Digestion and Absorption of Interesterified Lipids

Impact of Dietary FA on Post-prandial Inflammation

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Overview of digestion and absorption
   — lipases and FA specificity
   — absorption and processing by enterocytes

Specific effects of interesterified fats
   — medium vs long chain FA at sn-2 vs -1(3)
   — absorption efficiency
   — lymph vs portal fate
   — tissue targeting of energy and essential FA
   — clinical applications

Areas for potential investigation
Post-Prandial Inflammation:

Intestinal mast cell activation during lipid absorption
  — effects of specific FA
  — cytokine secretion

Circulating leukocytes post lipid absorption
  — monocyte and neutrophil activation
  — potential effects of specific FA
  — lessons from Npc1l1 knockout mice

Areas for potential investigation
Overview of lipid digestion

Lipolysis begins in the stomach by gastric lipase (GL), which generates FFA and DG and accounts for ~15% of TG digestion.

In the small intestine, lipid (food) is emulsified by bile salts and phospholipids, released when CCK stimulates gall bladder contraction after a meal, and by GL digestion products.

Pancreatic lipase (PTL)+ colipase performs bulk of lipolysis in duodenum and upper jejunum, releasing FFA and 2-mag.

Digested lipids are incorporated into mixed micelles with bile salts such that there is an oil phase → emulsion → micellar phase → aqueous phase continuum in the intestinal lumen.
PTL related protein-2 (PLRP2) is also secreted by pancreas and shows similar activity to PTL. Important for fat absorption in neonates and young infants but much less important in adults.

Bile salt stimulated lipase (BSSL, cholesterol esterase, carboxyl ester lipase, CEL) also secreted by pancreas and hydrolyzes TG completely to FFA and glycerol. Stimulated by tri-OH bile salts. Abundant in milk and important for fat absorption in neonates. Secondary to PTL and PLRP2 in adults (knockout mice).

Phospholipase A2 (PLA2) is also secreted by pancreas and generates FFA and lyso-PC from biliary and dietary PL. Intestine also makes a PL-B in lower jejunum which digests PL to FFA + phosphoryl choline. PLA2 not required for overall PL absorption but has large impact on metabolic disease. PLA2-ko mice are resistant to diet-induced diabetes and obesity.
GL shows a preference for FA in the sn-3 position. Some data suggest preference for short and medium chain FA but this may be due to preponderance of these FA in the sn-3 position of the substrate used. Chain length preference may be minimal.

PTL is specific for sn-1(3) FA and also shows some preference for related to chain length. Normally the amount of PTL in the lumen masks this substrate preference in vivo.

CEL has greater capacity than PTL to hydrolyze very long chain FA but also shows preference based on chain length.
Fatty acid uptake by enterocytes does not appear to be rate limiting for the typical dietary fatty acids. These flip-flop across the membrane and are subsequently esterified to CoA by one or more of the family of FATP proteins, which traps them in the cell and maintains a concentration gradient facilitating continued uptake. 2-MAG appear to also cross the membrane by a similar diffusion mechanism.

While a role for direct transporters such as CD36 has been implied, they may only be significant at low substrate levels or when FA are presented other than in micellar form, and most data are consistent with the flip-flop model.
Resynthesis of TG and formation of chylomicrons by enterocytes.

Exact roles of FABP's not clear but 2-MAG and long chain FA-CoA's are transported to ER where MGAT2 and DGAT1 resynthesize TG from luminal digestion products. Medium chain FA are primarily secreted into the portal circulation, though some are also incorporated into TG (10 & 12).

MGAT2 appears to be less active on 2-stearoyl-MAG than others species but does not show a distinct preference for FA-CoA's.

It is unclear whether DGAT1 has distinct specificity for FA substrates, however a preference for 18:1 as compared to 16:0, 18:3, or 20:4 was seen in competition assays.
Substrate specificities of MGAT2 expressed in mammalian cells

Chylomicron Assembly

MTP (micorsomal triglyceride transport protein) transfers lipid to Apo B 48 as the protein is synthesized, making a dense prechylomicron particle, which is transported to the smooth ER and subsequently enlarged by MTP transfer of oil droplet with apoAIV. During process through the Golgi, the nascent chylomicron is further modified by additional lipids as well as the addition of other apolipoproteins (AI, the C's). The mature particle is ultimately secreted by fusion with the basolateral membrane through which it enters the interstitial lacteal space, flows through the intestinal lymphatics and into the thoracic duct, and finally enters the circulation via the left subclavian vein.
Absorption and transport of fatty acids given as structured TG (STG) / interesterified (IE) lipids
• The IE lipids most characterized are those comprised of various mixtures of MCFA and LCFA.

• Several investigations have shown that absorption and lymphatic transport of FA from IE lipids is not the same as absorption from an equivalent mixture of the same oils used to derive the IE-TG.

• Other than the lipase preferences described above for natural oils and fats, there is no difference in lumenal lipolysis of the engineered fats, accept as the modifications affect solubility in mixed micelles.

• However, position on the glycerol backbone affects the rate and fate of absorption/transport of specific FA.
More efficient lymphatic transport of LCFA and MCFA from the sn-2 position.

From Ikeda et al. (1991) Lipids 26: 369-373
~ 3 fold greater lymphatic MCFA transport from structured TG than from mixtures of the parent oils when MCFA is in sn-2 position.

From Ikeda et al. (ibid)
Not all reports are consistent with these data. Mu & Hoy and others have shown that these effects vary depending on the MCFA. Also, if the total amount of FA available is low, TG synthesis and chylomicron secretion by the gut is slower (and lower), probably because endogenous FA are being diverted for TG production.

One consequence of greater lymphatic MCFA transport is better delivery of readily utilized FA energy source to peripheral tissues rather than predominantly liver, as is the case with portal transport. Both animal models and clinical studies suggest that interesterified fats provide a mechanism to deliver MCFA for energy utilization to peripheral tissues in patients with high stress such as burns, surgical trauma, or cancer thus diminishing nitrogen wasting and organ damage.

This phenomenon has been exploited for clinical nutrition needs such as malabsorption, and high energy needs to prevent tissue wasting and improve surgical outcome.
Increased lymphatic transport of MCFA and EPA from IE TG relative to oil mixtures in ischemia/reperfusion malabsorption model

IE TG derived from 55/45 mix of fish oil and MCT (56% 8:0, 44% 10:0)

Several studies in animal models as well as in humans indicate increased energy expenditure and diminished weight gain without changes in food intake when MCFA is given as IE lipids as compared to mixed TG.

Overall FA absorption (24 h) is minimally different between TG mixtures and IE fats of the same composition, except for saturated LCFA (16:0, 18:0) which form calcium soaps and are excreted to a greater degree when in the sn-1(3) position (Mu H, Porsgaard T (2005) Prog Lipid Res 44: 430-448)

Current data indicate only limited differences in long-term tissue fate of LCFA from mixed TG vs IE fats under normal conditions. Incorporation of DHA and EPA into brain phospholipids of rat dams or pups was not affected by dietary lipid structure, however EPA (but not DHA) delivery to splenocytes was increased if it replaced all 18:3 FA in the sn-2 position of fed TG.
Areas of Potential Investigation

There is little information about the effects of structured lipids on chylomicron size or apolipoprotein composition. Other than MCFA usage, there is little direct information about acute post-prandial tissue fate of lipids from interesterified vs mixed TG.

There are no reports of how these different lipids affect gut hormones (e.g. GlP, GLP-1) that play roles in insulin sensitivity and metabolic disease.
Post-prandial Effects of Lipid Absorption and Dietary FA on Inflammation

Potential relation to tissue inflammation and metabolic disease
Effect of Fat Absorption on Gut Inflammation

Intestinal mast cell activation

Yong Ji, Patrick Tso
Gut immune cells play an important role in host defense, and mast cells affect innate and adaptive immune responses by releasing mast cell mediators (histamine, PG's, cytokines) and other mechanisms. Mast cells are also implicated in GI disorders such as IBD/S and food allergies.

Dietary lipids (oleic acid) have been shown to affect lymphocyte migration and activation with effects that are relevant to inflammatory bowel disorders and food allergy.

Ji and Tso investigated the effects of fat absorption - including FA type - on protease, histamine and prostaglandin release from mast cells using the rat lymph fistula model.
Release of mast cell protease II (RMPCII) is stimulated by lipid absorption.
Activation requires LCFA and is greater with 18:2 than with 18:1 or 18:3
Histamine release parallels RMPCII in lymph during fat absorption
RMPC secretion is decreased when chylomicron secretion into lymph is blocked by Pluronic L-81.
Post-prandial Effects of Lipid Absorption and Dietary FA on Circulating Leukocyte Activation

Potential relation to peripheral tissue inflammation and metabolic disease
The post-prandial period when serum lipids are highest (3-6 h) is considered to be a period of high risk for cardiovascular (CVD) events, and reports from recent years indicate that risk may also be increased for other metabolic diseases, e.g. type II diabetes, due to lipid-induced inflammation.

In addition to the known chronic effects of specific dietary FA on CVD risk, they may also have effects during periods of acute hyperlipidemia. Long chain saturated FA (palmitate, stearate) are known to activate various signaling and stress pathways in a variety of cell types including macrophage.

Thus, dietary TG structure as well as FA composition may be important if it effects tissue targeting / metabolic fate.

Few studies have compared IE lipids or STGs to natural oils for this purpose, but some relevant data have been reported.
Postprandial monocyte activation in humans

- Normolipidemic subjects given a breakfast "challenge" of 2 sausage McMuffins with eggs, hash browns, and juice. Blood drawn before meal, 3.5 & 7 hr after meal. Serum parameters measured. Leukocytes assayed for activation by cell surface receptor stains and sorted by flow cytometry.

Transient elevation of cell surface activation markers on monocytes after a high fat meal


TNF-α, IFN-γ, IL-1β, IL-6, IL-8 elevated ~50% at 3.5 hr
Monocyte arrest on VCAM-1 increases postprandially in correlation to serum TG levels, and is dependent on CD11c.

Monocytes contain lipid droplets postprandially. Data indicate chylomicron remnant uptake via the LRP1 receptor.

Monocyte Activation In Vitro

Whole blood from human subjects incubated with different lipid emulsions - lipofundin, structolipid, omegaven, clinoleic, intralipid - at different doses for 2 hr.

Lyse red cells & isolate leukocytes; stain for surface markers; analyze by flow cytometry

Not a postprandial study but may model parenteral nutrition
Activation of leukocytes by parenteral lipid emulsions


L = intralipid
LM = lipofundin
FO = omegaven
Post-prandial monocyte activation and diet-induced adipose inflammation are reduced by lack of Npc1l1 function - possible effects of reduced saturated fat absorption.
Npc1l1 is the intestinal protein that plays a major role in cholesterol absorption. Its function is blocked by the drug ezetimibe (Zetia).

We demonstrated that Zetia-treated and Npc1l1 knockout mice are protected from diet-induced obesity and insulin resistance. (Labonte et al. (2008) AJPhys-Gastro)
Thus, blocking Npc1l1 function can have important metabolic effects beyond just blocking cholesterol absorption and reducing serum LDL levels.

Importantly, in the last year there have been three reports of small clinical studies that suggest this effect also occurs in humans - improved HOMA-IR index or improved waist:hip ratio with Zetia treatment that was independent of LDL lowering.
As part of our initial study, we measured fat absorption and found that Zetia treatment or Npc1l1 knockout reduces fat absorption, primarily of saturated fatty acids.

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<th>Control</th>
<th>Zetia</th>
<th>Npc1l1−/−</th>
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<tbody>
<tr>
<td>Laurate</td>
<td>98±1.6</td>
<td>94±2.8*</td>
<td>95±2.1*</td>
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<tr>
<td>Myristate</td>
<td>94±2.6</td>
<td>88±5.2†</td>
<td>85±4.8*</td>
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<td>Palmitate</td>
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<td>80±5.2*</td>
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<tr>
<td>Stearate</td>
<td>69±10</td>
<td>49±13*</td>
<td>50±10*</td>
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<tr>
<td>Oleate</td>
<td>96±0.6</td>
<td>91±3.7*</td>
<td>92±3.0*</td>
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<tr>
<td>Linoleate</td>
<td>97±0.3</td>
<td>95±2.0†</td>
<td>95±1.2*</td>
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*P < 0.045 vs. control †P < 0.06 vs. control..06 vs.
Fat absorption is measured by the non-absorbable tracer method developed by Ron Jandecek at UC. Sucrose polybehenate (used in Olestra) is added to the diet at a known concentration and the exact ratio of behenate to each FA in the diet is determined. Animals are fed the "tagged" diet for 2-5 days after which the ratio of behenate to each FA is determined in a small fecal sample. Behenic acid is present at trace or non-detectable levels in most oils used in experimental diets, and is virtually unabsorbed when given as the sucrose ester. Thus percent absorption of each FA can be calculated from the difference in behenate:FA ratio between diet and fecal samples. Method is widely used in animal models and is approved for human studies.
We have investigated the ramifications of lower saturated fat absorption by these mice, other than a potential contribution to decreased weight gain.

We postulated that lack of Npc1l1 function reduces postprandial inflammation resulting from saturated fat absorption. This may, in turn, reduce visceral adipose inflammation which may contribute to the observed protection from diet-induced insulin resistance.
**Approach:** Leukocytes are isolated from plasma taken after a 10-12 hr fast and again 3 hr after a gavage of either a lard or olive oil emulsion. After staining with fluorescently tagged antibodies, flow cytometry is used to quantitate the number and activation state of various cell types. Focus has been primarily on monocyte activation as measured by CD11c and or Ly6c. Monocytes are defined by forward / side scatter characteristics.

Fat depots were digested with collagenase to release stromavascular cells which were stained with F4/80 to identify macrophage, CD11c for M1 type, MRC2 for M2 type.

Ex vivo cytokine secretion from cultured adipocytes was measured by LincoPlex (MMPC). RNA isolated for Q-PCR.
Post-prandial monocyte activation following lard gavage is reduced in Npc1l1 knockout and Zetia-treated mice.

There was no drug / gene effect following olive oil gavage.
The profile of adipokine and cytokine secretion and gene expression by visceral adipose depots is less inflammatory in Npc1l1 knockout mice.

Ratio of adiponectin/leptin is greater in knockouts on both chow and high-fat diets. TNFa, Mip-1a, IL-6, IL10 are reduced.

Ex vivo cytokine secretion parallels gene expression
We've also investigated diet-induced adipose inflammation in these mice, using flow cytometry analysis of stromavascular cells to determine number and activation state of adipose tissue macrophage.

There are fewer total macrophage in visceral fat from knockout mice. Of those present, fewer are activated to M1 state and more to M2 state than in control mice.
Taken together, these and other data we've collected are consistent with the notion that post-prandial inflammation is exacerbated by dietary saturated fat - especially stearate and palmitate - and may be a contributing mechanism to their increased risk for metabolic disease.

Decreasing this acute effect in the plasma compartment may also protect against chronic inflammation of adipose and other tissues which can predispose patients to insulin resistance and diabetes.

Zetia treatment, or other means of Npc1l1 inhibition, may protect individuals from these risks by decreasing saturated fat absorption independent of cholesterol absorption.
Areas of Potential Investigation

There is limited information about effects of structured / interesterified lipids on postprandial inflammation.

Does FA composition or lipid structure of remnant particles affect the degree of monocyte or neutrophil activation?

How interesterified lipids vs. mixed oils affect gut immune and inflammatory function during the absorption process is also underinvestigated but may have local effects or may impact systemic inflammation as well.

Given the relevance of inflammation to insulin resistance and metabolic diseases, these may be important foci for future studies.