An Updated Perspective on the Dual-Track Model of Enterocyte Fat Metabolism
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Abstract

The small intestinal epithelium has classically been envisioned as a conduit for nutrient absorption, but appreciation is growing for a larger and more dynamic role for enterocytes in lipid metabolism. Considerable gaps remain in our knowledge of this physiology, but it appears that the enterocyte’s structural polarization dictates its behavior in fat partitioning, treating fat differently based on its absorption across the apical vs. the basolateral membrane. In this review, we synthesize existing data and thought on this dual-track model of enterocyte fat metabolism through the lens of human integrative physiology. The apical track includes the canonical pathway of dietary lipid absorption across the apical brush-border membrane, leading to packaging and secretion of those lipids as chylomicrons. However, this track also reserves a portion of dietary lipid within cytoplasmic lipid droplets for later uses, including the “second-meal effect”, which remains poorly understood. At the same time, the enterocyte takes up circulating fats across the basolateral membrane by mechanisms that may include receptor-mediated import of triglyceride-rich lipoproteins or their remnants, local hydrolysis and internalization of free fatty acids, or enterocyte de novo lipogenesis using basolaterally-absorbed substrates. The ultimate destinations of basolateral-track fat may include fatty acid oxidation, structural lipid synthesis, storage in cytoplasmic lipid droplets, or ultimate re-secretion, although the regulation and purposes of this basolateral track remain mysterious. We propose overall that the enterocyte integrates lipid flux along both of these tracks in order to calibrate its overall program of lipid metabolism.

Keywords: intestine; lipid absorption; lipid storage; Lipid transport; apical; basolateral; enterocyte; Triglyceride; chylomicron
Preface

In recent decades it has become apparent that intestinal fat handling extends beyond the purely absorptive. For one, the intestine senses and responds to bioactive signaling lipids, derived from the diet or the commensal microbes. But even as it pertains to bulk lipid homeostasis, we can now consider multiple facets of the enterocyte’s relationship with fat and how this intercalates into metabolism writ large. We therefore intend not to present an exhaustive recapitulation of the literature on this topic. Rather, we will synthesize the themes that emerge and analyze their potential connections to human health and disease. We focus on human data while using work from model systems to bridge knowledge gaps, and in both cases we emphasize studies that are physiologically integrative. For expert review of the mechanistic details of intestinal lipid uptake, deposition, and re-mobilization, we refer the reader to multiple recent publications [1-7].

Intestinal reverse lipid transport and the Dual-Track Hypothesis

The canonical anterograde lipid-absorptive pathways within enterocytes have been extensively studied given their obvious physiologic importance. However, as early as the 1950s, evidence of reverse intestinal lipid trafficking – that is, transcellular efflux of cholesterol from the circulation into the intestinal lumen – began to emerge [8-12]. The colocalization of cholesterol and triglycerides within lipoproteins naturally raises the question of a parallel pathway for fats – and, in fact, there is general agreement that intestinal epithelial cells take up circulating fat across their basolateral membrane. Yet, unlike cholesterol, circulating fatty acids are not known to undergo transintestinal excretion, calling into question the enterocyte’s purpose in taking them up.

The very existence of substantial basolateral fat uptake and storage by the human enterocyte suggests some degree of participation in systemic fat metabolism beyond its canonical role in enteral nutrient absorption and secretion. A wealth of animal-based evidence substantiates this inference [13-19], and suggests that lipid flux within the enterocyte operates on a dual-track system [13, 15, 16]. First, the canonical, or apical, pathway traffics in newly absorbed dietary fat, which may be directly secreted into lacteals as chylomicrons or deposited in dedicated cytoplasmic lipid droplets (CLD) for later use. Second, the apical track is joined by an ill-defined basolateral counterpart comprising fat taken up from the blood and also stored in CLD, but whose ultimate metabolic destiny remains largely unknown. Significantly, these two tracks appear to be segregated: lipids absorbed basolaterally seem to commingle little with those that the same cell has absorbed apically, suggesting distinct CLD and cellular machinery subserving them [13, 15, 16] (Figures 1, 2A).
The Apical Track

Although the apical track’s textbook role of importing dietary fat is obvious, closer examination reveals that the enterocyte is not merely a passive absorptive conduit. Rather, the enterocyte deliberately sequesters some of the fat that it brings on board within apical CLD (aCLD), implying a more dynamic role in the regulation of lipid metabolism.

The Second-Meal Effect

Suspicion that the healthy intestine siloes at least a portion of absorbed lipid for later release originated in the repeated observation of a “second-meal effect” (SME), in which one lipid-rich meal apparently triggers an early surge in circulating apoB-48-containing intestinal triglyceride-rich lipoproteins (TRL) during a second meal that occurs too quickly to be accounted for by the latter [20-27]. More compelling still, circulating chylomicron-TG levels still swiftly rise when the second meal is comprised solely of carbohydrate (i.e., fat free) [22, 28, 29]. Stable-isotope tracer studies have demonstrated that the chylomicron-TG appearing after later-meal ingestion may be derived from lipid intake more than 18 hours prior [30] and mirror the fatty acid composition of the first meal [21, 22, 24]. The discovery of duodenal [31] and jejunal [28] enterocytic CLD in human intestinal biopsy specimens corroborated the intestinal storage of dietary fat inferred from the second-meal effect. Two SME studies in humans documented fewer [31] and smaller [28, 31] CLD per cell on biopsy if subjects ingested a carbohydrate load versus water at 5-6 hours after a high-fat first meal, suggesting selective CLD depletion to serve the SME. However, biopsies were not taken after an overnight fast or in the immediate first-meal postprandial period to allow for direct comparisons of individuals’ CLD morphology over time [28, 31].

Alternative lipid sources may also contribute to the observed SME. For instance, enterocytes recover fatty acids derived from biliary phospholipid excretion for esterification to TG [32] and biliary phospholipids themselves are recycled as chylomicron coating [33, 34]. Thus, as duodenal entry even of pure carbohydrate triggers some bile excretion [35, 36], the SME might represent the reappearance of biliary phospholipid-derived fatty acids in TG. It is worth noting that only about 50% of bile is stored in the gallbladder during fasting and released with a meal; the other half drains into the duodenum, even during fasting [37]. Indeed, during fasting biliary lipids are the primary source of lipids in the mesenteric lymph [38]. Because glycerolipid hydrolysis–re-esterification likely proceeds too slowly for phospholipids from postprandial gallbladder emptying to account for the SME [25], SME TG may draw upon biliary lipids absorbed during fasting or a prior meal.
Although the *existence* of the SME is clear in a phenomenological sense, its biological basis and purpose remain hazy. This has been difficult to sort out experimentally as the SME has been difficult to model in mice [13]; we present a few hypotheses worth considering. First, early mobilization of reserved dietary fat may “prime the pump” to efficiently ramp up the postprandial chylomicron assembly line [25, 39]. This may be particularly important when fatty acid species enzymatically preferred – or perhaps even required – for TG esterification are limiting [40]. Another possible explanation for the SME is that constitutive low-level chylomicron production from aCLD [25, 41] protects other tissues from large TG excursions due to lipoprotein lipase saturation [25], although the SME would not be optimally timed for this purpose. On the other hand, intestinal lipid stockpiling may act as a defense against starvation. If so, then the renewed availability of dietary nutrients would temporarily relieve the intestine of this responsibility. In this vein, the SME may prove an exercise in self-defense if it represents a hurried divestiture of banked TG in anticipation of a glut of new dietary fat. Because the enterocyte can foresee neither the quantity nor the composition of fat that it will ultimately encounter over the course of the meal, it would be prudent to ensure optimal readiness of its finite machinery to process the incoming lipid load. Once it has averted the threat of acute fatty acid overload, the intestine can then restock its cache with fresh TG, perhaps to ration as needed during fasting. Note that each of these hypotheses involves a preparatory action on the part of the enterocyte; this would square with SME triggering by “cephalic-phase” nutrient sensing (*i.e.*, based on taste and smell), ostensibly as an early warning system of an impending food bolus [30, 42].

The apical track beyond the SME

Whatever its purpose, the SME casts aCLD as more than just a brief stopover for TG waiting their turn to be packaged in chylomicrons. As alluded to above, aCLD allow enterocytic participation in quantitative and qualitative fatty acid balance, a theme likely common to both tracks. Because of inherent enzymatic substrate preferences, the enterocyte scrambles the original FA composition of dietary TG in their reassembly for chylomicron packaging [40], hence the distinct FA composition of dietary versus circulating TG after a meal [43-45]. The enterocyte may therefore be able to call upon aCLD as a clearinghouse to mix and match FA for esterification as circumstances dictate [45-47].

We can look as well to the external signals that influence nutrient flux along the apical track for insights into its other roles. Bile acids promote secretion of incretin hormones and other neuropeptides by enteroendocrine cells [48-52] and fibroblast growth factor-19 (FGF-19) secretion by enterocytes [53]. Typifying the former, glucagon-like peptide-1 (GLP-1) receptor agonist administration both to healthy
volunteers [54] and to patients with type 2 diabetes [55] acutely diminishes postprandial secretion of apoB-48-containing TRL, while GLP-2 administration does just the opposite [56]. Attenuation of TG secretion by GLP-1 may, for example, help to coordinate the systemic after-meal switch to preferential utilization of glucose over fat [57], which in turn could entail temporary sequestration of a portion of dietary TG in aCLD. As human enterocytes do not express GLP receptors [56, 58], these observations reflect indirect regulation via some intermediary – perhaps through signals from neighboring cells or secondary to incretin-stimulated insulin secretion.

Alterations of gut hormones may represent only a portion of the lipid-regulatory activities of bile acids in the intestine. Treatment with chenodeoxycholic acid, an endogenous human bile acid species, lowers triglycerides in hyperlipidemic patients [59, 60] while bile acid-binding resins acutely lower circulating bile acids and raise TG [61, 62]. The molecular mechanism of this well-known observation remains enigmatic [61]; it may arise from bile acid regulation of chylomicron secretion [63] and/or from direct or indirect (cf. FGF-15/19) bile acid stimulation of hepatic de novo lipogenesis [64, 65].

**Laying the groundwork for a Basolateral Track**

**Origins of basolateral-track fats**

While dietary lipids are the source of apical track substrates, the source of lipids for the basolateral track is less clear. Before considering the purpose of a basolateral track, it would be useful first to establish that lipids would enter the basolateral track through an actively regulated, singular process in enterocytes. Such possibilities include (re)esterification of basolaterally absorbed free fatty acids, basolateral TRL-TG reuptake, or intestinal de novo lipogenesis from circulating carbohydrate precursors. Each of the possibilities is supported by existing data, albeit with varying degrees of directness.

*(Re)esterification of basolaterally absorbed FFA and glycerol:* Basolateral uptake of circulating fatty acids by enterocytes has been well established in animal models [13, 16, 17, 66, 67]. There is also human *in vivo* evidence of basolateral uptake as an ultimate source of TRL-TG: $^{14}$C-palmitate infused intravenously was recovered within minutes in jejunal biopsy homogenates [68], and IV-infused deuterated glycerol reappeared within circulating chylomicron-TG [69]. Additionally, PET imaging demonstrated duodenal and jejunal avidity for $^{18}$FTHA, a non-oxidizable palmitate analogue, in both lean and, to a greater extent, obese humans [70]. These important studies, however, lacked the spatial resolution necessary to localize their findings specifically to enterocytes *versus* the extra-enterocellular lamina propria [71].
The metabolic fate of FFA within enterocytes may differ by site of uptake, in keeping with the dual-track model. In both animals [16, 17] and humans [68], apical FFA are mainly esterified to TG, while the fate of basolateral FFAs has traditionally been seen primarily as oxidation or incorporation into phospholipids [1]. It is not necessarily surprising, however, that basolaterally absorbed FFA were found to be principally destined for oxidation or structural lipid synthesis, as studies were generally performed in the fasting state. As fasting may impel enterocytes, as other cells, to rely more heavily on β-oxidation of FA as an energy source, the destiny of basolateral FFA could coordinately differ under fed conditions [15, 16, 19]. Consistent with this possibility, intravenously infused tritiated oleate continued to be taken up by the intestinal mucosa during concomitant enteral administration of glyceryl trioleate in rats; intestinal mucosal specific activity was recovered almost entirely esterified within TG [15]. Only a small proportion of this basolaterally derived TG was ultimately incorporated into chylomicrons, again consistent with the dual-track hypothesis [15]. Although comparable human tissue-level data are not available, intravenous infusion of a lipid emulsion in fed healthy volunteers acutely ramped up apoB-48 production (i.e., chylomicron secretion) without affecting its catabolism [72]. Thus, to the extent that this rise in apoB-48 production reflects augmented availability of TG derived from basolaterally delivered FFA, the rat data suggest that the enterocyte stows away an even larger share of that newly esterified TG in bCLD than it secretes [15, 72]. The track-differential routing of FFA into TG synthesis may be reinforced by distinctive methods of re-esterification [73]: the apical track primarily utilizes the less common monoacylglycerol pathway [74, 75] while the basolateral track may favor the more widely used glycerol-3-phosphate pathway [16, 73, 76].

**Basolaterally absorbed lipoprotein-TG:** The enterocyte lipid pool may draw upon FA/TG taken up basolaterally from lipoproteins, perhaps in the form of recently secreted chylomicrons or recirculated chylomicron remnants [13, 18, 19]. As with other cells in the body, these lipoprotein-derived fats may undergo basolateral absorption as holoparticles entering the endo-lysosomal system, or as FFA locally derived from extracellular hydrolysis [13, 19]. Concordantly, both basolateral uptake of TRL/remnants and the constituent TG’s subsequent re-secretion as chylomicrons have been demonstrated in rodent enterocytes [14, 19]. Although these events have not yet been reported in humans, human enterocytes do express the LDL receptor on their basolateral surface [77, 78], suggesting the capacity for uptake of apoB100/E-containing TRL. That HDL does not represent a major source of cholesterol for transintestinal cholesterol excretion in mice further implicates basolateral uptake of non-HDL particles by enterocytes as a potential source of TG [79]. Yet, the failure of PCSK9 inhibitor treatment to meaningfully affect postprandial
chylomicron-TG levels or apoB-48 secretion in humans argues against a central role for enterocyte LDLR in this process [80-82].

**Enterocyte de novo lipogenesis (eDNL):** Enterocytes can also synthesize TG from precursor substrates and we surmise this includes those precursors taken up basolaterally from the circulation. Pure carbohydrate consumption increases circulating apoB-48 and chylomicron-TG levels in human volunteers, suggestive of human enterocyte DNL [83-85]. eDNL with coordinately increased chylomicron production has been conclusively demonstrated in rodent enterocytes [13, 86, 87] while human enterocytes at a minimum do show mRNA expression of the full suite of required enzymes, including acetyl coA carboxylase and fatty acid synthase [88]. Active DNL has not yet been specifically demonstrated in human enterocytes, but human duodenal explants do exhibit wholesale DNL [89], and in vivo stable-isotope tracer studies have documented incorporation of moieties derived from enteral fructose [90, 91] and from intravenous glycerol [92] in chylomicron-palmitate. The additional time required for eDNL from carbohydrate delays the secretion of resultant chylomicron-FA/TG and apoB-48, represented as a shoulder or second peak on the curve, after the absorption of the dietary lipid component of the original meal. Interestingly, the second peaks of apoB-48 and chylomicron-lipids coincide with, or even follow, the postprandial rise in hepatic apoB-100 and VLDL secretion [84, 85, 90, 91], and rates of hepatic and intestinal DNL appear to be very tightly correlated [92]. These observations leave open the possibility, mentioned above, that at least some of the second rise in chylomicron secretion attributed to eDNL may actually reflect basolateral uptake and reprocessing of recirculated, hepatically derived lipid.

**Distinguishing basolateral CLD**

If the basolateral track does contribute to the enterocyte lipid pool by any or all of the above mechanisms, dedicated basolateral-track CLD (bCLD) could, speculatively, enable spatiotemporal control over enterocyte lipid-metabolic processes. This, however, would require recognition and differential regulation of two coexisting CLD populations: aCLD versus bCLD. Mouse duodenal enterocytes have been found to contain CLD whose lipid makeup clearly differs from those in other cell types [47]. Enterocyte CLD also feature an adipocyte-like complement of lipid droplet-associated proteins [93, 94] that may further distinguish basolateral CLD from apical [13, 47]. Mouse enterocyte-specific knock-out of two major CLD-associated proteins, adipose triglyceride lipase (ATGL) and comparative gene identification-58 (CGI-58), results in massive enteral steatosis despite untrammeled dietary fat absorption and chylomicron secretion [13]. The selective enterocytic accumulation of intravenously administered fatty acids, on the other hand,
implies that the missing proteins specifically regulate bCLD [13, 95], consistent with a unique bCLD identity.

**Exploring the significance of the Basolateral Track**

*Housekeeping functions*

If we accept the above observations as evidence that basolateral track represents a discrete, organized process, we next consider its physiologic relevance (Figure 2A). The prevailing thinking on the matter, based largely on animal studies, tends to treat the basolateral track as subserving “housekeeping” functions within the enterocyte during fasting: providing a wellspring of energetic substrate to tide the cell over until the next meal and preferred FA species for structural-lipid synthesis [1, 13, 15-18, 68]. Although up to 30% of basolateral-track fatty acids may be oxidized during fasting [16], fatty acids do not appear to be major drivers of enterocyte ATP generation in either the fed [16, 96, 97] or fasted states [16, 98] on a background of normal dietary fat content. Moreover, to the extent that enterocytes do engage in FAO during energy-intensive meal absorption, most such fuel is apically derived [17].

We therefore confront a conundrum: enterocytes evidently possess a robust cellular machinery for uptake and oxidation of circulating fatty acids, yet they seem not to use it primarily for energy generation, as cells typically do [16]. We can envisage a few hypothetical explanations for a beefed up β-oxidative apparatus in enterocytes that encompasses a larger scale and/or a broader purpose than enterocytic energy independence during fasting [16]. For example, if the enterocyte were also a “professionally” lipolytic cell, it might disburse hydrolyzed fatty acids locally to support other epithelial or lamina propria cells during fasting. Occurrence of such a phenomenon in vivo (e.g., calculations based on portal-drained viscera) [99] or in explanted specimens of whole intestine would not necessarily have been experimentally localizable, and therefore prone to conflation with enterocyte-specific FAO. Beyond metabolism, intestinal FAO also affects epithelial proliferation and survival due to its collateral impact on the intracellular redox state [100]. This may be a particularly important regulatory consideration in the tumorigenic setting of constant cell turnover and exposure to environmental toxins.

*Fat sensing*

We conjecture that enterocytes harness FAO as a means of nutrient sensing (Figure 2B). Just as pancreatic β-cells co-opt glucose metabolism as a nutrient-sensing mechanism for autoregulation of insulin secretion, so may enterocytes reappropriate fatty acid metabolism as a barometer of systemic energy balance to which
they can couple their own metabo-regulatory activities [101]. This could occur, for example, through repurposing of FAO, which would also help to account for the enterocyte’s apparent excess capacity for FAO discussed above [16, 101]. As enterocytes can take up and oxidize fat from recirculated chylomicron remnants [13, 14, 18, 19], the internal calculus of basolateral- vs. apical-track FAO could constitute a fat-sensing circuit. Potentially consistent with this interpretation, augmenting enterocyte FAO in mice increases energy expenditure during high-fat diet feeding [95].

Whether through FAO or even non-oxidative means, the construction of such a fat-sensing circuit would allow the enterocyte to monitor the equilibrium between dietary (i.e., apical) fat supply and systemic demand, the latter inversely proportional to the basolaterally returning chylomicron-remnant TG not already siphoned by other tissues. This could underpin, for example, the intestine’s observed role in feedback regulation of food intake [101]. Although this concept is attractive and comports with some animal physiologic data [13, 16, 75, 102-104], particularly the independent regulation of apical vs. basolateral FAO based on feeding status [16], it remains a hypothesis in need of further testing [70, 101].

Enterocyte signaling of nutrient status by any means likely requires cooperation with enteroendocrine cells, whose role in satiety and facilitation of insulin-mediated nutrient disposal is well established. For example, enterocyte nutrient-status feedback may be mediated in part via cholecystokinin production by enteroendocrine cells [105, 106]. Bile acids may also play a role, given their dual functions of fat emulsification and digestion-timed signaling in multiple intestinal cell types. Bile acids reabsorbed into the circulation by ileal enterocytes go on to activate their cell-surface receptor GPBAR1 (a.k.a. TGR5) on the basolateral membranes of enteroendocrine cells (including L-cells, also enriched in the ileum) and various neurohormonal cells of the lamina propria [48-50]. In this way, bile acids contribute to the human intestine’s regulated secretion of the key gut hormones GLP-1 and peptide YY [48-50, 107]. Although not proven experimentally, this regulatory scheme implicates the enterocyte as well in its capacity as a dynamic gatekeeper mediating the enterohepatic circulation of bile acids [108, 109]. However, the manner and extent to which these extra-enterohepatic processes play into enterocyte lipid metabolism remains unclear.

Fatty acid balance
The enterocyte has access to a range of FA species from the diet that can be further strategically enriched by basolateral uptake of circulating lipoproteins and de novo lipogenesis [13, 86, 87]. This may be important intracellularly to manage the high phospholipid throughput required for enterocytes’ constant cellular turnover [110]. As FA composition governs phospholipids’ essential structural properties [111], we predict that this fundamental task requires active balancing of the cell’s fatty acid repertoire.
Balancing the fatty acid reserve may also be important to curate appropriately structured phospholipids that sustain lipoprotein secretion [33, 34, 111], and we speculate that ultimately it may influence the composition of the circulating FA/TG pool. In order to engage meaningfully in such a process, the basolateral track would need to influence not only the uptake of fats at the basolateral surface but also their secretion or re-secretion. This process could entail some crossover of fat from the basolateral to the apical track [15, 69, 72]. In so doing, basolaterally derived lipid might serve a constitutive, low-grade pump-priming function to maintain the readiness of the chylomicron assembly line and/or may even give rise to the second-meal effect [13-15, 25, 41]. Cross-tracking of bCLD lipid also potentially buffers the cytoplasmic concentrations of specific FA species to optimize esterification of chylomicron-bound TG according to enzymatic substrate preferences [40].

(Re)secretion of basolaterally derived fat could also reflect a separate basolateral lipid-secretory pathway, perhaps based on VLDL [112]. Although the chylomicron is the small intestine’s canonical TRL, enterocytes are also capable of secreting apoB48-containing VLDL (i.e., distinguished operationally based on sedimentation rate), particularly during fasting [41, 113-115]. This enterocyte VLDL production may occur independently of chylomicron production and the resulting lipoproteins can differ in lipid composition [113-118]. As many lipoprotein-kinetic studies have reported effects on TRL without distinguishing between particles of differing size or density, they may inadvertently have conflated two separate processes [41].

Enterocyte lipid storage in metabolic disease

The apparent spatial and functional separation of bCLD from those containing newly absorbed dietary fat (aCLD) [13, 15, 16] makes it unclear a priori if bCLD represent metabolic friend or foe. In other words, should this distinct bCLD pool be accorded the generally detrimental reputation of extra-adipocellular “ectopic” lipid accumulation? Patients with chylomicron retention disease develop relatively enterocyte-specific massive apical CLD overload due to impaired intracellular trafficking of nascent chylomicrons [119, 120]. However, they do not appear syndromically prone to diabetes and, interestingly, have normal serum TG, ostensibly due to augmented hepatic de novo lipogenesis [119, 120]. On the other hand, there are no known human disorders specifically of enterocyte bCLD metabolism. In fact, correlative human data suggest a beneficial role for enterocyte CLD; SME magnitude, presumably reflecting the extent of prior-meal fat storage in CLD, associates positively with insulin sensitivity [25]. Congruently, a mouse model of specific bCLD accumulation manifests lower plasma TG and protection from hepatic steatosis versus
control, although any potential effects on glucose metabolism were not reported [13]. However, reduction of CLD by intestine-specific transgenic augmentation of lipolysis in mice does not affect TG levels [95].

If the enterocyte represents a qualified “safe haven” for short-term lipid banking, we imagine it, like adipose tissue or liver [121], will come to fail in the face of chronic fat excess and its attendant insulin-desensitizing repercussions [25]. Insulin resistance and diabetes do appear to be associated with dysregulated intestinal lipid handling [69, 70, 122-132]. Fat consumption produces exaggerated spikes in postprandial chylomicron-TG in patients with insulin resistance or type 2 diabetes versus healthy controls [25, 69, 122, 130, 133-135] and improved diabetes control attenuates postprandial chylomicron excursions [127, 136]. Mechanistically, these findings appear to result both from increases in apoB-48 production and decreases in its clearance, as well as enhanced uptake and esterification of basolaterally (re)absorbed FFA, in the setting of insulin resistance and diabetes [69, 70, 129, 131, 135, 137-139]. The finding that insulin resistance is associated with inflated postprandial TG excursions appears to conflict with the previous mentioned positive correlation between SME magnitude and insulin sensitivity. Insulin resistance thus may impair the enterocyte’s ability to siphon dietary fat for storage as CLD during active absorption. Consequently, a greater proportion of that dietary lipid directly would enter the circulation in the prandial/post-prandial period while less would remain within enterocyte CLD to resurface during the next SME.

Speculation as to the relationship between insulin resistance and dysregulated intestinal lipid metabolism calls up the question of mechanism. Although the intestine is not generally considered a classic insulin target tissue, some evidentiary support exists for a direct effect of insulin on intestinal lipid handling [89, 140]. For example, insulin treatment of human fetal jejunal explants decreased the quantity of chylomicrons secreted without affecting their composition [141]. On the other hand, in the setting of preexisting insulin resistance, duodenal explants from humans undergoing biliopancreatic diversion for weight control exhibited increased rates of DNL and apoB-48—TRL secretion in concert with decreased basal AKT phosphorylation versus controls [89].

Several potential mechanisms have been proposed to account for the postulated intestinal resistance to insulin. Unsuppressed FFA themselves may produce insulin-desensitizing lipotoxic effects [121], as may their derivatives, notably ceramides [142]. The small intestine of patients with the metabolic syndrome may also feature a pro-inflammatory milieu that, by analogy to the prevailing view in obese adipose tissue, could exacerbate insulin resistance [89]. Nevertheless, we must also consider the possibility of indirect intestinal effects of insulin resistance elsewhere. Based on the previous discussion of a role for basolateral uptake of plasma FFA in enterocyte TG synthesis, it follows that exogenous infusion of FFA
during hyperinsulinemic-euglycemic clamp prevents insulin’s suppression of apoB-48 secretion in healthy volunteers [140] but not in the chronically hyperlipidemic setting of type 2 diabetes [126]. Moreover, although surgical treatment of obesity-associated insulin resistance reduced apoB-48—TRL pool size and production rate relative to the pre-operative state, even in the setting of constant feeding, this may have been secondary to improvements afield, as apoB-100—TRL pool size decreased to the same extent [139].

These direct and indirect effects of insulin resistance on intestinal lipid metabolism are not mutually exclusive, and may even reinforce one another. For example, insulin resistance appears to drive up the proportion of chylomicron-TG derived from recirculated (basolaterally reabsorbed) FFA vs. enteral (apically absorbed) FFA [69]. Increased intestinal TRL secretion may then provide further substrate for TG lipolysis to FFA, including with subsequent derivatization to insulin-desensitizing ceramides [142].

Although such data implicate the intestine in the maintenance – if not also the genesis – of diabetic dyslipidemia, they do not elucidate the enterocellular goings-on between luminal fat input and chylomicron output, particularly as regards the behavior of CLD. As yet, we lack direct human evidence that insulin resistance or diabetes impact on apical or basolateral CLD physiology; circumstantial data generally support the notion but have presented interpretive difficulties. In a study of severely obese patients undergoing weight-loss surgery, triglyceride and apoB-48 levels diverged markedly in blood (both higher) versus in jejunal explants (both lower) in patients with diabetes relative to those without it [71]. These findings could suggest a role for the enterocyte as a buffer against dyslipidemia that fails in the run-up to diabetes [25]. Confounding this interpretation, however, the bulk of the stained jejunal-wall neutral lipid resided in the lamina propria, likely in the form of apoB48-TRL, rather than within enterocytes proper; electron microscopic analysis was not presented [71]. Surrogate measures also have not provided straightforward results. For example, small intestine specimens from insulin-resistant humans have shown both increased [89, 143] and decreased [144] expression of MTP versus control specimens. Duodenal expression of several other genes involved in lipoprotein synthesis were lower despite greater apoB-48 (i.e., chylomicron) production rate and pool size in obese humans with vs. without insulin resistance [144]. This dissociation may result from differential impacts of hyperglycemia versus hyperinsulinemia or insulin resistance per se [145, 146]. A cell-autonomous effect of hyperglycemia itself on human enterocellular lipoprotein production has yet to be demonstrated, but studies of its effects on whole-body apoB-48—TRL kinetics have yielded mixed results [126, 143, 146-148].

Finally, we once again consider bile acids given their tight correlation with insulin resistance [149-152] and the antidiabetic effects of BA sequestrants [153, 154]. Levels of FGF-19, a classical surrogate of
intestinal BA action, are lower despite higher serum bile acid levels in IR [155, 156], although the effect of improved IR on these parameters appears to depend on the treatment modality [151, 157-161].

**In-conclusions**

We have attempted to update and streamline a dual-track model of enterocyte lipid handling, with a particular emphasis on human physiology in health and disease. Although others have also proposed elements of a dual-track model [13, 15, 16], it remains largely conceptual due to incomplete understanding of the two tracks and their relationship with one another. Both apical and basolateral tracks can silo their respective fats in distinct cytosolic lipid droplets. Both apical- and basolateral-track CLD seem capable of participating in similar processes: fatty acid oxidation, structural lipid synthesis, lipoprotein (re)secretion, and storage of other lipid-soluble molecules. However, the ends for which they are employed is where the trail starts to go cold.

A central question raised by this hypothesis is the extent to which these two tracks interact. That is, do they carry out their activities purely in parallel or do they functionally intersect? We have speculated on interactions between the two tracks, including as an integrated energy-sensing circuit or as a hedge against systemic TG overload. However, these remain hypotheses in want of further testing. Key questions that remain unanswered include the precise mechanism of basolateral fatty acid uptake (i.e., as hydrolyzed FFA vs. as remnant TRL), the reason for the apparent dissociation between basolateral track’s β-oxidative potential relative to its demand, and why the enterocyte stores fat from each track in distinct CLD.

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Figure 1. Dual-track model of enterocyte fat metabolism. The apical track is illustrated in yellow on the left side of the schematized enterocyte and the basolateral track on the right, in purple. Both tracks feature uptake of TG- or de novo lipogenesis (DNL)-derived fatty acids that are used to synthesize phospholipids (PL), oxidized, or re-esterified to TG via the G3P (predominant in basolateral track) and GPAT (predominant in apical track) pathways. Re-esterified TG are stored in cytosolic lipid droplets in both tracks, and although bCLD express a different complement of CLD-associated proteins than do aCLD, these droplets or their contents may “cross-track”. The source of basolateral-track fatty acids – via uptake of VLDL or chylomicron remnants for intracellular hydrolysis versus extracellular TRL hydrolysis – remains unclear. Bile acids reabsorbed from the intestinal lumen also regulate enterocyte gene expression patterns before recirculating to the liver. Cholesterol taken up from the circulation is excreted via the basolateral track through the transintestinal cholesterol excretion (TICE) pathway.

Figure 2. Functional interaction of the dual tracks. (A) Summary of known functions and interplay of the two enterocyte lipid tracks. The apical track, illustrated in yellow on the left side of the schematized enterocyte, demonstrates the absorption and utilization of dietary lipid, including re-esterification for storage in aCLD and/or chylomicron synthesis and secretion, phospholipid (PL) synthesis, and fatty acid (FA) oxidation. Additional apical track lipid is provided by de novo lipogenesis (DNL) from non-lipid precursors (e.g., carbohydrates) and perhaps “cross-tracking” of basal-track fats. These functions appear to be largely mirrored in the basolateral track, illustrated in purple at right, demonstrating a largely similar array of fates for circulating fats following basolateral uptake. However, there remain unknown aspects of basolateral track function remain. (B) Hypothetical energy-sensing circuit, a model for interaction of apical and basolateral tracks. Fats traffic along pathways indicated by black arrows, while small quantities may be diverted along the red arrows for oxidative (i.e., via FA oxidation) or non-oxidative energy sensing to compare fat inputs and outputs as a barometer of systemic energy needs. An “energy sensor” may then control flux of fats along storage vs. oxidation pathways on either track according to its homeostatic needs.
A. Summary of known function of dual tracks

- Dietary & biliary TG→FA
- FA→TG re-esterification
- aCLD TG storage
- Chylomicron assembly
- DNL
- TRL-TG circulation

- FA oxidation
- "Cross-tracking"?
- bCLD TG storage
- PL synthesis

- FA→TG re-esterification
- SME
- Circulating TG→FA

B. Hypothetical energy-sensing circuit

- Dietary fats
- FA oxidation
- Oxidative sensing
- Nonoxidative sensing
- Energy sensor

- aCLD storage
- "Cross-tracking"?
- Systemic fat output
- Systemic fat return

- Systemic energy needs
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