

**Switching triglyceride palmitoyl and oleoyl positions in sn-2 and sn-3 changed
body weight gain rates and hepatic gene expressions in mice fed a high-fat diet**

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Abstract

Alterations of triacylglycerol (TAG) are associated with metabolic diseases such as obesity and diabetes. The fatty acyl groups in a TAG molecule determine its characteristics from melting temperature to digestion and absorption. We hypothesize that the TAG fatty acyl position affects the body weight (BW) gain and lipid metabolism in mice fed a high-fat diet (HFD, 36 % w/w diet). Six-week-old male C57BL/6J mice were randomly assigned to 3 dietary groups, 1,2-dipalmitoyl-3-oleoylglycerol (PPO), 1,3-dipalmitoyl-2-oleoylglycerol (POP), and chow control. The food intake and BW were measured daily during 6 weeks of treatment. The PPO group had significantly higher food intake, caloric intake, and BW gain rate but lower liver-to-body weight ratio than the POP group. The chow group had higher food intake in grams, but lower caloric intake, liver and epididymal fat weight, and body weight gain rate than the PPO, but not the POP group. The PPO group had higher blood glucose levels than the POP and chow groups. The chow group had lower cholesterol levels than the PPO and POP groups. The blood TAG levels are not different among the three groups. The hepatic levels of insulin receptor substrate 1, fatty acid synthase, and phosphorylated acetyl CoA carboxylase of the chow group were higher than those of the PPO or POP groups. The stearoyl CoA desaturase 1 level in the POP group was lower than in the PPO and chow groups. We conclude that the TAG fatty acyl locations affect the food intake, BW gain rate, and lipid homeostasis in mice.

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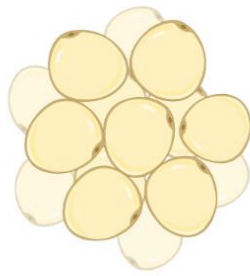
Chapter 1

1.1 Lipids

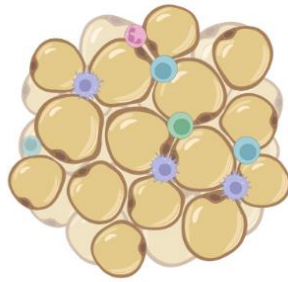
1.1.1 Definition of lipids

Macronutrients contain carbohydrates, fats, and proteins that provide energy to maintain body and cellular structures and systems functioning. According to the dietary reference intake (DRI) published by the US department of health and human services, three dietary macronutrients, carbohydrate protein and fat (or lipid), should fulfill 45-65%, 10-35%, and 20-35% of one's daily calories intake, respectively (1). Lipids are defined as biomolecules that are soluble in non-polar solvents. LIPID MAPs classified lipids molecule into eight categories, including fatty acids, glycerophospholipids, glycerolipids, prenols, polyketides, saccharolipids, sphingolipids, and sterols (2). One of the primary functions of lipids is to act as a stored form of energy that supports human daily activities. When energy intake is more than the energy expenditure, the excess energy is stored as triacylglycerol (TAG) in the adipose tissue (3). Compared to other forms of energy storage, such as glycogen made of glucose coming from carbohydrates, TAG, as a major lipid molecule, does not need water to be stored in the adipose tissue and contains high energy per unit of mass, which will provide longer-term of energy reserve and a greater number of calories (4).

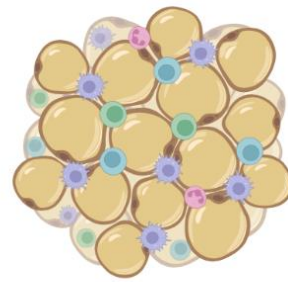
The adipose tissue, the place where TAG is stored, is located in different parts of the body (5). Precursor cells differentiate into adipocytes and from adipose tissues, which can be categorized into three types, white, brown, or beige adipose tissues, as shown in Figure 1. White adipose tissue (WAT) is the major one in the human body; it functions as the primary energy storage site and helps insulate the body. Brown adipose burns more energy and



White adipose
tissue



Beige adipose
tissue



Brown adipose
tissue

Figure 1. White, beige, and brown adipose tissue.

generates heat to maintain body temperature. Even though beige adipose tissue is genetically different from both white and brown adipose tissue, it also can burn energy and generate heat just like brown adipose tissue, and it can be derived from WAT (5). Brown and beige adipocytes can boost heat production under cold conditions (6). The color difference among all three types of adipose tissues comes from the number of blood vessels and the presence of iron-containing mitochondria within the tissue. Energy conversion takes place in mitochondria, which is the reason that with increased color, adipose tissues increase their ability to burn fuels (7). Visceral fat is located in the abdominal cavity and helps protect important organs like the heart, liver, and kidney (8). Subcutaneous fat is found underneath the skin and contributes to thermal regulation in the body in response to the changes in the environment temperature (9).

Other lipid molecules such as eicosanoids and sterols hormones can function as signaling molecules to regulate a variety of functions from appetite, and fuel homeostasis, to the blood clotting (10). Lipids such as phospholipids and cholesterol also act as major structural components of the cell membrane. Fat depots in the body may also contribute to the storage of other lipophilic molecules such as fat-soluble vitamins (11).

1.1.2 Classification

The most common way to classify lipids is through their structural differences. Lipids can be separated into three major groups, simple, complex, and derived lipids. Simple lipids are defined as the fatty acid ester form of different alcohols and do not carry any other substance. The main simple lipids include TAGs and wax esters. Complex lipids are defined as lipids containing three or more chemical identities, including fatty acid and glycerol, plus other substrate groups such as phosphate or nitrogenous base group. The representatives of complex

lipids include phospholipids, glycolipids, and lipoproteins. The third major lipid category is derived lipids, which are defined as the hydrolysis product of simple and compound lipids. Derived lipids include fatty acids, glycerol, and sterol lipids.

1.2 Triacylglycerol

TAG is a simple lipid and major dietary fat. Most human dietary fat intake is in the form of TAG, which can be found in many fat-rich foods, including both animal and plant oil, and is made up to 95% of dietary lipid in the food (12). A TAG molecule contains three fatty acyl groups esterified to the glycerol backbone. The physical, chemical and physiological characteristics of the TAG are determined by the properties of the three acyl groups. Therefore, TAG molecules are classified according to their structural differences (13).

a. Simple vs. complex TAG

When a TAG contains three identical acyl groups, it is identified as a simple TAG, which is very rare in nature. One TAG with two or three different acyl groups is classified as complex or mixed TAG. The difference in the acyl groups can be caused by different chain lengths, numbers, positions, configurations of the double bonds, or stereochemical structures.

b. Saturated, monounsaturated, polyunsaturated fatty acids

Complex TAGs can be further classified based on their saturated status of the acyl groups. The saturated acyl group is classified as a fatty acid side chain that contains no double bond. The monounsaturated acyl group has one double bond, and the polyunsaturated contains more than one double bond.

1.2.1 Triacylglycerol Structure modifications

Over the years, it has been recognized that the fatty acid composition of TAGs can be very different. The second position of the glycerol is unique as it can be a chiral center, and it is not always recognized that the two primary positions are stereo-chemically different. Therefore, TAGs exist in the form of enantiomers. These positions are defined by the "stereo numbering" (sn) system as sn-1, sn-2, and sn-3, and in native triacyl-sn-glycerol, each position has a unique fatty acid composition (14). Native TAG molecule structures are largely different in animal oil or plant oil (15). Most TAG molecules in animal adipose tissue have a saturated fatty acid in the sn-1 position and an unsaturated fatty acid in the sn-2 position; the fatty acid in the sn-3 position could vary among different animal oils. In plant oils, saturated fatty acid mainly occurs in the sn-1 position. At the same time, unsaturated fatty acids like oleic and linoleic are more commonly found in plant oils than in animal oils (15).

To further study any impacts of structural differences of TAGs on biochemical and physical property changes and related lipid metabolism pathways, scientists started to modify their structures and generate artificial ones. Early studies demonstrated that modified dietary fat could be beneficial in mice by improving nitrogen balance, oxygen consumption, and energy expenditure (16). By doing this, scientists can create TAGs that are only different in only one position, which can be used to further study the physical, chemical, and physiological properties of those TAGs due to their structural differences.

1.2.2 Fatty acid interesterification

Fatty acid interesterification in a TAG molecule can be defined as a re-distribution of the fatty acid regarding its position over the glycerol backbone. The reaction of interesterification in

the early years relies on the different crystallization temperatures of different fatty acids. When a high melting TAG is allowed to crystallize, the major saturated fatty acids will be left in the liquid phase, so the distribution of the lipid phase fatty acids will be organized. The continuation of this re-distribution of the residual fatty acid generates TAG with an even higher melting point (1, 13, 17). This allows scientists to obtain TAGs that are only different in one location to further investigate their functions.

Interesterification is now widely used as an alternative to partial hydrogenation of fats and oils. The process uses a chemical approach to catalysis to randomize all three stereospecific positions of fatty acids in natural edible oils at low temperatures. The enzymatic method can be either random or specific, depending on the enzyme used in this process. The distribution of fatty acids on the glycerol backbone depends on the location of fatty acids within or between TAGs. If interesterification contains TAG seeds found in the same dietary fat, the fatty acid composition will not change (18).

Inter-esterified TAGs share the same fatty acid components compared to their natural forms, besides the positions of the fatty acid groups. Even though the calory content for Inter-esterified TAG is considered similar compared to its natural form, the position change would alter the metabolism outcomes. Fatty acid at the sn-2 position is better absorbed than at the sn-1 or sn-3 position for both long chain and medium chain fatty acid. Previous research showed that in lymph-cannulated rats, both caprylic and linoleic fatty acid in the sn-2 position could be better absorbed than in the sn-1 or sn-3 position (19). It is the same for long chain saturated acid, which can be better absorbed at the sn-2 position but better excreted at the sn-1/3 position. Therefore, since the fatty acid at the sn-2 position is more absorbed than those

at the sn-1/3 positions, it is profoundly related and more crucial to lipid metabolism outcomes, including lipidemia, platelet aggregation, and other plasma fatty acids parameters compared to fatty acids at sn-1/3 positions, regardless of the saturation status of the fatty acid (20). For example, unsaturated fatty acid at the sn-2 position can be efficiently uptake by hepatic tissue as 2-MAG and is easier to be further covert to very long chain PUFA (21).

1.2.3 Fatty acid randomization

Unlike fatty acid interesterification which needed to use the catalyzation of a regiospecific lipase, fatty acid randomization occurs when a non-specific lipase is used (22). In this case, the specific distribution of fatty acids in a natural TAG is interrupted and changed to a random distribution. Randomization allows component fatty acids to be esterified equally to all three glycerol carbon atoms. In contrast, the distribution is specific and unequal in naturally occurring fats (11). Randomized fats have diverse applications in both the food industry and clinical applications. In the food industry, since randomized fats have a different melting point and crystallization point compared their natural forms, it is wildy used in bakery and confection products field field (23).

1.2.4 Structure modification application

In clinical applications, randomization provides an energy-rich, well-absorbed maturity for parenteral, enteral, and infant feeding Fields(24). For example, optimal randomization of medium-chain fatty acids on sn-1 and sn-3 positions of TAG allows direct absorption into the portal circulation after digestion (25). This product can be used to help patients with insufficient fat absorption (26). Structurally modified TAGs can help improve lymphatic absorption and lead to increased absorption rate for both TAG and cholesterol as they share the same transport

pathway in healthy or ischemia and reperfusion injured rats. All of these indicate that modified fats can be used to improve tolerance and lower gastrointestinal complications for patients with malabsorption syndrome (27). Structured lipid emulsions for parenteral nutrition in metabolically stressed patients are said to promote systemic fat oxidation and nitrogen balance (28).

The specific localization of C16:0 sn-2 in breast milk fat has a biological function by enhancing the fat absorption (29). Likewise, infant formula contains Betapol™, a C16:0 interesterification fat at sn-2. This increases fat absorption and decreases calcium excretion in infants (30). In contrast, decreased fats absorption can be engineered to promote weight loss, such as randomizing C18:0 to short-chain fatty acids (C2:0 to C4:0)(31).

1.3 Dietary TAG metabolism pathways

The metabolism of TAG in the body refers to all biochemical processes related to the absorption, transport, usage, and storage of TAG.

After the intake, foods are partially digested in the stomach first before the food bolus enters the small intestine. The digestion of TAGs by gastric lipase reduces the sizes of the lipid droplets in the stomach, which are further digested in the small intestine lumen by pancreatic lipase (32). The action of pancreatic lipase requires digestion and absorption, which starts in the upper segment of the jejunum (33). The pancreatic lipase hydrolyzes the ester bonds on a TAG molecule's sn-1 and sn-3 positions and breaks them down into 2-monoacylglycerol (2-MAG) and two free fatty acids (FFAs). Further hydrolysis of 2-MAG by pancreatic lipase in bile acid micelles results in the formation of glycerol and 3 FFAs (34). At the same time, the degradation of TAG will cause gall bladder emptying and cholecystokinin releasing, as well as increase pancreatic

lipase secretion (35). A TAG molecule must be hydrolyzed into mono/diacylglycerols and fatty acids, which are solubilized in the mixed micelles and allowed to be transported through passive diffusion and active transport mechanism across the apical membrane from the intestine lumen into the enterocytes (36).

1.3.1 Monoacylglycerol pathway

Inside the enterocytes, 2-MAG and fatty acids are re-esterified in the smooth endoplasmic reticulum (ER) into TAGs. There are two different intracellular metabolic pathways to re-esterify fatty acids. The first one is the MAG pathway, which is a sequential acylation of MAG in the presence of acyl-CoA. 2-MAG is re-acylated first by monoacylglycerol acyltransferase to generate diacylglycerols, then with diacylglycerol acyltransferase, diacylglycerols get further acylated into a TAG molecule. The enzymes involved in these MAG pathways are present in a complex called TAG synthetase, which is located on the cytosolic surface of the ER. Therefore, TAGs are first formed at the cytosolic surface of the ER, and then they gain access to the lumen of the ER.

1.3.2 Glycerol 3-phosphate pathway

The second one is the glycerol 3-phosphate pathway. This involves the enzymatic specific and stepwise acylation of glycerol 3-phosphate to form 1-acyl-glycerol-3-phosphate (lysophosphatidic acid), 1,2-diacyl-glycerol-3-phosphate. Glycerol 3-phosphate is generated after dihydroxyacetone phosphate (DHAP) reduction by glycerol-3-phosphate dehydrogenase. In the presence of acyl-CoA, glycerol 3-phosphate is acylated into 1-acyl-glycerol 3-phosphate, and then 1,2-diacyl-glycerol-3-phosphate. After that, in the presence of phosphatidic acid phosphatase, phosphatidic acid is hydrolyzed to release inorganic phosphate and to form

diacylglycerol, which is further esterified to form TAG. Under normal lipid absorption condition in the enterocytes, the MAG pathway is preferred due to the sufficient supply of 2-monoacylglycerol and fatty acids. However, if 2-MAG is lacking or insufficient, the glycerol-3-phosphate pathway becomes the major metabolism pathway to the synthesis TAGs (37).

1.4 *De novo lipogenesis*

Dietary TAG absorption is not the only source of TAGs in mammals. De novo lipogenesis is another source of TAGs, which synthesizes fatty acids from excess carbohydrates, typically after intake of carbohydrate-rich food. This process primarily occurs in the liver and is considered a mechanism to maintain the blood TAGs homeostasis (38). Carbohydrates are broken down into glucose, which enters the cells via glucose transporter 2 (39). Figure 2 shows that glucose is metabolized in glycolysis to generate pyruvate. The first step of glycolysis is catalyzed by the hexokinases that transfers a phosphate group from ATP to glucose to produce glucose-6-phosphate. Glucose-6-phosphate is then converted into its isomer, fructose-6-phosphate, with phospho-glucose isomerase. Then another phosphate group is transferred from ATP to fructose-6-phosphate, making fructose-1,6-bisphosphate with phosphor-fructose kinase. Fructose-1,6-bisphosphate is then broken to form two three-carbon sugars: dihydroxyacetone phosphate or glyceraldehyde-3-phosphate with the presence of fructose biphosphate aldolase. However, only glyceraldehyde-3-phosphate will continue to the next step of glycolysis. Glyceraldehyde-3-phosphate dehydrogenase converts glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate and reduces NAD^+ to NADH and H^+ .

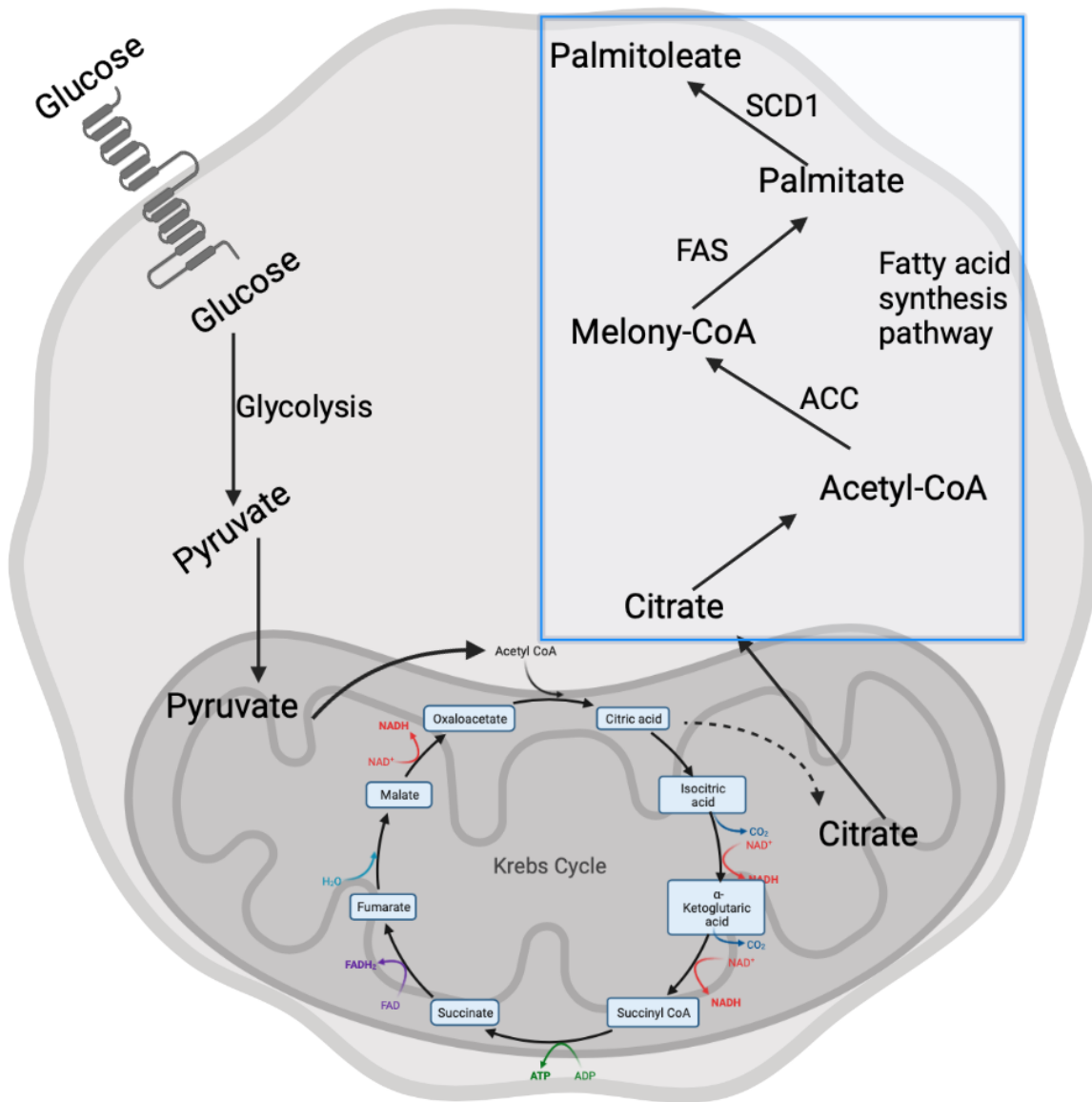


Figure 2. De novo lipogenesis pathway, using glucose to generate palmitoleate.

1,3-bisphosphoglycerate then loses one of its phosphate groups to ADP to generate an ATP and 3-phosphoglycerate, catalyzed by phosphoglycerate kinase. 3-phosphoglycerate is converted into its isomer, 2-phosphoglycerate with phosphoglycerate mutase as the enzyme, and 2-phosphoglycerate is then dehydrogenized to phosphoenolpyruvate with enolase as an enzyme. Finally, phosphoenolpyruvate loses its phosphate group to ADP to generate another ATP and pyruvate, which is the end product of glycolysis. The pyruvate then enters mitochondria and is converted into acetyl CoA, which is used in the tricarboxylic acid (TCA) cycle, also known as the Krebs cycle. First, pyruvate is oxidized in the presence of NAD^+ from acyl-CoA. This reaction is carried by a large enzyme complex called pyruvate dehydrogenase complex (40). Then the acetyl-CoA starts the TCA cycle to condense with a four-carbon molecule called oxaloacetate, to lose the CoA and results in a 6-carbon molecule called citrate in the presence of the enzyme citrate synthase. Citrate is then converted into its isomer, isocitrate, by the enzyme aconitase. Then isocitrate is oxidized in the presence of NAD^+ to release one carbon dioxide and then becomes α -ketoglutarate by the enzyme isocitrate dehydrogenase, which is one of the rate-limiting enzymes in the TCA cycle. α -ketoglutarate is further oxidized and reduced to generate one molecule of NAD^+ to NADH and lose one carbon dioxide molecule and the four carbon succinyl CoA. The enzyme responsible for this reaction is another rate-limiting enzyme of the TCA cycle called α -ketoglutarate dehydrogenase. Then in the presence of ADP, the CoA is lost from the succinyl CoA to make one GTP (equivalent to one ATP) and generate succinate. The enzyme for this reaction is called succinyl CoA synthetase. Succinate is further oxidized in the presence of FAD^+ to generate FADH_2 and fumarate in the presence of the enzyme succinyl dehydrogenase. Fumarate is then hydrogenated with one water molecule and converted into

malate by the enzyme fumarase. Finally, malate is further oxidized by the enzyme malate dehydrogenase in the presence of NAD⁺ to generate one NADH and one oxaloacetate, which is the final product of the TCA cycle (38).

The citrate produced by the TCA cycle leaves the mitochondria and enters the cytosol, where it is converted back into acetyl-CoA and oxaloacetate by the enzyme ATP-citrate lyase. This acetyl-CoA is further converted to malonyl-CoA by the enzyme Acetyl-CoA carboxylase (ACC). Malonyl-CoA is then utilized as a building block with acetyl CoA to generate a 16-carbon saturated palmitate by the enzyme fatty acid synthase. The main product of *de novo* lipogenesis is palmitate, which can be elongated and desaturated further (41).

Insulin stimulates hepatic lipogenesis through the induction of the expression levels of enzymes involved in the fatty acid synthesis (42). The insulin receptor is a heterodimer containing two α and two β subunits. The insulin binding to its receptor causes the phosphorylation of β -subunits, and tyrosine kinases. After the activation of β -subunits, components in a cascade of signaling pathways are activated. This leads to changes in expression levels and activation states of enzymes involved in glucose and lipid metabolism. Insulin stimulation induces the expression levels of lipogenic genes such as fatty acid synthase (FAS) and acetyl CoA carboxylase (ACC).

1.4.1 Fatty acid elongation

The main product of *de novo* lipogenesis is palmitate with 16 carbons. However, at least 60% of fatty acids in TAGs have 18 carbons. Palmitoyl CoA is elongated to stearoyl CoA after two carbons from a malonyl CoA group are added to the carboxyl-terminal by elongases (43). Acyl-CoA synthase converts palmitic acid to palmitoyl-CoA in the cytoplasm, then carnitine

acyltransferase transfer palmitoyl-CoA into the mitochondria. In the mitochondria, a two-carbon unit is added to the palmitoyl-CoA by reverse beta-oxidation, when NAD⁺ reductase replaces FAD-dehydrogenase in the first step of beta-oxidation. In this reaction, acetyl-CoA is used as a carbon source, and NADH is used as a reducing equivalent. Unsaturated fatty acids are preferred in this reaction, and the products are used mainly for the mitochondrial membrane (41).

Fatty acid elongation can also occur in microsomal. After palmitoyl-CoA is produced in the cytoplasm, elongases in the endoplasmic reticulum help add a 2-carbon unit as in the fatty acid biosynthetic pathway. However, acetyl-CoA instead of not acyl-ACP is used in this reaction, and malonyl-CoA instead of acetyl-CoA is used as the substrate (44). Both NADH and NADPH can be used as reducing equivalents. Almost all fatty acids can be elongated via this pathway. Different elongase isozymes were used for other substrates, and very long chain fatty acids (ELOVL) 1,3, and 6 are used to elongate saturated and monounsaturated fatty acids. In contrast, ELOVL 2,4 and 5 are used to elongate polyunsaturated fatty acids (41).

1.4.2 Fatty acid desaturation

Fatty acid desaturation is a reaction that introduces a carbon-carbon double bond to an acyl chain in TAG combined with one oxygen molecule completely reduced to water and often alternates with fatty acid elongation (45). Desaturation can be classified with delta nomenclature, indicating that the double bond is generated at a certain position related to the carboxyl end of the fatty acid. Desaturases can appear in all organisms, but only four desaturases occur in humans, at delta4, 5, 6, or 9 position (46). The rule is that if the substrate is fully saturated (without any double bond) or is a trans-fatty acid, the first double bond will be

generated at the Cthe 9 position. If the substrate already contains at least one double bond, then the new double bond will be generated between the carboxyl group and the nearest double bond (47). Desaturation reaction usually produces *cis*-double bonds in TAGs.

Stearoyl-Coenzyme A desaturase (SCD) is one major desaturase that is located the in endoplasmic reticulum (48). Delta 9 desaturase, also known as SCD1, can be found in the liver, and SCD2 can be found in adipose tissue; as many as 5 SCD genes can be found in the human body. SCDs contain flavoprotein and cytochrome b5 or P-450. All saturated fatty acids and many trans-fatty acids can be desaturated by SCDs (49). In this reaction, oxygen is reduced by NADH and generates an enzyme bounded superoxide radical to further oxidize stearoyl-CoA. SCD1 is used to desaturate stearic acid and generate oleic acid, a monounsaturated fatty acid that is universal in all human cells (48).

1.5 Transport of TAG

As shown in Figure 3, the transport of TAG has primarily relied on chylomicrons. Therefore, chylomicrons are primarily generated and synthesized in the small intestine. Broadly, chylomicrons carry re-synthesized dietary TAGs from the small intestine to the heart first and then travel to the skeleton muscle to provide energy, and to the adipose tissue, where the TAGs in the chylomicrons are hydrolyzed into fatty acid for usage and storage (50). In addition, the TAG derived from the tic lipogenesis is packed into very low-density lipoprotein (VLDL), released from the liver, and delivered to the skeletal muscle and adipose tissue as well (51).

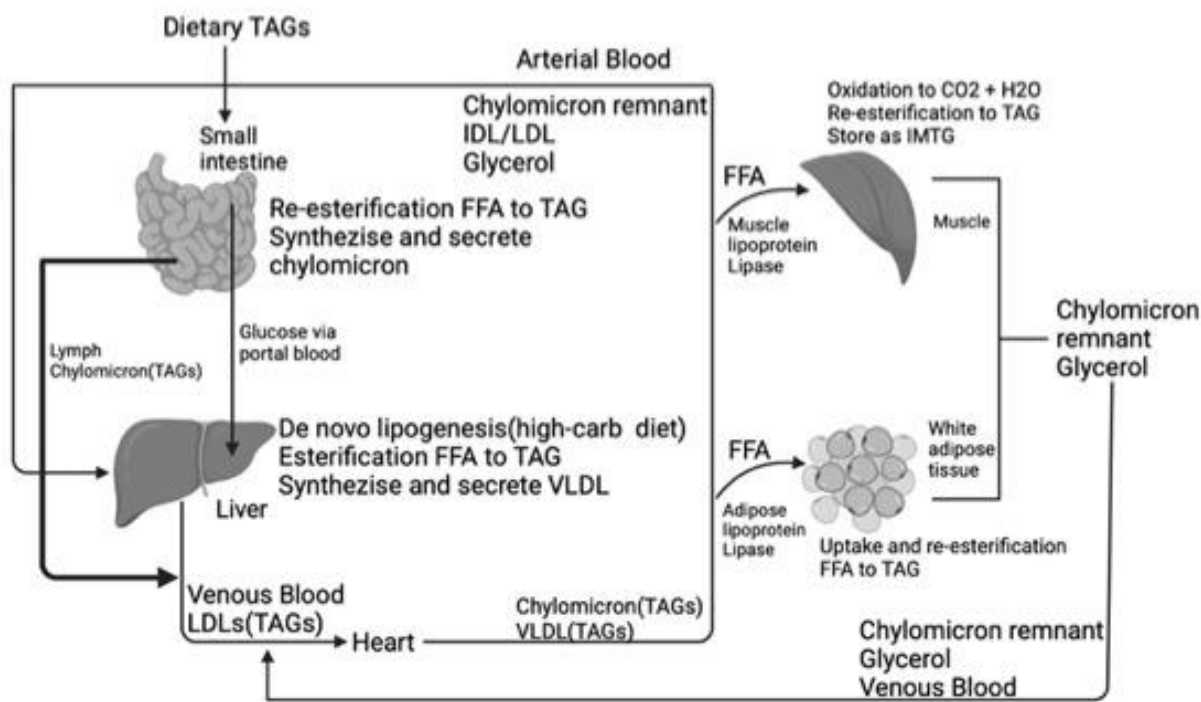


Figure 3. Dietary TAG metabolism and transport pathways in the human body in the fed state.

1.5.1 Chylomicrons transport pathway

Chylomicrons are large particles produced in the small intestine that carry dietary lipids and lipophilic vitamins. Chylomicrons are not only involved in the transport of dietary TAGs, but they also carry cholesterol and lipophilic vitamins to the peripheral tissues (52). Chylomicrons carry one copy of each of several apolipoproteins (Apo, the protein parts of a lipoprotein), including Apo AI, Apo B48, Apo CIII, and Apo E (53). Apo B48 acts as the core structural protein, and each chylomicron particle contains one copy of Apo B-48 molecule (54). Before picking up TAGs, nascent chylomicrons first lose around 70 to 80 percent of apo AI, which goes to make nascent HDL (55). While in circulation, chylomicrons acquire apo CII, apo CIII, and apo E from HDL. The secreted chylomicrons enter the blood via the lymph, to the subclavian vein, via the thoracic duct. Chylomicron particles appear in the blood within 1-2 hours after a meal and continuously enter into the blood from lymph for 8-10 hours (4). The size of chylomicrons varies based on dietary fat intake. A fat-rich meal will lead to the formation of larger chylomicron particles since more TAGs are packed onto one particle for transport. In the fasting state, smaller chylomicron particles may be formed to carry a smaller number of TAGs. When the starvation process continues and the body has no TAGs to be transferred from the small intestine, there will be no chylomicron particles (56).

Inside an enterocyte, the movement of TAGs from the ER to Apo-48 is mediated by microsomal triglyceride transfer protein (MTP). The newly made chylomicrons are delivered to the systematic circulation to reach peripheral tissues rather than the liver first, so the skeleton muscle and adipose tissue can pick up TAGs more efficiently (57). When chylomicron particles containing TAGs travel in the blood vessel, they meet the heart and lung first, where lipoprotein

lipase (LPL) is expressed (53, 58). Glycosyl phosphatidylinositol HDL Binding Protein 1(GPIHBP1) is critical for LPL binding, and action help to anchor chylomicrons to the endothelium. LPL is found along the capillary beds. It is secreted from cells (myocytes and adipocytes) in the sub-endothelium and binds to endothelial cells via heparin sulfate proteoglycans and GPIHBP1. It depletes TAG from chylomicrons and leads to generating FFAs and MAG, which are further hydrolyzed by a mono acyl glyceride lipase on the plasma membrane to FFAs and glycerol. Then tissues, including skeleton muscles and adipose tissue, take up FFA. Glycerol will return to the liver bound to the albumin (53). During hydrolysis, a substantial portion of phospholipids, apo A, and apo Cs are removed and transferred to HDL. All of these lead to the production of chylomicrons remnants that contain Apo E from HDL (59).

Loss of TAGs from chylomicrons following LPL action leads to the production of chylomicron remnants that are rich in cholesterol but poor in TAGs and contain Apo E as their pre-dominate protein. Chylomicrons remnants are cleared by the liver via the perisinusoidal space. This is a receptor-mediated process, including low-density lipoprotein (LDL) receptor, LDL receptor-related protein (LRP), and Apo E receptor, with enzyme hepatic lipase (HL) (60). Excess phospholipids are removed from chylomicrons and used for the formation of new HDL particles via phospholipid transfer protein (PLTP) by interacting with the circulating Apo AI (53). Around 80-90% of The AG of chylomicrons is removed before their clearance from the blood (4).

1.5.2 VLDL transport pathway

The vehicle responsible for the transport of newly synthesized TAGs in the liver is VLDL; this process is called endogenous lipoprotein pathway (53). TAGs and cholesterol esters in the liver are transferred to ER and loaded on the newly synthesized Apo B-100. Just like the chylomicron

pathway, this transfer is also mediated by MTP. The synthesis rate of VLDL is primarily determined by the availability of TAGs. If the number of TAGs that need to be transferred from the liver is low, Apo B-100 will be rapidly degraded. Therefore, the rate of VLDL formation is determined by available TAGs. Adding TAGs to Apo B-100 particles at an early stage required MTP (53). When additional TAGs are added via this pathway, MTP is no longer needed. Loss of function or mutation in Apo B-100 or MTP results in the inability to produce VLDL and markedly lower plasma TAG and cholesterol levels (61). However, the pathway of VLDL particles generated in the hepatocytes and transported to the circulation remains unknown.

Just like the chylomicron pathway, VLDL particles are traveled to peripheral tissues, and LPL hydrolyzes TAGs and releases fatty acids. Since the metabolism of VLDL and chylomicrons are very similar, there is competition between those two pathways (53). A higher level of chylomicrons can inhibit the clearance of VLDL. When the TAG is removed from the VLDL, it will lead to the formation of VLDL remnants, called intermediate density lipoproteins (IDL). IDL particles are also rich in cholesterol esters and contain Apo E obtained from HDL. IDL particles can be removed from circulation by the liver. This is also a receptor-mediated process. IDL is removed by binding Apo E to LDL and LRP receptors; however, different from the chylomicron remnants clearance pathway, only a certain amount of IDL particles can be cleared, approximately 50%, but it may vary due to individual differences. The TAGs remaining in the IDL will be further hydrolyzed by HL and lead to further decrease in TAGs content. Leftover exchangeable apolipoproteins will be transferred from IDL particles to other lipoproteins and form LDL. IDL can pick up cholesterol esters from HDLs in exchange for TAG via cholesterol ester

transfer protein (CETP). Eventually, IDL will be converted to LDL. LDL particles also contain cholesterol ester and Apo B-100 and are considered as the product of VLDL metabolism (53).

In the TAGs transport system, LPL plays a very important role. LPL can be synthesized in the muscle, heart, lung, and adipose tissue. Then it is secreted and attached to endothelium cells of the adjacent blood capillaries (53, 62). LPL hydrolyzes the TAGs within chylomicrons and VLDL to fatty acid and glycerol, so the tissues can pick up FFA from their vehicles. Apo C-II is required as a co-factor of this enzyme. Apo A-V oversees the activation of this enzyme. Apo C-III and Apo A-II inhibit the activity of LPL. Insulin also can stimulate the expression and activity of LPL. At the same time, LPL is less active in diabetes patients (53). In the fed stage, synthesis and secretion of LPL in adipose tissues are increased. In the the fasting state, its synthesis and secretion in muscle are increased (63).

The two major destinations of circulation TAGs are the skeleton muscle and adipose tissue (53). When TAGs arrive at the skeleton muscle, TAGs will be hydrolyzed by LPL to release FFAs into the muscle cell. FFA incellscle cell will go through beta-oxidation to generate acetyl-CoA, which enters the Krebs cycle, and produces CO₂ and NADH and one GTP to provide energy for the cell. Excess FFAs can be re-esterified and stored as intramuscular TAG. In adipose tissue, TAGs are broken down to glycerol and FFA by the enzyme adipose LPL. Similar to that in the muscle, excess FFA is re-esterified to TAG and stored for further use (53). In both, leftover chylomicron remnants or IDL/LDL will be moved back to the liver.

1.6 Lipolysis

As shown in Figure 4, during fasting, plasma insulin concentration decreases during fasting, which the riggers lipolysis process in WAT, and releases FFA to plasma that can be picked up by

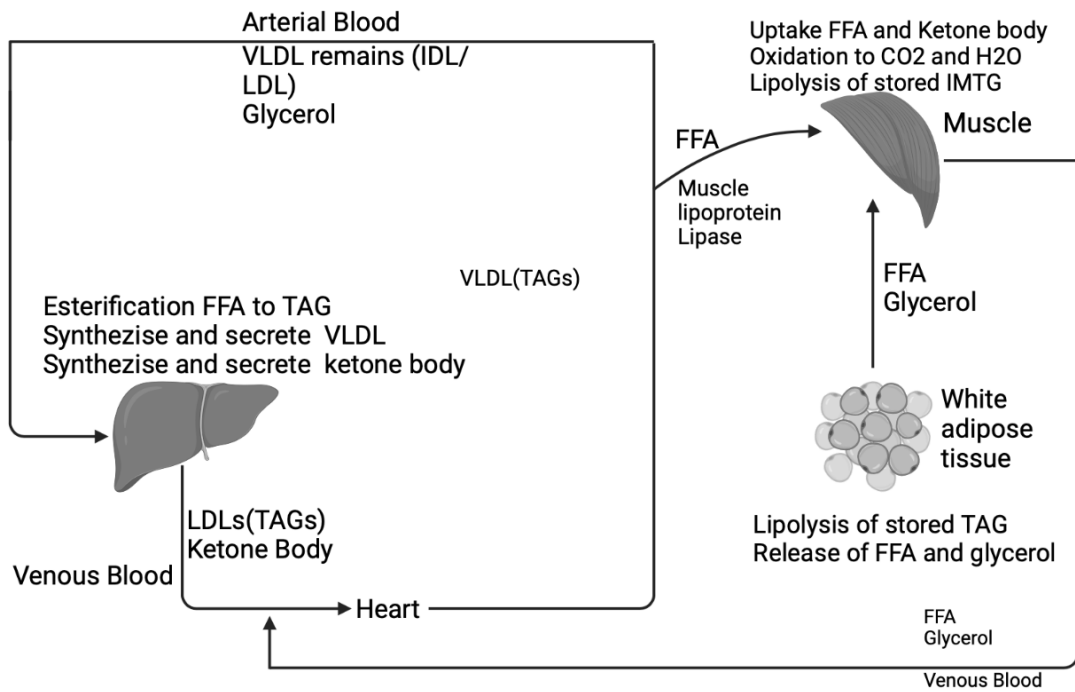


Figure 4. TAG lipolysis in the liver and adipose tissue in the fasting state.

the liver. FFAs from becomesasma become the primary source of making hepatic TAGs. In the adipose tissue, TAGs are stored in the cytoplasm as intracellular lipid droplets, and this is where the lipolysis process takes place (64, 65). Lipolysis is mediated by lipases and starts with phosphorylation of the highly regulated lipase enzymes access to lipid droplets. These lipase enzymes work sequentially, and TAGs in lipid droplets are eventually broken down through multiple steps of hydrolysis to 3 FFAs and one glycerol. Each hydrolytic step releases one fatty acid. The first step of lipolysis is the rate-limiting and is carried out by adipose triglyceride lipase (ATGL). This enzyme catalyzes the hydrolysis of one TAG to one diacylglycerol and one FFA. The second step is carried out by hormone-sensitive lipase (HSL) that catalyzes the hydrolysis of one diacylglycerol to generate one MAG and one FFA. The final step is carried out by monoacylglycerol whichpase (MGL), which catalyzes the hydrolysis of one MAG to release the last fatty acid and one glycerol as the final product (65).

Once FFA and glycerol enter the venous blood vessel, they will be circulated to the heart and enter the arterial blood vessel. FFAs in the plasma will be uptake by the skeletal muscle cells and used through β -mitochondrial fieldmitochondria (66). In the liver, glycerol is converted into glycerol 3-phosphate, which is used in the re-esterification of fatty acids to reproduce TAGs. TAGs are packed into VLDL and secreted from the liver. In addition, the fatty acid is converted into acyl CoA by Acyl CoA synthetases entry into mitochondria requires carnitine palmitoyl transferase 1 (CPT-1). Once inside the mitochondria, fatty acyl CoA is broken down into acetyl CoA through β -oxidation. The acetyl CoA can be used in the TCA cycle to generate

NADH and CO₂ or the production of ketone bodies. When excessive acetyl CoA is created due to elevated flux of fatty acid into β -oxidation, acetyl-CoA then will be condensed into acetoacetyl-CoA with the enzyme acetyl coenzyme A acetyltransferase (ACAT). Then, the mitochondrial form of HMG-CoA synthase combines one acetoacetyl-CoA and one acetyl Co A to create an HMG-CoA, which is hydrolyzed by HMG-CoA lyase to generate one acetoacetate and one acetyl CoA. In the presence of NADH, acetoacetate is converted into β -hydroxybutyrate and NAD⁺ by β -hydroxybutyrate dehydrogenase. In addition, acetoacetate can be spontaneously converted into acetone. The production of ketones, acetoacetate, β -hydroxybutyrate, and acetone is called ketogenesis and occurs in the mitochondria of hepatocytes (67). As acetoacetate and β -hydroxybutyrate are acids, ketoacidosis is a characteristic of patients with uncontrolled diabetes (68).

Both acetoacetate and β -hydroxybutyrate are ketone bodies and can be used for energy in peripheral tissues (69). In extrahepatic tissues, β -hydroxybutyrate is converted to acetoacetate and one NADH with the enzyme β -hydroxybutyrate dehydrogenase. Then, the enzyme β -ketoacyl-CoA transferase uses succinyl CoA and acetoacetate to generate succinate and acetoacetyl-CoA which is broken down by thiolase into two acetyl CoA, which enter the TCA cycle. Acetate can produce 22 ATP per molecule after the oxidative phosphorylation process. Since acetone cannot be used to produce acetyl-CoA, it will be excreted either through exhale or urine (69). Even though ketogenesis can be regulated by many hormones, including cortisol, glucagon, catecholamines, and thyroid hormones, it is primarily regulated by insulin level in the body (70).

1.7 Health effects

TAG, as one of the major forms of dietary fat and source of energy, is deeply related to human health. The major impact of too much TAGs intake would be metabolic syndrome.

1.7.1 TAG and metabolic syndrome

Metabolic syndrome is a combination of insulin resistance, hyperglycemia, hypertension, dyslipidemia, and obesity. Metabolic syndrome is related to an increased risk of multiple metabolic diseases, including cardiovascular disease, stroke, and type 2 diabetes (T2D) (71, 72). The World Health Organization (WHO) first developed the definition of metabolic syndrome in 1998, and this is also the most used definition to identify metabolic syndrome by health professionals. Since insulin resistance is the key to developing obesity, hypertension, and dyslipidemia, it is the required criteria for metabolic syndrome (72, 73). The definition also include obesity (waist-hip ratio > 0.9 for male or > 0.85 for female, or BMI > 30kg/m²), hyperglycemia (insulin resistance), dyslipidemia (TAG level equal or higher than 150 mg/dl, or HDL-C less than 35mg/dl for male or 39mg/dl for female), hypertension (equal or higher than 130/80 mmHg), and microalbuminuria. An individual needs to meet at least two of the criteria and insulin resistance or T2D to be identified as having a metabolic syndrome (73). Metabolic syndrome can be used as a clinical assessment to identify patients at higher risk of having T2D or CVD. However, it is not the only way to identify individuals with higher risk. For example, a family history of T2D will also contribute to an increased risk of an individual having a T2D (74).

Atherogenic dyslipidemia is the reason why TAG is related to metabolic syndrome. The definition of atherogenic dyslipidemia includes high plasma TAG level, low HDL-cholesterol level, and high small dense LDL level (75). Both low HDL-cholesterol levels and high small dense

LDL levels indicate that the TAGs transfer in the blood is relatively slower and less efficient compared to individuals with higher HDL-C levels and lead to increased plasma TAGs levels (53).

Visceral obesity and insulin resistance also contribute to atherogenic dyslipidemia (76, 77). Insulin is especially important since it can regulate lipidemia in several ways. First, insulin can suppress lipolysis in adipose tissue, therefore decrease in insulin signaling will lead to increased lipolysis and result in increased FFA levels in the blood. After FFA arrives in the liver, it can be used as a substrate to generate TAGs (77, 78). At the same time, since FFAs can promote the production of Apo B100, which is the dominant lipoprotein for VLDL particles, increased FFA level will lead to increase VLDL production. Insulin also can regulate Apo-B lipoprotein levels promote Apo-B degradation through the PI3K-dependent pathway, and insulin resistance will also directly lead to increased VLDL production (78). Finally, since insulin can promote the LPL production and activity, especially in adipose tissue, via the increase in its expression, it becomes the rate-limiting mediator that mediates VLDL clearance, and insulin resistance will cause delayed VLDL clearance (79).

In conclusion, insulin resistance contributes to hypertriglyceridemia via both increased VLDL production and decreased VLDL clearance. VLDL will be metabolized into lipoprotein remnants and small dense LDL, both can lead to the formation of atheroma. TAGs in the VLDL are then released to HDL in exchange for cholesteryl esters through CETP, leading to TAGs-rich HDL particles and cholesterol-rich VLDL particles (53). TAGs-rich HDL is preferred so it can be cleared more quickly from the circulation and a decrease faster decreased in plasma TAGs levels. A few left-over HDL particles will help reverse cholesterol transport from the vasculature (80).

1.7.2 TAG and cardiovascular disease

Cardiovascular disease (CVD) has been the primary cause of morbidity and mortality in America for a very long time. Increased atherogenic TAG-rich remnant lipoproteins level has been identified as a risk factor for CVD (81). On the one hand, individuals with high plasma TAGs level also exhibit other risk factors, like insulin resistance, which contribute to the increased chance of having CVD. In addition, elevated plasma TAG level is associated with low HDL-Cholesterol, leading to an increased risk of CVD (82).

Unlike TAGs that can be consumed and degraded by most cells, it is difficult for cholesterol to be degraded by any cell. The remnant cholesterol in TAGs-rich lipoproteins contributes more to atherosclerosis and CVD than elevated TAGs themselves. Even though cholesterol is what accumulated in intimal foam cells and atherosclerotic plaques, chylomicrons that contain a large number of dietary TAGs are too large to enter arterial intima. After lipoprotein remnants enter the arterial intima, remnants can be trapped with LDL since it has a bigger size and higher chance to be transferred to extracellular proteoglycans via attachment. LPL functions on the surface of remnants at the vascular endothelium or within the intima and results in hydrolyzation of TAGs and release of FFAs and MAGs, which may cause local injury and inflammations. Also, remnants can be directly taken up by macrophages and lead to the formation of foam cells. Therefore increased TAGs concentration indicates the raising of cholesterol-rich remnants and leads to low-grade inflammation, which promotes foam cell formation and atherosclerotic plaques and eventually causes CVD and increased death (83).

1.7.3 TAG and Stroke

Stroke is the third leading of death in America, ranking behind CVD and all kinds of cancers. It is also the leading cause of long-term serious disability in America (84). Ischemic stroke is the most common type of stroke and contributes to 80% of the total stroke cases in America, caused by the interrupted blood supply to the brain. The cause of ischemic stroke is when fatty deposits in arteries break off and traveled to the brain area or are caused by blood clotting in the brain area. Increased TAGs levels will lead to increased fat deposits in arteries and increase the chance of having a stroke, with a similar mechanism as it contributes to increased risk of CVD (85). Hemorrhagic stroke is a less common type of stroke, normally caused by burst or leaking blood vessels in the brain area, often related to high blood pressure and frequent use of blood thinner medicine (86).

1.7.4 TAG and Obesity

WAT is the primary energy storage site, not only in human but also in all higher eukaryotes. The main purpose of WAT is to synthesize and store TAG during the energy excess period, as well as hydrolysis TAG to generate fatty acid for other organs to use as energy during energy deprivation. WAT also can secrete adipokines that help regulate energy intake and metabolism (87). Excess dietary fat intake leads to increase TAGs levels, which then leads to excess energy that needs to be stored, eventually causing obesity due to excess WAT. Since obesity is closely related to hypertension, atherosclerosis, and T2D, it has become a serious global public health problem (88). Obesity caused by adipocyte hypertrophy occurs when TAGs synthesis, from either dietary TAGs or de novo TAGs, esterify overcomes TAGs breakdown, which is lipolysis, results in increased TAG storage in WAT. In other words, when

the human body contains more TAGs than it could use, it will lead to obesity. Increased adipocyte size also contributes to insulin resistance, which is one of the major causes and symptoms of T2D (89). Meanwhile, the selective loss of adipose tissue from regions of the body, called lipodystrophies, is associated with metabolic abnormalities that are very common in obesity. Some metabolic complications may result from excess storage of TAGs in tissues that normally do not function as TAGs storage sites, including liver and muscle (90). Even though plasma TAGs level does not contribute to the development of T2D, it is one of the five major accepted criteria for defining T2D. However, it does represent the general ability of the human body to turn food into an energy (91). Insulin not only supervises the use of glucose in the human body as energy but also plays an important role in TAGs synthesis and metabolism. A fasting TAG level of ≥ 150 mg/dl indicates that the human body cannot properly use dietary fat as energy which becomes a sign of insulin resistance, which is closely related to T2D (91).

In conclusion, dietary TAG is deeply related to human health, excess TAGs intake may lead to metabolic syndromes, including increased risk for cardiovascular disease, stroke, and T2D. The structures of TAGs vary in different dietary lipids. At the same time, artificial processed TAGs via fatty acid interesterification or fatty acid randomization are widely used in the food industry and clinical setting. Often due to the different physical or physiological properties of modified TAGs compared to their natural form. However, TAGs metabolism, transportation, and absorption are involved in several organs, including the liver, skeletal muscle, and adipose tissue. It is also regulated by multiple enzymes and hormones like insulin. Therefore, it is important to understand how TAG structure changes would lead to different metabolic outcomes in vivo.

Chapter 2

2.1 Introduction

Dietary fat, as a major macronutrient, is deeply related to human health and can be obtained from both animal and plant sources (92). On one hand, the proper amount of dietary fat intake is necessary for maintaining normal functions in the body and providing energy for daily activities. The DRI of fat is about 20% to 35% of one's daily calory intake (1). In addition, fat also provides support to the immune system, hormone regulation, and cellular structure (92). On the other hand, excessive dietary fat intake will also lead to several adverse health events. First, elevated dietary fat ingestion indicates an increase in energy intake, which will increase the energy stored in WAT, the primary storage site of fat. When excessive fat is stored in the adipose tissue, obesity develops, which is associated with the development of the T2D (71). Second, increased dietary fat intake will lead to increased plasma TAG level and cause hyperlipidemia, a risk factor for the formation of foam cell and atherosclerotic plaques, and then eventually increases the risk of cardiovascular disease, stroke, and other metabolic syndromes (81, 83).

TAG, also called triglyceride, is a major lipid species. Excessive energy is stored as TAGs in WAT. A TAG molecule contains three fatty acyl groups esterified to the glycerol backbone. Those three fatty acyl groups can be identical or different in chain length, number, and position of double bonds. The positions of fatty acyl groups are defined by the "stereo numbering" (sn) system as sn-1, sn-2, and sn-3, and in native triacyl-sn-glycerol. Each position has a unique fatty acid composition (14).

The difference in TAG structure will first change its physical property, including melting and crystallization temperature (18). TAG structural differences also change their biochemical features, such as melting temperature and fatty acids released after hydrolysis, which may affect metabolism. For the dietary TAG, the breakdown is necessary for its absorption (50). The digestion process is associated with emptying of the gall bladder to release bile juice for the formation of micelles and release of cholecystokinin to increase secretion of pancreatic juice that contains digestive enzymes, including pancreatic lipase, the major player in TAG digestion in the small intestine lumen. The complete hydrolysis of a TAG releases three FFAs and one glycerol backbone. During the digestion in the small intestine, the ester bonds at the sn-1 and sn-3 position of the TAG molecule are hydrolyzed first by the pancreatic lipase, generating one 2-MAG and 2 FFAs. The fatty acyl group at the sn-2 position of the 2-MAG will then be further hydrolyzed by pancreatic lipase in bile acid micelles which leads to the production of one more FFA and one glycerol (53). Dietary TAGs have to be hydrolyzed into MAGs and fatty acids before they can be absorbed to cross the apical membrane (brush border membrane) from the intestine lumen into the enterocytes then they are re-esterified, packed into chylomicrons, and transported to the rest part of the body for energy production and storage. Dietary TAGs are primarily transported in chylomicrons. The TAGs on chylomicrons are hydrolyzed by LPL to generate FFAs and glycerol and chylomicron remnants that are eventually taken by the liver (52).

Since the metabolism of TAGs starts with the release of fatty acyl group at sn-1 and sn-3 positions first, the fatty acid at the sn-2 position of the MAG can be metabolized differently. Results of previous studies in rats show that stearic acid at the sn-2 position of a TAG molecule

is better absorbed than at the sn-1 or sn-3 position (93). Since MAG can be absorbed directly into the body, then the fatty acid at the sn-2 position can significantly affect lipemia, platelet aggression, and related plasma fatty acid composition (93). Another study also showed that the long-chain saturated fatty acid at the sn-2 position is better absorbed than those at the sn-1 or sn-3 position (25). The position of the saturated fatty acid not only can affect the absorption rate of TAGs but also affect the clearance rate (20). It has been shown that saturated fatty acid at the sn-2 position will affect the metabolism of dietary TAGs by delaying the clearance of chylomicrons from circulation (94). In addition to the absorption and transport of TAGs, the saturated fatty acid at the sn-2 position also significantly affects the appetite and body weight (BW) in mice (95). However, the possible mechanism remains unknown.

The epidemic of obesity has become a concern of global health for the past few decades. The obesity rate in the United States has increased from the 30.5% in the year 2000 to 41.9% in the year 2017 to 2020. On the one hand, obesity contributes to metabolic syndromes, including CVD, stroke, and certain types of cancer. On the other hand, an increased obesity rate also leads to increased annual medical costs for obesity was 173 billion dollars in the U.S. in the year 2019 (96). Generally, when an individual's body mass index is higher than 30, this individual is considered obese. Uncontrolled obesity increases the risk of diabetes. Around 37.3 million Americans were diagnosed with diabetes in 2021, and about 96 million American adults have prediabetes (97). The environmental causes of obesity are often excessive energy intake and lack of physical activity. Therefore, it is important to understand how foods contribute to this process and what we can do to delay the onset of obesity or T2D.

To investigate the effect of saturated fatty acid on the sn-2 position, fatty acid randomization and interesterification were developed to modify the structure of TAGs and generate artificial fat through partial hydrogenation. Depending on the amount of saturated fatty acid at the sn-2 position of the natural fat, the randomized or interesterification forms of fat may have increased or decreased the amount of saturated fatty acids in their sn-2 position (13). Even though artificial fat is widely used in the food industry and clinical settings, the health impacts derived from fatty acid randomization and interesterification need to be studied in vivo.

1,2-Dipalmitoyl-3-Oleoylglycerol (PPO) and 1,3-Dipalmitoyl-2-Oleoylglycerol (POP) are two dietary TAGs with very similar structures. POP is purified from commercial palm oil in its natural form, with a monounsaturated fatty acid at the sn-2 position. PPO is generated and purified from POP by fatty acid randomization, with a monounsaturated fatty acid at the sn-3 position. Both PPO and POP are solid fat at 37°C. The only difference between these two TAG molecules is that the positions of palmitic and oleoyl acids are switched (95).

We hypothesize that the position of the saturated and monounsaturated fatty acyl groups in the TAG will affect the metabolic outcomes in vivo. This study aims to investigate the role of TAG structure on BW and hepatic gene expression in mice fed a high-fat diet. We report that different positions of oleic acid groups in the dietary TAG structure affected mouse energy intake, BW gain rate during the treatment period, and blood parameters, body composition, and hepatic gene expression levels after six weeks of the treatment.

2.2 Materials and Methods

2.2.1 Reagents

Table 1 shows the antibodies used in this study and their sources. Table 2 shows the reagents and materials used in this study and their sources. Table 3 shows the composition of POP and PPO used in the study. PPO fat was randomized with 0.6% sodium methoxide and then catalyzed at 80°C for 30 minutes. Then the product was washed with water to remove sodium methoxide from the final product. POP fat is synthesized by Xiaosan Wang (associate professor of the School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi, Jiangsu, 214122, PR China)

2.2.2 Animals

Male C57BL/6J mice aged 5 weeks old were obtained from the Jackson Laboratory. All mice were placed in a temperature and humidity-controlled cubicle with a 12-hour light-dark cycle. After arrival, 27 mice were allowed to acclimate to the condition in the animal facility for one week, then were randomly separated into three groups. Nine mice were fed a regular chow diet (the control group). Another 9 mice were fed the chow diet supplemented with 36% (w/w) 1,2-dipalmitoyl-3-oleoylglycerol (PPO group). The last nine mice were fed the chow diet supplemented with 36% (W/W) 1,3-Dipalmitoyl-2-oleoylglycerol (POP group). The PPO and POP had been used previously in a mouse study (95). Mice housed individually had free access to water and the respective diet during the study. Food intake and BW were measured daily for six weeks. At the end of the study, all mice fasted over night (about 12 hours). The mice were anesthetized using isoflurane, and blood samples were collected by cardiac puncture. After

Table 1, Antibodies used in this study for western blot

Name	Company	Buffer	Dilution
Insulin Receptor Substrate 1 (IRS1) (L3D12)	Cell Signaling Technology (Danvers, Massachusetts)	5% w/v non-fat dry milk	1: 500
Insulin Receptor Substrate 2 (IRS2) (L1326)	Cell Signaling Technology (Danvers, Massachusetts)	5% w/v non-fat dry milk	1: 500
Stearoyl-CoA desaturase 1 (SCD1) (C12H5)	Cell Signaling Technology (Danvers, Massachusetts)	5% w/v BSA	1: 500
FAS(C18C12)	Cell Signaling Technology (Danvers, Massachusetts)	5% w/v non-fat dry milk	1:1000
Insulin Receptor β (IR- β) (L55B10)	Cell Signaling Technology (Danvers, Massachusetts)	5% w/v non-fat dry milk	1:1000
Akt (pan) (11E7)	Cell Signaling Technology (Danvers, Massachusetts)	5% w/v BSA	1:1000
Phospho-Glycogen Synthase (PGS) (Ser641)	Cell Signaling Technology (Danvers, Massachusetts)	5% w/v BSA	1:1000
Glycogen Synthase (15B1) (GS)	Cell Signaling Technology (Danvers, Massachusetts)	5% w/v BSA	1:1000
Cluster of differentiation 36 (D8L9T) (cd36)	Cell Signaling Technology (Danvers, Massachusetts)	5% w/v BSA	1:1000
Acetyl-CoA Carboxylase (C83B10) (ACC)	Cell Signaling Technology (Danvers, Massachusetts)	5% w/v BSA	1:500
Phospho-Acetyl-CoA Carboxylase (Ser79) (p-ACC)	Cell Signaling Technology (Danvers, Massachusetts)	5% w/v BSA	1:500

Table 2. Sources of reagents and materials used in this study

Company	Reagent(s)
Jackson Laboratory (Farmington, Connecticut)	Male C57/BL6J mice
ENVIGO (Indianapolis, Indiana)	8604 Rodent chow diet
Thermo Scientific (Waltham, MA)	Pierce BCS protein assay kit, Tris-HCl,
Fisher scientific (Hampton, NH)	acrylamide: bis-acrylamide, ammonium persulfate, Glycerol, DTT, Bromophenol Blue, NaCl, Tween-20
GE (Boston, Massachusetts)	0.2 um PVDF membrane
Thermo fisher	TEMED
TCI	Glycine
EKF link	Stanbio Glucose LiquiColor kit, Stanbio Cholesterol LiquiColor kit, Stanbio triacylglycerol LiquiColor kit

Table 3. POP and PPO composition

TAG included	POP	PPO (chemical randomization)
OOL (Oleic/Oleic/Linolenic acid)	—	0.73
POL (Palmitic/Oleic/Linolenic acid)	1.58	0.97
OOO (Oleic/Oleic/Oleic acid)	—	1.97
OOP (Oleic/Oleic/Palmitic acid)	—	20.62
POP/PPO	91.58	45.81
PPP (Palmitic/Palmitic/Palmitic acid)	—	22.55
OOS (Oleic/Oleic/Stearic acid)	—	0.68
POS (Palmitic/Oleic/Stearic acid)	6.84	3.65
PPS (Palmitic/Palmitic/ Stearic acid)	—	3.03

that, animals were euthanized by cervical dislocation for tissue collection. After collection, the tissue samples were frozen immediately in liquid nitrogen and stored at -80°C until used. The liver and epididymal fat pads were weighed and recorded. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Tennessee at Knoxville (Protocol #2761).

2.2.3 Body composition analysis

The BW gain for each mouse was calculated using initial and final BW. The liver to BW ratio and epididymal fat pads to BW ratio was calculated at the end of the experiment. Total caloric intake was calculated based on the control diet energy density (3.0 kcal/g) provided by the manufactory protocol. The energy density of the PPO and POP diet was calculated as 5.16 kcal/g.

2.2.4 Liver protein sample preparation

About 50 mg of liver tissue was homogenized in 1 mL 1x lysis buffer (1% Triton x-100, 10% Glycerol, 1% NP-40, 50mM HEPES at pH7.5, 10mM EDTA) using a tissue homogenizer (International, Kennesaw, GA) at maximum speed and kept on ice for 30 minutes. Then, the lysate was centrifuged at 15,000 x g for 10 minutes, and the supernatant was collected and defined as the total lysate. The BCA protein assay was performed using the Pierce BCA protein assay kit to determine the total protein concentration in the total lysate. According to the manufacturer's protocol, 5 µL of each total lysate sample was added to 495 µL of ddH₂O. A standard curve was created using BSA protein standards at 0, 5, 10, 20, 40, and 80 µg/µL. The working reagent was generated by mixing reagent A and reagent B (50:1), and a 500 µL working reagent was added to each reaction. Reaction tubes were incubated at 37°C for 30

minutes. The absorbance values of optical density (OD) at 560nm of all reactions in triplicates were measured in 96-plates using a GloMax Multi detect system (Promega, Madison, Wisconsin). The OD value of the blank reaction was subtracted from that of all samples for further processing. The average OD reading of the triplicates and the protein concentrations of standard samples were plotted to generate a standard curve, which results in a formula to determine the protein concentrations of the total lysates. This formula and corresponding average OD reading for each sample were used to calculate the protein concentration of the total lysate.

2.2.5 Western Blot

To make one 10% SDS-polyacrylamide gel, 3 mL ddH₂O, 2.5 mL 30:1 w/v acrylamide: bis-acrylamide, 1.9 mL 1.5M Tris-HCl at pH 8.8, 80 μ L 10% SDS and 80 μ L ammonium persulfate were mixed, and then 5 μ L TEMED were added and mixed thoroughly. A 4% stacking gel was made by mixing 2.2ml ddH₂O, 0.5 ml 30:1 w/v acrylamide: bis-acrylamide, 950 μ L 1.5M Tris-Cl (pH 6.8), 40 μ L 10% SDS, 80 μ L ammonium persulfate and 2 μ L TEMED. The final total protein concentration in the 6 x SDS loading buffer (0.5M Tris-HCl, 30% Glycerol, 100mM DTT, 10% SDS, 0.012% Bromophenol Blue) was adjusted to 2 μ g/ μ L, and 30 μ L (60 μ g) of each sample was separated in a 10% SDS-polyacrylamide gel. The separated proteins in the gel were transferred onto a 0.2 μ m PVDF membrane under 100-volt for 90 minutes in transfer buffer (2.5mM Tris-base, 19.2mM Glycine). The membrane was blocked in 1x TBST (50mM tris-base, 150 mM NaCl (FisScientific, Hampton, NH), and 1% Tween 20) containing 5% fat-free milk for 1 hour. The membrane was then briefly washed in 1X TBST and incubated with the indicated primary antibody at the working concentration as shown in Table 1 at 4 °C overnight. After that, the

membrane was washed 3 times in 1X TBST for 5 minutes each and incubated in the goat anti-rabbit HRP conjugated secondary antibody at a 1:5000 (Upper state) for 1 hour at room temperature. The membrane was then rewashed in 1X TBST 3 times for 5 minutes each. Pierce ECL Western blotting substrate was used to cover the membrane for 3 minutes to visualize the protein band. FUJI medical x-ray film and Konica SRX 101A Film Processor were used to develop the film. The film was then scanned into digital form and quantified with ImageJ software. The densities of the protein bands were compared with their corresponding beta-actin density in the same sample.

2.2.6 Measurements of glucose, TAG, and cholesterol in serum samples

The blood glucose level was measured using the Stanbio Glucose LiquiColor kit (EKF link, Reference No. 1070). 1.0 mL working reagent (included in the kit) was incubated at 37 °C for 5 minutes, and 5 µL of plasma samples or standard (included in the kit) was added to the 1.0 mL working reagent. The mixture was incubated at 37°C for another 5 minutes. The OD at 500 nm absorbance of each reaction in triplicates was read in 96-well plates with the GloMax Multi detect system. The glucose level was calculated using the equation included in the manufacturing protocol.

$$\text{Glucose (mg/dl)} = \text{Au (OD for unknown)} / \text{As (OD for standard)} * 100$$

The plasma TAG level was measured using the Stanbio Triglyceride LiquiColor kit (EKF link, Reference No. 2200). 10 µL of plasma sample or standard (included in the kit) were mixed with 1.0 mL working reagent (included in the kit) and then incubated at 37°C for 5 minutes. The OD at 500 nm absorbance of each reaction in triplicates was read in 96 well plates with the

GloMax Multi detect system. The blood TAG level was calculated using the equation including the manufacturing protocol:

$$\text{Serum triacylglycerol (mg/dl)} = \text{Au (OD for unknown)} / \text{As (OD for standard)} * 200$$

The plasma cholesterol level was measured using the Stanbio cholesterol LiquiColor kit (EKF link, Reference No. 1010). 10 μ L of serum samples or standard (included in the kit) was mixed with 1.0 mL working reagent (included in the kit) and then incubated at 37°C for 5 minutes. The OD at 500 nm absorbance of each reaction in triplicate was read in 96 well plates with The GloMax Multi detect system. The serum cholesterol level was calculated using the equation, which included manufacturing protocol.

$$\text{Serum total cholesterol (mg/dl)} = \text{Au (OD for unknown)} / \text{As (OD for standard)} * 200.$$

2.2.7 Statistics analysis

For the statistical analysis, a One-way Aposthoch LSD posthoc statistical analysis was performed with SPSS statistics software, version 28.0.0.0 (IBM, Armonk, NY, U.S.A.). Data were presented as means $\pm$ S.E.M. When the p-value is less than 0.05, the result is considered significantly different between groups.

2.3 Results

2.3.1 Food intake, body weight, and accumulated percentage of body weight gain

As shown in Figure 5A, mice in the PPO group started to have significantly higher BW than that in the control group at week five and then that in both control and POP groups at week 6. The PPO group had significantly higher calory intake than the POP and control groups at week 2, and the POP group also had significantly higher calory intake than the control group

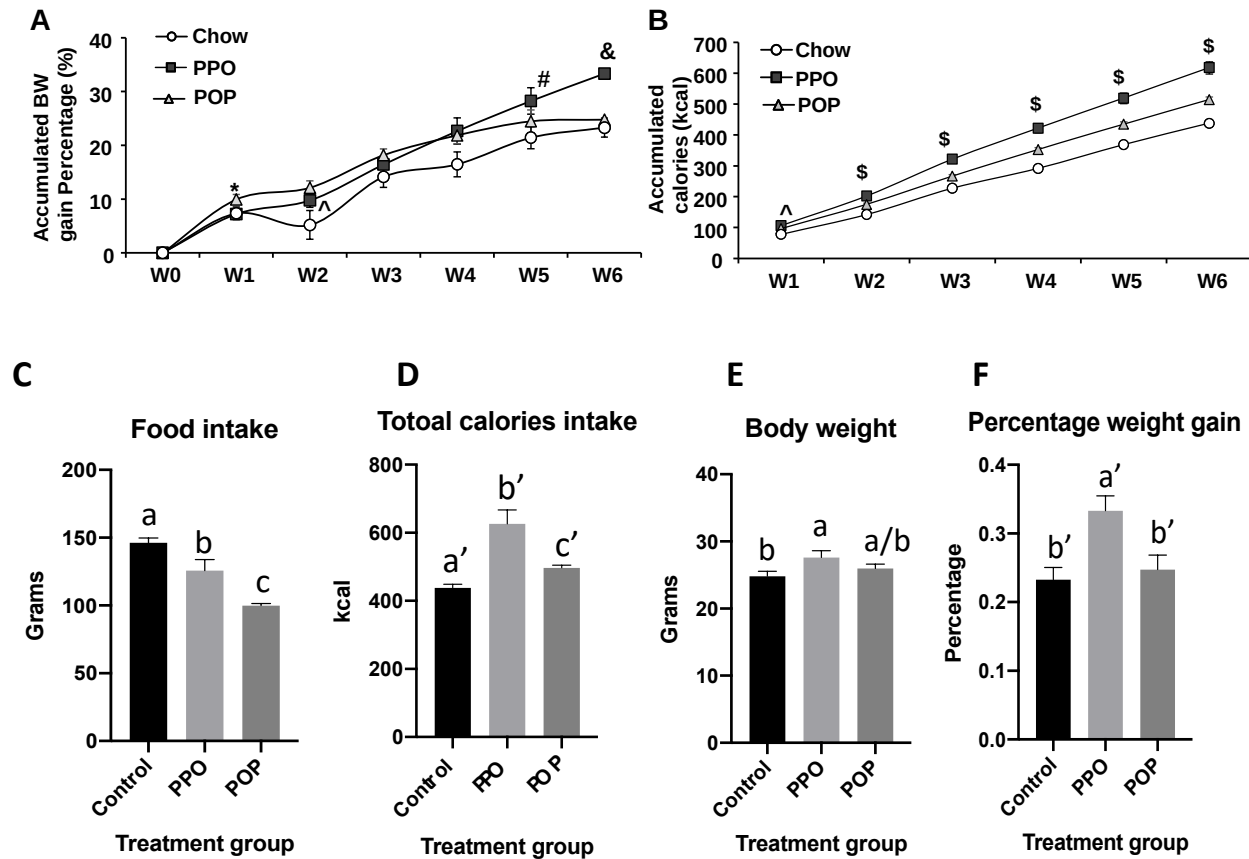


Figure 5. Accumulated body weight (BW) gain percentage (A), accumulated calories intake (B), food intake (C), total calories intake (D), final BW (E), body growth percentage of mice fed the control, PPO, or POP diet for six weeks. A. The accumulated BW gain percentage was calculated by subtracting the BW at week 0 from the value at the indicated week and divided the week 0 BW, and presented as a percentage (* For PPO>POP, ^ for PPO>control, # for PPO>Control, & for PPO>POP/control). B. The accumulated calories were determined by timing the caloric density of chow (3.0kcal/g), PPO (5.16kcal/g), or POP (5.16kcal/g) to the food intakes in grams of the indicated week (^ for PPO>chow, \$ for PPO>POP>chow). C. The total food intake in grams over 6 weeks (a for Control > PPO and POP, b for PPO > POP).calorietotal calories intake (be for PPO > ca for POP > a' for control). E. The final BW at the end of 6 weeks of treatment (a for PPO > b for control). F. The percentage weight gain over six weeks of treatment (a' for Control > PPO and POP). Data were presented as mean $\pm$ SEM, n=9 per group, $p < 0.05$.

The Control group had the highest food intake in weight among the three groups. Mice in the PPO group had a higher food intake weight than the POP group (Figure 5C). After calculating the final calory intake for all three groups, the PPO group had the highest calory intake, followed by a POP group than the control group (Figure 5D). As shown in Figure 5E, mice in the PPO group had significantly higher final BW than the control group, but there was no difference between PPO and POP group or the POP and control group. The BW gain percentage was calculated using final weight minus initial BW and then divided by initial BW. We found that the hat PPO group had significantly higher BW gain than both the control and POP groups, and there was no difference between these two groups (Figure 5F).

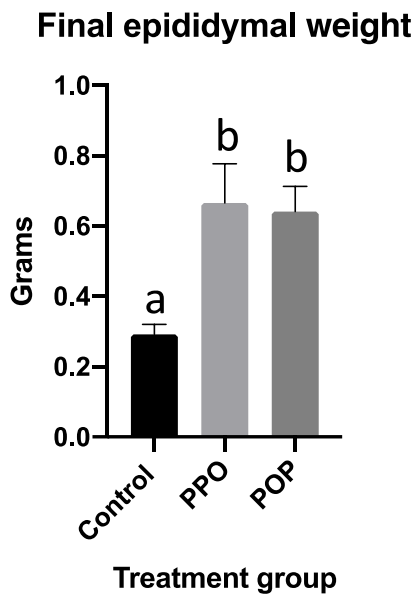
2.3.2 Tissue parameters

Mice in PPO and POP had significantly higher epididymal weight and lower liver weight than that in the control group, as shown in Figures 6A and 6C, respectively. The epididymal fat to BW ratio of the control group was lower than that of the PPO and POP groups, which were not different between them, as shown in Figure 6B. The liver to BW ratio of the PPO group was significantly lower than that of the POP group, which was lower than that of the control group, as shown in Figure 6D.

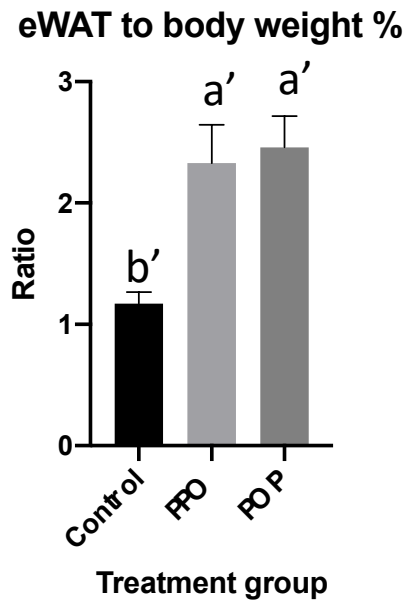
2.3.3 Plasma parameters Glucose, cholesterol, and triacylglycerol

As shown in Figure 7A, the plasma cholesterol levels of mice in the POP and PPO groups were similar but were higher than that in the control group. The blood glucose levels of mice in the PPO group were significantly higher than that in the POP and control groups, which were not different between them (Figure 7B). Even though both POP and PPO groups had a trend of

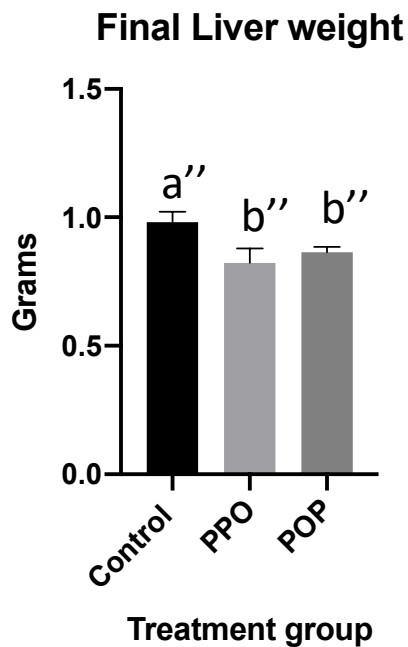
A



B



C



D

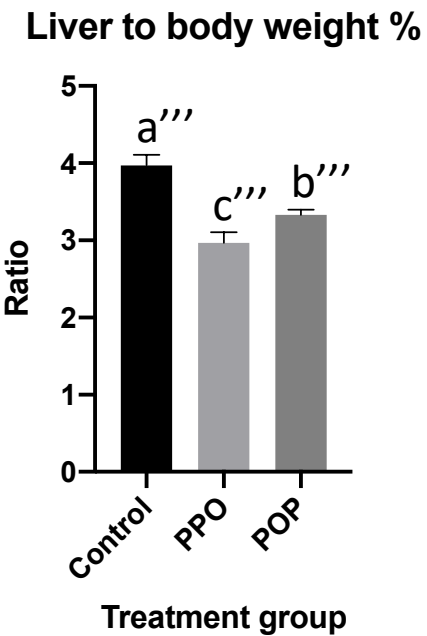


Figure 6. Final epididymal fat weight (A), epididymal fat to BW percentage (B), final liver weight and (C), liver to BW percentage (D) after 6-week treatment. A. The final BW at the end of treatment (a for Control > b for PPO and POP). B. The epididymal fat weight to BW ratio in percentage (a' for PPO and POP > b' for control). C. The final liver weight (a'' for control > b'' for PPO and POP). D. liver to BW ratio in percentage (a''' for Control > b''' for POP > c''' for PPO).

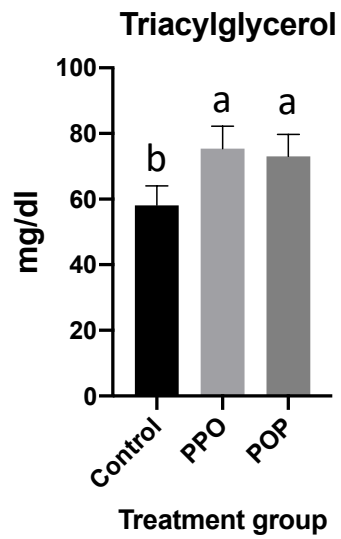
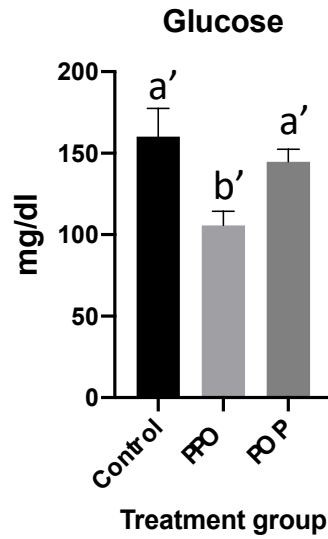
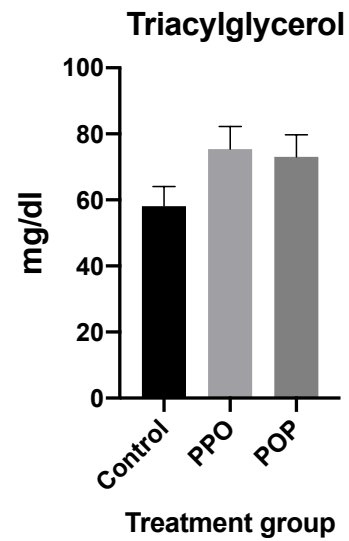
A**B****C**

Figure 7. Serum total cholesterol level (A), Serum glucose level (B), and serum Triacylglycerol (C) after 6-week treatment. A. Serum total cholesterol level (a for PPO and POP > b for Control). B. The serum glucose level (a' for Control and POP > b' for PPO) C. The serum triacylglycerol level.

having increased plasma TAG levels than the control group, the results did not reach statistical significance.

2.3.4 Hepatic lipogenic gene expression

As shown in Figure 8, the hepatic ACC expression in the control group was significantly higher than that in the PPO group, which was higher than that in the POP group. On the other hand, the Phospho-ACC level in the control group was higher than that in the POP group, which was higher than that in the PPO group. The expression levels of CD36 and FAS in the control group were significantly higher than that in the PPO and POP groups, which were not different between them. Interestingly, the SCD1 expression levels in the POP were lower than those in the control and PPO groups, which were not different between them.

2.3.5 Hepatic Insulin signaling, glycogen, cholesterol gene expression

As shown in Figure 9, the IRS1 protein expression level in the control group was significantly higher than in the POP and PPO groups. The GS level in the control group was significantly higher than that in the PPO group, whereas that in the POP group was not different from that in the control and PPO groups. However, the phosphor-GS levels in the POP and control groups were significantly higher than in the PPO group. The gene expression for IR- β subunit, AKT, and HMG-CoA reductase showed no significant difference among all three groups.

2.4 Discussion

This study aims to investigate the role of saturated fatty acid at the sn-2 in regulating metabolism in vivo and the health effects of the randomized fat on the BW gain and energy intake in mice. As shown in the results, we found the different PPO and POP effects. It indicates

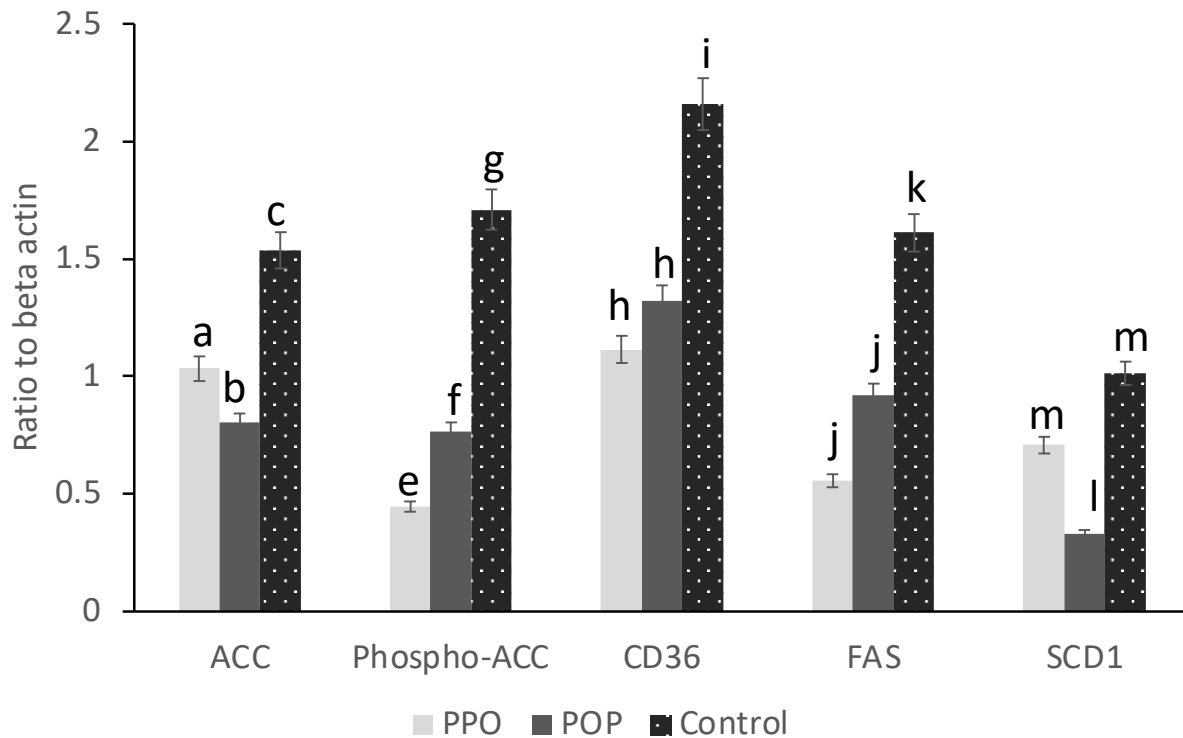
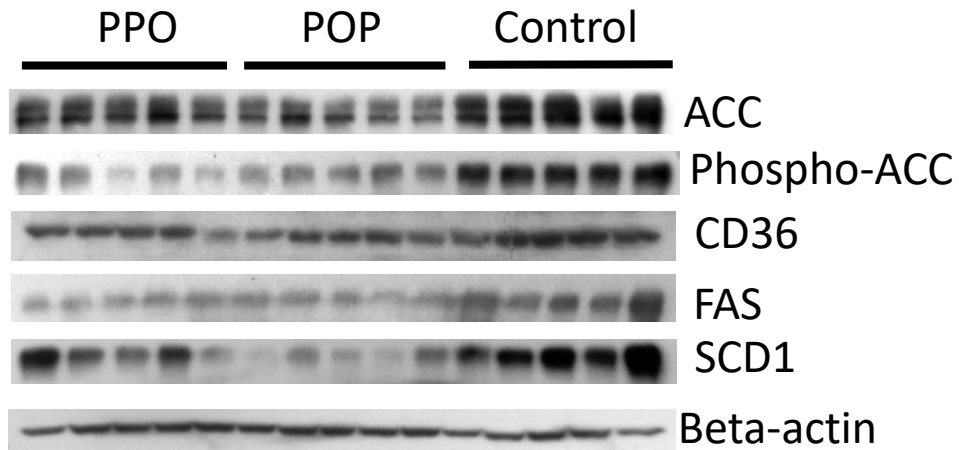


Figure 8 Protein expression of hepatic lipogenic gene expression. ACC (Acetyl-CoA carboxylase): (c for control >a for PPO >b for POP), phospho-ACC: (g for Control >f for POP > e for PPO), CD36 (Cluster of differentiation 36): (i for control > h for PPO and POP), FAS (Fatty acid synthase): (k for control > j for POP and PPO), SCD1(Stearoyl-CoA desaturase-1): (m for control and PPO > l for POP).

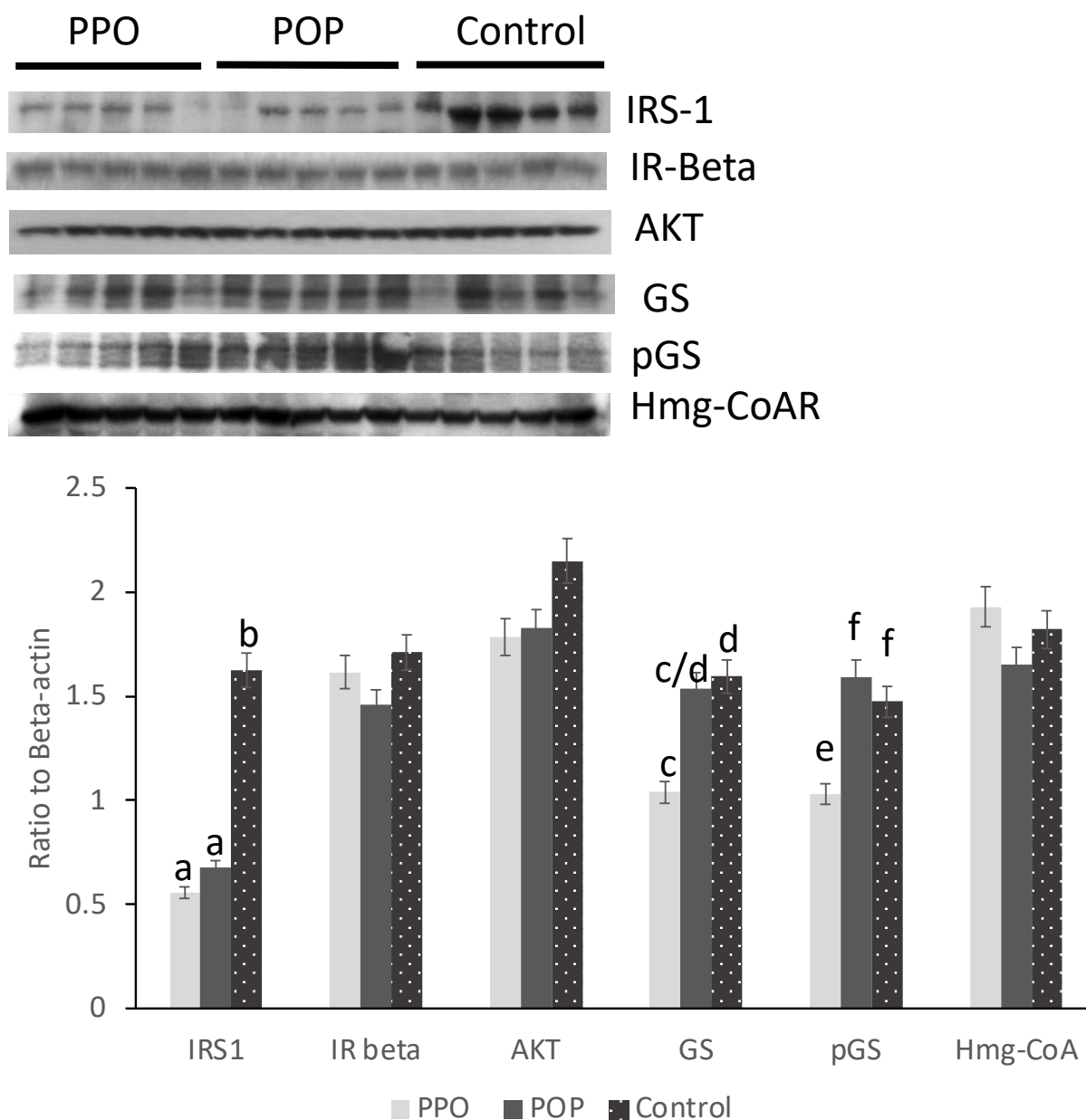


Figure 9 Protein expression of hepatic *Insulin signaling, glycogen, cholesterol gene expression*. IRS-1 (insulin receptor substrate), IR-beta (insulin receptor beta subunit), AKT (Protein kinase B), GS (Glycogen synthase), pGS (phospho-glycogen synthase, HMG-CoA R (HMG-CoA reductase). $b > a$ for comparing the control with the PPO and POP groups; $d > c$ for comparing the control with the PPO group; $f > e$ for comparing the POP and control groups with the PPO group.

that a TAG molecule's fatty acyl location can change its physiological functions, including appetite, which leads to significant differences in food intake and BW in mice. Mice in the PPO group started to have higher accumulated caloric intake than that in the POP group since week 2, indicating that PPO or other ingredients in the PPO diet might promote appetite as early as two weeks after the dietary treatment. Interestingly, based on the results of the accumulated BW during the experiment, the difference in BW gain rates between the PPO and POP groups did not show any difference until five weeks into the treatment. Both energy intake and expenditure play a role in the energy homeostasis and, in turn, the BW gain. The discrepancy may be caused by the differential energy expenditure rates between the PPO and POP. Whether POP mice had reduced energy expenditure to maintain the same BW rate gain as the PPO mice or not is worth investigating.

The switch of the location of the fatty acyl group also affects the liver to BW ratio. The liver to BW ratio of mice in the POP group is lower than that in the PPO group, which may be caused by less fat/energy stored in the liver. At the same time, the PPO group also has a lower blood glucose level compared to the POP group. This indicates that although the PPO group had higher energy intake than the POP group, the PPO diet can lower plasma glucose levels. Whether this is due to the increases in glucose disposal and usage in mice in the PPO group remains to be determined. It appears that a diet with TAG in the form of PPO might benefit patients with T2D since it can maintain the blood glucose at a lower level compared to the fat in POP form.

The 36% PPO or POP in the diet leads to decreased liver weight, increased epididymal fat (WAT), and increased plasma cholesterol levels compared to the control group in C57BL/6J

mice. Moreover, the 36% increase in dietary fat content affects the expression levels of hepatic genes involved in lipid metabolism. The hepatic expression levels of ACC, Phospho-ACC, CD36, and FAS in the control group are higher than those in the POP and PPO groups. The results demonstrate that feeding a high-fat diet for six weeks suppresses the de novo fatty acid synthesis (ACC and FAS) and fatty acid uptake (CD36) in the liver of C57BL/6J mice. When the dietary TAG level is high, it is reasonable to reduce the expression levels of proteins for lipogenesis. It will be interesting to find out what the underlying mechanism is.

Interestingly, PPO and POP diets differentially impact the expression levels of ACC, phospho-ACC, and SCD1. Both POP and PPO groups have significantly lower ACC and phospho-ACC than the control group. The POP group has significantly lower ACC but higher phospho-ACC levels than the PPO groups. The feeding of the POP diet seems to promote the phosphorylation of ACC. Interestingly, the PPO diet group has significantly higher SCD1 levels than the POP group. This indicates that the intake of the PPO diet is more vital in influencing the expression of SCD1 expression, which initiates the first double bond at the $\Delta 9$ position of saturated fatty acid. Since the palmitic acid group is at the sn-2 position of the PPO, it appears that the liver needs more SCD1 to introduce a double bond on it. Whether there is a system sensing the elevated saturated fatty acid group at the sn-2 position remains to be determined. Consequently, whether there is an elevation of palmitoleic acid (16:1), oleic acid (18:1), or both in this experimental setting should be further investigated. All of these suggest that the locations of the fatty acyl groups can influence the lipogenic gene expression in a high-fat diet setting.

Regarding insulin signaling and glucose metabolism, we saw a significant decrease in the IRS-1 gene expression in the PPO and POP groups compared to that in the control group, suggesting a reduction in insulin signaling. Excess dietary TAG intake has been shown to lead to poor insulin signaling and may lead to insulin resistance (98). In addition, even though the GS expression levels are similar between the two high-fat diet groups, mice in the POP group have significantly higher phospho-GS levels than that in the PPO diet group, suggesting less GS activity in the POP group. Here, mice in POP groups probably should not synthesize glycogen in the liver when their blood glucose level is low.

In conclusion, our research shows that feeding an HFD with different TAGs can influence the BW gain rate differently in mice, which may be attributed to their effects on the hepatic expressions of lipogenic genes and the phosphorylation of GS. More experiments are warranted to determine the underlying mechanisms.

Chapter 3

3.1 Conclusion

TAG is the major form of dietary lipids and stored form of excessive energy and plays a crucial role in human lipid metabolism. The synthesis of TAGs is regulated by multiple enzymes and hormones (53). Since the structures of TAGs vary, it is very important to understand how the structural difference of the dietary TAGs leads to different physiological outcomes. The breakdown of one TAG molecule starts with the cleavage of the fatty acyl groups at the sn-1 and sn-3 positions and leads to the production of 2-MAG, which can be absorbed together. Then the 2-MAG can be directly used as the backbone for esterification of fatty acids in the synthesis of TAG, which is the primary pathway for incorporating dietary TAG in the small intestine. 2-MAG can be broken-down again into one more FFA and glycerol, which has to be activated before functions as the backbone for esterification in the synthesis of TAG. Therefore, the fatty acyl group at the sn-2 position may be more likely to impact the metabolism of TAG than the fatty acyl groups at the sn-1/3 positions.

In this thesis, we demonstrated that the uptake of the TAG with a saturated fatty acid at the sn-2 position (PPO) led to a significantly more increase in food intake and BW gain than the one with a monounsaturated fatty acid at the sn-2 position (POP), which is a natural TAG found in palm oil and has saturated fatty acids at sn-1/3 positions. In addition, intake of the TAG with a saturated fatty acid at the sn-2 position also lowered the plasma glucose level in mice. These differences may be caused by different fatty acids and/or 2-MAG released from the TAG after hydrolysis due to different TAG structures, as shown in Figure 10. The 2-MAG from PPO and

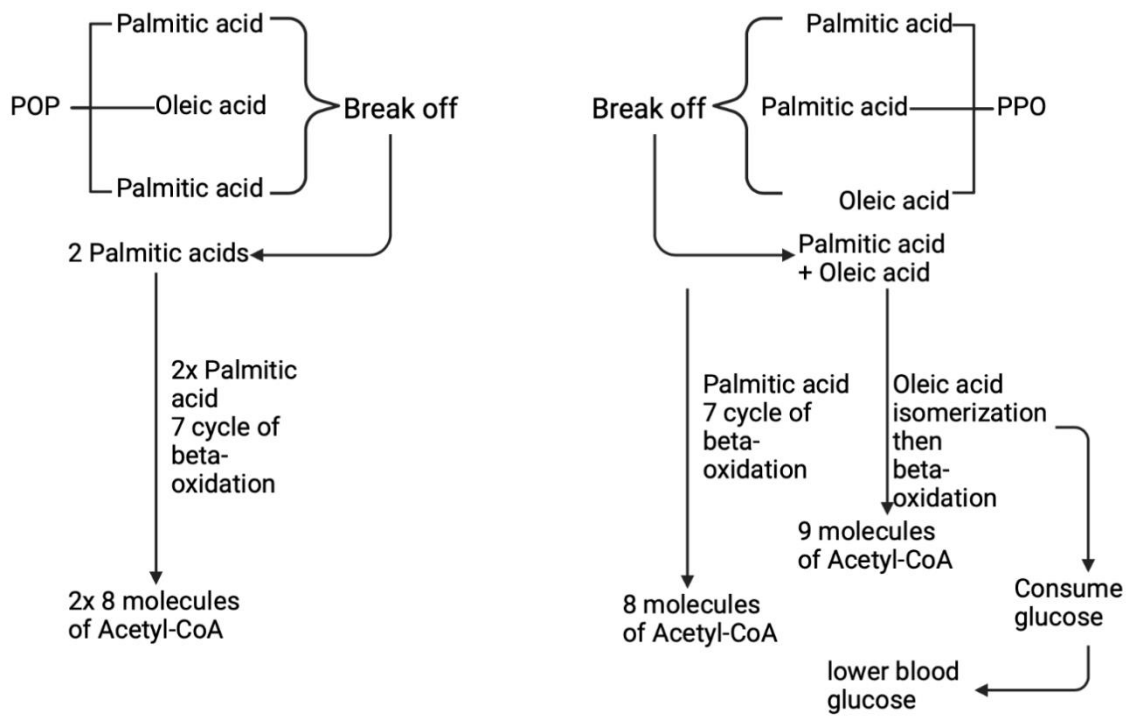


Figure 10. Palmitic acid and Oleic acid synthesis pathway in mammals.

POP is 2-palmitoylglycerol and 2-oleoylglycerol, respectively. The fatty acids released from PPO are one palmitic acid and one oleic acid, whereas those from POP are two palmitic acids. Each palmitic acid will generate 7 FADH₂ after seven rounds of β -oxidation.

On the other hand, the complete oxidation of the oleic acid needs one enoyl CoA isomerase to move the double bond into the position between α and β carbons. Then, acetyl CoA can be oxidized into the TCA cycle. The total β -oxidation for oleic acid needs to go through 8 rounds, but palmitic acid only needs to go through 7 rounds (99). In this case, more glucose may be used for energy to compensate for the relative extra steps for the oxidation of oleic acid. Therefore, mice in the PPO group have lower plasma glucose levels than those in the POP group if we only consider the uses of the fatty acids released from the sn-1 and sn-3 positions. On the other hand, the net energy produced from one molecule of palmitic acid oxidation is 106 moles of ATP. Still, the net ATP production for oleic acid oxidation is 146 moles of ATP, which may result in excessive energy production and, in turn, increased BW (100).

Our research results also demonstrate that the POP diet decreased the hepatic expression levels of SCD1 in mice. Previous research showed that knockout out of SCD1 in mice leads to activation of AMPK and results in a decrease in ACC in the liver tissue (101). Our results showed that mice in the POP group have significantly lower ACC protein and higher phospho-ACC levels compared to that in the PPO group. These results demonstrate that the POP diet can promote the phosphorylation of ACC in liver tissue, suggesting the reduction of de novo lipogenesis. Meanwhile, SCD1 expression is also related to the insulin signaling pathway and insulin resistance. SCD1 knockout mice have increased insulin-mediated glucose uptake in the skeleton muscle compared to the controls (102). Our western blot results also show that mice

in the POP diet group have increased glycogen synthase (GS) and phosphor- glycogen synthase (PGS) levels more than those in the PPO group, which suggests a possible increase in insulin sensitivity.

A previous study also focused on the metabolism outcome differences between PPO and POP dietary lipids. Their study showed that the PPO group had a significantly lower food intake and BW gain (95), which is the opposite of our study result. It might cause by different PPO synthesis methods; the PPO fat used in our study is synthesized by a chemical approach with 0.6% sodium methoxide compared to an enzymatic approach with 6.6% Lipozyme 435. The different randomization processes may cause different residue products and produce different results. At the same time, the purity of the PPO fat is also different. In the previous study (95), the PPO fat used was purified, and 82.5% of fatty acid at the sn-2 position was palmitic acid, and 17.5% was oleic acid. The purity of PPO fat used in our study (refer to table 3) is 45.81%. The two other major products are OOP (20.62%), and PPP (22.55%), meaning only 68.36% of the fatty acid at the sn-2 position is palmitic aciReferringfer to figure 10, the synthesis products are different for oleic acid (9 molecules of acetyl-CoA) and palmitic acid (8 molecules of acetyl-CoA), may also contribute to the differences in the two studies. However, both studies showed that the PPO diet group has significantly lower blood glucose, indicating that palmitic acid at the sn-2 position could decrease blood glucose levels.

3.2 Future studies

Based on the results of previous research done in our lab, we found out that dietary vitamin A, retinol, interacts with dietary fat metabolism (103). Excessive vitamin A intake will lead to increased insulin resistance, blood glucose, and development of T2D in ZDF rats fed a

standard or high-fat diet. The reduction of vitamin A intake also contributes to the decreased SCD1 protein expression in the liver (103). It is essential to understand whether the structure of dietary TAGs would impact the dietary vitamin A absorption and metabolism in vivo and how it would impact the lipogenesis pathway in the high-fat diet setting.

We also found that the PPO group has a significantly lower liver to BW ratio. To understand the cause of this difference, more studies need to be done regarding the hepatic fat content of the mice fed the POP and PPO diets. At the same time, the skeletal muscle is another major organ in mammals that use FFAs from TAGs as a source of energy. Due to decreased SCD1 protein expression in the POP group, it is possible that mice fed the POP diet would have decreased glucose uptake by the skeletal muscle. It is essential to investigate whether the structure of TAGs would impact muscle fatty acid uptake and oxidation.

The limitation of this study is that we only investigated lipogenesis protein expression in the liver. Still, the skeletal muscle and adipose tissues are also major organs that use TAGs as a source of energy. Also, the experiment started when mice were six-weeks-old, considered very close to mature adult mice (104). In this case, we don't know how dietary TAG structure would impact mouse development. At the same time, we only compared one pair of TAG structures, commercial palm oil, compared to its randomized form; more studies could be done on other dietary lipids and their synthetic forms. At the same time, the purity of the TAG within the comparison is different, and the by-products from TAG randomization also will affect the metabolism outcomes.

With more and more people with metabolic problems such as obesity and diabetes, more nutritional studies should be done to understand the roles of TAG structures in regulating glucose and fatty acid metabolism.

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Vita

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