Postprandial dyslipidemia: pathophysiology and cardiovascular disease risk assessment

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ABSTRACT

Although the fed state predominates over the course of a day, the fasting lipid profile has traditionally been used to assess cardiovascular disease (CVD) risk. The nonfasting lipid profile may be more reflective of the daily circulating plasma lipids and simplifies lipid monitoring for patients, laboratories, and clinicians. Nonfasting triglyceride levels are also independently associated with cardiovascular events, leading to several clinical guidelines (e.g. in Denmark, the UK, Europe, and Canada) now recommending nonfasting lipid testing in the primary prevention setting.

Obese and insulin resistant states are associated with intestinal chylomicron overproduction and subsequent remnant lipoprotein accumulation, leading to development of postprandial dyslipidemia in the fed state. Postprandial dyslipidemia is thought to be a major contributor of atherogenesis and shown to be an important CVD risk factor. As intestinal peptides (e.g. glucagon-like-peptide 1; GLP-1) have been shown to regulate chylomicron output, alterations in these signaling pathways in insulin resistant states may play a role in the development and/or progression of postprandial dyslipidemia.

Although several advances have been made in understanding postprandial dyslipidemia in insulin resistance and its association with CVD, several limitations remain. Although nonfasting lipid measurements (i.e. random blood sampling) are now recommended in some countries, a more functional assessment of postprandial lipemia involves ingestion of a high-fat meal with subsequent blood collection over a specified time period (i.e. oral fat tolerance test). However, oral fat tolerance test methodology remains largely unstandardized and reference values to interpret postprandial values remain to be accurately established. Development of standardized methodologies and biomarker profiles for assessment of postprandial dyslipidemia in clinical practice will enable early and accurate identification of those at risk for CVD.

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Abbreviations

apoB-48: *apolipoprotein B-48*

apoB-100: *apolipoprotein B-100*

CM: *chylomicron CVD: cardiovascular disease*

DGAT: *diacylglycerol acyltransferase*

DPP-4: *dipeptidyl peptidase-4*

FA: *fatty acid*

HDL-C: *high-density lipoprotein cholesterol*

GLP-1: *glucagon-like peptide-1*

GLP-2: *glucagon-like peptide-2*

LDL-C: *low-density lipoprotein cholesterol*

LPL: *lipoprotein lipase*

MCP-1: *monocyte chemoattractant protein-1*

MTP: *microsomal triglyceride transfer protein*

OFTT: *oral fat tolerance test*

PAI-1: *plasminogen activator inhibitor-1 (PAI-1)*

PCOS: *polycystic ovary syndrome*

RLP: *remnant lipoprotein*

T2D: *type 2 diabetes*

TG: *triglyceride*

TRL: *triglyceride-rich lipoprotein*

VLDL: *very low-density lipoprotein*

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POSTPRANDIAL LIPID METABOLISM

With the current eating patterns in Western societies, the fed state predominates over the course of a day, with the typical individual only in the fasted state for a few hours in the early morning (1). Nevertheless, the fasting lipid profile has been a standard assessment of cardiovascular disease (CVD) risk. There are two primary reasons for traditionally measuring fasting triglycerides (TG): to reduce the variability in TG concentration following meal ingestion and to accurately calculate low-density lipoprotein cholesterol (LDL-C) using the Friedewald equation. However, nonfasting (i.e. random blood sample measurement irrespective of time since last meal) TG levels have been reported to fluctuate only modestly within the same individual (2). Additionally, calculated LDL-C has been shown to change minimally after food intake (3) and measured and calculated LDL-C are highly correlated between fasting and nonfasting states (4,5). As nonfasting TG levels are independently associated with cardiovascular events (6), a paradigm shift towards assessing lipid parameters in the nonfasting or postprandial (i.e. blood sample measurement at specified time points following a standardized meal) state is occurring (7). In fact, postprandial TG levels obtained after consuming a standardized high-fat meal, better predict coronary artery disease compared to fasting TG levels (8). Several clinical guidelines have included nonfasting lipid testing in the primary prevention setting, including Denmark in 2009 (3), UK in 2014 (9), as well as the European Atherosclerosis Society (EAS) and European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) (7) and the Canadian Cardiovascular Guidelines in

2016 (10). The nonfasting lipid panel has become the clinical standard in Denmark, offering physicians the option to measure fasting lipids when TG > 4 mmol/L (3), while in Canada it is recommended to obtain a fasting measurement when TG > 4.5 mmol/L (10). Furthermore, the EAS/EFLM guidelines state that nonfasting and fasting measurements should be complementary and not mutually exclusive (7). The option of nonfasting lipid testing has also been included in The 2011 National Heart, Lung, and Blood Institute (NHLBI) Guidelines specific for the pediatric population (11). Assessing the postprandial lipid profile can provide a better indication of an individual's capacity to metabolize lipids following a meal, reflecting their metabolic efficiency.

Following ingestion of a fat-containing meal, cholesterol, monoacylglycerol, and fatty acids (FAs) are absorbed by the small intestine and re-esterified in the enterocyte. Triglyceride-rich lipoproteins, termed chylomicrons (CMs) are formed within the enterocyte, comprised of cholesterol, TG, phospholipids, and apolipoproteins (12). These TG-rich lipoproteins (TRLs), containing apolipoprotein B-48 (apoB-48), are subsequently secreted into lymphatic vessels, entering the blood via the thoracic duct. As CMs travel through the circulation, TGs are hydrolyzed by lipoprotein lipase (LPL), releasing FAs for subsequent uptake by peripheral tissues. As CMs become TG-depleted, they form smaller, cholesteryl ester- and apoE-enriched particles, termed CM remnants. CM remnants then compete with hepatic-derived TRL (i.e. apoB-100-containing very-low density lipoprotein (VLDL)) remnants for clearance by the liver through apoE binding to the LDL receptor or LDL-receptor-related protein. Accumulation of remnant lipoproteins (RLPs), often present in obese and insulin resistant states, is a major CVD risk factor. In fact, T2D patients have a three- to fourfold increased risk of death due to CVD (13),

accounting for the majority of deaths in these patients (14). Patients with type III hyperlipoproteinemia, a genetic disorder characterized by RLP accumulation as a result of impaired RLP removal, are also predisposed to premature atherosclerosis and CVD risk (15).

This state-of-the-art review discusses the current scientific knowledge of postprandial dyslipidemia. The dysregulation of postprandial lipid metabolism in insulin resistant states and subsequent CVD risk, regulation of postprandial lipids by the brain-gut neuroendocrine axis, clinical assessment of postprandial dyslipidemia, as well as current limitations and future research directions will be discussed.

POSTPRANDIAL LIPIDS AS MARKERS OF CARDIOVASCULAR DISEASE RISK

The traditional fasting lipid profile used to assess CVD risk includes TG, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and LDL-C. However, evidence is lacking that fasting measurements of lipid parameters are superior to nonfasting measurements. Rather, several notable advantages are recognized for nonfasting measurements, including a more reflective measure of the daily average of plasma lipids, simplification of blood sampling for patients, laboratories, and clinicians, as well as improvement of patient compliance with lipid testing (7). Although fasting dyslipidemia is strongly associated with CVD risk, only 47.5% of patients with acute coronary syndrome present with fasting dyslipidemia (16). Additionally, it has been suggested that nonfasting and postprandial lipid parameters may better predict CVD risk compared to fasting measurements (6,17).

The potential atherogenicity of TGs and TRLs in the postprandial state was first proposed by Zilversmit in 1979 (18), stating that LPL-mediated lipolysis of CMs results in

cholesteryl ester-enriched CM remnants, which are subsequently retained by arterial smooth muscle cells (18). This widely accepted hypothesis regarding the contribution of nonfasting lipids to CVD risk has been further supported by numerous prospective studies further evaluating this link. An 11.4 year follow-up study in women found that postprandial TG levels, but not fasting TG levels, are independently associated with incident cardiovascular events (6). Additional prospective studies have reported an association between nonfasting TG levels and increased risk of coronary heart disease (19), ischemic stroke (2), as well as myocardial infarction, ischemic heart disease, and death (20). Furthermore, Langsted et al reported a significant association between nonfasting levels of TG and cholesterol with myocardial infarction risk, as well as a significant association between nonfasting TG and total mortality (21). Additional postprandial lipid parameters, including markers of intestinally-derived lipoproteins (i.e. apoB-48) and RLPs, have also been reported to be significantly associated with CVD risk. Karpe et al reported postprandial levels of CM remnants (apoB-48 in the Svedberg flotation (Sf) 20-60 subfraction) following ingestion of a mixed-meal correlate with 5-year progression of coronary atherosclerosis in postinfarction patients (22). Postprandial RLP cholesterol (RLP-C) (i.e. total remnant cholesterol, including intestinal and hepatic derived) was more strongly associated with common carotid artery intima-media thickness (i.e. CIMT, a surrogate marker for atherosclerosis) compared to fasting RLP-C in healthy middle-aged men (17). Similarly, Higashi and colleagues found significantly higher RLP-C in patients with coronary artery disease compared to those without (23).

Accumulation of RLPs is thought to be a main contributor to the link between postprandial lipid measures and CVD risk. RLPs less than 70 nm in diameter are able to contribute to atherosclerosis due to their ability to deliver cholesterol to the arterial wall (24). It has been widely reported that CM remnants are able to penetrate the arterial wall and are subsequently retained in the subendothelial space (25–28). Fully hydrolyzed CM remnants contain approximately forty times more cholesterol than LDL particles (29,30) and may be preferentially retained relative to other lipoproteins (26,31). Thus, it is not surprisingly that CM remnants substantially contribute to the cholesterol deposition within the intima. Lipolysis products of TRLs (i.e. RLPs) have been shown to increase endothelial permeability through rearrangement of tight and adherens junctions of endothelial cells (32). Increased permeability may in turn contribute to increased diffusion of RLPs and LDL into the subendothelial space via paracellular transport (32). Upon entry to the subendothelial space of arterial walls, CM remnants induce the development of atherosclerotic lesions (31,33). RLPs may augment atherosclerosis via several mechanisms including stimulation of the inflammatory state through increased expression of leukocyte activation markers and proinflammatory genes, as well as complement system activation (34). Inflammation is a key characteristic of atherosclerotic progression and requires monocytes to adhere to endothelial cells and enter the vascular wall (35). CM remnants in rats have been shown to induce mRNA expression and protein secretion of monocyte chemoattractant protein-1 (MCP-1), which stimulates monocyte migration, and thus plays a critical role in atherosclerosis development (36). Endothelial cell apoptosis (37), as well as increased production of plasminogen activator inhibitor-1 (PAI-1) in endothelial cells, an important regulator of thrombus formation (38), have also been reported in response to CM remnants. Therefore, understanding the regulation of CM metabolism and how CM remnant accumulation associates with CVD risk in insulin resistant states is critical to slow atherosclerotic CVD progression.

THE ROLE OF THE COMPLEX BRAIN-GUT AXIS IN REGULATING POSTPRANDIAL LIPID METABOLISM

The intestine was once thought to be a relatively passive organ in regards to lipid handling, however intestinal lipid absorption and lipoprotein secretion are now recognized as complex, regulated processes. The significant changes in gut hormone secretion and reversion of T2D following gastric bypass surgery (39,40) further highlights the critical role of the intestine in metabolic regulation. Two hormones secreted in equimolar amounts from enteroendocrine L-cells following nutrient ingestion, glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2), paradoxically pose opposite effects on intestinal lipoprotein output (41). While GLP-1R agonists have been shown to blunt CM output in animal (42) and human studies (43,44), administering GLP-2 has been shown to stimulate secretion of stored TG and preformed CMs, in a process requiring nitric oxide signaling (45,46).

GLP-1, a potent incretin, mediates several effects involved in regulating glycemia, including glucose-dependent insulin secretion (47). Pharmaceutical agents have thus been developed to exploit these beneficial effects by either preventing endogenous GLP-1 degradation through inhibiting dipeptidyl peptidase-4 (DPP-4), or stimulating GLP-1 receptors (GLP-1R). In the scientific community, these agents have been used as tools to elucidate functions and subsequent mechanisms of GLP-1. Animal and human studies using these agents have reported profound effects, not only on glycemic regulation, but on lipid metabolism. Hsieh et al reported that injecting a GLP-1R agonist intraperitoneally reduced postprandial TRL-TG and apoB-48 concentration compared to vehicle-treated controls in both healthy mice and hamsters (42). Furthermore,

3 weeks of DPP-4 inhibitor treatment in an insulin-resistant hamster model reduced TG excursions following a fat-load (42), supporting the inhibitory effects of GLP-1 on postprandial lipemia in both healthy and insulin resistant states. GLP-1-mediated lipid regulation has also been confirmed in healthy and T2D humans. A study of normolipidemic, normoglycemic men found that a GLP-1R agonist significantly reduced TRL-apoB-48 accumulation in the postprandial state (44). A single oral dose of the DPP-4 inhibitor sitagliptin decreased chylomicron production in healthy men, independent of changes in insulin, glucagon, glucose, and free FAs (48). Furthermore, a double-blind cross-over study of T2D patients treated with a DPP-4 inhibitor had reduced postprandial TG and apoB-48 after an oral lipid tolerance test compared to placebo-treated controls (49).

The mechanisms by which GLP-1 regulates lipid metabolism are incompletely understood. Improvements in postprandial lipemia with GLP-1R agonists and DPP-4 inhibitors could result from a number of mechanisms, including reduced lipid absorption, decreased CM secretion, and/or enhanced clearance from the circulation. Although GLP-1 is a potent stimulator of insulin secretion and insulin decreases CM secretion (50,51), the effects of the GLP-1R agonist exenatide on postprandial CM production are maintained under pancreatic clamp conditions (44). Regulation of intestinal lipoprotein production by exenatide or a GLP-1 infusion can also occur independent of changes in gastric emptying as demonstrated by bypassing the stomach (44,52). Additionally, fractional catabolic rates of intestinal lipoproteins in response to exenatide and DPP-4 inhibitors do not appear to be significantly affected, supporting the view that reduced CM accumulation is not due to impaired clearance (44,48,53). GLP-1R stimulation may regulate lipid metabolism by decreasing the rate of intestinal lipid absorption

and subsequently limiting lipid availability within the enterocyte for CM production. Reduced absorption of radiolabeled dietary TG has been observed in hamsters (41) and rats (52) that received a GLP-1 infusion. This may result from a reduction in pancreatic exocrine secretions including lipases (54).

More recently, a brain-gut axis has been proposed to explain the effects of GLP-1R agonists on postprandial lipoprotein production. GLP-1-producing neurons have been identified in the nucleus of the solitary tract of the brain stem, which project to hypothalamic nuclei that express the GLP-1R, including the arcuate (ARC), paraventricular (PVN), and dorsomedial (DMH) nuclei (55). A recent study demonstrated that injecting the GLP-1R agonist exendin-4 into the third ventricle of the brain reduced plasma and TRL-TG and TRL-apoB48 levels in fat-loaded hamsters (56). These effects could be prevented by adrenergic receptor blockers as well as central delivery of an antagonist to melanocortin-4 receptors (MC4R), which are known to activate the intermediolateral nuclei (IML) of the spinal cord to mediate sympathetic outflow to the periphery (57). These findings support the involvement of MC4R signaling and sympathetic pathways in the brain-gut axis that GLP-1R agonists may use to regulate postprandial lipid output (56). This study further demonstrated that GLP-1R agonists may work by reducing jejunal FA absorption as well as decreasing microsomal triglyceride transfer protein (MTP) activity, which is required for apoB-48 lipidation (56). Despite the potential for a brain-gut axis for intestinal lipid regulation, peripheral exendin-4 was found to sufficiently reduce CM production, independent of central GLP-1R signaling (56).

Postprandial lipid regulation by GLP-1 has primarily been studied using pharmaceutical agents which either mimic GLP-1 action or pharmacologically increase its concentration by blocking its inhibition. However, it is important to understand if physiological levels of endogenous GLP-1 are also able to regulate intestinal lipoprotein production. GLP-1R antagonism in chow-fed hamsters augmented TRL-apoB-48 120min after a fat load (42), indicating that endogenous GLP-1R signaling is able to modulate postprandial lipid metabolism. Furthermore, GLP-1R-/- mice had enhanced plasma and TRL-TG compared to GLP-1R+/+ littermate controls, despite similar gastric emptying rates (42). However, current evidence for the ability of physiological levels of GLP-1 to signal through the proposed central pathway is limited. Central treatment with the DPP-4 inhibitor MK-0626 reduced TRL-TG and this effect was negated by central GLP-1R antagonism, implicating endogenously produced central GLP-1(56). However, there is a lack of evidence for the presence of DPP-4 in the brain, and central GLP-1R antagonism alone has no effect of CM secretion compared to vehicle-treated controls (56). There is some evidence that DPP-4 inhibitors may have additional mechanisms of action beyond inhibiting endogenous GLP-1 degradation, such as the stimulatory effects of sitagliptin on L-cell GLP-1 secretion (58). Similar DPP-4-independent mechanisms may account for the effects of central MK-0626 treatment on postprandial lipemia – however, further studies are needed to delineate the role of endogenous central GLP-1 in regulating chylomicron secretion.

DYSREGULATION OF POSTPRANDIAL LIPID METABOLISM IN INSULIN RESISTANT STATES

Dyslipidemia is commonly present in insulin resistant and T2D patients, which as previously discussed, leads to an increased risk of CVD due to the presence of a pro-atherogenic environment (59). It is therefore not surprising that CVD accounts for the majority of deaths in T2D patients (60). In fact, increased CVD risk is already

present in non-diabetic subjects with impaired glucose tolerance or impaired fasting glucose (61). While dyslipidemia associated with T2D has traditionally been described as a combination of elevated TG, reduced HDL-C, and a shift towards small, dense LDL particles, more recent studies describe increased intestinally-derived lipoproteins in T2D patients (62). As nonfasting dyslipidemia independently predicts CVD events (6), it is critical to understand the link between insulin resistance and dyslipidemia in the postprandial state.

Postprandial dyslipidemia in insulin resistant states has been examined in both human and animal studies. Postprandial elevations of hepatic and intestinal lipoproteins are evident in T2D patients, despite normal TG levels in the fasting state (63). Duez et al reported an increased production rate of intestinal apoB-48-containing lipoproteins in hyperinsulinemic men compared to those with normal insulin levels, although no significant difference in CM clearance was evident (64). An increased CM production rate was similarly seen in T2D patients, however, decreased catabolism of CMs was also evident (65). Furthermore, a recent study by Wang et al reported elevated postprandial TG concentration in insulin resistant, abdominally obese adults, compared to abdominally obese adults without insulin resistance and non-abdominally obese controls (66), further highlighting the association between insulin resistance and postprandial lipid abnormalities. In addition to elevated postprandial TG concentrations, a prolonged elevation of TG concentration has also been seen in T2D patients. For example, after an oral fat load, TG levels peaked at 2 hours in healthy controls, but were still elevated at 4 hours in T2D patients (67).

Several animal studies provide further insight into potential mechanisms associated with postprandial dyslipidemia in insulin resistance. Dysregulation of insulin signaling at the level of the enterocyte may contribute to postprandial dyslipidemia, as CM secretion is reduced from fetal jejunal explants in response to insulin (68). Indeed, Federico et al observed cellular changes in the insulin receptor signaling pathway, as well as a lack of response to insulin-induced downregulation of CM secretion in a fructose-fed hamster model of insulin resistance (69). Several additional changes accompanying CM over secretion may occur at the level of the enterocyte through altered CM assembly. CM assembly involves lipidation of apoB-48, mediated by MTP, which subsequently prevents apoB-48 degradation. Insulin resistant hamsters exhibited increased intracellular apoB-48 stability, enhanced intestinal *de novo* lipogenesis, and increased MTP mass, compared to chow-fed controls (70). Diacylglycerol acyltransferase (DGAT) is another enzyme involved in TG synthesis in the enterocyte, and thus CM assembly. Activity and expression of DGAT has also been shown to be elevated in insulin resistant hamster models (71). Enhanced dietary lipid absorption may also be upregulated in insulin resistant states through upregulation of various FA transporters to provide increased substrates for CM assembly (72). Taken together, intestinal lipoprotein production is a highly regulated process that becomes significantly altered in insulin resistant states.

CLINICAL ASSESSMENT OF POSTPRANDIAL LIPIDS IN HUMANS

Non-fasting lipid measurement is a simple approach to assess postprandial lipids, however it does not allow for a complete functional assessment of postprandial lipid excursion and potential abnormalities in insulin resistant states. An oral glucose tolerance test (OGTT) is a well-established method used clinically to assess glucose intolerance in pre-diabetic and diabetic states (73). A similar method to assess lipid parameters at fixed time points following ingestion

of a high-fat meal (i.e. oral fat tolerance test (OFTT)) to examine the efficiency of lipid metabolism is not currently performed routinely in the clinic. This is mainly due to a lack of standardized methodology and reference values for result interpretation. Nevertheless, postprandial lipid responses to fat-containing meals have been examined in research settings in human subjects for the past 30 years (74). Assessing postprandial lipid metabolism provides indications of an individual's capacity to process dietary lipids from digestion and absorption of lipids through secretion and clearance of lipoproteins. A wide range of methodologies have been used, varying in pre-test meal conditions, the size, ingredients, nutrient composition, and frequency of the meal, time of blood collection, and selection of circulating markers to measure (74). Additionally methodology will differ depending on the research question. For example, OFTT studies can be used to compare postprandial lipid responses between different nutrient mixtures, between subjects with different habitual diets, between subjects with and without disease, or even to assess the relationship between postprandial response and other markers of disease risk (e.g. fasting lipids, inflammatory markers). Several factors affect TG response to a fat-containing meal, including the amount of fat consumed, consumption of alcohol before or during the meal, fiber content, contents of other macronutrients, and physical activity (reviewed in (74) and (75)).

Postprandial lipemia studies in adults using OFTT

Previous studies have analyzed the healthy postprandial lipid profile following ingestion of a fat-containing meal. For example, Tanaka et al performed an OFTT study in 19 healthy adults to observe the normal postprandial profile of RLPs, which were found to remain elevated even after 8 hours of OFTT and display a similar profile to

total serum TG (76). Others have assessed differences in the postprandial profile between healthy and diseased populations. For example, OFTT cream (Jomo Food Industry) was provided to T2D patients (normoinsulinemic and hyperinsulinemic) and healthy volunteers at a dose of 17g fat/m² body surface area and blood samples were collected at fasting, 2 and 4 hours following the oral fat load (66). Although TG levels did not differ between the three groups, RLP-TG and RLP-C were higher in hyperinsulinemic T2D patients compared to the other two groups, with no difference between normoinsulinemic T2D and healthy subjects. This suggests that hyperinsulinemia, rather than the presence of T2D, may be the causal link between postprandial dyslipidemia and CVD risk. More recently, Larsen et al found high peak and delayed clearance of serum and CM-TG in obese compared to healthy adults 6 hours after consumption of a weight-adjusted (1g fat per kg body weight) high-fat meal (70% calories from fat) (77).

Postprandial lipemia studies in pediatrics using OFTT

Although limited, studies have assessed postprandial lipemia in the pediatric population (78–82) (Table 1). The rate of pediatric obesity is increasing significantly worldwide and one in five children with a BMI above the 95th percentile is hypertriglyceridemic, which is a 7-fold higher rate than for nonobese children (83). Furthermore, vascular pathophysiological changes begin soon after birth and accelerate in adolescence (84). Thus, examining abnormalities in postprandial lipid metabolism in children may allow for early identification of those at risk for additional metabolic co-morbidities. Recent studies found no significant difference in postprandial TG between overweight and normal weight adolescents at fasting, 2 and 4 hours following a high-fat meal (78,79). However, when Sahade et al examined central

Table 1	Studies assessing postprandial lipemia after ingestion of a fat-containing meal in adolescents with obesity and/or associated co-morbidities				
Reference	**Population**	**Fat-containing meal**	**Time points**	**Blood parameters measured**	**Key findings**
Couch et al Am J Clin Nutr 2000 (82)	60 adolescents (M/F: 27/33, mean age: 14.0y) from families with or without history of premature CHD	52.5g fat, 24g carbohydrates, 16g protein per m^2 body surface area	0 (fasting), 3, 6, 8 hours	*Lipids/ Lipoproteins:* TC, TG, HDL-C, LDL-C, retinyl palmitate, apoE genotyping	Delayed postprandial TG was associated with the combination of high fasting TG and low HDL-C.
Moreno et al J Pediatr Endocrinol Metab 2001 (80)	12 obese adolescents (M/F: 5/7, mean age: 12.8y) 12 normal weight adolescents (M/F: 5/7, mean age: 12.7y)	39.0g fat, 47.7g carbohydrates, 18.5g protein	0 (fasting), 2, 4, 6 hours	*Lipids/ Lipoproteins:* TC, TG, HDL-C, LDL-C, apoAI, apoB *Other:* glucose, insulin	Postprandial TG positively correlated with central obesity.
Umpaichitra et al J Pediatr Endocrinol Metab 2004 (81)	12 T2D obese adolescents (M/F: 5/7, mean age: 14.0y) 15 non-diabetic obese adolescents (M/F: 9/6, mean age: 13.2y) 12 healthy adolescents (M/F: 5/7, mean age: 14.9y)	117g fat, 41.5g carbohydrates, 0.5g protein	0 (fasting), 2, 4, 6 hours	*Lipids/ Lipoproteins:* TC, TG, HDL-C, LDL-C *Other:* glucose, insulin, C-peptide, HbA1c	Postprandial TG in T2D obese adolescents was associated with the presence of insulin resistance.

Sahade et al Lipids Health Dis 2013 (78)	49 overweight adolescents (M/F: 20/29, med(IQR) age: 12.0 (11.0-14.0)y) 34 normal weight adolescents (M/F: 17/17, med age: 13.0 (11.0-15.3)y)	25g fat, 25g carbohydrates, 0g protein	0 (fasting), 2, 4 hours	_Lipids/ Lipoproteins:_ TC, TG, HDL-C, LDL-C _Other:_ glucose, insulin	Only overweight adolescents with insulin resistance and fasting hyper-triglyceridemia had higher post-prandial TG. Waist circumference positively correlated with 4h TG.
Schauren et al J Dev Orig Health Dis 2014 (79)	38 overweight adolescents (M/F:18/20, mean age: 14.2y) 24 normal weight adolescents (M/F: 9/15, mean age: 14.2y)	64g fat, 69g carbohydrates, 37g protein	0 (fasting), 4, 6 hours	_Lipids/ Lipoproteins:_ TC, TG, HDL-C, LDL-C _Other:_ glucose, insulin, fibrinogen, leukocyte count, hsCRP	BMI z-score significantly correlated with postprandial TG.
Vine et al J Clin Endocrinol Metab 2017 (85)	12 obese-PCOS female adolescents (mean age: 15.3y) 18 obese female adolescents (mean age: 14.9y) 10 normal weight female adolescents (mean age: 15.6)	0.61g fat, 0.66g carbohydrates, 0.16g protein per kg of body weight	0 (fasting), 2, 4, 6, 8 hours	_Lipids/ Lipoproteins:_ TC, TG, apoB48, apoB100 _Other:_ hormone profile	Obese female adolescents with and without PCOS have exacerbated postprandial TG and apoB-48 compared to normal weight controls.

*apoA-I: apolipoprotein A-I; apoB-48: apolipoprotein B-48; apoB-100: apolipoprotein B-100; apoE: apolipoprotein E; BMI: body mass index; CHD: coronary heart disease; HbA1c: haemoglobin A1c; HDL-C: high-density lipoprotein cholesterol; hsCRP: high-sensitivity C-reactive protein; LDL-C: low-density lipoprotein cholesterol; PCOS: polycystic ovary syndrome; TC: total cholesterol; TG: triglycerides

obesity specifically, 4 hour TG levels were significantly higher in subjects with central obesity (78). Similarly, Moreno et al reported that adolescents with central obesity had higher postprandial TG levels compared with subjects with a peripheral pattern of fat distribution (80). Umpaichitra et al examined postprandial TG (fasting, 2, 4, and 6 hours after a high-fat meal) in obese subjects with and without T2D, as well as non-diabetic, non-obese controls (81). They found that the degree of insulin resistance, rather than the presence of T2D, determines the degree of postprandial lipemia. However, additional studies in adolescents are warranted which measure markers more indicative of intestinal lipoprotein metabolism (e.g. apoB-48, RLP-C), as well as potential relationships with regulators of lipid metabolism (e.g. GLP-1, GLP-2). One recent study measured postprandial apoB-48 in adolescents following an OFTT (0.61g fat/kg body weight) in obese females with and without polycystic ovary syndrome (i.e. PCOS; a metabolic-endocrine disorder associated with obesity and insulin resistance), as well as normal weight controls (85). Obese adolescent females with and without PCOS were found to have elevated postprandial TG and apoB-48 compared to controls, and these levels were associated with indices of adiposity and insulin resistance (85). These data suggest apoB-48 may be a good marker of metabolic disturbances associated with insulin resistance in adolescents, necessitating further studies to confirm these results, as well as investigate relationships with fasting parameters and hormones involved in intestinal lipoprotein regulation.

Postprandial biomarkers to measure in OFTT

In addition to the traditional lipid profile (i.e. TC, LDL-C, HDL-C, TG), it is important for OFTT studies to measure laboratory markers that are more reflective of postprandial dyslipidemia and intestinal lipid metabolism. For example,

measuring apoB-48 (i.e. a measure of CM particle number) and TRL remnants can provide a more complete indication of pro-atherogenic remnant accumulation (34). ApoB-48 measurement allows a direct measure of CMs, as this protein is specific to intestinally-derived lipoproteins in humans. ApoB-48 is indicative of CM particle number, with each CM or CM remnant particle containing one apoB-48 molecule that does not transfer to other lipoproteins (86). ApoB-48 can be determined through commercially available sandwich ELISA kits (87). Remnant-like particles can also be measured by commercially available kits. Japan Immunoresearch Laboratories produce a kit to measure remnant-like particles that uses an immunoaffinity gel with immobilized anti-apoAI monoclonal antibodies and a unique anti-apoB-100 monoclonal antibody that does not bind apoE-rich apoB-100 lipoproteins (88). Lipoproteins that do not bind to the immunoaffinity gel resemble apoE and cholesteryl ester-enriched CM and VLDL remnants, called remnant-like particles, from which cholesterol can be quantified (88). A more recent assay for serum remnant lipoprotein cholesterol (RemL-C) directly solubilizes and degrades remnants using surfactant and phospholipase-D. This assay can be performed on an automated analyzer in only 10 minutes (89). Although RLP-C measurements detect remnants of both intestinal and hepatic origin, examining postprandial RLP-C in populations with various cardiometabolic conditions can provide an indication of the magnitude and duration of RLP-C in the circulation following a meal.

Furthermore, measuring markers secreted following meal ingestion, which are involved in regulation of intestinal lipoprotein metabolism, may aid in early detection of postprandial dyslipidemia and CVD risk. For example, it has been suggested that postprandial GLP-1 may be an early biomarker of obesity-associated metabolic dysfunction. After consuming a mixed-meal

(34% energy from fat), T2D patients had significantly reduced active GLP-1 at 75, 90, and 120 minutes compared to healthy controls (90). Furthermore, following ingestion of a mixed meal (42% energy from fat), fasting and postprandial (60-150min) GLP-1 concentration was significantly lower in T2D compared with healthy controls (91). However, others found no difference in postprandial GLP-1 between subjects with and without the metabolic syndrome (i.e. a cluster of CVD risk factors including central obesity, fasting dyslipidemia, impaired glucose tolerance/impaired fasting glucose, and hypertension) (92). Overall, pathophysiological changes in endogenous GLP-1 concentration in obese/insulin resistant states remains controversial, with some studies indicating an increase (93,94), decrease (95,96), or unaltered (97) GLP-1 concentration in obese/insulin resistant subjects. Although studies have measured postprandial GLP-1, they are often in the context of abnormal glucose metabolism, rather than in relation to postprandial dyslipidemia. Further studies are warranted to assess changes in endogenous GLP-1 concentration in obese and insulin resistant subjects, and examine associations with postprandial dyslipidemia.

FUTURE DIRECTIONS

A paradigm shift towards measuring postprandial, as opposed to fasting lipids has occurred in recent decades. Some countries have already adopted nonfasting lipid testing (i.e. measured on a random blood sample irrespective of time since last meal) in routine practice, including Denmark in 2009 (3), the UK in 2014 (9), as well as Europe (7) and Canada (10) in 2016. Although several advances have been made in understanding dysregulation in intestinal lipid metabolism in insulin resistant states and its association with CVD, several limitations remain. Assessment of the functional postprandial lipid profile (i.e. lipid measurements at specified time points following a standardized meal) is the preferred methodology to ensure optimal comparability between test subjects. However, OFTT methodology remains largely unstandardized, and thus more studies are required to develop standard procedures which are able to distinguish between healthy and at-risk populations, including population-specific meal sizes, nutrient composition, blood sampling time points, and markers to measure. Additionally, robust reference values, which are critical to interpret postprandial parameters, remain to be accurately established. However, these must be specific to the methodology of the OFTT used. These limitations become exacerbated in the pediatric population even though the genesis of these metabolic disturbances often occur early in life. As quantifiable outcome measures are often harder to detect early in life, it is also difficult to assess predictive ability of biomarkers in children and adolescents at risk early enough to prevent disease development later in life.

With the obesity and diabetes epidemics upon us, it is becoming imperative to develop more effective approaches to assess metabolic abnormalities that increase CVD risk. Recent studies have clearly highlighted the importance of intestinal lipid dysfunction in pathogenesis of insulin resistant and diabetic states. Translating important new findings from basic research studies into the clinic is essential to improve clinical assessment of postprandial dyslipidemia, increasingly recognized as a major contributor to development of atherosclerosis and future CVD. Further studies are also warranted to elucidate mechanisms of postprandial dyslipidemia associated with insulin resistant states. A more complete understanding of the underlying pathobiology will allow subsequent development of standardized methodologies and biomarker profiles to be used in clinical practice for early and accurate identification of those at risk for CVD.

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