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REVIEW

Methodology for studying postprandial lipid metabolism

D Lairon[1,2,3], J Lopez-Miranda[4] and C Williams[5]

[1]*INSERM, 476 'Nutrition Humaine et lipides', Marseille, France;* [2]*INRA, 1260, Marseille, France;* [3]*Univ Méditerranée Aix-Marseille 2, Faculté de Médecine, IPHM-IFR 125, Marseille, France;* [4]*Lipids and Atherosclerosis Research Unit, Department of Medicine, Hospital Universitario Reina Sofía, University of Cordoba, Córdoba, Spain and* [5]*Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, University of Reading, Whiteknights Reading (Berks.), UK*

Background: Postprandial lipid metabolism in humans has deserved much attention during the last two decades. Although fasting lipid and lipoprotein parameters reflect body homeostasis to some extent, the transient lipid and lipoprotein accumulation that occurs in the circulation after a fat-containing meal highlights the individual capacity to handle an acute fat input. An exacerbated postprandial accumulation of triglyceride-rich lipoproteins in the circulation has been associated with an increased cardiovascular risk.
Methods: The important number of studies published in this field raises the question of the methodology used for such postprandial studies, as reviewed.
Results: Based on our experiences, the present review reports and discuss the numerous methodological issues involved to serve as a basis for further works. These aspects include aims of the postprandial tests, size and nutrient composition of the test meals and background diets, pre-test conditions, characteristics of subjects involved, timing of sampling, suitable markers of postprandial lipid metabolism and calculations.
Conclusion: In conclusion, we stress the need for standardization of postprandial tests.
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Introduction

Postprandial lipid metabolism: characteristics and importance
Unlike circulating carbohydrates that normally show only transient elevations (as glucose) following a meal, circulating triglycerides show pronounced elevation (postprandial lipaemia) within an hour of meal ingestion and can remain elevated for 5–8 h following consumption of a typical fat-containing meal (30–60 g fat). Since most people consume fat-containing meals at regular 4–5 h intervals as well as fat-containing snacks, it is clear that the usual state of triglyceride metabolism for most humans is a postprandial one (Roche and Gibney, 1995; Lairon, 1996; Williams, 1996; Frayn, 2002).

Correspondence: Dr D Lairon. UMR INSERM 476 'Nutrition Humaine et lipides', Faculté de médecine, 27 Bd Jean Moulin, 13385 Marseille Cedex 05, France.
E-mail: denis.lairon@medecine.univ-mrs.fr
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The specificity of the postprandial period is a physiological transient accumulation (traffic jam) of lipoprotein particles in the circulation as provided by both the liver (roughly as in the fasting state) and the small intestine (specifically after lipid digestion and absorption). The capacity of individuals to regulate circulating triglyceride levels and clear triglyceride-rich lipoproteins (TRLs) is obviously an important reflection of their metabolic efficiency, which can be modulated by various gene polymorphisms (Ye and Kwiterovich, 2000; Ordovas, 2001; Vincent *et al*., 2002).

It is now recognized that elevated postprandial lipaemia is a characteristic metabolic abnormality of a number of life style-related diseases and conditions that are associated with increased morbidity and mortality (type II diabetes, metabolic syndrome, obesity, hypertriglyceridaemia). Furthermore, the high-fat, high-sugar diets and sedentary life styles that typify the late twentieth century are predisposed to cause pronounced circulating triglyceride levels, even within normal healthy subjects. For this reason research in this area has been greatly stimulated in recent years.

The importance of the postprandial period can be summarized as follows:

It reflects the capacity of the body to undertake the efficient processing of a fat-containing meal (fat) through the complex regulation of digestion and absorption of lipid nutrients in the gut, and secretion and clearance of lipid-transporting particles in the circulation.

- It represents the early acute metabolic response to a meal (i.e., mixed foods) and nutrients.
- Most of the time is spent in the postprandial state, given the usual sequences of meals during daytime and the usual long duration of the postprandial hyperlipidemic period (5–8 h).
- Through this process, adipose tissue is filled with lipid moieties while remnants particles should be cleared from the circulation.
- Triglycerides and cholesterol moieties can be exchanged between lipoproteins; this is important in reverse cholesterol transport mediated by high-density lipoprotein (HDL) particles and cholesterol homeostasis but can also cause the generation of atherogenic particles.
- Exacerbated postprandial lipaemia has been repeatedly associated with cardiovascular risk.

This review will extensively discuss the most important methodological aspects of postprandial lipaemia studies such as the approaches used to study individuals and groups, the factors that influence variations within and between individuals, main confounding factors and standardization issues that need to be addressed to obtain optimized results.

Aim of postprandial lipaemia tests

Over the past 20 years there has been extensive investigation of the postprandial response of human subjects to meal-fat ingestion. The nature of the studies undertaken and the protocols used vary according to the aims of the individual studies. These can be summarized under a number of headings:

Comparative check of postprandial lipid responses to specific foodstuffs or nutrient mixtures in healthy subjects

The aim of this kind of study is to evaluate how a given foodstuff or nutrient or nutrient mixture affects the postprandial lipid response. As compared with a control test meal or another test meal, the studied component will be incorporated in the experimental test meal. This will make possible to evaluate the effect of the studied component on the amplitude and/or kinetics of the postprandial lipid response in subjects with normal metabolic homeostasis (Cara *et al.*, 1992; Zampelas *et al.*, 1994a, b; Murphy *et al.*, 1995; Dubois *et al.*, 1998; Roche *et al.*, 1998a, b; Fielding *et al.*, 2000; Harbis *et al.*, 2001, 2004; van Wijk *et al.*, 2001; Sauvant *et al.*, 2003; Jackson *et al.*, 2005).

Comparative check of postprandial lipid responses to habitual/chronic diets in healthy subjects or patients

In this kind of study, the aim is to investigate how the composition of the habitual diet, or chronic enrichment of habitual diet with a given foodstuff or nutrient, influences markers of postprandial lipid metabolism. The diet(s) to study can be either the specific habitual diet of a given group of subjects (either healthy subjects or selected patients) or a recommended diet in the context of an intervention study. At least two diets should be compared (type A and type B; so-called control and experimental diets). The test meals used to evaluate the postprandial lipid response can either reflect the habitual background diet or a standard meal can be used to compare how different background diets alter the postprandial metabolic capacity (Lovegrove *et al.*, 1997; Weintraub *et al.*, 1988; Zampelas *et al.*, 1998; Roche *et al.*, 1998b; Silva *et al.*, 2003; Finnegan *et al.*, 2003).

Check of foodstuff/nutrients–subject interactions in subjects at disease risk (vs. healthy controls)

A fraction of the whole population is at risk for some disease. This is generally the result of interactions between genetic traits linked to gene polymorphisms and environment, especially diet. That means that it could be particularly worthwhile to evaluate the effects of given foodstuffs or nutrients in those subjects with particular susceptibility. This can be done by selecting, on screening healthy subjects or selected patients with specific gene, polymorphisms or other kinds of defined syndromes. In that case, a similar standardized test meal is to be used in every subject. (Weintraub *et al.*, 1988, 1989; Lopez-Miranda *et al.*, 1997, 2000, 2004; Patsch *et al.*, 1992; Mekki *et al.*, 1999; Minihane *et al.*, 2000; Tiret *et al.*, 2000; Sharrett *et al.*, 2001; Robertson *et al.*, 2002; Jackson *et al.*, 2003; Harbis *et al.*, 2004).

Evaluation of relationships of postprandial responses to other markers of disease risk at fasting

This approach is used to evaluate the possible links between the postprandial lipid response elicited by a specific foodstuff, nutrient or diet and some other(s) marker(s) of disease risk evaluated at fasting (i.e., body mass index (BMI), insulin sensitivity, triglyceridaemia, etc). It is usual to employ a standard fat-containing meal although there is debate as to the size of the fat content of the meal as discussed below. (Patsch *et al.*, 1983; Karpe *et al.*, 1998; Smith *et al.*, 1999; Mekki *et al.*, 1999; Guerci *et al.*, 2000; Marcoux *et al.*, 2000; Tiret *et al.*, 2000; Verges *et al.*, 2001; Jackson *et al.*, 2003; Finnegan *et al.*, 2003; Harbis *et al.*, 2004).

Meal size and composition

As detailed below, the amount and type of macronutrients, cholesterol, dietary fibre, energy, alcohol and physicochemical composition of a meal can profoundly affect the

postprandial triglyceride response in individual subjects. Since these are rarely standardized across different studies it is not surprising that findings differ between laboratories and even within laboratories that use different meal protocols on different occasions. Many of these issues are still debated in the scientific community and we will try to deal with the different opinions and propose a way forward.

Meal size

Definition. The first aspect to consider is how to define meal size. One way is to consider that every subject would ingest the same amount of nutrients/energy in the test meal, irrespective of bodyweight/blood volume. Practically, it is most suitable and minimizes errors during the experiment. In groups of subjects where there is homogeneous body weight (healthy or patients) it may be the best choice (references in Table 1).

When groups are studied where there is greater variability in body weight (i.e., with large range of body weights and therefore blood volumes), it may be preferable to use the alternative approach in which the amount of nutrients/energy in the test meal is estimated as unit amount per kg body weight (Cohn *et al.*, 1988a, b, 1993; Lopez-Miranda *et al.*, 1997; Shishebor *et al.*, 1999) or body surface area (Weintraub *et al.*, 1988; Karpe *et al.*, 1993). Although this may be considered a more valid approach because it takes account of greater blood volume in larger subjects, it should be considered that in adults the main variable influencing body weight is variation in fat mass. In significantly obese subjects this could cause an overestimate of additional amounts required to standardize intake relative to blood volume. In relation to meal-fat content this can introduce a bias into the findings. A further complication is that postprandial fat clearance reflects the amount and activity of adipose tissue lipoprotein lipase (LPL), which will be increased in subjects with greater adipose tissue depots. It could be argued that elucidation of defects in fat clearance because of defective LPL activity or other adipose-related factor(s), could best be revealed via administration of fat load according to total adipose tissue mass, rather than body weight *per se*. However, no study to date has employed such an approach, nor has there been a systematic attempt to compare the findings from test meals in which fat load is either fixed or is varied according to subject body weight or fat mass. Much of the existing literature is based on studies that have employed fixed test meals in subject groups with heterogeneous body weights. Such studies have clearly shown this approach allows observation of differences in postprandial lipid metabolism in subjects of varying age, degree of obesity, body fat distribution and insulin sensitivity (Lewis *et al.*, 1990; Couillard *et al.*, 1998; Mekki *et al.*, 1999; Guerci *et al.*, 2000, 2001; Dallongeville *et al.*, 2002; Harbis *et al.*, 2004). In this kind of study with fixed test meals, introducing adjustment for body weight or fat mass when analysing the data would be a worthwhile approach to take into account such variables.

Table 1 Examples of fat contents of test meals used in relevant studies

References	*Fat (g)*
Patsch *et al.* (1983) and (1992)	65.2
Cohen *et al.* (1988)	40–120
Weintraub *et al.* (1988)	50 g/m^2 body surface area
Cohn *et al.* (1988a, 1993)	1 g/kg body weight
Schneeman (1990)	43
Cara *et al.* (1992)	70
Karpe *et al.* (1993)	50 g/m^2 body surface area
Dubois *et al.* (1994a, b)	31/42, 45
Zampelas *et al.* (1994)	40
Frayn *et al.* (1994)	80
Murphy *et al.* (1995)	20–80
Fielding *et al.* (1996)	61
Lopez-Miranda *et al.* (1997)	1 g/kg body weight
Lovegrove *et al.* (1997)	82
Roche and Gibney, 1997; Roche *et al.*, 1998a, b	40
Dubois *et al.* (1998)	15–50
Couillard *et al.* (1998)	60/m^2
Shishebor *et al.* (1999)	0.5 g/kg body weight
Guerci *et al.* (2001)	80
Harbis *et al.* (2001)	40
Dallongeville *et al.* (2002)	70
Harbis *et al.* (2004)	28–29

Meal size and amount of fat. During day life, we are used to have meals of highly variable size, depending on culture, season, kind of day and time during the day. That means that there is no standard size *per se* and implies that we need to define it for each study purpose.

As shown in Table 1, during the early sets of postprandial studies in the 1980s, investigators used test meals providing high amount of nutrient/energy, usually more than 50 g fat per test meal (Cohn *et al.*, 1988a, 1993; Patsch *et al.*, 1983, 1992; Weintraub *et al.*, 1988). These were called 'fat load' or 'fat-tolerance tests' and resembled the glucose-tolerance tests used to detect underlying defects in glucose metabolism in diabetes. The amplitude and duration of plasma triglyceride responses (in studies up to 12 h) were enormous. Large fat loads were practically feasible because the test meals used were mostly milk shakes. Some recent studies have also been performed with very large amounts of fat in the test meals (Lovegrove *et al.*, 1997; Guerci *et al.*, 2000, 2001; Dallongeville *et al.*, 2002). The aim of the early studies was mostly to differentiate responses in healthy subjects and some kinds of patients with metabolic disorders. The concept behind this type of approach is that a large fat load will overwhelm the normal capacity of lipoprotein handling postprandially and thus, will reveal those subjects with reduced capacity (linked to disturbed metabolism and/or diet), whereas a smaller meal may be insufficient to challenge the metabolic capacity to its limit.

Then, early in the 1990s, several groups of nutritionists (Redard *et al.*, 1990; Cara *et al.*, 1992; Williams *et al.*, 1992; Frayn *et al.*, 1994; Lopez-Miranda *et al.*, 1994a; Roche and Gibney, 1995) started to select test meals with more regular amounts of energy (about 700–1000 kcal) and fat (about 40–50 g/test meal) (see Table 1). These authors advocated that these levels are more comparable with daily meals and to the normal metabolic capacities of the subjects (gastric emptying, lipid digestion and absorption, lipoprotein handling and clearance). The aim of such studies was to understand the normal fluctuations and variations in circulating triglycerides that could be seen throughout the day and in response to meals of varying nutrient composition. The rationale is that some subtle differences could be observed depending on subjects and/or test meals or diets because metabolic responses stay within a normal or above normal range.

Some studies have been performed with increasing doses of dietary fat to determine how meal-fat size impacts on metabolic capacity in normal subjects (Cohen *et al.*, 1988; Dubois *et al.*, 1994, 1998; Murphy *et al.*, 1995). The data revealed that (i) very low (5 g) or low doses (15 g) of dietary triglycerides do not significantly increase triglyceridaemia postprandially, (ii) moderate doses (30–50 g) cause dose-dependent increases in postprandial triglyceridaemia and (iii) very high doses (80 g and above) exaggerate postprandial triglyceridaemia but with a tendency for triglycerides to show a plateaux and less clear dose dependence than with more moderate fat loads (see Table 2 for summary).

This kind of mixed test meal with moderate energy (2500–4200 kJ; 600–1000 kcal) and amount of dietary fat (40–50 g; 1500–1900 kJ, 360–450 kcal) is most generally used now, especially in the nutrition community. This would reflect the level of intake of a moderate-sized lunch or dinner, although mostly such studies are conducted in the morning after an over night fast.

Mixed meals

As stated before, the early studies on postprandial lipid metabolism used simplified test meals, that is essentially milk shakes. Nutritionists in the early 1990s aimed to have more true meals, that is mixed liquid–solid meals (Table 3). It is clear that this kind of meal has several advantages, given they elicit so-called normal eating behaviour and feeling, gastric emptying (regulated by the composition and physicochemical properties of the meal), concomitant digestion and absorption of nutrients with normal rates, concomitant occurrence of nutrients in the blood circulation or colon and thus, regular physiological and hormonal responses and subsequent nutrient metabolisms. In particular, recognition of the importance of insulin in triglyceride metabolism emphasized the importance of including significant amounts of carbohydrate in test meals to ensure effective insulin-dependent postprandial processing of dietary triglycerides.

The relative proportions of protein/carbohydrate/fat and amount of fibre can be somewhat variable depending on the study aim. It is recommended that extreme ranges of macronutrients and energy are not employed since the relevance of the findings from such studies has been criticized. The specific amounts and types of macronutrients employed will vary according to specific aims of different studies and should be justified on an individual basis.

Dietary fatty acids

Since the early time of postprandial studies, the question of the effect of the kind of dietary fatty acids on postprandial lipid metabolism has been addressed.

Table 2 Factors affecting the posptrandial triglyceride response to a test meal

Characteristics of the test meal	*Effect on TAG response*
Increase in fat content of meal	Increases TAG AUC
Alcohol consumption before the meal and with the meal	Increases TAG AUC
Polyunsaturated fatty acid composition of meal	Increases postprandial peak TAG
Cholesterol addition to meal	Delays time to return to baseline TAG concentration
Fibre addition	Generally decrease the TAG response
Palatability	Butter increase or decrease TAG response
Ratio of fat to protein and carbohydrate	Addition of glucose to meal increases TAG AUC
Patient characteristics that alter the response	
Baseline TAG concentration	Higher fasting concentrations lead to higher postprandial TAG
Lipoprotein phenotype	Phenotypes with higher baseline TAG concentrations are associated with higher postprandial TAG. CAD patients with apoE2/E3 have significantly increased TAG AUC
Age of patient	Older patients have higher TAG concentrations, but could be simply an effect of higher baseline TAG
Hydration status	Dehydration causes blood concentrations to be higher
Time of last exercise bout	Recent exercise reduces the postprandial response
Time of day test is administered	Late-night testing shows delayed return to baseline

AUC, area under the cueve; CAD, coronary artery disease; TAG, triglyceride.

Table 3 Examples of mixed meals used during postprandial studies

	Food products	*Protein*	*Carbohydrate*	*Fat*
Dubois *et al.* (1998)	French bread (30 g), cooked pasta (100 g), tomato sauce (130 g), one non-fat yogurt (125 g), a cup of coffee (100 ml) and the tested fat or not (sunflower oil)	22.1 g	125.1 g	Negligible (≅1 g) 15 g (18.6% energy) 30 g (31.4% energy) 40 g (37.9% energy) 50 g (43.3% energy)
Karpe *et al.* (1998)	Emulsion consisting of soybean oil, glucose, egg-white protein, dried egg yolk and 200 ml water prepared with some lemon flavour	13.3% energy	26.5% energy	60.2% energy
Pedersen *et al.* (1999)	Rice, beef, vegetable, white bread. Oil 15 g for breakfast and 55 g for lunch	Breakfast: 9.1 g Lunch: 32.3 g	Breakfast: 42.8 g Lunch: 152.8 g	Breakfast: 17.4 g Lunch: 63.6 g
Harbis *et al.* (2001)	Tomato sauce, olive oil, egg white, white bread or spaghetti or white kidney beans	33 g	0 or 90 g	40 g
Mekki *et al.* (2002)	French bread (two slices), 150 g cooked pasta, 50 g tomato sauce, one no fat yogurt, tested fat (olive oil, butter or sunflower oil).			0 (≅1.3 g) or 40 g
Jackson *et al.* (2002a, b)	Breakfast: skim milk (250 g), skim milk powder (15 g), milk shake powder (10 g), cereal (30 g), banana (100 g), test oil (40 g): palm oil, safflower oil, fish oil and safflower oil Lunch: cheese and tomato pizza (200 g), lettuce (30 g), cucumber (20 g), cherry tomatoes (45 g)	Breakfast: 16.9 g Lunch: 20.7 g	Breakfast: 78.4 g Lunch: 68.4 g	Breakfast: 41.4 g Lunch: 5.7 g
Harbis *et al.* (2004)	Plain biscuit or extruded wheat flakes, unsweetened yogurt (0% fat), fresh cheese, whole milk, sugar, palm oil homogenized in dairy product	18 or 24g	94 or 91 g ≅55% energy	28 or 29 g ≅38% energy
Jackson *et al.* (2005)	Skimmed milk (150 g), Nesquick (15 g), skimmed milk powder (15 g), white bread (105 g), jam (30 g), test oil (50 g), mixture of palm oil (29 g) and cocoa butter (21 g), safflower oil, olive oil	23.1 g	128.6 g	53.2 g

We will summarize the data available by dealing first with effect of fatty acids present in the test meal and, secondly, with the effect of fatty acid content of the background diet.

Fatty acids present in the test meal. To summarize the important number of studies performed (reviewed by Williams, 1997; Roche *et al.*, 1998a; Thomsen *et al.*, 1999; Mekki *et al.*, 2002), short or medium dietary fatty acids lower postprandial lipid response by mostly entering the portal route instead of chylomicron secretion. Dairy fats contain significant amounts of short- and medium-chain fatty acids. Most studies have shown that saturated, monounsaturated and *n*-6 polyunsaturated fatty acids (PUFAs) do not generally elicit markedly different postprandial lipid responses (de Bruin *et al.*, 1993; Lichtenstein *et al.*, 1993; Tholstrup *et al.*, 2001; Mekki *et al.*, 2002), while some studies report exacerbated (Thomsen *et al.*, 1999) or reduced (Mekki *et al.*, 2002) responses after intake of saturated butter fat. More recently, studies that have measured changes in different lipoprotein subfractions have revealed important differences with lipemic responses being of the order saturated fatty acid (SFA) > monounsaturated fatty acid (MUFA) > PUFA (Jackson *et al.*, 2002a, b; Jackson *et al.*, 2005). It is important to note that the physicochemical characteristics of the meal-fat mixture, linked to the fatty acid composition, could be a highly determinant of the rate of digestion and absorption, thus on the extent and/or kinetic of the postprandial lipid response (Sakr *et al.*, 1997; Armand *et al.*, 1999). *N*-3 PUFAs can lower the postprandial triglyceride response if sufficient amount is present within the test meal (Zampelas *et al.*, 1994a), but levels used were far greater than those which would be consumed by most populations.

The influence of the positional distribution of fatty acids within the dietary triglyceride moieties on postprandial lipaemia has been investigated with some showing an effect (Jensen *et al.*, 1994) but others not (Zampelas *et al.*, 1994b).

Fatty acid content of the background diet. Much less data have been published on this aspect (Weintraub *et al.*, 1988; Williams *et al.*, 1992; Lovegrove *et al.*, 1997; Zampelas *et al.*, 1998; Roche *et al.*, 1998b; Silva *et al.*, 2003; Finnegan *et al.*, 2003). Diets rich in monounsaturated or *n*-6 PUFAs tend to lower the postprandial lipid response as compared with SFAs and in the case of MUFA there is evidence of an alteration in the kinetics of the postprandial responses (Weintraub *et al.*, 1988; Zampelas *et al.*, 1994a, b, 1998). Diets rich in *n*-3 PUFAs lower the postprandial triglyceride response at intake levels that would be equivalent to daily consumption of oily fish (2.7–4 g/day) (Williams *et al.*, 1992; Minihane *et al.*, 2000). Lower doses than 1.5 g/day have not been shown to cause marked attenuation of postprandial triglycerides (Roche *et al.*, 1996; Finnegan *et al.*, 2003). Compared with a saturated fat-rich diet, a MUFA diet led to a marked reduction in apolipoprotein (apo)B-48 production following

the test meal with no difference for postprandial lipaemia (Silva *et al.*, 2003).

Dietary cholesterol

The limited data available indicate that important doses of dietary cholesterol (280 or 700 mg per meal) tend to exacerbate the postprandial increase in triglyceridaemia and especially chylomicrons, while a moderate dose (140 mg) does not generate noticeable change after a single meal (Dubois *et al.*, 1994). Nevertheless, the fact that ingested dietary cholesterol occurs in plasma chylomicrons during three subsequent postprandial periods (Beaumier-Gallon *et al.*, 2001) raises questions about the overall influence of dietary cholesterol on postprandial lipid metabolism. In view of the variable effects of cholesterol on postprandial lipaemia and possible prolonged duration of absorption, the question of standarization of meal cholesterol content, as well as intake in days preceding the tests meal, is an important consideration. In addition, habitual intake of plants sterols may need to be accounted for.

Dietary carbohydrates

Several studies have shown that the amount or nature of carbohydrate in an individual meal alters postprandial lipid metabolism. Data obtained after addition of glucose (50 and 100 g) to fatty test meals have not been proved consistent in healthy subjects (Cohen and Berger, 1990), whereas addtion of sucrose (Grant *et al.*, 1994) or fructose (Jeppesen *et al.*, 1994) increases postprandial triglyceridaemia. In healthy subjects, starchy foods (white bread, pasta, beans) do not induce noticeable alterations in the overall postprandial triglyceride response but can alter apoB48-containing chylomicrons (Harbis *et al.*, 2001). In subjects with insulin resistance, ingestion of mixed meals with different glycaemic index can modulate the postprandial accumulation of apoB100 and apoB48 containing triacylglycerol-rich lipoproteins (Harbis *et al.*, 2004). Adding various digestible carbohydrates to a test-meal can elicit biphasic response of postprandial lipaemia (Shishebor *et al.*, 1999; Harbis *et al.*, 2004).

Dietary fibres

Addition of some dietary fibre sources into mixed test meals (Cara *et al.*, 1992; Lia *et al.*, 1997) at the level of 4–10 g/meal can moderately reduce postprandial triglyceridaemia or chylomicron lipids as generated by a mixed meal (Table 2). Sources of soluble viscous fibres (i.e., oat bran) or with hypotriglycerdemic properties (i.e., wheat germ) were shown to display such an effect in line with the interference they generate on fat lipolysis within the gut.

Dietary proteins

Essentially no information is available so far regarding the influence of the amount or nature of dietary proteins on postprandial lipid responses.

Alcohol

Another important meal component is alcoholic beverages. Clearly, ethanol consumed with a meal elevates total plasma and very-low-density lipoprotein (VLDL)–triglycerides. In a recent study (Fielding *et al.*, 2000) , the addition of 47.5 g of alcohol to a high-fat meal (54% of energy) was associated with approximately a 60% increase in the peak plasma triglycerides concentration as compared with a meal consumed without alcohol.

Effects of drugs

Several drugs can induce modifications in the postprandial lipoprotein metabolism. Gemfibrozil, bezafibrate, fenofibrate and cipofibrate were shown to increase the clearance of postprandial lipoproteins in normal and hypertriglyceridaemic patients. Furthermore, statin (atorvastatin, pravastatin, simvastatin and lovastatin) and niacin treatment in addition to its beneficial effect on hypercholesterolaemia, was as effective as fibrates in reducing postprandial accumulation of TRLs in patients with type II diabetes and combined dyslipidaemia (Karpe, 2002).

Physicochemical properties of test meals

Physicochemical properties of nutrients and test meal can modulate the postprandial lipid responses. Gastric emptying of liquid meals is faster than that of solid meals or liquid–solid meals, with fat output being delayed unless mixture is fully homogenized. This is particularly true for dietary lipids, which are basically insoluble in the aqueous medium present in most foodstuffs and in the digestive tract. Thus, lipid moieties are organized in different kind of complex structures (emulsified droplets with different compositions and sizes, multilamelar structures, vesicles) that will determine the rate of fat digestion by gut lipases (Armand *et al.*, 1996, 1999). This can affect the rate of gastric emptying as well as kinetics of postprandial occurrence of chylomicrons in the circulation (Sakr *et al.*, 1997; Armand *et al.*, 1999).

Great care should be taken on this aspect in designing test meals and postprandial studies. Comparison of findings from different studies is often prevented by the fact that although the meals used may be similar in terms of their nutrient and energy compositions, they differ in their physicochemical characteristics.

Amount of nutrient of interest to test

In the field of nutrition, the soundness of studies is often related to the amounts of nutrients tested that should probably keep within a physiologically acceptable range. Considering that the average daily intake of a given nutrient in the population is 1, the range of amounts to test should probably be between 0.5 and 1 for macronutrients and between 0.5 and 2 for micronutrients. Alternatively, it could be better related to average meal size/content.

Single or multiple meals

It is known that a previous meal can affect the metabolic response after a second meal, the so-called second meal effect (Fielding *et al.*, 1996; Evans *et al.*, 1998; Burdge *et al.*, 2003; Silva *et al.*, 2005). This is particularly the case when postprandial lipaemia does not come back to baseline level after a first meal. Thus, it could be of interest in some cases to look at sequences of test meals to test for more complex metabolic patterns (Minihane *et al* 2000; Robertson *et al* 2002). An extreme situation of this kind is when a daylong follow-up is performed after a series of sequential of test meals (van Wijk *et al.*, 2001). Despite considerable demand on subjects and investigators, this type of approach can provide a sound reflection of day life dietary patterns and metabolic responses and can reveal underlying defects in postprandial efficacy not revealed by single-meal challenges. A marked difference (i.e., less suppression in circulating levels) in the postprandial non-esterified fatty acid (NEFA) response can be observed following a second meal but this was not found in all studies (Fielding *et al.*, 1996; Burdge *et al.*, 2003). In addition, the second insulin response can be more elevated or not compared with the insulin response to the same nutrient intake consumed after an overnight fast. Because the relationship between triglyceride metabolism and insulin variations are complex, the mechanisms behind the somewhat conflicting observations made after sequential meals are not clearly known.

In conclusion, it appears worthwhile to test sequential meals to more deeply investigate the complex relationship existing between food intake and metabolic homeostasis.

Pre-test meal conditions

It is known that some conditions prevailing before the day of postprandial study can have some influence on some parameters and responses on the experiment day. Particularly when a series of repeated test meal challenges are being undertaken in the same subjects over a period of time it is important to standardize the conditions before each study day.

The kind of meal ingested the day before the postprandial test especially, the dinner meal, can have some influence. A light, low-fat meal should be preferred with no alcohol intake for the 24 h preceding the study day (Alcohol section). Moreover, physical exercise, even light exercise such as walking for 30–60 min is known to lower fasting and postprandial triglyceridaemia on the day following the exercise (Hardman, 1998). This means that subject selection needs to consider this aspect (inclusion/exclusion criteria) and that subjects should be advised to avoid any intensive physical exercise during the 2 days before a postprandial test to avoid confounding the study findings.

Time-of-day administration

The effect of time-of-day administration of the postprandial test has been investigated (Romon *et al.*, 1997; Hadjadj *et al.*, 1999; Burdge *et al.*, 2003). Compared with breakfast time, the same meal consumed at lunch elicited a twofold less rise in triglycerides. Compared with a morning or mid-day test in the same patients, the nocturnal test showed a later or increased peak triglycerides concentration and a delayed return to baseline concentrations, The nocturnal test was better tolerated in the patients because it did not disrupt their normal activity and eating patterns, as morning tests often do. Some other studies have been conducted overnight following an evening meal (Williams *et al.*, 1992; Zampelas *et al.*, 1994a, b) and with standardization of the preceding days intake. Overnight studies allow blood sampling to be continued for 12–14 h and demonstrate that triglycerides return towards 'fasting' levels only in the early hours of the morning (0400–0500 hours) after a meal the night before (1900 hours).

Use of tracers

For particular purposes specific tracers can be added into the test meals. These tracers are generally enriched with stable isotopes (deuterium or 13 carbon). This is particularly the case for the follow-up of dietary fatty acids versus endogenous or for the specific follow-up of individual exogenous fatty acids (Demmelmair *et al.*, 1997), for the follow-up of dietary cholesterol versus endogenous (Beaumier-Gallon *et al.*, 2001) or for studying fat oxidation with a breath test (Votruba *et al.*, 2001). The bioavailability of fat-soluble vitamins or carotenoïds can be studied through the postprandial follow-up after enriched test meals (Tyssandier *et al.*, 2002; Sauvant *et al.*, 2003).

Characteristics of the subjects

The selection of subjects to be involved depends on the study target and aim (see Aim of postprandial tests section). In most cases, adult subjects are involved. For more specific studies, elderly people could be enrolled. Children have not been classically involved. Healthy subjects are regularly involved in studies with the first two aims of the Aim of postprandial tests section. Patients or subjects at risk for some pathology are involved in studies with the last three aims of the Aim of postprandial tests section (see Table 2 for summary).

Healthy subjects

They are classically defined on the basis of absence of known pathology or syndrome and with anthropometric, clinical and biochemical parameters in the normal range. With careful selection, homogeneous groups can be achieved, but they may not be representative of the normal population and these subjects are not prone to marked metabolic imbalance given that they generally demonstrate well-controlled lipid homeostasis. Many studies are performed

in young male adults, especially students. Studies that have compared young and middle aged subjects reveal marked effects of age on postprandial lipaemia (Jackson *et al.*, 2003) Few of the early studies were performed in women, although recently studies have been specifically performed in female subjects (Murphy *et al.*, 1995; Mekki *et al.*, 1999; Lovegrove *et al.*, 2002) to alleviate the lack of information in relation to sex differences. It is noteworthy that for a given meal, the postprandial lipid response is lower in women than men, owing to a higher clearance capacity (lipoprotein lipase activity). Following menopause, the postprandial lipoprotein response becomes more like that observed in middle-aged men.

Subjects at disease risk or patients

Several conditions can be found as follows: *Subjects with borderline or moderate cardiovascular risk*. Fasting parameters (triglyceridaemia, cholesterolaemia, glycaemia) can be moderately elevated but with absence of any drug therapy. Compared with healthy subjects who demonstrate well-regulated lipid homeostasis, these subjects can be especially sensitive to meal challenges (Patsch *et al.*, 1983; Mekki *et al.*, 1999; Sharrett *et al.*, 2001; Finnegan *et al.*, 2003).

Subjects with frank dislipidaemia. Fasting parameters are above normal levels (hypertriglyceridaemia without or with hypercholesterolaemia) and because many such subjects are under drug therapy, their existing treatment can complicate recruitment especially when comparisons are undertaken with normal healthy untreated subjects (Aim of postprandial lipaemia tests section). Subjects with fasting hypertriglyceridaemia are known to display exaggerated and prolonged postprandial lipid responses (Mekki *et al.*, 1999; Marcoux *et al.*, 2000; Minihane *et al.*, 2000; Weintraub *et al.*, 1988).

Overweight and obese subjects. According to WHO standards, normal weight is with BMI <25, overweight is with BMI >25 and <30, obesity being with BMI $\geqslant 30$. Elevated BMI alone is not enough to well characterize these subjects because elevated waist circumference or waist/hip ratio are associated with visceral obesity and associated metabolic disorders only (see below for metabolic syndrome) (Couillard *et al.*, 1998; Mekki *et al.*, 1999; Guerci *et al.*, 2000; Verges *et al.*, 2001). Selection of subjects should take into account this well-known heterogeneity.

Subjects with the metabolic syndrome. According to an acknowledged definition (WHO or NCEP III) these subjects exhibit a complex syndrome with three disturbances among the following: elevated waist circumference (>102 cm in men and >88 in women); elevated triglyceridaemia (>1.7 mmol/l); low HDL cholesterol (<1.0 mmol/l in men and 1.3 mmol/l in women); elevated glycaemia (>6 mmol/l); hypertension (systolic blood pressure >130; diastolic blood pressure >85 mm Hg). Given the complexity of this syndrome involving insulin resistance, great care should be given to selection of subjects because of their metabolic heterogeneity. Some studies have been performed in obese/insulin-resistant subjects (Jeppesen *et al.*, 1994; Couillard *et al.*, 1998; Mekki *et al.*, 1999; Guerci *et al.*, 2000; Lovegrove *et al.*, 2002; Harbis *et al.*, 2004).

Subjects with diabetes. While type I diabetes is characterized by impaired insulin secretion and elevated glycaemia requiring insulin infusion, type II diabetes is mainly characterized by insulin resistance and elevated insulin concentrations. Most nutritional studies performed in this field are performed with type II diabetes patients (Chen *et al.*, 1993; Syvanne *et al.*, 1994; Mero *et al.*, 1998; Tentolouris *et al.*, 2007). According to WHO standards, they show elevated glycaemia (>7 mmol/l) and some degree of hyperinsulinaemia to compensate for acquired insulin resistance. Many type II diabetes patients exhibit a metabolic syndrome (see above), needing great care in subject selection to avoid to large heterogeneity.

Subject genotyping

Healthy subjects, subjects at risk or patients can display either usual or less frequent polymorphisms of key gene involved in lipid handling and more generally, metabolic homeostasis. This is the widespread genetic basis of individual susceptibility to disorders or responses to diet (Ye and Kwiterovich, 2000; Ordovas, 2001; Vincent *et al.*, 2002). For some of these genes, some polymorphisms have been well studied and subjects with some specific allelic forms can show particular responses to diet as shown in references cited above. For example, in this field, compared with subjects with the dominant apoE-E3 allele, subjects with the minor allele apoE-E4 are prone to hypercholesterolaemia, while those with the apoE-E2 allele are prone to hypertriglyceridaemia.

Given genotyping is becoming easy routine laboratory work, it could be advisable to genotype potential subjects (for some key single nucleotide polymorphisms) to be included in a protocol to ensure maximum homogeneity of the subject group. Alternatively, it could be worthwhile to evaluate interactions between different genotypes and postprandial responses in groups or larger cohorts (Minihane *et al.*, 2000; Lopez-Miranda *et al.*, 1994b, 1997, 2004; Moreno *et al.*, 2003).

Markers of postprandial lipid metabolism

While triglyceridaemia is the cornerstone marker of postprandial lipid metabolism, several other markers can be used for studying postprandial lipid metabolism that will be reviewed below. Clearly, the markers to select strictly depend on the aim of the study and the extent of investigation desired.

Plasma triglycerides

By essence, the first and main change elicited in the circulation after intake and digestion of a fatty meal is a

Table 4 Proposal for design of postprandial studies

Item	*Proposal for standardization*	*Comment or alternative*
Time	Morning	Other period possible
Test meal	Mixed solid—liquid	Simplified meal
Energy	700–1100 kcal	More or less for specific purpose
Amount of fat	Fixed amount of fat 30–60 g, 50–70% energy	Expressed in g/kg body weight when subjects with different weights. In studies with normal subjects, 0.5–1 g/kg body weight. More or less for specific purpose
Type of fat	Emulsified triglycerides made of long-chain fatty acids	Other kinds for specific purpose
Amount of cholesterol	Below 200 mg	In first studies, 5–7 mg/kg body weight with normal subjects More for specific purpose
Vitamin A	50–80 000 IU/m^2 surface area if necessary	Not compulsory
Amount of carbohydrate	20–40% energy	More or less for specific purpose
Duration	6–8 h	Extended follow-up
Sampling	Baseline and hourly	Every 2 h; for glucose and insulin, every half hour until 3 h
Numbers of subjects	10 or above depending on power calculation	Depends on study aim (food testing, clinical study, cohort follow-up, etc)
Markers	Triglycerides, cholesterol, phospolipids, TRL, apoB-48 apoB-100, apoAIV, retinyl palmitate	To select depending on study aim

TRL, triglyceride-rich lipoprotein.

transient elevation of triglyceridaemia resulting from occurrence and accumulation of TRLs. For this reason and because triglyceride assay is routine, inexpensive and accurate, plasma triglycerides is the first marker to use.

Postprandial changes in triglyceridaemia will thus reflect the overall body response to the meal. Nevertheless, a more sound understanding of specific changes elicited in TRL metabolism necessitates the use of more specific markers.

TRLs

As mentioned above, postprandial changes in triglyceridaemia are essentially because of TRLs, that is, both intestinally derived chylomicrons and hepatically derived VLDL (Cohn *et al*., 1993; Schneeman *et al*., 1993; Karpe *et al*., 1993, 1995; Roche *et al*., 1998a; Mekki *et al*., 1999; Harbis *et al*., 2001, 2004). The two main components of these TRL species are triglycerides and apoB. Assaying TRL triglycerides will reflect the amount of triglycerides carried out by the TRL species, while assaying apoB will reflect the number of TRL particles present because one apoB moiety is associated to one TRL particle. Calculating the triglycerides/apoB ratio could thus provide an estimate of the mean size of the particles. This is of interest because particle size is a reflection of secretion and lipolysis rates as well as indication of the clearance rate of TRL. Thus, such two determinations will give a sound view of TRL status postprandially.

Chylomicrons and VLDL can be distinguished from one another using two approaches. For research purposes it is preferable to combine both methods to ensure the best possible characterization of TRL as either of dietary (chylomicron) or hepatic (VLDL) origin.

In theory, ultracentrifugation can be used to separate the two types of particles, because the density of large chylomicron particles is lower and their size is larger than that of VLDL. Chylomicrons are classically floated on the top of a plasma sample in the form of a cloudy layer after a short ultracentrifugation at moderate speed (Dubois *et al*., 1994, 1998). Then in a second run, VLDL can be floated after adjusting plasma sample density to 1.006 and ultracentrifugation for several hours at moderate/high speed. After collection, chylomicrons and VLDL triglycerides (and other lipids) are assayed in the two fractions.

The limit of this approach is that native chylomicrons are highly heterogeneous and much of the apoB-48 (marker of chylomicrons) present following a meal is found in the VLDL fraction (Jackson *et al*., 2003). This means that the VLDL fraction is significantly 'contaminated' with small chylomicron remnants. Measurement of apoB-100 (VLDL marker) and apoB-48 (chylomicron marker) can help to quantify the relative proportion of endogenous and exogenous TRL, but does not allow interpretation of the remnant population, which is frequently of interest.

The quantification of apoproteins specifically associated with the TRLs reflects the number of TRL particles. Early studies that measured apoB concentrations postprandially, employed protein separation by SDS-PAGE and quantification by staining or blotting (Smith *et al*., 1997). New methods have been developed based on immunochemistry of apoB species as reviewed (Jackson and Williams, 2004). Indeed, the key apoprotein of TRLs is apoB but in humans the liver packages VLDL with the full form of apoB called apoB-100, while the small intestine delivers chylomicrons packaged with a truncated form of apoB called apoB-48 (48% of the full form). Thanks to the use of specific antibodies that recognize specifically apoB100 or apoB48, the assay of the two different apoproteins can be performed using nephelometry (apoB-100) or ELISA method (Lovegrove *et al*., 1996; Lorec *et al*., 2000; Harbis *et al*., 2001, 2004) reflecting the number of each kind of particles present.

The SDS-PAGE strategy is highly time consuming, while the limit of the immunochemical method is that few laboratories run ELISA assay of apoB-48 with no commercial kit available yet. A recent review has dealt with the continuing difficulty of quantifying apoB-48 that is complicated by lack of available pure protein for standardization (Jackson and Williams, 2004). In addition, given apoB-100 is also a key component of LDL particles, the specific assay of VLDL apoB-100 requires previous isolation of TRL or VLDL faction as above.

Alternatively, immobilized antibodies can be used to isolate specifically apoB-100 or apoB-48 containing TRL from a total TRL sample allowing further assay of lipid components in these fractions (Cohn *et al.*, 1988a; Schneeman *et al.*, 1993; Mekki *et al.*, 1999). The limit is that antibodies specific of apoB-48 are available in few laboratories only.

It could be of interest to measure the mean size of the chylomicron or TRL particles that is increased postprandially and could be somewhat altered by the meal composition. The best routine way to make such non-destructive measurements is by using a particle-size analyser based on photon correlation spectroscopy (Mekki *et al.*, 1999, 2002).

Chylomicrons remnants

As a result of the very efficient endovascular triglyceride lipolysis, chylomicrons are very quickly transformed to chylomicron remnants with reduced triglyceride content and particle size. The accumulation of these remnants in the circulation (owing to meal characteristics and/or subject clearance defect) is thought to be one of the most detrimental consequence of the postprandial period. Thus, it is of clear interest to determine the level of the chylomicron remnants postprandially.

The classical and widely used way is to add retinol (vitamin A) into the test meal (20–50 000 IU) and to assay retinol palmitate in total plasma (or separately TRL and non-TRL fractions) (Weintraub *et al.*, 1987, 1988; Cohn *et al.*, 1993; Karpe *et al.*, 1995; Guerci *et al.*, 2001). Indeed, retinol palmitate is specifically accumulated and secreted into native chylomicrons and minor exchanges with other kinds of lipoproteins are thought to occur.

The limit of this approach is that limited exchange to other lipoprotein particles (VLDL and LDL) does occur in the late postprandial period and that the kinetic of retinol palmitate does not strictly follow that of triglycerides. In addition, the fatty acid composition of the test meal can modulate the relative levels of the different chylomicron retinyl esters (Sauvant *et al.*, 2003), meal fatty acids being used to re-esterify free retinol within the enterocyte.

A new measurement of remnant lipoproteins is available through the use of the remnant-like particle kit made by Japan Immunoresearch Laboratories (Takasaki, Japan). This immunoaffinity assay takes advantage of two antibodies, one that binds apoA1 and a specific mouse monoclonal antibody (JI-H) that binds apoB100. When serum is incubated with the antibodies, HDL and LDL are bound, leaving in the supernatant, apoE-rich VLDL- and TRL-containing apoB48 particles. The cholesterol in the supernatant is measured by enzymatic assay and thus gives the cholesterol concentration of fraction of lipoproteins that is highly enriched in remnants (Marcoux *et al.*, 2000).

LDL and HDL

Because all classes of lipoproteins are interrelated, every kind of lipoprotein can be altered postprandially (Patsch *et al.*, 1983; Weintraub *et al.*, 1987; Cohn *et al.*, 1988a, b; Karpe *et al.*, 1993, 1995; Dubois *et al.*, 1994, 1998). If the full pattern of lipoprotein parameters, including LDL and HDL particles, is required, these can be separated from plasma samples by a more prolonged ultracentrifugation and components assayed (triglycerides, cholesterol and phospholipidsapolipoproteins). Direct assay of LDL or HDL total cholesterol can also be achieved by precipitation of apoB-containing lipoproteins. However, this method is only valid if triglyceride concentrations are below 5 mmol/l and this may not be the case during the postprandial period especially for subjects with an elevated postprandial response.

As fasting LDL size has been related to cardiovascular risk in some subjects, it could be worthwhile to measure LDL size postprandially. Commercial methods are available but are time consuming.

Plasma cholesterol, phospholipids and NEFAs

Given plasma total cholesterol is an acknowledged marker of lipid metabolism in the fasting state, it is tempting to measure it in postprandial samples. In fact, total cholesterol is the sum of two different molecular entities present in the plasma, that is free cholesterol and esterified cholesterol. It is striking to observe that free cholesterol temporarily increases, whereas esterified cholesterol drops after a fatty meal (Dubois *et al.*, 1994, 1998). As a result, total cholesterol does not show any marked change or somewhat decreases (Cara *et al.*, 1992; Dubois *et al.*, 1998). This means that measuring total plasma cholesterol postprandially will not add new important information.

- Phospholipids are important surface components of lipoproteins and plasma phospholipids transiently increase postprandially. After a zero-fat meal, phospholipids smoothly decrease, while triglycerides and cholesterol do not change. It is thus a sensitive marker of postprandial changes (Dubois *et al.*, 1998). Commercial kits allow determination of plasma phosphatidylcholines (lecithins) only.
- NEFAs show a transient and marked decrease after a mixed test meal in which carbohydrate as well as fat is given. The suppression of NEFA is a result of inhibition of lipolysis within adipocytes as elicited by the increased secretion of

insulin, inhibition of hormone-sensitive lipase and therefore decreased release of free fatty acids into the circulation (Frayn *et al.*, 1994). This is clearly an effect generated by glucose and not by dietary fatty acids. Free fatty acids that are generated from dietary triglyceride by endovascular lipolysis at the chylomicron surface are transported across the adipocyte membrane and further esterified for storage. Normally, only a minor part of the NEFA released from circulating chylomicrons and VLDL escapes re-esterification and storage into the adipocyte triglyceride. However, under certain circumstances, for example, after a high-fat meal and in subjects with impaired capacity to re-esterify the released fatty acids, NEFA may 'escape' back into the circulation and contribute to a higher than normal NEFA concentration. This abnormality is frequently observed in subjects with type II diabetes and those demonstrating the lipid disturbances typical of the metabolic syndrome.

Plasma apoproteins

Among the numerous apoproteins associated to lipoproteins in the plasma, some of them are of importance regarding postprandial metabolism.

- ApoB-100 and apoB-48 have already been referred to. ApoA1 does not show significant changes postprandially.
- ApoA-IV is specifically secreted by the small intestine and show variations postprandially (Verges *et al.*, 2001). It is expected to play several roles that need to be substantiated *in vivo*. ApoA-V is a newly discovered apoprotein associated to TRL particles and fasting triglyceridaemia (Pennacchio *et al.*, 2001) and its role is being studied.
- ApoC2 is an activator of lipoprotein lipase and thus of endovascular lipolysis and clearance, while ApoC3 is an inhibitor. The levels of fasting triglyceridaemia and TRL have been associated in several studies with the fasting levels of these apoproteins, while the amplitude of postprandial triglyceridaemia has been positively associated with fasting levels of ApoC3 (Mekki *et al.*, 1999). These two apoproteins are of potential interest but their relevance for postprandial studies remains to be better documented.
- ApoE is one of the most important apoprotein associated to TRL with a key role for tissue uptake through the ApoB/E receptor pathway. Nevertheless, the relationship between apoE plasma levels and postprandial lipid metabolism is not yet fully documented (Jackson *et al.*, 2005).

Plasma and chylomicron fatty acid composition

The fatty acid composition of postprandial plasma total lipids and of VLDL shows some concordance with the meal fatty acid composition, while in the chylomicron fraction there is high concordance between meal and chylomicron fatty acid composition. (Mekki *et al.*, 2002). When an unusual meal fat is consumed (e.g., fish oil) analysis of fatty acid species such as eicospaentaenoic and docosahexaenoic acids (currently done by gas–liquid chromatography of extracted lipids) in the chylomicron and VLDL fractions, can provide a useful means of studying the kinetics of the postprandial chylomicron appearance and clearance and of the rate of incorporation of dietary fatty acids into VLDL.

Plasma glucose and insulin

Given mixed meals are used, the glucose units provided by digestible carbohydrate will transiently increase glyacaemia and insulinaemia postprandially. As mentioned previously, sugars and/or insulinaemia can alter postprandial lipaemia. It is thus important to determine plasma glucose and insulin during such studies performed either in normal subjects or in subjects at risk or patients. Commercial kits are available.

Tracers with stable isotopes

As already mentioned, some specific studies can be performed by adding tracers enriched with stable isotopes in test meals. From blood, plasma or lipoprotein samples, fatty acids (Bickerton *et al.*, 2007), cholesterol (Beaumier-Gallon *et al.*, 2001) or fat-soluble vitamins (Sauvant *et al.*, 2003) or alternatively apoB-100 or apoB-48 (Welty *et al.*, 1999) can be extracted and specific analyses made using various methods involving mass spectroscopy (GC-MS, GC-C-MS, LC-MS/MS-MS, etc). Determination of isotopic ratio in expired air (breath test) can also be done. Such determinations are highly specific and allow definitive answer to particular questions about the metabolic fate of lipid nutrients in humans.

The limit is the high price of tracers and mass spectroscopy analyses with costly and sophisticated equipments as well as laboratory skills.

Some specific markers

Gastric emptying: When one would like to be able to assess the rate of gastric emptying (for well-controlled study or as a purpose of the study), an acknowledged marker of gastric emptying (i.e., paracetamol) could be added to the meal and further assayed in the plasma by high-performance liquid chromatography method (Johansson *et al.*, 2003).

Intestinal absorption: To check that intestinal lipid absorption is not altered or to compare absorption after different meals, ^{13}C octanoate can be added to the meal and breath test collections made over time with analysis of carbon isotopic enrichment in expired CO_2 by mass spectroscopy (Groot and Hulsmann, 1973).

Fibrinolysis and hemostasis: Some markers are classically measured as they are acknowledged markers of thrombosis risk and can display measurable changes postprandially related to the fact that TRL interact with the endothelium and promote the activation of the fibrinolysis and haemostatic systems. Coagulation factor VII, plasminogen activator

inhibitor-1 (PAI-1) and plasminogen are the most frequently used markers during postprandial studies. Routine assays are available for the most commonly used markers.

Inflammatory markers: Inflammation is increasingly considered as a risk factor involved in cardiovascular disease and metabolic diseases. Several acknowledged markers can be routinely measured from blood samples such as C-reactive protein (CRP), IL-1, IL-6 and tumour necrosis factor α. Interesting changes have been reported after acute meal challenge (Nappo *et al.*, 2002; Schaefer *et al.*, 2005; Blackburn *et al.*, 2006).

Vascular reactivity: This is a new marker of the dynamic capacity of the blood vessel wall to adapt to metabolic changes and thus, of cardiovascular risk. Although numerous methods have been developed for studying vascular tone *in vivo*, two non-invasive measures have received the most widespread use and recent attention. These are flow-mediated dilation of the brachial artery, a marker of endothelial health and integrity and, to a lesser extent, total peripheral vascular resistance, a measure of systemic vascular constriction and an index of myocardial workload (after load). More recently, a non-invasive technique using increased shear stress to stimulate release of nitric oxide and dilation of the brachial artery has been developed to assess endothelial function (Raitakari and Celermajer, 2000).

Oxidative stress: The postprandial state is generally associated with elevated triglyceride and glucose concentrations as well as increased oxidative reactions. Damage to cellular structures such as proteins, carbohydrates, nucleic acids and lipids can result from oxidative processes during this time. Increased postprandial oxidation is a major contributor to atherosclerosis and endothelium dysfunction (Bae *et al.*, 2001). Today, many *in vitro* and *ex vivo* assays exist for the assessment of oxidative stress and have been described in detail elsewhere (Moore and Roberts, 1998). Simple and inexpensive *in vitro*/*ex vivo* antioxidant capacity assays are highly accessible and easily automated for high throughput. It has been reported that the increased consumption of fruits, vegetables and vitamin C can increase the serum/plasma antioxidant capacity as measured using various systems such as the oxygen radical absorbance capacity assay, trolox-equivalent antioxidant capacity assay and ferric-reducing ability assays.

Fasting markers of postprandial lipid metabolism

As postprandial studies are time consuming and costly, it is tempting to search for reliable markers that can be measured in the fasting state to predict the level of postprandial lipid response.

Up to now, elevated fasting triglyceridaemia is the only fasting marker clearly associated with exacerbated and possibly delayed postprandial lipid response (Patsch *et al.*, 1983; Cohn *et al.*, 1988b; Mekki *et al.*, 1999). Nevertheless, the amplitude of postprandial lipid response cannot precisely be predicted by fasting triglyceride levels. Also, several studies report that some fasting normotriglyceridaemic subjects display an abnormal exacerbated postprandial response (Couillard *et al.*, 1998; Mekki *et al.*, 1999).

Interesting but somewhat less investigated reproducible relationships have been found for fasting plasma HDL cholesterol (Patsch *et al.*, 1983; Sharrett *et al.*, 2001), fasting apoA-IV level (Verges *et al.*, 2001), fasting apoCIII level (Mekki *et al.*, 1999; Tiret *et al.*, 2000) or fasting apoB-48 level (Smith *et al.*, 1997).

Timing of sampling and measurements

Fasting check

At entry to the investigation unit, subjects lie in a rested position for a short period (10 min). An indwelling catheter is placed into the antecubital vein to allow frequent blood sampling with minimal discomfort for the subject.

It is essential to have accurate measurements of parameters of interest at baseline, usually after an overnight fast. One (or preferably two) fasting samples are collected within 15 min before the intake of the test meal.

Standard single test

After the ingestion of the test meal in well-controlled conditions and fixed time (15–20 min), repeated blood samples are collected over a number of hours. The length of the postprandial test remains an open question. In healthy subjects with meals providing up to 60 g fat, plasma triglyceride values rise within 2–3 h reaching a plateaux at 3–4 h and returning towards baseline by 6 h. Most studies employ at least 6 h since this is necessary for triglyceridaemia to come back to baseline levels (Lopez-Miranda *et al.*, 1997; Williams, 1997; Dubois *et al.*, 1998). In at-risk subjects or patients with lipid disturbances, delayed postprandial triglyceridaemia is usually found, necessitating the follow-up period to be extended up to 8 h. Unlike plasma triglycerides, glucose and insulin responses to meal ingestion are very rapid and thus necessitate sampling at least every half-hour until 2–3 h (depending on the kind of meal), then every hour.

For assessment of triglyceridaemia, TRL parameters, lipids and apoprotein levels sampling every hour is usually done as a good practice. Sampling every 2 h has been done in some studies with 8-h follow-up but is obviously less precise. In some studies, lipoproteins (LDL and HDL) have been measured at baseline, postprandial peak (3 h) and the end of follow-up only (6 h) (Dubois *et al.*, 1994, 1998).

Simplified single test

When a large number of subjects are to be investigated (e.g., for an epidemiological study) it is not feasible to perform a full standard postprandial test. It is however valuable in such a context to have a simplified postprandial test. This has

been already done on some occasions and some comparative validation has been provided. Indeed, after a meal with a rather large amount of fat/energy, measurements made at baseline and after 4 h (expected peak) and 8 h postprandially correlate well with data obtained using more frequent time points (Guerci *et al*., 2001). Although this study suggests a simplified procedure may be feasible for large studies, more validation is required.

Calculations and data interpretation

There are several ways of calculating and expressing the measurement data obtained during postprandial studies.

The raw measurements of the circulating parameter of interest allows graphical representation of absolute concentration over time and reflects the metabolic response to meal ingestion.

The incremental or decremental concentrations are obtained by subtracting baseline values from postprandial ones (baseline value becomes 0), which is the best way to represent the relative changes from baseline at the different time points during the follow-up. Moreover, this approach has the advantage of (i) normalizing baseline values which are always somewhat different between subjects and thus, reduces inter-individual variability and (ii) amplifies the capacity to identify potential differences between the meals compared. An alternative when baseline values are not significantly different is to introduce an adjustment for baseline values when analysing postprandial values.

- Postprandial variations are characterized by the occurrence of a peak of change during the study period: the postprandial peak value for a given parameter or the time to peak response can provide an additional useful indicator of the individual/group response to a meal.
- By definition, the postprandial period lasts a prolonged period of time. This means that calculation of the overall variation during the follow-up for a given parameter is a valid way of evaluating the whole response; this is classically made by calculating the area –under –the curve (AUC), either absolute or incremental/decremental. The trapezoidal method is generally used for that purpose. Calculating AUCs, which sums time-point differences, generally allows for identifying small differences between meal responses. Contrary to glycaemia, it is uncommon that lipid parameters decrease below baseline values postprandially; when occurring, one can take into account this negative shift or not given the two options are sounded.
- The postprandial change in triglyceridaemia (or some other parameters) is usually a bell-shaped curve. The two parts of the curve have different meanings: the early rising part best reflects the occurrence and accumulation of TRL within the circulation (dominant secretion phase), while the late decreasing part is mostly determined by the lipolysis and uptake capacity (dominant clearance phase). In subjects at cardiovascular risk or in some patients, an enlarged and delayed postprandial response has repeatedly been observed. The slope of these two curves or the AUCs of the two periods is a reflection, but not a true measure, of the efficiency of the two processes. More validation of this approach would be welcome.
- It is not uncommon to observe a biphasic curve for some parameters in some individuals instead of the more general uniphasic bell-shaped curve. This could result from some discontinuation in some process involved (i.e., gastric mixing, gastric emptying, small intestine motility, secretion rate of TRL, hormonal regulations, etc). Unfortunately, specific emphasis has not been put on this aspect, which remains largely speculative.
- Finally, data interpretation should take into account the kind and aim of the study as referred to above (Aim of postprandial lipaemia tests section).

Proposal for postprandial lipid studies

The review presented above clearly illustrates that noticeable differences are found between studies in different laboratories and even within the same investigation centre. The reasons for this are clear when the number of variables that influence the postprandial response are taken into account. Since the nature of the fat in the habitual background diet can influence the response to a standard test meal it is not appropriate to specify a particular fat composition for use in clinical investigations in different countries. However, it may be appropriate to advise that the fat composition of the typical diet of that region should be used. A test-meal fat content of 40–60 g could be recommended on the basis that this is sufficient to stimulate significant challenge to the lipid-metabolizing pathways but not sufficient to overload the system. A mixed meal should be used including protein and carbohydrate, with the latter comprising at least 50–100 g to ensure an adequate insulin response.

As summarized in Table 4, we would propose some guidelines for performing postprandial lipid studies in humans, in the nutrition area.

It is important to consider that such a protocol should allow investigation of specific foodstuffs. Because other nutrient (especially carbohydrate and fibre) than fat can alter the postprandial lipid response great care should be given to study design to avoid confounding factors. Specific foodstuffs should be better incorporated into mixed meals to mimic day-life situation but overall nutrient composition should be kept comparable in compared meals. Search for an agreeable reference meal control is encouraged.

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