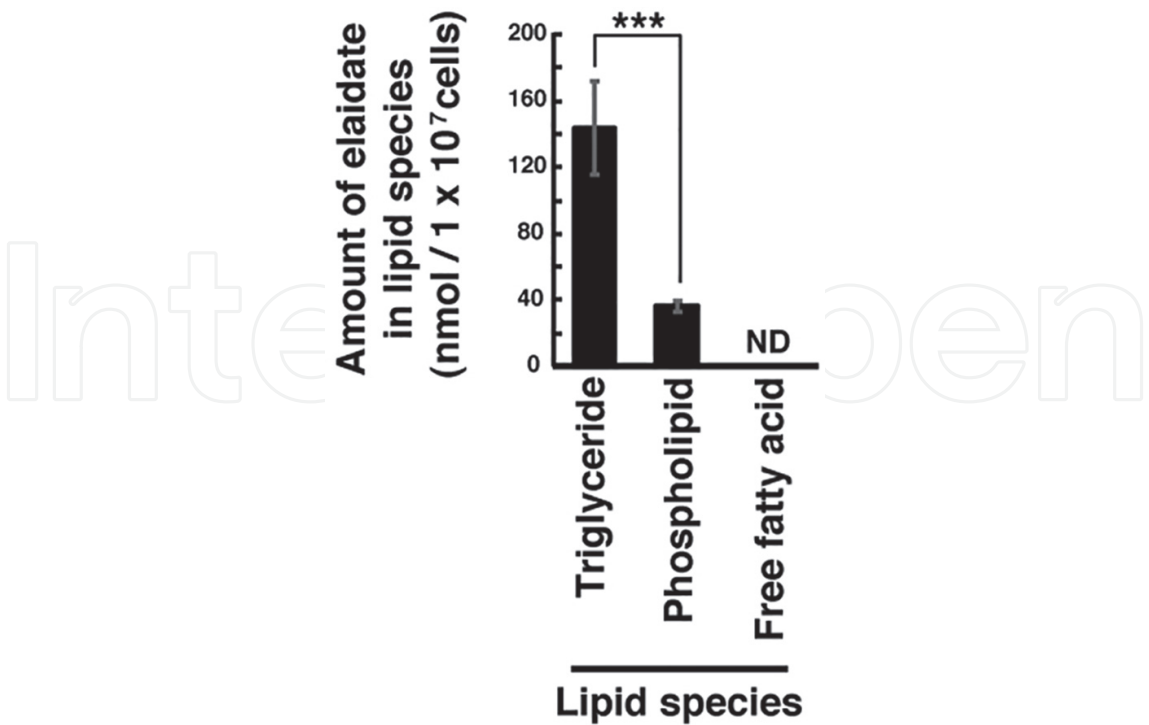
## **2.2. Results and discussion**

### *2.2.1. Extracellular elaidate is incorporated into adipocytes and esterified into triglyceride and phospholipid*

To examine whether incorporated extracellular elaidate is esterified into lipid species of adipocytes, total lipid was extracted from adipocytes exposed to elaidate for 18 days, and it was separated by TLC followed by analyzing with GC-MS. In major lipid species of adipocytes was triglyceride, phospholipid and free fatty acid, and diacylglycerol and monoacylglycerol were in trace amounts. Therefore, we analyzed the amount of elaidate in triglyceride, phospholipid and free fatty acid and revealed that elaidate was contained in triglyceride and phospholipid (**Figure 1**). On the other hand, the non-esterified form of elaidate was not detected (**Figure 1**, free fatty acid). Moreover, the amount of elaidate in triglyceride was higher than that in phospholipid. These results suggest that incorporated extracellular elaidate is mainly esterified into triglyceride and phospholipid in adipocytes.

### *2.2.2. Elaidate-esterified phospholipid is distributed in various organelles membrane*

Since phospholipids are a component of membranes of various organelles [35], we further examined intracellular distribution of elaidate-contained phospholipids using subcellular fractionation methods. Cells were homogenized and organelles in the lysates were enriched by centrifugation (**Figure 2A**). TLC and GC-MS analysis revealed that elaidate was contained in phospholipid of nuclear, mitochondrial, cytosolic and plasma membrane fraction (**Figure 2B**). The amount of elaidate in phospholipid of mitochondrial fraction was higher than that of nuclear, cytosolic and plasma membrane fraction, suggesting that elaidate-contained phospholipids are mainly distributed in mitochondria.

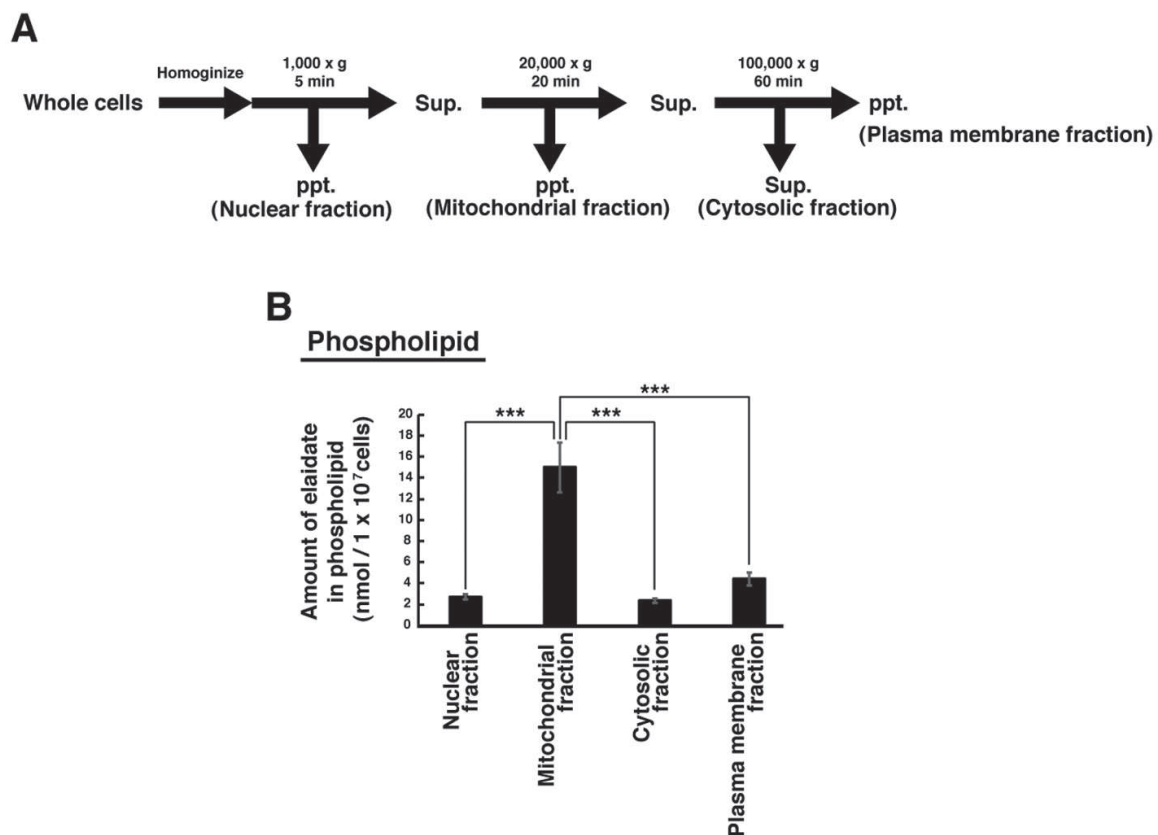


**Figure 1.** Extracellular elaidate is incorporated into adipocytes, and esterified into triglyceride and phospholipid. 3T3-L1 pre-adipocytes were cultured for 10 days and differentiated into adipocytes for 8 days with 10  $\mu$ M elaidate. Amount of elaidate in triglyceride, free fatty acid and phospholipid were analyzed by GC-MS after separated by TLC as described in Section 2.1. Results are represented as means  $\pm$  S.D., n = 5 independent experiments. ND means not detected. Asterisks indicate a significant difference (\*\* $p < 0.001$ ) calculated by Student's *t*-tests.

2.2.3. Amount of elaidate-contained phospholipids in organelles is different from that of stearate or oleate-contained phospholipids

Is the intracellular distribution of elaidate-contained phospholipids specific to elaidate or not? Therefore, we compared elaidate (trans-C18:1) with stearate (C18:0) which has similar steric structure to elaidate, or oleate (cis-C18:1) which is cis form of elaidate (**Figure 3A**). For trace of incorporated extracellular stearate and oleate, we used deuterium-labeled stearate (stearate-d35) and oleate (oleate-d17). As shown in **Figure 3B**, incorporated extracellular stearate-d35 (dark gray bars) and oleate-d17 (light gray bars) were esterified into phospholipid of each organelle, and stearate-d35 or oleate-d17-contained phospholipids were mainly distributed in the mitochondria fraction. It is reported that extracellular [<sup>3</sup>H]-raveled arachidonate is incorporated into cells and the distribution ratio of [<sup>3</sup>H]-raveled arachidonate in the mitochondria is lower than that in other organelles at several minutes after addition of [<sup>3</sup>H]-raveled arachidonate into the medium [36]. However, the distribution ratio of [<sup>3</sup>H]-raveled arachidonate dramatically rises at 24 h. Since cells were exposed to fatty acid for 18 days, incorporated elaidate, stearate-d35 and oleate-d17 may be transferred from other organelles to the mitochondria. Therefore, the mitochondrial distribution of elaidate is not specific to elaidate.

Intriguingly, although the amount of elaidate was similar to that of stearate-d35 and higher than that of oleate-d17 in the nuclear, mitochondrial and cytosolic fraction, the amount of elaidate in the plasma membrane fraction was significantly higher than that of both stearate-d35

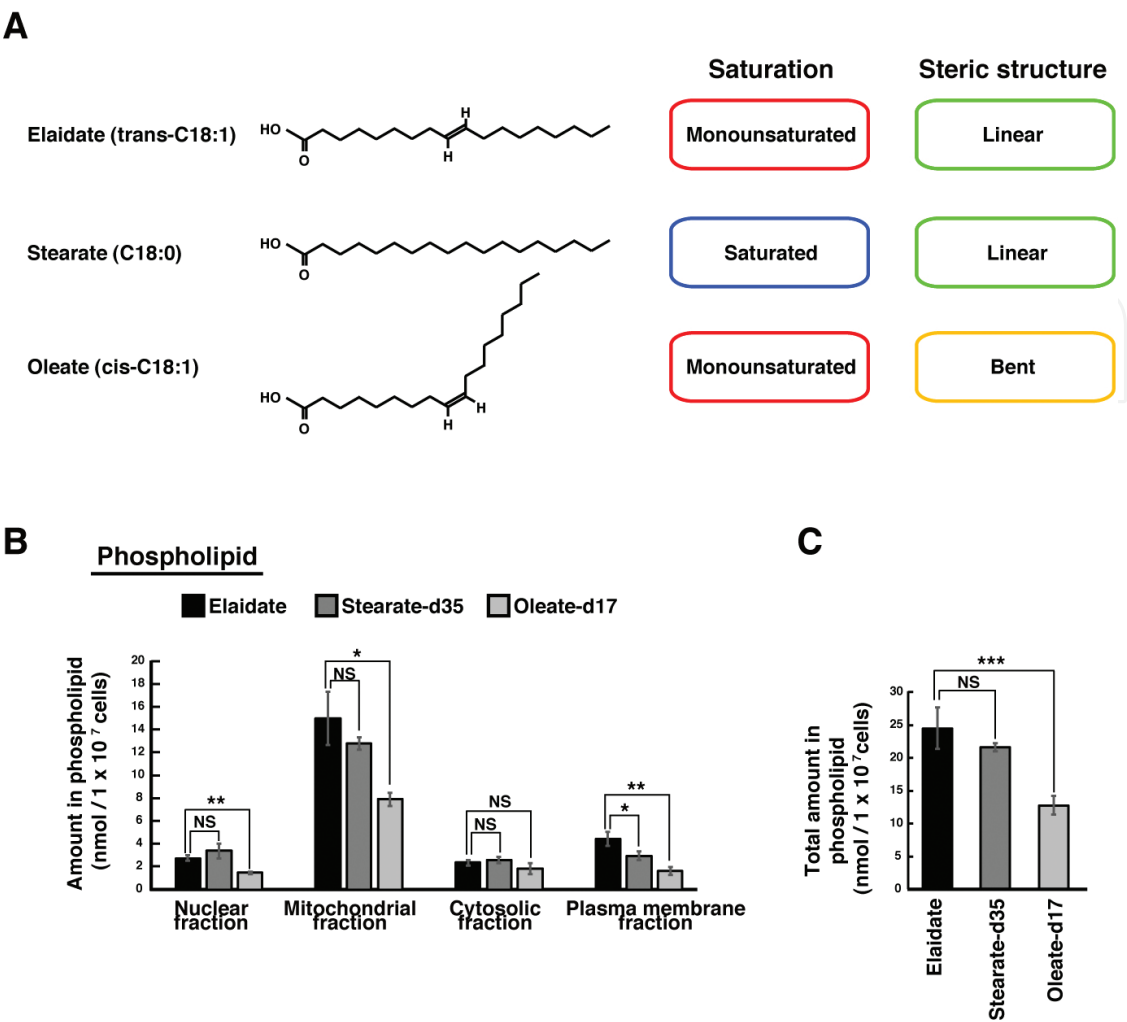


**Figure 2.** Elaidate-esterified phospholipid is distributed in various organelles membrane. 3T3-L1 pre-adipocytes were cultured and differentiated into adipocytes as described in **Figure 1**. (A) Each fraction was prepared by centrifugation from cell lysates. (B) Amount of elaidate in phospholipids of each fraction was analyzed using GC-MS. Results are represented as means  $\pm$  S.D.,  $n = 3$  independent experiments. Asterisks indicate a significant difference ( $***p < 0.001$ ) calculated by Student's *t*-tests.

and oleate-d17. What causes the difference in the amount of these fatty acids in organelles? As shown in **Figure 3C**, the total amount of elaidate in phospholipid was higher than that of oleate-d17, but not stearate-d35. There were several possibilities that oleate-d17 was degraded through  $\beta$ -oxidation pathway, the amount of incorporated oleate-d17 into adipocytes was lower than that of elaidate, and oleate-d17 tend to be esterified into other lipid species such as triglyceride or to be removed from phospholipid by phospholipases compared with elaidate. It is reported that  $\beta$ -oxidation rate of oleate is higher than that of elaidate in rat heart homogenate [37]. Moreover, our unpublished data showed that the amount of oleate-d17 in triglyceride is higher than that of elaidate at 6 h after addition of those fatty acids into the culture medium of adipocytes, although there was no difference in total amount of oleate-d17 and elaidate. Since oleate and elaidate have a different steric structure, it may result in a difference between oleate and elaidate in metabolism and esterification into or removal from phospholipid.

Although steric structure of elaidate is similar to that of stearate, there was a difference in the amount of elaidate and stearate-d35 in the plasma membrane fraction (**Figure 3B**). Moreover, the amount of stearate-d35 was not significantly but slightly low in the mitochondrial fraction and high in the nuclear and cytosolic fraction compared with that of elaidate (**Figure 3B**).





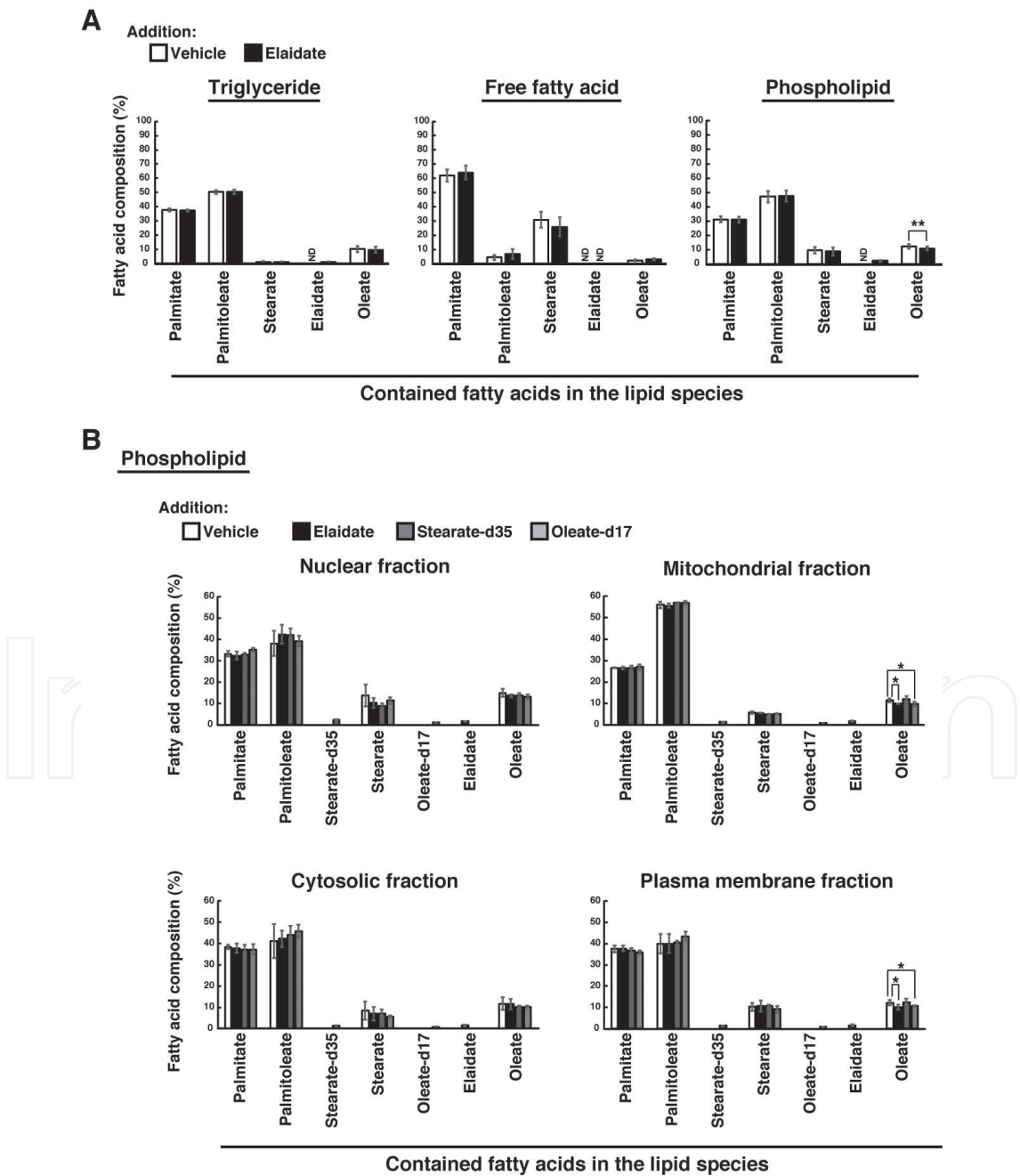
**Figure 3.** Amount of elaidate-contained phospholipids in organelles is different from that of stearate or oleate-contained phospholipids. (A) Structure of elaidate, stearate and oleate. (B) 3T3-L1 pre-adipocytes were cultured for 10 days and differentiated into adipocytes for 8 days with 10  $\mu$ M elaidate (black bar), stearate-d35 (dark gray bar) or oleate-d17 (light gray bar). Amount of elaidate, stearate-d35 or oleate-d17 in phospholipid of each fraction were analyzed using GC-MS. (C) Total amount in phospholipids was calculated by summing up the amount of elaidate, stearate-d35 or oleate-d17 in each fraction. Results are represented as means  $\pm$  S.D.,  $n = 3$  independent experiments. Asterisks indicate a significant difference (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; NS, not significant) calculated by Student's  $t$ -tests.

It is known that stearate is desaturated by stearoyl-CoA desaturase-1 (SCD-1; n-9 desaturase) and converted into oleate [38]. We detected that exposure to stearate increases the amount of oleate but not stearate in adipocytes (unpublished data), suggesting that stearate is converted into oleate in adipocytes. Since elaidate is already desaturated at n-9 position, elaidate may be less likely to be metabolized by SCD-1. However, the different amount of elaidate and stearate in phospholipid was not only explained by the difference in the metabolism, suggesting that there are differences between elaidate and stearate in esterification into or removal from phospholipid. Therefore, the differences in both metabolism and esterification into or removal from phospholipid may result in different amounts of elaidate and stearate in phospholipid of organelles.

Thus, since elaidate has a double bond but exhibits linear-shaped structure, it may cause unique distribution of elaidate in phospholipid of various organelles membrane.

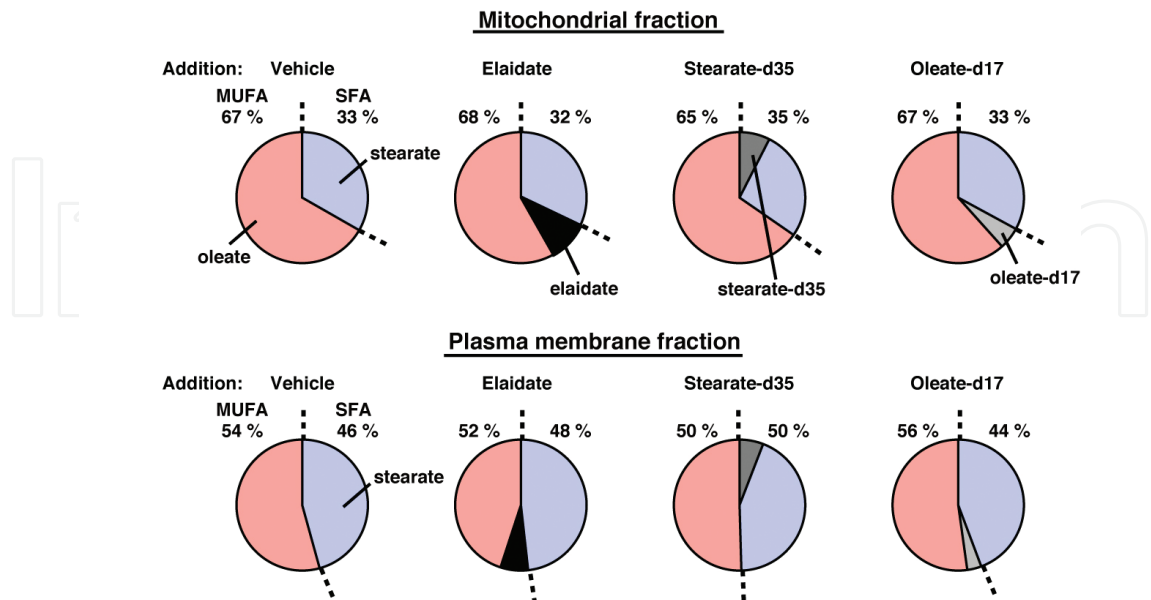
2.2.4. Fatty acid composition of phospholipid is altered in adipocytes exposed to elaidate

Next, we examined whether incorporation and esterification of elaidate into lipid species may disturb the equilibrium state of metabolism or distribution of other fatty acids. Therefore, we analyzed the amount of palmitate, palmitoleate, stearate and oleate, which were mainly contained in total lipid of adipocytes and calculated fatty acid composition (%). On the other hand, the amount of linoleate (C18:2), linolenate (C18:3) and arachidonate (C20:4) may be trace amount in 3T3-L1 adipocytes and we could not detect these fatty acids. As shown in **Figure 4A**, there was no difference in the fatty acid composition of triglyceride and free fatty acid. On the other hand, the percentage of oleate in phospholipid was slightly but significantly low in adipocytes exposed to elaidate compared with vehicle (**Figure 4A**).

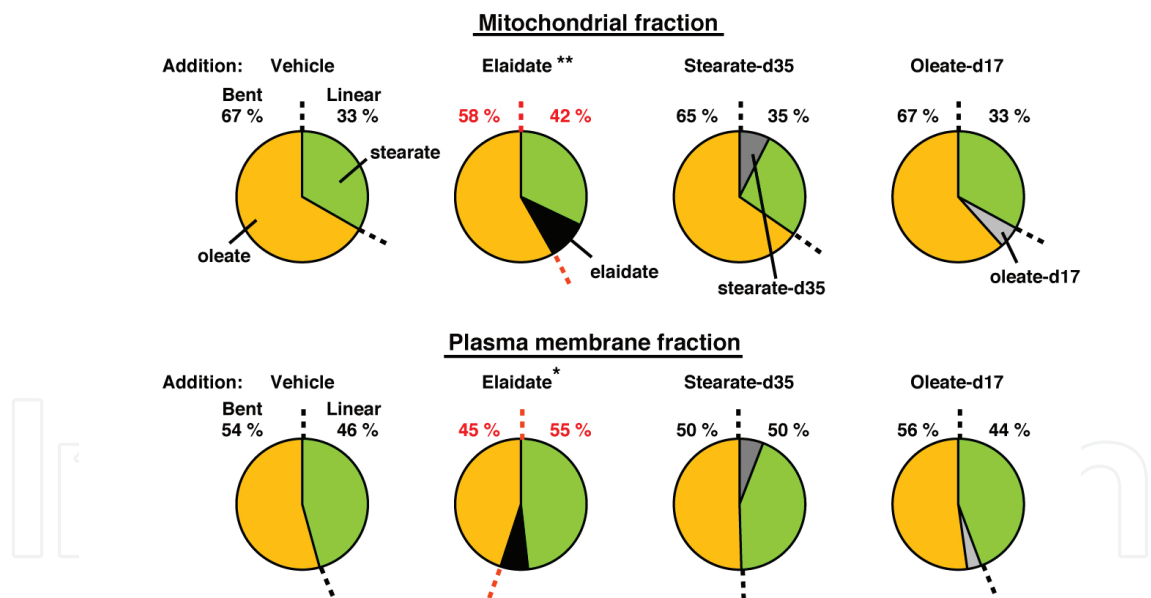


C

Saturation (unsaturated or saturated)



Steric structure (bent shaped or linear shaped chain)



**Figure 4.** Fatty acid composition of phospholipid is altered in adipocytes exposed to elaidate. (A) Analysis of fatty acid composition of lipid species in adipocytes cultured with vehicle (white bars) or elaidate (black bars). Fatty acid composition (%) was calculated by the amount of each fatty acid dividing by total amount of palmitate, palmitoleate, stearate, elaidate and oleate. Results are represented as means  $\pm$  S.D.,  $n = 5-8$  independent experiments. ND means not detected. (B) Analysis of fatty acid composition of phospholipid in organelles in adipocytes cultured with vehicle (white bars), elaidate (black bars), stearate-d35 (dark gray bars) or oleate-d17 (light gray bars). Fatty acid composition (%) was calculated by the amount of each fatty acid dividing by total amount of palmitate, palmitoleate, stearate-d35, stearate, oleate-d17, elaidate and oleate. Results are represented as means  $\pm$  S.D.,  $n = 3$  independent experiments. (C) Fatty acid equilibrium (%) between monounsaturated fatty acid (MUFA) and saturated fatty acid (SFA) or bent shaped and linear shaped chain fatty acid in 18-carbon fatty acid. Results are represented as means,  $n = 3$  independent experiments. Asterisks indicate a significant difference ( $*p < 0.05$ ;  $**p < 0.01$ ) calculated by Student's  $t$ -tests.

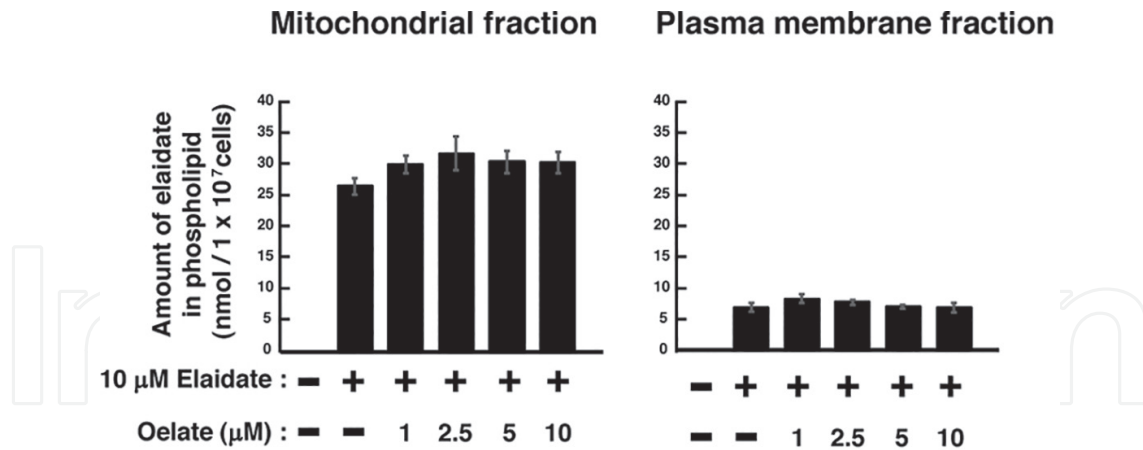
Further analysis with subcellular fractionation revealed that the percentage of oleate was low in phospholipid of mitochondrial and plasma membrane fraction in adipocytes exposed to elaidate (**Figure 4B**, compare white bars with black bars). Moreover, this alteration was observed in both mitochondrial and plasma membrane fraction of adipocytes differentiated with oleate-d17 but not stearate-d35 (**Figure 4B**, see dark and light gray bars). Since there was no difference in the percentage of 16-carbon fatty acids (palmitate and palmitoleate), we surmised that elaidate limitedly affects the equilibrium of 18-carbon fatty acids. The equilibrium between monounsaturated fatty acid (MUFA) and saturated fatty acid (SFA) in 18-carbon fatty acids was maintained in adipocytes exposed to elaidate, stearate-d35 and oleate-d17 in both mitochondrial and plasma membrane fraction (**Figure 4C**, upper panels). However, when the steric structure was considered, elaidate has similar linear-shaped structure to stearate, and raises the percentage of linear-shaped chain fatty acid (**Figure 4C**, lower panels). Thus, in phospholipid of adipocytes exposed to elaidate, the equilibrium between bent and linear-shaped chain fatty acid was disturbed, although the equilibrium between MUFA and SFA was maintained.

The alteration was small compared with the total amount of fatty acid in phospholipid. However, since activation of insulin signaling occurs in the local area of the plasma membrane such as raft [39], elaidate may locally disturb the equilibrium of fatty acid of phospholipid and partially suppress the insulin signaling.

#### 2.2.5. Elaidate does not compete with oleate

How does incorporated and esterified elaidate affect the equilibrium of fatty acid of phospholipid? There were possibilities that elaidate competes with incorporation of extracellular oleate into adipocytes or esterification of oleate into phospholipids, or elaidate indirectly reduces the amount of oleate through alteration of synthesis or metabolism of intracellular oleate. To examine the possibility of competitive inhibition, cells were exposed to 10  $\mu$ M elaidate plus 1 to 10  $\mu$ M oleate, which was expected oleate to competitively inhibit incorporation or esterification of elaidate into phospholipid and reduce the amount of elaidate. However, there was no difference in the amount of elaidate between cells exposed to elaidate and elaidate plus oleate (**Figure 5**). Since incorporated extracellular oleate and elaidate may exhibit different intracellular dynamics (see Section 2.2.3), elaidate does not compete with extracellular oleate in an incorporation into adipocytes or esterification into phospholipid. These results suggest that elaidate may alter synthesis or metabolism of intracellular oleate which results in a reduction of the amount and percentage of oleate in phospholipid.

Intracellular oleate is synthesized from stearate by SCD-1, and it is reported that SCD-1 prefers stearate than palmitate [40]. Therefore, reduced activity of SCD-1 may lead to reduced amount of oleate but not palmitoleate. However, it may raise the amount of stearate, although persistent exposure to elaidate did not alter the amount and percentage of stearate (**Figure 4**). Since the amount of palmitate and palmitoleate in triglyceride was significantly high in adipocytes exposed to elaidate compared with control (data not shown), reduced supplementation of palmitate from triglyceride to phospholipid may result in the prevention of increased amount of stearate, and maintain the equilibrium of MUFA and SFA, but not bent and linear-shaped chain fatty acid.



**Figure 5.** Elaidate does not compete with oleate. The amount of elaidate in phospholipid of mitochondria and plasma membrane fraction in cells differentiated with 10  $\mu$ M elaidate plus 1, 2.5, 5 or 10  $\mu$ M oleate. Results are represented as means  $\pm$  S.D.,  $n = 3$  independent experiments. Significant difference was calculated by Student' s  $t$ -tests.

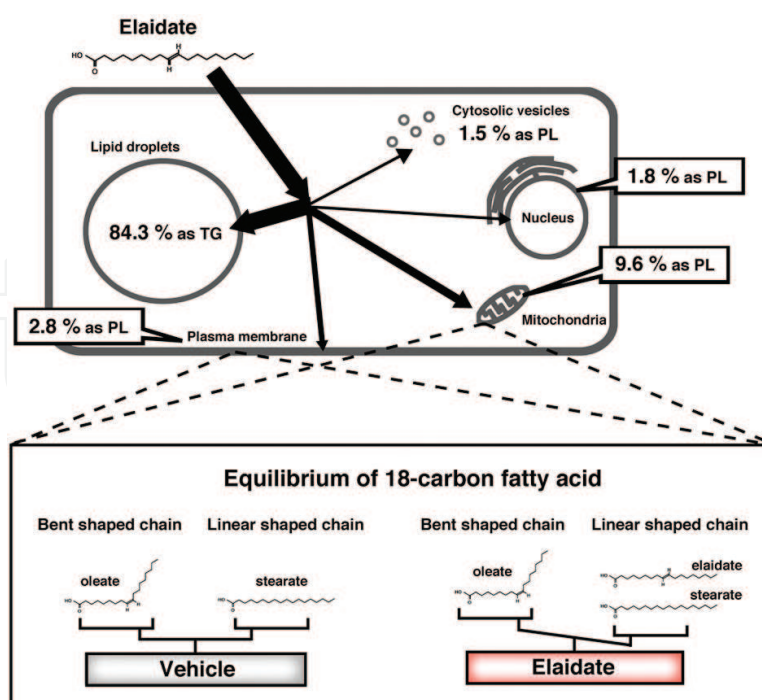
2.2.6. Intracellular distribution of elaidate and its effects on fatty acid composition of lipid species

Our results were illustrated in **Figure 6**. In adipocytes, incorporated extracellular elaidate is mainly esterified into triglyceride. The rest is esterified into phospholipid of various organelles such as mitochondria, plasma membrane, nucleus and cytosolic vesicles. Moreover, distribution of elaidate into phospholipid is not competitively inhibited by oleate and stearate (data not shown), suggesting that elaidate may exhibit unique intracellular dynamics or incorporation into cells is not a rate-limiting step. Furthermore, elaidate reduces the amount of oleate which results in the reduction of the percentage of oleate in phospholipid of mitochondria and plasma membrane. Intriguingly, esterification of elaidate and reduction of oleate disturbs the equilibrium between bent and linear-shaped chain fatty acid in phospholipid but not unsaturated and saturated fatty acid. Therefore, elaidate-esterified phospholipid may be recognized as oleate-esterified phospholipid.

To clarify the molecules which may recognize elaidate as oleate-esterified phospholipid and command to maintain the equilibrium of MUFA and SFA, we should separate phospholipid classes such as phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine and phosphatidylinositol and analyze its fatty acid composition, since enzymes which are involved in synthesis and metabolism of phospholipids (e.g. acyl-CoA synthases, acyltransferases and phospholipases) have substrate specificity to lipid classes and its fatty-acyl chain [41]. Thus, the maintenance of the equilibrium between MUFA and SFA in our results cannot be explained without a recognition system of fatty acids. Therefore, further analysis also enables us to elucidate the recognition system of fatty acids.

Note that certain phospholipids such as phosphatidylinositol and phosphatidylcholine have the potential to affect insulin signaling [25, 26]. Further analysis of alteration of fatty acid composition in phospholipid classes will lead to elucidate the mechanism for suppression of the insulin signaling.





**Figure 6.** Intracellular distribution of elaidate and its effects on fatty acid composition of lipid species. Incorporated extracellular elaidate is esterified into triglyceride (TG) and phospholipid (PL) of organelles. Elaidate reduces the amount of oleate in phospholipid of mitochondria and plasma membrane, and disturbs the equilibrium between bent shaped and linear shaped chain fatty acid in phospholipid.

### 3. Conclusions

Our recent study reveals the deteriorative effects of elaidate on insulin responsiveness of adipocytes. In this chapter, we show for the first time that elaidate alters fatty acid composition of lipid species of adipocytes. Since lipid species may have important roles in regulation of insulin signaling, we should consider the effects of elaidate on lipid species to analyze the mechanism for an impairment of insulin responsiveness.

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### Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this chapter.

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## References

- [1] Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Trans fatty acids and cardiovascular disease. *The New England Journal of Medicine*. 2006;**354**(15):1601-1613. DOI: 10.1056/NEJMra054035
- [2] Sommerfeld M. Trans unsaturated fatty acids in natural products and processed foods. *Progress in Lipid Research*. 1983;**22**(3):221-233
- [3] Angulo J, Hiller B, Olivera M, Mahecha L, Dannenberger D, Nuernberg G, Losand B, Nuernberg K. Dietary fatty acid intervention of lactating cows simultaneously affects lipid profiles of meat and milk. *Journal of the Science of Food and Agriculture*. 2012;**92**(15):2968-2974. DOI: 10.1002/jsfa.5709
- [4] Vesper HW, Kuiper HC, Mirel LB, Johnson CL, Pirkle JL. Levels of plasma trans-fatty acids in non-Hispanic white adults in the United States in 2000 and 2009. *Journal of the American Medical Association*. 2012;**307**(6):562-563. DOI: 10.1001/jama.2012.112
- [5] Martin CA, Milinsk MC, Visentainer JV, Matsushita M, de-Souza NE. Trans fatty acid-forming processes in foods: A review. *Anais da Academia Brasileira de Ciências*. 2007;**79**(2):343-350
- [6] Arcand J, Scourboutakos MJ, Au JT, LAbbe MR. Trans fatty acids in the Canadian food supply: An updated analysis. *The American Journal of Clinical Nutrition*. 2014;**100**(4):1116-1123. DOI: 10.3945/ajcn.114.088732
- [7] Rippin HL, Hutchinson J, Ocke M, Jewell J, Breda JJ, Cade JE. An exploration of socioeconomic and food characteristics of high trans fatty acid consumers in the Dutch and UK national surveys after voluntary product reformulation. *Food & Nutrition Research*. 2017;**61**(1):1412793. DOI: 10.1080/16546628.2017.1412793
- [8] Mori K, Ishida T, Yasuda T, Hasokawa M, Monguchi T, Sasaki M, Kondo K, Nakajima H, Shinohara M, Shinke T, Irino Y, Toh R, Nishimura K, Hirata K. Serum trans-fatty acid concentration is elevated in young patients with coronary artery disease in Japan. *Circulation Journal*. 2015;**79**(9):2017-2025. DOI: 10.1253/circj.CJ-14-0750
- [9] Mensink RP, Katan MB. Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *The New England Journal of Medicine*. 1990;**323**(7):439-445. DOI: 10.1056/NEJM199008163230703

- [10] Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Sampson LA, Hennekens CH. Intake of trans fatty acids and risk of coronary heart disease among women. *Lancet*. 1993;**341**(8845):581-585
- [11] de Souza RJ, Mente A, Maroleanu A, Cozma AI, Ha V, Kishibe T, Uleryk E, Budylowski P, Schünemann H, Beyene J, Anand SS. Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: Systematic review and meta-analysis of observational studies. *BMJ*. 2015;**351**:h3978. DOI: 10.1136/bmj.h3978
- [12] Brouwer IA. Effect of Trans-fatty Acid Intake on Blood Lipids and Lipoproteins: A Systematic Review and Meta-regression Analysis. Geneva: World Health Organization; 2016. ISBN: 978 92 4 151060 8
- [13] Micha R, Khatibzadeh S, Shi P, Fahimi S, Lim S, Andrews KG, Engell RE, Powles J, Ezzati M, Mozaffarian D. Global, regional, and national consumption levels of dietary fats and oils in 1990 and 2010: A systematic analysis including 266 country-specific nutrition surveys. *BMJ*. 2014;**348**:g2272. DOI: 10.1136/bmj.g2272
- [14] Wanders AJ, Zock PL, Brouwer IA. Trans fat intake and its dietary sources in general populations worldwide: A systematic review. *Nutrients*. 2017;**9**(8). pii: E840. DOI: 10.3390/nu9080840
- [15] Barnard ND, Bunner AE, Agarwal U. Saturated and trans fats and dementia: A systematic review. *Neurobiology of Aging*. 2014;**35**(Suppl 2):S65-S73. DOI: 10.1016/j.neurobiolaging.2014.02.030
- [16] Golomb BA, Bui AK. A fat to forget: Trans fat consumption and memory. *PLoS One*. 2015;**10**(6):e0128129. DOI: 10.1371/journal.pone.0128129
- [17] Chavarro JE, Mínguez-Alarcón L, Mendiola J, Cutillas-Tolín A, López-Espín JJ, Torres-Cantero AM. Trans fatty acid intake is inversely related to total sperm count in young healthy men. *Human Reproduction*. 2014;**29**(3):429-440. DOI: 10.1093/humrep/det464
- [18] Osso FS, Moreira AS, Teixeira MT, Pereira RO, Tavares do Carmo M, Moura AS. Trans fatty acids in maternal milk lead to cardiac insulin resistance in adult offspring. *Nutrition*. 2008;**24**(7-8):727-732. DOI: 10.1016/j.nut.2008.03.006
- [19] Louheranta AM, Turpeinen AK, Vidgren HM, Schwab US, Uusitupa MI. A high-trans fatty acid diet and insulin sensitivity in young healthy women. *Metabolism*. 1999;**48**(7): 870-875
- [20] Lovejoy JCI, Smith SR, Champagne CM, Most MM, Lefevre M, DeLany JP, Denkins YM, Rood JC, Veldhuis J, Bray GA. Effects of diets enriched in saturated (palmitic), monounsaturated (oleic), or trans (elaidic) fatty acids on insulin sensitivity and substrate oxidation in healthy adults. *Diabetes Care*. 2002;**25**(8):1283-1288
- [21] Granados N, Amengual J, Ribot J, Palou A, Bonet ML. Distinct effects of oleic acid and its trans-isomer elaidic acid on the expression of myokines and adipokines in cell models. *The British Journal of Nutrition*. 2011;**105**(8):1226-1234. DOI: 10.1017/S0007114510004885

- [22] Ishibashi K, Nehashi K, Oshima T, Ohkura N, Atsumi G. Differentiation with elaidate tends to impair insulin-dependent glucose uptake and GLUT4 translocation in 3T3-L1 adipocytes. *International Journal of Food Sciences and Nutrition*. 2016;**67**(2):99-110. DOI: 10.3109/09637486.2016.1144721
- [23] Haeusler RA, McGraw TE, Accili D. Biochemical and cellular properties of insulin receptor signalling. *Nature Reviews. Molecular Cell Biology*. 2018;**19**(1):31-44. DOI: 10.1038/nrm.2017.89
- [24] Jaldin-Fincati JR, Pavarotti M, Frendo-Cumbo S, Bilan PJ, Klip A. Update on GLUT4 vesicle traffic: A cornerstone of insulin action. *Trends in Endocrinology and Metabolism*. 2017;**28**(8):597-611. DOI: 10.1016/j.tem.2017.05.002
- [25] Koeberle A, Shindou H, Koeberle SC, Laufer SA, Shimizu T, Werz O. Arachidonoyl-phosphatidylcholine oscillates during the cell cycle and counteracts proliferation by suppressing Akt membrane binding. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;**110**(7):2546-2551. DOI: 10.1073/pnas.1216182110
- [26] Gan X, Wang J, Su B, Wu D. Evidence for direct activation of mTORC2 kinase activity by phosphatidylinositol 3,4,5-trisphosphate. *The Journal of Biological Chemistry*. 2011;**286**(13):10998-11002. DOI: 10.1074/jbc.M110.195016
- [27] Hirasawa A, Tsumaya K, Awaji T, Katsuma S, Adachi T, Yamada M, Sugimoto Y, Miyazaki S, Tsujimoto G. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nature Medicine*. 2005;**11**(1):90-94. DOI: 10.1038/nm1168
- [28] Schaffer JE, Lodish HF. Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein. *Cell*. 1994;**79**(3):427-436
- [29] Coburn CT, Knapp FF Jr, Febbraio M, Beets AL, Silverstein RL, Abumrad NA. Defective uptake and utilization of long chain fatty acids in muscle and adipose tissues of CD36 knockout mice. *The Journal of Biological Chemistry*. 2000;**275**(42):32523-32529. DOI: 10.1074/jbc.M003826200
- [30] Coleman RA, Lewin TM, Van Horn CG, Gonzalez-Baró MR. Do long-chain acyl-CoA synthetases regulate fatty acid entry into synthetic versus degradative pathways? *The Journal of Nutrition*. 2002;**132**(8):2123-2126
- [31] Egwim PO, Kummerow FA. Incorporation and distribution of dietary elaidate in the major lipid classes of rat heart and plasma lipoproteins. *The Journal of Nutrition*. 1972;**102**(6):783-792
- [32] Schrock CG, Connor WE. Incorporation of the dietary trans fatty acid (C18:1) into the serum lipids, the serum lipoproteins and adipose tissue. *The American Journal of Clinical Nutrition*. 1975;**28**(9):1020-1027
- [33] Rangel R, Dobroff AS, Guzman-Rojas L, Salmeron CC, Gelovani JG, Sidman RL, Pasqualini R, Arap W. Targeting mammalian organelles with internalizing phage (iPhage) libraries. *Nature Protocols*. 2013;**8**(10):1916-1939. DOI: 10.1038/nprot.2013.119

- [34] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*. 1959;**37**(8):911-917. DOI: 10.1139/O59-099
- [35] Rouser G, Nelson G, Fleisher S, Simon G. Lipid composition of animal cell membranes, organelles, and organs. In: Chapman D, editor. *Biological Membranes*. New York: Academic Press Inc.; 1968. pp. 5-69
- [36] Neufeld EJ, Majerus PW, Krueger CM, Saffitz JE. Uptake and subcellular distribution of [<sup>3</sup>H]arachidonic acid in murine fibrosarcoma cells measured by electron microscope autoradiography. *The Journal of Cell Biology*. 1985;**101**(2):573-581
- [37] Lanser AC, Emken EA, Ohlrogge JB. Oxidation of oleic and elaidic acids in rat and human heart homogenates. *Biochimica et Biophysica Acta*. 1986;**875**(3):510-515
- [38] Paton CM, Ntambi JM. Biochemical and physiological function of stearoyl-CoA desaturase. *American Journal of Physiology. Endocrinology and Metabolism*. 2008;**297**(1):E28-E37. DOI: 10.1152/ajpendo.90897.2008
- [39] Reis-Sobreiro M, Roué G, Moros A, Gajate C, de la Iglesia-Vicente J, Colomer D, Mollinedo F. Lipid raft-mediated Akt signaling as a therapeutic target in mantle cell lymphoma. *Blood Cancer Journal*. 2013;**3**:e118. DOI: 10.1038/bcj.2013.15
- [40] Miyazaki M, Bruggink SM, Ntambi JM. Identification of mouse palmitoyl-coenzyme A Delta9-desaturase. *Journal of Lipid Research*. 2006;**47**(4):700-704. DOI: 10.1194/jlr.C500025-JLR200
- [41] Yamashita A, Hayashi Y, Nemoto-Sasaki Y, Ito M, Oka S, Tanikawa T, Waku K, Sugiura T. Acyltransferases and transacylases that determine the fatty acid composition of glycerolipids and the metabolism of bioactive lipid mediators in mammalian cells and model organisms. *Progress in Lipid Research*. 2014;**53**:18-81. DOI: 10.1016/j.plipres.2013.10.001



