Review of Triacylglycerol Digestion, Absorption, and Metabolism with Respect to SALATRIM Triacylglycerols

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The SALATRIM family of structured triacylglycerols is composed of fats containing acetic, propionic, butyric, and stearic acids esterified to a glycerol backbone. These triacylglycerols contain at least one short-chain fatty acid and stearic acid. The scientific literature shows that people are regularly exposed to SALATRIM components and SALATRIM-like structures from both dietary and metabolic sources. The digestion, absorption, and metabolism of SALATRIM fats are similar to those of other fats and proceed by well-known mechanisms and metabolic pathways. These aspects of fats are reviewed with respect to SALATRIM structured triacylglycerols.

INTRODUCTION

Fats occur in natural products as triacylglycerol mixtures with different fatty acids esterified to a glycerol backbone. Specific fats can be defined by their unique fatty acid content and distribution. Physicochemical properties of triacylglycerols are dictated by their fatty acid constituents. Fatty acids are divided into several classifications, such as saturated and unsaturated and long, medium, and short chain. Saturated fatty acids contain no double bonds, whereas unsaturated fatty acids contain one or more double bonds. Short-chain fatty acids (SCFA) range from C_2 to C_5, medium-chain fatty acids (MCFA) range from C_6 to C_{12}, and long-chain fatty acids (LCFA) range from C_{14} to C_{24}.

A family of novel low-calorie fats named SALATRIM has been developed by the Nabisco Foods Group. These triacylglycerols contain at least one SCFA and one stearic acid. The remaining acyl group can be either a SCFA or a LCFA. Members of the SALATRIM family are made from hydrogenated natural vegetable fats and are composed of a mixture of triacylglycerols with a random positional distribution of SCFA and LCFA. The predominant differentiating factors between members of the SALATRIM family and other fats is the presence of a significant amount of SCFA and a preponderance of stearic acid. A SALATRIM composition contains from 33% to 66% SCFA on a molar basis with the balance being primarily stearic acid.

OCCURRENCE OF TRIACYLGLYCEROLS IN THE DIET

Roughly 100–160 g of fat, primarily triacylglycerols, are consumed on a daily basis in the average Western diet (Carey et al., 1983; Glickman, 1980; Van Dyke, 1989). More than 100 different fatty acids are found in dietary triacylglycerols. The distribution of typical fatty acid intake by humans is illustrated in Table 1 (Hashim and Babayan, 1978).

Table 1. Fatty Acids in the Western Diet

fatty acid	% of total dietary fatty acids	fatty acid	% of total dietary fatty acids
medium and short chain	3	linoleic	15
palmitic	20	stearic	5
oleic	50	long chain $>C_{18}$	7

Both plant and animal fats exhibit characteristic fatty acid distributions and positional specificity for the fatty acids on the glycerol backbone. In fats from 28 species of plants, palmitic and stearic acids and fatty acids with C >18 have been shown to be esterified predominantly at the primary positions (1, 3) of glycerol (Mattson and Volpenhein, 1963). Similarly, in animal fat depots, the shorter and more unsaturated fatty acids tend to occupy the 2-position, while stearic and palmitic acids tend to occupy the 1- and 3-positions (Brockerhoff et al., 1966).

Members of the SALATRIM family are composed predominantly of the LCFA stearate and one or more of the following SCFA: acetate, propionate, and butyrate. Each of these components is found in the human diet, where propionate appears as a free fatty acid, while stearate is found esterified to glycerol. Both acetate and butyrate can be present in the diet in either form. Unlike most fats, the stearic acid in a SALATRIM fat can be randomly esterified among the three positions on glycerol, as can the SCFA.

PROCESSES OF TRIACYLGLYCEROL DIGESTION, ABSORPTION, AND METABOLISM

Triacylglycerol digestion, absorption, and metabolism are efficient, relatively well-defined processes. The major phases of these processes are listed in Table 2. Since the SALATRIM family is composed of triacylglycerols, it can be predicted that members of this family should be digested, absorbed, and metabolized by the same mechanisms as other dietary fats and oils.

Two major variables, fatty acid chain length and degree of unsaturation, profoundly affect digestion, absorption, physiological transport, and metabolism of dietary fats (Hashim and Babayan, 1978). Enzymatic hydrolysis of

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Table 2. Major Phases of Triacylglycerol Digestion, Absorption, and Metabolism

intraluminal digestion
micellar solubilization
permeation across cell membrane
chylomicron formation
chylomicron release into lymphatics
transport in lymph
lipolysis at tissue sites
fatty acid oxidation

triacylglycerols is initiated in the mouth and stomach by lingual lipase and possibly gastric lipase (Hamosh and Scow, 1973; Hamosh et al., 1975; Hamosh, 1979). Approximately 30% of total dietary triacylglycerols may be digested in the stomach (Carey et al., 1983). However, more complete hydrolysis and absorption, especially of LCFA, require less acidic conditions, more appropriate lipases (primarily pancreatic) and detergents (bile salts), and specialized absorptive cells. This environment is provided by the lumen and mucosa of the proximal small intestine (Carey et al., 1983). Over 95% of dietary lipids are digested and absorbed in the upper intestine (Borgstrom, 1986; Carey et al., 1983; Glickman, 1980; Gurr, 1983; Patton, 1981; Thomson and Dietschy, 1981; Tso, 1985; Van Dyke, 1989).

Triacylglycerol Digestion and Absorption in the Stomach. Lingual lipase (Carey et al., 1983; Hamosh, 1984; Hamosh and Burns, 1977; Jensen et al., 1982; Nelson et al., 1977; Van Dyke, 1989) and, perhaps, gastric lipase (Cohen et al., 1971; Gargouri et al., 1986) are responsible for gastric triacylglycerol hydrolysis. Lingual lipase is much more active on triacylglycerols with SCFA and MCFA than on those with LCFA (Fernando-Warnakulasuriya et al., 1981; Staggers et al., 1981). It is relatively specific for the hydrolysis of fatty acids in the 1- and 3-positions of the glycerol backbone. Fatty acids at the 3-position are preferentially hydrolyzed compared to the fatty acids at the 1-position by a 2:1 ratio (Paltauf et al., 1974). Gastric lipase is thought to originate in the gastric glands and demonstrates hydrolytic specificity for SCFA, although both SCFA and LCFA have been shown to be hydrolyzed (Gargouri et al., 1986). Complete hydrolysis of triacylglycerols to glycerol in the stomach has not been reported (Bezard and Bugaut, 1986).

The preferential release of SCFA and MCFA in the stomach allows these fatty acids to be rapidly absorbed into the gastric mucosa (Aw and Grigor, 1980; Bugaut, 1987; Carey et al., 1983; Fernando-Warnakulasuriya et al., 1981; Greenberger and Skillman, 1969; Staggers et al., 1981). The pK_a of the SCFA and MCFA ranges from 4.75 for acetic acid to 4.9 for decanoic acid, and these acids typically are protonated at the gastric pH. The protonated SCFA are solubilized in the bulk aqueous media in the stomach and diffuse to the gastric mucosa gel. This gel is composed of expanded, highly hydrated glycoproteins. The SCFA can diffuse through this protective layer to the gastric mucosa, where they passively diffuse into the epithelial cells. There is currently no evidence of any carrier-mediated transport mechanism for SCFA in the gastric epithelial membrane. Because of their molecular size, water solubility, and shorter chain length, SCFA should be more rapidly absorbed in the stomach than MCFA. The SCFA serve as a preferred energy source for the gastric mucosa. After absorption, any SCFA not utilized by the gastric mucosa are transported through the cytosol without being esterified. They enter the portal circulation and are transported to the liver, where they are a ready energy source.

A significant amount of LCFA at the 1- or 3-position of glycerol also should be cleaved in the stomach. Hydrophobic LCFA, like stearic acid, that are released in the stomach by lingual and gastric lipases are predominantly protonated at stomach pH. These protonated LCFA readily partition into the hydrophobic triacylglycerol/diacylglycerol emulsion droplets that are further metabolized in the small intestine before absorption (Carey et al., 1983; Friberg et al., 1971). The products remaining in the stomach after hydrolysis of triacylglycerols by lingual and gastric lipases, principally LCFA, diacylglycerols, and monoacylglycerols contained in emulsion droplets, are propelled through the pylorus into the duodenum.

As can be seen from the above, digestion and absorption of SALATRIM are predicted to be initiated in the stomach. A significant portion of the SCFA will be hydrolytically cleaved and occur in the free form in the bulk aqueous phase. These will be absorbed by the gastric mucosa, enter into the portal circulation, and be taken up by the liver for further anabolic and catabolic metabolism. Because the SCFA serve as a preferred substrate for the gastrointestinal mucosal cells, a significant portion will be metabolized for energy by these cells. Rapid absorption and metabolism of acetic and propionic acids from SALATRIM have been demonstrated by Hayes et al. (1994) and indicate these fats are metabolized and absorbed as predicted. Stearic acid produced by hydrolysis of SALATRIM fats in the stomach will partition into the fat droplets that are released into the small intestine. There should be little or no absorption of stearic acid by the stomach. This prediction appears to be confirmed for SALATRIM fats (Hayes et al., 1994).

Triacylglycerol Digestion, Absorption, and Metabolism in the Intestine. In the small intestine, especially the jejunum, triacylglycerols in the emulsion droplets are hydrolyzed by pancreatic lipase (Borgstrom, 1986; Brockerhoff, 1970; Brockerhoff and Jensen, 1974; Carey et al., 1983; Fernando-Warnakulasuriya et al., 1981; Mattson and Volpenhein, 1964; Patton, 1981; Staggers et al., 1981; Van Dyke, 1989). Intestinal digestion and absorption of fat appear to be calcium-dependent because pancreatic lipase requires calcium as an enzyme cofactor (Patton, 1981). Pancreatic lipase acts in conjunction with colipase and bile salts to digest triacylglycerols. Hydrolysis proceeds from the triacylglycerol to the diacylglycerol and finally to the 2-monoacylglycerol, with the concomitant release of free fatty acids. Pancreatic lipase, like lingual lipase, is specific for primary ester bonds of triacylglycerols regardless of whether the fatty acids are short or long chain. However, hydrolysis appears to be faster with triacylglycerols containing SCFA. LCFA in the 1- and 3-positions are completely hydrolyzed from the glycerol backbone. Under alkaline or slightly acidic conditions, calcium ions can react with these long-chain protonated fatty acids, causing the release of protons and the formation of insoluble calcium soaps (Benzonana and Desnuelle, 1968). These soaps may comprise from 5% to 50% of fecal fat (Bliss et al., 1972; Sammons and Wiggs, 1960).

Free fatty acids diffuse away from the fat emulsion droplet during lipolysis. The LCFA and monoacylglycerols become solubilized in bile salt micelles. Remaining SCFA and MCFA partition into the aqueous phase depending upon their water solubility. The SCFA components of the SALATRIM fats, acetate, propionate, and butyrate, are water-soluble and will occur as free fatty acids, while the stearate moiety will become associated with the bile salt micelles.

For absorption to take place, the monomeric SCFA in the aqueous phase and the micellular LCFA and 2-monoacylglycerols must pass two barriers, the unstirred water layer adjacent to the intestinal mucosal cells and the mucosal cell membrane. The unstirred water layer is believed to be continuous with the bulk aqueous phase in the intestinal lumen but has very different physical characteristics. The mucous layer over the intestinal epithelium participates in the formation of the unstirred water layer and is a component of it (Dietschy et al., 1971; Smithson et al., 1981). The smaller molecular weight fatty acids can diffuse rapidly from the aqueous phase of the lumen (diffusion coefficient at 37 °C of butyric acid = 12.1 $\times 10^6$ cm^2/s), whereas the high molecular weight fatty acids diffuse slowly (diffusion coefficient at 37 °C of stearic acid = 6.04 $\times 10^6$ cm^2/s) and the large mixed bile acid micelles diffuse much more slowly.

LCFA are not absorbed as the bile salt micellular aggregate but as monomers that must diffuse from the micelle through the unstirred water layer and through the mucosal cell membrane. This process appears to be diffusion-limited, and no evidence has been found for carrier proteins participating in either active or facilitated transport. No energy dependency has been shown for LCFA uptake. LCFA appear to simply partition among the aqueous phase, the mucosal cell membrane, and the cytosol on the basis of their carbon chain length and diffusion coefficient.

Stomach, small intestine, colon, and rumen have the capacity to absorb SCFA (Cummings et al., 1987). Although SCFA concentrations within the small bowel generally are low, the small intestinal mucosa can absorb these acids as efficiently as colonic and rumen epithelium (Smyth and Taylor, 1958). Since intestinal SCFA are produced mainly by endogenous bacteria as a byproduct of anaerobic fermentation of carbohydrate, SCFA concentrations normally are highest where the bacterial population is most abundant. "Interestingly, in the large intestine of the human, SCFA concentrations are roughly equivalent to those found in the rumen (approximately 75 mM acetate, 30 mM propionate, and 20 mM butyrate), reflecting the similarity in bacterial flora between these two organs" (Rombeau and Kripke, 1990).

Although the permeability of the SCFA through the mucosa epithelial cell membrane is lower than that of MCFA and LCFA, it is higher than would be expected on the basis of their hydrophilic nature. SCFA absorption appears to be by passive diffusion and linear with increasing concentration, and there is little evidence of a carrier-mediated transport. As Rombeau and Kripke (1990) have noted, the exact mechanism of SCFA absorption is not known, but several potentially important characteristics are known: (1) SCFA can be absorbed in either the ionized or nonionized states; (2) absorption is characterized by accumulation of the bicarbonate ion in the lumen of the intestine; (3) SCFA absorption stimulates sodium absorption, and the SCFA themselves may provide the energy required for sodium absorption; (4) a source for hydrogen ions in the lumen is important for absorption from the lumen.

Absorption of the protonated state of the SCFA can take place in the stomach, but the pH of the intestinal contents does not favor the protonated form. However, it has been proposed that a mucosal anion countersystem exists that exchanges the bicarbonate anion for the SCFA anion (Lamers, 1975; Lamers et al., 1975; Schmitt et al., 1977). However, this mechanism has been disputed (Ruppin et al., 1980). Alternatively, several investigators have proposed that absorption of the protonated SCFA may be by a Na^+/H^+ exchange mechanism (Argenzio and Whipp, 1979; Bustos-Fernandez et al., 1976; Turnberg et al., 1970a,b; Umesaki et al., 1979; Wilson and Kazyak, 1957). Hydrogen ions may be actively transported across the epithelial cell membrane in exchange with sodium ions. The hydrogen ions accumulate on the luminal side of the mucosal cell providing an acidic microenvironment where the SCFA are protonated and absorbed. This accounts for the absorption of sodium ions noted by several investigators and provides a mechanism for the formation of protonated SCFA for absorption. These potential mechanisms may vary in different regions of the gastrointestinal tract and may vary between species depending upon anatomy, physiology, and the specific diet consumed by the animal. Whatever the specific mechanism, SCFA are readily absorbed by the gastrointestinal tract with no differences in the rate of absorption of acetic, propionic, and butyric acids (Rombeau and Kripke, 1990).

Once absorbed, the SCFA are readily metabolized by the gastrointestinal mucosal epithelial cells (Cummings, 1981). In human colonocyte suspensions of either proximal or distal colon mucosa, about 75% of the oxygen consumed can be attributed to butyrate oxidation when butyrate was the only available substrate, and glucose oxidation was suppressed by about 50% in the presence of butyrate (Roediger, 1980). Butyrate was also shown to be preferentially oxidized by colonocytes *in vitro* over several common substrates with the order of utilization being butyrate > acetoacetate > *l*-glutamine > *d*-glucose (Ardawi and Newsholme, 1985; Roediger, 1982). Human colonocytes also produced ketone bodies, primarily acetoacetate from *n*-butyrate (Roediger, 1980).

Less is known about the metabolism of acetate and propionate by colonocytes, but approximately 50% of the propionate and 30% of the acetate produced in the colon appear to be utilized by the mucosa itself (Bergman and Wolff, 1971; Bergman, 1975). Acetate appears to be largely unmetabolized by the proximal large intestine of rabbits but is extensively metabolized to glutamate and aspartate by the distal colon (Marty et al., 1985).

SCFA not metabolized by the mucosal epithelium are transported via the portal blood to the liver, which provides a substantial clearance function (Hoverstad, 1986). Portal blood concentrations of the SCFA are 4–10 times higher than systemic levels (Dankert et al., 1981). Net acetate uptake by the liver is directly related to its portal concentration in both rats and humans (Buckley and Williamson, 1977; Herrmann et al., 1985). Acetate may be cleared from portal blood by the liver as effectively as butyrate and propionate, but the liver also releases endogenous acetate that contributes to normal systemic acetate levels (Lazarus et al., 1988). Since the liver almost quantitatively removes the propionate and butyrate from the portal blood, acetate comprises 90–98% of the SCFA present in arterial and peripheral venous blood.

The enzymatic activation of SCFA by formation of acetyl-CoA compounds seems to be the most important mechanism for regulating the different rates of uptake of the individual SCFA by various tissues. The relative activities of acetyl-CoA, propionyl-CoA, and butyryl-CoA synthetases in different tissues appear to ensure that, in most species, butyrate is metabolized mainly in the intestinal epithelium, propionate in the liver, and acetate in peripheral tissues (Annison and White, 1962; Ash and Baird, 1973; Cook et al., 1969).

In contrast to the rapid absorption of SCFA, stearic acid and its esters have been found to be poorly digested

and absorbed. As early as 1890, Arnschink found tristearin only 9–14% digestible in dogs, with nearly all of the undigested tristearin being found in the feces as triacylglycerol. In contrast, Lyman (1917) found that dogs utilized 95% of dietary tripalmitin and 82% of dietary palmitic acid. Hoagland and Snider (1943) also found tristearin and stearic acid to be poorly absorbed, while tripalmitin and palmitic acid were much more digestible.

Studies using a limited number of fats led Mattil and Higgins (1945) and Calloway et al. (1956) to conclude that stearic acid absorption depended, at least in part, on the nature of the fatty acids making up the rest of the triacylglycerol molecule. In general, stearic acid was better assimilated when fed as a mixed triacylglycerol than when fed as tristearin.

Mattson (1959) fed rats a series of fats that varied in the amount of stearic acid and the distribution of the stearic acid among simple and mixed triacylglycerols. The coefficients of absorption of these fats were determined. The results showed that the stearic acid of tristearin was not absorbed, whereas the stearic acid of distearin–monounsaturated or monostearin–diunsaturated triacylglycerols was almost completely absorbed. The theory that the coefficient of absorption of fat was a function of its content of saturated fatty acids containing 18 or more carbon atoms was proven untenable. Instead, the coefficient of absorption of a fat was inversely proportional to its content of simple triacylglycerols made up of saturated fatty acids having chain lengths of 18 or more carbon atoms and was influenced by the amount of saturated fatty acids only if they are present as saturated triacylglycerols.

Mattson et al. (1979) further demonstrated that the absorption of stearic acid depended on its position in the triacylglycerol molecule and on whether the diet contained calcium and magnesium. In these studies (Mattson et al., 1979), rats were fed diets containing triacylglycerols with oleate and stearate as the sole fatty acids. The triacylglycerols were 1-stearoyldiolein (SOO), 2-stearoyldiolein (OSO), 2-oleoyldistearin (SOS), 1-oleoyldistearin (OSS), and triolein (OOO). Two diets, one sufficient and the other deficient in calcium and magnesium, were used. The absorption of the fatty acid component was measured by a fat balance technique. Oleic acid was absorbed almost completely from all triacylglycerols. Absorption levels of the stearate component in the presence and absence of divalent cations, respectively, were 98% and 99% for OSO, 55% and 96% for SOO, 37% and 70% for SOS, and 59% and 60% for OSS, respectively. These absorption patterns demonstrated that if the stearic acid is esterified at the 2-position of the triacylglycerols, the resulting 2-monostearin is readily absorbed. If, however, it is esterified at the 1- or 3-position, it is released as free stearic acid, and in the presence of calcium and magnesium it is poorly absorbed. Gacs and Barltrop (1977) also demonstrated that calcium absorption from the rat small intestine is impaired by stearic acid and is accompanied by a significant increase in calcium soap formation in both the intestine and the feces. Finley et al. (1994a,b) have shown that rats and humans consuming SALATRIM fats have higher fecal stearic acid concentrations than when consuming control fats. The fecal stearic acid concentration increases as the amount of dietary SALATRIM increases. These studies further support the hypothesis that free stearate is poorly absorbed.

Variations in lipid solubilization and absorption also influence cholesterol homeostasis. It has been repeatedly reported that dietary fats rich in saturated fatty acids raise serum cholesterol, whereas fats rich in polyunsaturated fatty acids lower it. Gram for gram, saturated fatty acids were found to be twice as potent in elevating serum cholesterol as polyunsaturated fatty acids were in bringing about a decrease (Keys et al., 1965). However, there appear to be exceptions to this general rule, and it is now clear that certain saturated fatty acids are hypocholesterolemic while others are not.

In estimating hypercholesterolemic effects of fatty acids, Keys et al. (1965) assigned a neutral role to stearic acid. The lack of effect of stearic acid on cholesterol was confirmed by Grande et al. (1970). They showed that palmitic acid caused a rise in serum cholesterol compared to stearic acid, which had a neutral or slightly lowering effect. Hegsted et al. (1965), using neutral fats, concluded that stearic acid, like the short-chain saturated fatty acids, had no specific effect on serum cholesterol. Bonanome and Grundy (1988) confirmed the observation in a carefully controlled study. When semisynthetic fats were tested, stearic acid appeared to be nearly as hypercholesterolemic as palmitic acid (McGandy et al., 1970). Vergroesen and DeBoer (1971) reported similar findings. However, the interactions are complex, and the issue is still not resolved.

Iodine value, defined as grams of iodine taken up per 100 g of fat or oil, is used as an index of unsaturation, with larger values resulting from a greater degree of unsaturation. Cocoa butter has an iodine value of about 37 but has a hypocholesterolemic effect comparable to that of olive oil and peanut oil, which have iodine values of about 90 (Bergstedt et al., 1990). The hypocholesterolemic effect of cocoa butter has been demonstrated in animals and humans (Grande et al., 1970; Kritchevsky and Tepper, 1965; Kritchevsky et al., 1982) and may be explained by the relatively high content of stearic acid (about 35% of total fatty acids) in cocoa butter and the poor absorption of fats rich in stearic acid by the small intestine (Mattson, 1959).

Bergstedt et al. (1990) showed that tristearin is not only poorly digested by the small intestine but also taken up and transported by the small intestine less efficiently than triolein. This observation also led these investigators (Bergstedt et al., 1990) to believe that the lack of cholesterolemic effect of cocoa butter was due to the poor digestion, uptake, and transport of stearic acid.

Bonanome and Grundy (1988) compared the effect of three separate diets rich in palmitic acid (C16:0), stearic acid (C18:0), or oleic acid (C18:1) on plasma lipid and lipoprotein levels. While they reported no significant difference in absorption of the three diets on the basis of fecal fat excretion, they showed the diet rich in stearic acid resulted in significantly lower plasma cholesterol and low-density lipoprotein when compared with the feeding of a palmitic acid-enriched diet. The feeding of the oleic acid-enriched diet had effects similar to those of feeding the stearic acid-enriched diet. This led Bonanome and Grundy (1988, 1989) to conclude that "stearic acid appears to be as effective as oleic acid in lowering plasma cholesterol levels when either replaces palmitic acid in the diet". They proposed that the apparent resemblance in the cholesterolemic effect between stearic acid and oleic acid was because stearic acid is converted to oleic acid, as evidenced by the rise in the oleic acid content of plasma triacylglycerols and cholesteryl esters. Hayes et al. (1994) found that up to 50% of the absorbed stearic acid from SALATRIM is converted to oleic acid. Another possible explanation is that stearic acid is poorly absorbed and, therefore, has little or no effect on serum cholesterol (Apgar et al., 1987).

Bergstedt et al. (1991) demonstrated with three groups of intestinal lymph fistulated rats, given either radiolabeled

tristearin, radiolabeled tristearin with triolein added, or radiolabeled tristearin with tripalmitin added, that the lymphatic radioactivity and triacylglycerol outputs were significantly lower in rats given tristearin alone than in rats given tristearin with triolein or tripalmitin. Significantly more tristearin remained in the intestinal lumen of rats given tristearin alone, and the majority of radioactivity in the lumen was still in the tristearin form in all three groups, indicating poor lipolysis. Once the fatty acid and monoglyceride were absorbed, however, the reesterification process was shown to be similar in all three groups. The rats given tristearin alone were less able to transport the absorbed lipid into the lymph than rats given tristearin with either triolein or tripalmitin. The presence of triolein or tripalmitin greatly enhanced the lymphatic transport of tristearin. This enhancement was shown to be mediated by better lipolysis and also by more efficient packaging of absorbed fat into triacylglycerol-rich lipoproteins.

SALATRIM triacylglycerols contain at least one stearic acid that is randomly distributed among the three ester sites. Esters with stearic acid at the 1- and/or 3-positions will be cleaved by the lipases, resulting in free stearic acid that is poorly absorbed. This stearic acid is most likely excreted in the feces as either calcium or magnesium soaps. Stearic acid that occurs at the 2-position will result in the 2-monoacylglycerol that will be absorbed into gastrointestinal mucosal cells and enter into pathways associated with fat metabolism. A portion of the stearic acid that occurs at the 2-position of the monoacylglycerol may migrate to the 1- or 3-position by ester migration and can then be cleaved by the lipases, resulting in glycerol and free stearic acid. The high stearate content of SALATRIM fats and the high content of SCFA, therefore, contribute to their lower caloric availability (Finley et al., 1994a).

Triacylglycerol Resynthesis and Chylomicron Formation. Once inside the enterocyte, triacylglycerols are resynthesized from a few MCFA, LCFA, monoacylglycerols, and glycerol and are packaged into chylomicrons for export (Tso and Balint, 1986). There is no evidence for reesterification of SCFA. Roughly 75% of the LCFA originally at the 2-position in the triacylglycerols are maintained there (Akesson et al., 1978; Mattson and Volpenhein, 1964). The fatty acids reesterified to the 1- and 3-positions, in contrast, have been randomized during digestion, absorption, and intracellular resynthesis.

Triacylglycerol resynthesis takes place in the apical portion of the enterocyte mediated by fatty acyl-CoA ligase, monoacylglycerol acyltransferase, and diacylglycerol acetyltransferase which are located in a complex on the cytoplasmic face of the smooth endoplasmic reticulum (SER) (Grigor and Bell, 1982; Mattson and Volpenhein, 1964). Cytosolic transport of the fatty acids to the SER is facilitated by an intracellular fatty acid binding protein (FABP) (Ockner and Manning, 1974, 1976). Within the SER, triacylglycerols are predominantly resynthesized directly from 2-monoacylglycerol by the addition of two molecules of fatty acid (Kayden et al., 1967). Alternatively, resynthesis occurs by the α-glycerophosphate pathway that adds three molecules of fatty acid to glycerol 3-phosphate, yielding a triacylglycerol.

Once the triacylglycerol droplet has been formed within the SER, chylomicrons are assembled in the endoplasmic reticulum (ER)–Golgi region of the enterocyte. The chylomicrons are large lipoprotein particles (800–5000 Å) with a density of less than 1.006 g/mL (Fredrickson et al., 1978). The particles are composed mainly of triacylglycerol (86–92%) and cholesterol ester (0.8–1.4%), free cholesterol (0.8–1.6%), phospholipids (6–8%), and protein (1–2%) (Glickman, 1976). Triacylglycerol composition largely reflects dietary fatty acids, while the other chylomicron lipids bear little relationship to dietary fat. Structurally, triacylglycerol forms the apolar core of the chylomicron, while the protein, phospholipid, and free cholesterol form a polar surface around the triacylglycerol core. There is sufficient phospholipid to cover almost the entire chylomicron surface (>80%), while protein covers approximately 20% of the surface (Zilversmit, 1965).

Although apoproteins comprise only 1–2% of the mass of chylomicrons, they are a complex mixture and have important roles in chylomicron clearance and metabolism. Half of the chylomicron apoprotein is immunologically related to high-density lipoprotein (HDL) (Glickman and Kirsch, 1973; Glickman et al., 1972). Most of the known plasma apolipoproteins are present and include apo B (10%), apo A-IV (10%), apo E (5%), apo A-I (15–35%), and apo C (45–50%) (Bisgaier and Glickman, 1983). Apo A-II, a minor apoprotein in the rat, is present on human chylomicrons.

Phospholipids, largely derived from dietary and biliary phospholipids that are hydrolyzed in the intestine, absorbed passively by enterocytes as lysophospholipids, reacylated on the SER membrane, and transported into the SER lumen by a specific carrier protein (Bishop and Bell, 1985), serve as a source of chylomicron phospholipids. Phospholipid can also be synthesized *de novo* in the SER via the α-glycerophosphate pathway when biliary phospholipid availability is limited. The phospholipids form a coat around the triacylglycerol particle and, with the apolipoproteins, stabilize the particle.

Cholesterol, both free and as cholesterol ester, is the other component of chylomicrons. After transfer from the intestinal lumen to the enterocyte cytoplasm, free cholesterol is esterified in the SER by acyl-CoA cholesterol acyltransferase (ACAT) (Clark and Tercyak, 1984) prior to incorporation into chylomicrons.

After assembly in the ER, chylomicrons move to the Golgi apparatus (Sabesin and Frase, 1977) for glycosylation (Kessler et al., 1975) and final processing. From the Golgi apparatus, secretory vesicles, each containing several chylomicrons, bud off and move to the basolateral enterocyte plasma membrane. The chylomicrons are released into the intercellular space by exocytosis and subsequently enter intestinal lacteals and are transported in lymph (Sabesin and Frase, 1977). Microtubules may be involved in the selective directional movement of secretory vesicles to the basolateral membrane (Pavelka and Gangl, 1983).

SALATRIM fats should be included in chylomicron formation in a manner similar to other dietary triacylglycerols. The SCFA from SALATRIM fats will not be reesterified to produce triacylglycerols in the enterocyte and will not be a component of the chylomicrons. Also, the stearic acid freed by lipolysis in the gastrointestinal tract will be poorly absorbed and will be available for reesterification and chylomicron metabolism to only a limited extent. The stearic acid absorbed as the free acid or as the monoacylglycerol will be transformed to triacylglycerol and packaged into chylomicrons. Also, a portion of stearic acid (as much as 50% in the rat) will be converted to oleic acid (Hayes et al., 1994).

Chylomicron Metabolism. Current understanding of chylomicron metabolism suggests that, following their formation by the intestine and excretion in the lymph, they pass from the thoracic duct to the general circulation,

where they attach to capillary endothelial cells (Blanchette-Mackie and Scow, 1971). Here, following activation by the apoprotein C-II (Bier and Havel, 1970), they are exposed to the enzyme lipoprotein lipase that is abundant in many extrahepatic tissues, including the membranes of adipose cells. This enzyme hydrolyzes about 80% of the triacylglycerols of the chylomicrons into fatty acids and glycerol.

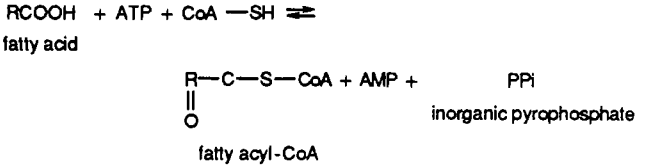
Following removal of the triacylglycerols, the surface of the chylomicron is altered to allow the chylomicron remnant to be recognized by hepatic parenchymal cells. Surface constituents of the chylomicron such as phospholipid and apoproteins A-I and A-II are rapidly distributed to the HDL fraction of plasma (Redgrave and Small, 1979; Schaefer et al., 1978; Tall et al., 1979). The remnant is then rapidly cleared via receptor-mediated uptake by the liver, which recognizes apo E and perhaps intestinal apo B (Shelburne et al., 1980; Windler et al., 1980). Once inside the hepatic cell, lysosomal catabolism of the lipid and protein components occurs. The cholesterol and cholesterol esters are utilized almost completely by the liver. The dietary cholesterol esters are hydrolyzed to release cholesterol or are oxidized to bile acids, both of which are secreted into the bile. Cholesterol can also be incorporated into hepatogenous lipoproteins.

Fatty Acid Utilization. At the adipose tissue, the fatty acids hydrolyzed from the chylomicrons by lipoprotein lipase are released in part into the blood plasma, where they are bound to serum albumin for transport to skeletal muscles and the heart. Here they are absorbed, oxidized, and utilized as the major fuel source. Free fatty acids released by lipoprotein lipase also diffuse into the adipocytes, where they are resynthesized into triacylglycerols for storage. Adipocytes actively store triacylglycerols, particularly after meals rich in fat. Triacylglycerols already stored in adipocytes are not attacked by lipoprotein lipase, which is located on the outer surface of the cells, but are attacked by intracellular lipases to release free fatty acids, which may be delivered into the blood, where they are bound by serum albumin. Each molecule of serum albumin can bind two molecules of long-chain fatty acid very tightly and one or two more molecules loosely. Because of its high concentration in the blood plasma, serum albumin is the major carrier of fatty acids in the blood. They are carried to skeletal muscles and the heart, which use most of the free fatty acids bound to serum albumin.

The liver also plays a major role in lipid metabolism. Fatty acids hydrolyzed from chylomicrons that enter the liver may be either converted to triacylglycerols or directly oxidized for energy. Some of the triacylglycerols are processed and distributed to other organs and tissues.

Fatty Acid Oxidation. The final stage of fat metabolism is fatty acid oxidation. These pathways are well-known and are reviewed in a number of textbooks (Lehninger, 1982; Stryer, 1988) and will be mentioned only briefly here. Free fatty acids are metabolized to CO_2 and H_2O, and in the process, adenosine triphosphate (ATP) is produced. Studies by Knoop (1905) were among the first to elucidate the mechanism of fatty acid oxidation. He deduced that fatty acids were degraded two carbon atoms at a time by oxidation at the 2- or β-carbon.

Free fatty acids in the cytosol cannot pass through the mitochondrial membranes as such and must be activated before they enter the mitochondrial matrix (Berg, 1956). Three enzymatic reactions occur in this process. First, acyl-CoA synthetase in the outer mitochondrial membrane activates the fatty acid to the acyl-CoA derivative:



Energy from ATP is used to form the thioester bond between the fatty acid carboxyl group and the thiol group of coenzyme A, resulting in fatty acyl-CoA. The pyrophosphate formed in the activation reaction is hydrolyzed by pyrophosphatase, which drives the equilibrium toward formation of fatty acyl-CoA.

Carnitine acyltransferase I, on the outer surface of the inner mitochondrial membrane, catalyzes the conversion of fatty acyl-CoA to fatty acyl–carnitine:

fatty acyl—S—CoA + carnitine ⇄ fatty acyl—carnitine + CoA—SH

The fatty acyl–carnitine ester then passes through the inner membrane into the internal matrix of the mitochondria. Carnitine acyltransferase II, present on the inside surface of the inner mitochondrial membrane, transfers the fatty acyl group from carnitine to intramitochondrial CoA:

fatty acyl—carnitine + CoA—SH ⇄ fatty acyl—S—CoA + carnitine

This three-step process keeps the cytosolic and intramitochondrial pools of CoA separated. The mitochondrial pool is largely used in oxidation of pyruvate, fatty acids, and some amino acids, whereas the cytosolic pool is used in fatty acid biosynthesis.

The fatty acid component of fatty acyl-CoA is oxidized by enzymes in the mitochondrial matrix by four reactions: oxidation by flavin adenine dinucleotide (FAD), hydration, oxidation by NAD^+, and thiolysis by CoA. The fatty acyl chain is shortened by two carbons as a result of these reactions, and $FADH_2$, NADH, and acetyl-CoA are generated.

$$C_n\text{-acyl CoA} + FAD + NAD^+ + H_2O + CoA \rightarrow C_{n-2}\text{-acyl CoA} + FADH_2 + NADH + \text{acetyl-CoA} + H^+$$

Three ATP are generated when each NADH is oxidized by the respiratory chain, whereas two ATP are formed for each $FADH_2$. Therefore, five molecules of ATP are formed per molecule of acetyl-CoA.

In the second stage of fatty acid oxidation, the acyl residues of acetyl-CoA are oxidized in the mitochondria to CO_2 and H_2O via the citric acid cycle. Acetyl-CoA derived from fatty acid oxidation enters a common pathway of oxidation with acetyl-CoA coming from glucose via pyruvate oxidation.

Oxidation of unsaturated fatty acids is the same as for saturated fatty acids except two additional enzymes, an isomerase and an epimerase, are involved. The acyl-CoA ester undergoes a number of passes through the fatty acid oxidation cycle inside the mitochondrial matrix until the cis double bond of the unsaturated fatty acid is reached. At this point, an isomerase converts the cis double bond to a trans double bond. The subsequent reactions are those of the saturated fatty acid oxidation pathway. Another enzyme, epimerase, is needed for the oxidation of polyunsaturated fatty acids.

Odd-carbon fatty acids are oxidized by the same pathway as the even-carbon fatty acids, beginning at the carboxyl end of the chain. However, propionyl-CoA and acetyl-

CoA, rather than two molecules of acetyl-CoA, are produced in the final cycle of degradation. Acetyl-CoA is oxidized via the citric acid cycle, while propionyl-CoA is carboxylated to methylmalonyl-CoA by propionyl-CoA carboxylase. Bicarbonate is the precursor of the new carboxyl group, and ATP furnishes the energy required to form the new covalent bond. D-Methylmalonyl-CoA is epimerized to its L-stereoisomer by methylmalonyl epimerase. The L-methylmalonyl-CoA rearranges to succinyl-CoA catalyzed by methylmalonyl-CoA mutase and its coenzyme deoxyadenosylcobalamin. Therefore, the –CO–S–CoA group migrates from C2 to C3 in exchange for a hydrogen atom. Succinyl-CoA is an intermediate of the citric acid cycle and ultimately leads to oxaloacetate.

Once acetyl-CoA has been formed by fatty acid oxidation in liver mitochondria, it may either be oxidized to CO_2 via the citric acid cycle or converted to ketone bodies and circulated to the peripheral tissues.

Ketone Body Formation. Acetyl-CoA formed in fatty acid oxidation enters the citric acid cycle only if fat and carbohydrate degradation are appropriately balanced. This is because the entry of acetyl-CoA into the citric acid cycle depends on the availability of oxaloacetate for the formation of citrate. If carbohydrate is unavailable or improperly utilized, such as in fasting or diabetes, oxaloacetate is diverted to form glucose. Under these conditions, acetyl-CoA is diverted to form acetoacetate and D-3-hydroxybutyrate. Acetoacetate, D-3-hydroxybutyrate, and acetone are referred to as ketone bodies.

Acetoacetate is formed by the condensation of 2 mol of acetyl-CoA to form acetoacetyl-CoA. Acetoacetyl-CoA reacts with acetyl-CoA and water to yield 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) and CoA. 3-Hydroxy-3-methylglutaryl-CoA is cleaved to acetyl-CoA and acetoacetate.

3-Hydroxybutyrate is formed by the reduction of acetoacetate by D-β-hydroxybutyrate dehydrogenase in the mitochondrial matrix. The ratio of hydroxybutyrate to acetoacetate depends on the mitochondrial NADH/NAD^+ ratio. Acetoacetate can also undergo a slow spontaneous decarboxylation to acetone. Acetoacetate and 3-hydroxybutyrate are produced primarily in the liver. However, they are not further oxidized in the liver but diffuse from the liver mitochondria into the blood and are transported to peripheral tissues.

Ketone bodies were at one time regarded as degradation products of little physiological value. However, the work of Cahill (1970) revealed that these derivatives of acetyl-CoA are important molecules in energy metabolism. Acetoacetate and 3-hydroxybutyrate are normal fuels of respiration and are quantitatively important as sources of energy. Heart muscle and the renal cortex use acetoacetate in preference to glucose. In contrast, glucose is the major fuel for the brain and red blood cells in well-nourished persons on a balanced diet.

In the peripheral tissues, acetoacetate can be activated by the transfer of CoA from succinyl-CoA in a reaction catalyzed by a specific CoA transferase. Acetoacetyl-CoA is then cleaved by thiolase to yield two molecules of acetyl-CoA which can enter the citric acid cycle for complete oxidation. The liver supplies acetoacetate to other organs because it lacks this particular CoA transferase.

Overview of the Predicted Digestion, Absorption, and Metabolism of SALATRIM Fats. Because SALATRIM fats contain a significant amount of SCFA, the stomach will be the first site for their digestion. Through the action of lingual lipase and gastric lipase, a portion of the acetate, propionate, and butyrate moieties will be hydrolyzed from the glycerol backbone. As noted above, the SCFA should be rapidly absorbed by the gastric mucosa. Since the gastric mucosa appears to lack the ability to esterify SCFA to glycerol (Clark et al., 1969), the SCFA will pass through the mucosa to the gastric vein. Some of the SCFA, especially butyrate, will be utilized by the gastric mucosa as an energy source for anabolic metabolism. From the gastric blood vessels the SCFA will travel to the liver, where they will be extracted from the blood and enter the hepatic metabolism system. SALATRIM metabolism studies in rats support these predictions (Hayes et al., 1994).

The stearic acid released from SALATRIM fats by lingual lipase and gastric lipase will partition into the fat emulsion droplets in the stomach, will not be absorbed by the gastric mucosa, and will be carried into the small intestine for further processing. In the small intestine, the remaining SCFA will be preferentially cleaved by pancreatic lipase. Additionally, fatty acids, regardless of chain length, will be hydrolyzed from the 1- and 3-positions of the glycerol backbone. Monostearin, glycerol, and free acetic, propionic, butyric, and stearic acids will be produced. The free short-chain fatty acids will diffuse freely across the intestinal mucosa and will enter the portal circulation in the free form. Free stearic acid, on the other hand, will be poorly absorbed (Carey et al., 1983; Hashim and Babayan, 1978; Jensen et al., 1982). A portion of the stearate will be excreted in the feces as calcium and magnesium salts along with free stearate (Benzonana and Desnuelle, 1968; Bliss et al., 1972; Gacs and Barltrop, 1977; Sammons and Wiggs, 1960).

Monostearin and free stearic acid that are absorbed by the enterocytes will be reesterified to form triacylglycerols and will undergo the complex processes associated with chylomicron formation before appearing in the lymphatic circulation. A portion of the absorbed stearic acid will be converted to oleic acid (Hayes et al., 1994).

The relatively poor absorption of stearate and the lower number of calories provided by the short-chain fatty acids compared to the long-chain fatty acids result in a lower caloric availability for members of the SALATRIM family as compared to other dietary fats such as corn oil.

CONCLUSION

The components of SALATRIM, SCFA, stearic acid, and the glycerol backbone, are normal metabolites handled daily by the human body. There is a vast knowledge base that supports the predictability of the metabolic fate of these components. Hence, it can be concluded that the components of the family of triacylglycerols represented by SALATRIM are metabolized by well-understood metabolic pathways and that these compounds should not represent any significant safety concern.

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