

The Summer Meeting of the Nutrition Society was held at the University of Surrey on 29 June–2 July 1998

Macronutrient Group Symposium on ‘Dietary determinants of lipoprotein-mediated cardiovascular risk’

Lipoprotein atherogenicity: an overview of current mechanisms

Bruce A. Griffin

Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford, Surrey GU2 5XH, UK

Raised serum cholesterol does not adequately explain the increased risk of CHD within populations or the relationship between diet and CHD. Nevertheless, the principal transport vehicle of cholesterol in the circulation, LDL, must still be regarded as the most atherogenic lipoprotein species, but not because of its contribution to serum cholesterol. The atherogenic potential of LDL in the majority of individuals arises from an increase in the number of small dense LDL particles and not from its cholesterol content *per se*. There is now a wealth of evidence from cross-sectional and prospective studies to show that LDL particle size is significantly associated with CHD and predictive of increased coronary risk. Moreover, there are a number of credible mechanisms to link small dense LDL with the atherogenic process. The rate of influx of serum lipoproteins into the arterial wall is a function of particle size, and will thus be more rapid for small dense LDL. Components of the extracellular tissue matrix in the intima, most notably proteoglycans, selectively bind small dense LDL with high affinity, sequestering this lipoprotein in a pro-oxidative environment. The oxidation of LDL promotes the final deposition of cholesterol in the arterial wall, and numerous studies have shown small dense LDL to be more susceptible to oxidative modification than its larger and lighter counterparts. An increase in the number of small dense LDL particles may originate from a defect in the metabolism of triacylglycerol-rich lipoproteins. One mechanism may involve the overproduction and increased residence time of large triacylglycerol-rich VLDL in the postprandial phase, a situation thought to arise through pathways of insulin resistance.

Triacylglycerols: Small dense LDL: CHD: Postprandial metabolism

Atherosclerosis is an insidious disease characterized by the development, over 30–40 years, of fibrofatty lesions in the intimal lining of large and medium-sized arteries, most typically in the coronary circulation. Coronary arteries suffer an impaired blood supply through occlusion of their lumen and a predisposition to thrombosis, which can lead to fatal myocardial infarction. Whilst atherosclerosis has a multifactorial aetiology, the presence of a yellowish fatty substance in the atheromatous lesions of rabbits was reported over 90 years ago, long before the chemical recognition of cholesterol (Anitschkow, 1913). The cholesterol in the arterial wall originates from circulating lipoproteins, principally LDL, which can be isolated from human arterial tissue in amounts directly related to its concentration in serum (Smith & Slater, 1972).

The cholesterol hypothesis

The cholesterol hypothesis was born on the strength of evidence from large-scale epidemiological surveys such as the Multiple Risk Factor Intervention Trial (Stamler *et al.* 1986) which showed a continuous positive relationship between serum cholesterol levels and mortality rate from CHD. These findings confirmed the earlier cross-cultural observations of Keys (1970) which established firm links between a raised intake of saturated fat, elevated serum cholesterol and CHD. At about this time, the cholesterol hypothesis was underpinned by two major discoveries. First the Nobel Prize-winning elucidation of the LDL-receptor pathway, impairment of which through genetic mutation or dietary manipulation was shown to result in raised LDL

Abbreviations: apo, apoprotein; TAG, triacylglycerol.

Corresponding author: Dr Bruce A. Griffin, fax +44 (0)1483 259724, email B.Griffin@surrey.ac.uk

levels and premature atherosclerosis (Brown *et al.* 1975). Second, the discovery that LDL oxidation is a major prerequisite for the uptake and deposition of LDL-cholesterol in the artery wall (Goldstein *et al.* 1979). These findings led to a unified hypothesis of atherosclerosis which encompassed the infiltration and oxidation of LDL in the arterial wall. These events, which at the time were believed to arise entirely from an elevated level of LDL-cholesterol, initiated the formation of the early lesion or 'fatty streak'. This was coupled, through the damaging effects of oxidized lipid and other agents on the endothelium, to further lipid deposition, cell proliferation and the formation of a mature fibrous lesion (Steinberg & Witztum, 1990). Together, these events have formed the mechanistic backbone for the role of LDL in atherogenesis for the last decade. More recently, the cholesterol hypothesis has withstood rigorous testing, in the form of cholesterol-lowering drug intervention trials, which proved beyond any doubt that reducing serum cholesterol in individuals with existing CHD or raised cholesterol reduces the risk of suffering a coronary event (Scandinavian Simvastatin Survival Study Group, 1994; Shepherd *et al.* 1995). This considerable weight of evidence in favour of the cholesterol hypothesis is difficult to reconcile with the fact that serum cholesterol is not an adequate predictor of coronary risk within populations such as the UK. Moreover, variations in total serum cholesterol or LDL-cholesterol levels do not provide an adequate scientific basis by which to explain the interrelationships between diet and CHD. As such, lowering serum cholesterol on a grand scale, even if achievable, would not provide the most rational approach to the primary prevention of CHD.

The cholesterol paradox

To shed light on this paradox, it is important to appreciate the distinction between 'absolute risk' and 'attributable risk'. A recent Franco-Scottish interpretation of the Framingham study, a prospective study of CHD with a 26-year follow-up, illustrates this point well (Fruchart & Packard, 1997). The frequency distribution of the diseased and non-diseased population from Framingham shows bell-shaped curves which overlap considerably, producing what clinicians sometimes call a 'grey area' of risk which includes the majority of individuals (Fig. 1). The absolute risk associated with a raised cholesterol of 7.5 mmol/l, as calculated from mortality curves, is about 90%. This would be impressive if it were not for the fact that only 3% of individuals with CHD have a serum cholesterol at or above this level. On the other hand, the risk associated with a cholesterol of 5.2 mmol/l is only about 20%, but approximately 45% of CHD patients fall into this category. Thus, the risk that can be attributed to raised serum cholesterol (90% of 3) is considerably less than that attributed to factors other than serum cholesterol (20% of 45). Hence, for the majority of individuals serum cholesterol cannot discriminate between diseased and non-diseased groups because of the relative frequency distributions of this variable in both groups. This is not a new finding. Gofman *et al.* (1950) reported that a 'tremendous number of people who suffer the consequences of atherosclerosis show blood cholesterol in the accepted normal range'. This group

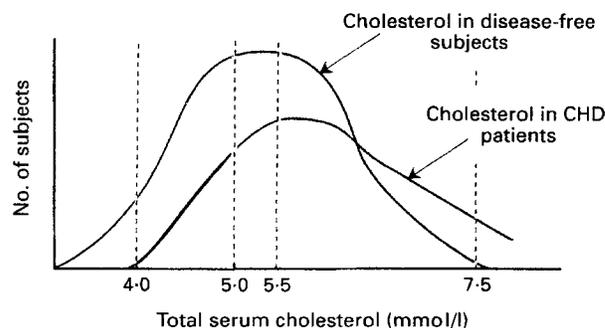


Fig. 1. Total serum cholesterol is an inadequate predictor of CHD status within a population as illustrated by the frequency distributions of serum cholesterol in diseased and disease-free groups. The overlapping distributions create a 'grey area' within which the level of serum cholesterol does not discriminate CHD from health. (Data adapted from the Framingham study (Castelli, 1984) by Fruchart & Packard, 1997.)

pioneered the separation of serum lipoproteins in the analytical ultracentrifuge, and was the first to identify the importance of lipoprotein heterogeneity as a major determinant of atherosclerotic risk. Significant atherogenic potential was attached to raised levels of specific apoprotein (apo) B-containing lipoproteins, which included triacylglycerol (TAG)-rich VLDL, IDL and LDL. Of these atherogenic candidates, LDL is still considered to be the most atherogenic lipoprotein, but not because of its contribution to raised serum cholesterol. In the intervening years it has become apparent that the atherogenicity of LDL in the majority of individuals arises from its small size and increased density and particle number, and not from its cholesterol content *per se*. There is now a convincing body of evidence to suggest that these abnormalities in LDL are the products of defective TAG metabolism which may arise, in part, through the impaired action of insulin (Despres & Marette, 1994).

Serum triacylglycerol and lipoprotein atherogenicity

Following the original exclusion of serum TAG as a risk factor for coronary disease on the grounds that it co-segregated with other risk variables such as HDL, serum TAG has now re-emerged as an independent risk factor for CHD. This development came about largely on the strength of evidence from a meta-analysis of seventeen prospective population-based studies (Hokanson & Austin, 1996), and also because of our increased knowledge of the mechanisms which underlie this risk association. In retrospect, it is perhaps ironic that the relationship between TAG and CHD derives part of its strength from its association with HDL, since this was the reason for the original statistical downgrading of TAG as a risk factor. Moderately-raised serum TAG is associated with a constellation of abnormalities in serum lipoproteins which predispose to increased cardiovascular risk, known collectively as an atherogenic lipoprotein phenotype (Austin *et al.* 1990). These abnormalities include a reduced level of HDL, a predominance of small dense LDL and, in certain circumstances, an over-

production of apoB, and thus raised number of LDL particles. Whilst the latter feature did not form part of the original definition of an atherogenic lipoprotein phenotype, which included the 'lipid triad' (moderately-raised TAG, low HDL and small dense LDL), increased LDL particle number has emerged in recent years as a major source of atherogenicity associated with small dense LDL, which should now perhaps be regarded as an important subgroup within this definition. An atherogenic lipoprotein phenotype is also characterized by an intolerance to dietary TAG, resulting in a delayed clearance of TAG-rich lipoproteins.

Framingham (Castelli, 1984) and other more recent studies such as PROCAM (Assman *et al.* 1996) have firmly established HDL to be one of the most significant markers of CHD risk. HDL and CHD share an inverse relationship which is consistent with the role of HDL in the cellular efflux and elimination of cholesterol from the body. Although raised levels of HDL are typically found in low-risk groups (including premenopausal women and athletes), suggesting HDL to be putatively cardioprotective, there is greater evidence to implicate a reduced level of HDL in the disease process. Hence, moderately-raised serum TAG is associated with HDL levels below 1 mmol/l, a level at which the removal of cholesterol from peripheral sites may be compromised.

A predominance of small dense LDL (LDL subclass III, particle size <25.5 nm, density >1.04 g/ml) has been associated with a 3-fold or even greater risk of CHD in a collection of cross-sectional studies (Austin *et al.* 1988; Griffin *et al.* 1994). There is now prospective evidence to show that small dense LDL is as predictive of CHD as many of the more traditional risk factors such as smoking and blood pressure (Gardner *et al.* 1996; Stampfer *et al.* 1996). Between 40 and 50 % of all patients with CHD have small dense LDL. In these patients total serum cholesterol or LDL-cholesterol will be unremarkable and uninformative with respect to the distribution of LDL size and density. In contrast, serum TAG measured in the fasting state will invariably be in excess of 1.5 mmol/l, which represents a threshold value beyond which LDL becomes small and dense (Fig. 2). The other most important determinant of LDL atherogenicity is an increased number of LDL particles. Since there is a single apoB moiety in each LDL particle the concentration of this protein serves as a marker of LDL particle number. Sniderman *et al.* (1980) were the first to suggest that a raised number of LDL particles, as measured by the total serum concentration of apoB (serum apoB >1.3 g/l represents hyperapo-B) was the most common source of lipid-mediated cardiovascular risk in man. Taken together, what seems to be important is not simply the proportion of LDL that exists in a small and dense form, but the predominance or concentration of small dense LDL as expressed by the number of small dense LDL particles. Of patients with CHD, 30 % demonstrate hyperapo-B. In these patients LDL will always be small and dense. However, a predominance of small dense LDL is not always accompanied by hyperapo-B, and in fact we have evidence to show a high prevalence of small dense LDL in free-living individuals with the traditional lipid triad (raised serum TAG, low HDL, small dense LDL) but with apoB levels below the clinically significant cut-off of 1.3 g/l.

Whether the formation of small dense LDL precedes the development of hyperapo-B in certain individuals or even carries significant risk at a level below 1.3 g/l has yet to be established. What seems likely is that these phenomena either share or have distinct metabolic origins depending on the metabolic phenotype or genotype of an individual.

Why should an increased number of small dense LDL particles be so atherogenic?

There is a series of credible biochemical mechanisms which render small dense LDL more potentially atherogenic than its larger and lighter counterparts. First, there is evidence from cell-culture studies that small dense LDL binds with lower affinity to the LDL receptor (Nigon *et al.* 1991). This is supported by studies on the structural conformation of apoB in small dense LDL which show modifications in the binding region of the polypeptide (Galeano *et al.* 1994). This will effectively increase the residence time of small dense LDL in serum for interaction and infiltration of the endothelial barrier. The rate of influx of serum lipoproteins across the endothelium and into the subendothelial space is a function of particle size (Nordestgaard & Nielsen, 1994). Thus, small dense LDL will infiltrate at a faster rate than either large LDL, IDL or VLDL, and would also pass out at a similar rate if it were not for its selective binding to components of the extracellular tissue matrix, chiefly arterial proteoglycans that are in abundance in the subendothelial space (Anber *et al.* 1996). There is as yet no clear explanation for this preferential binding of small dense LDL to proteoglycans, although the exposure of basic amino acids and positive charge on the surface of small dense LDL must influence binding to these highly-electronegative proteoglycans to some degree. Thus, small dense LDL is sequestered in a cellular and pro-oxidative environment where its atherogenicity will depend on its capacity to resist oxidation. In this respect, small dense LDL shows increased susceptibility to oxidation, certainly *in vitro* (Tribble *et al.* 1992), as a result of the unsaturation of its fatty acids in surface monolayer phospholipids (Tribble *et al.* 1995), and possibly through interactions of its phospholipid with apoB (LDL can be protected against oxidation by antioxidant supplementation or the replacement of dietary polyunsaturated fat with monounsaturated fat). All these processes work together in sequence to confer increased risk on small dense LDL (Table 1).

The importance of triacylglycerols in the generation of atherogenic small dense LDL

In the bulk of literature on this topic, the relationship between small dense LDL and serum TAG is, not surprisingly, defined in the post-absorptive state, largely because this is clinical requirement. However, postprandial events and the nature and metabolism of TAG-rich lipoproteins are critical to our understanding of the underlying metabolic processes which generate small dense LDL. At the outset, it is important to recognize that the liver can synthesize and secrete a spectrum of VLDL particles which vary in density and size purely as a result of their TAG content

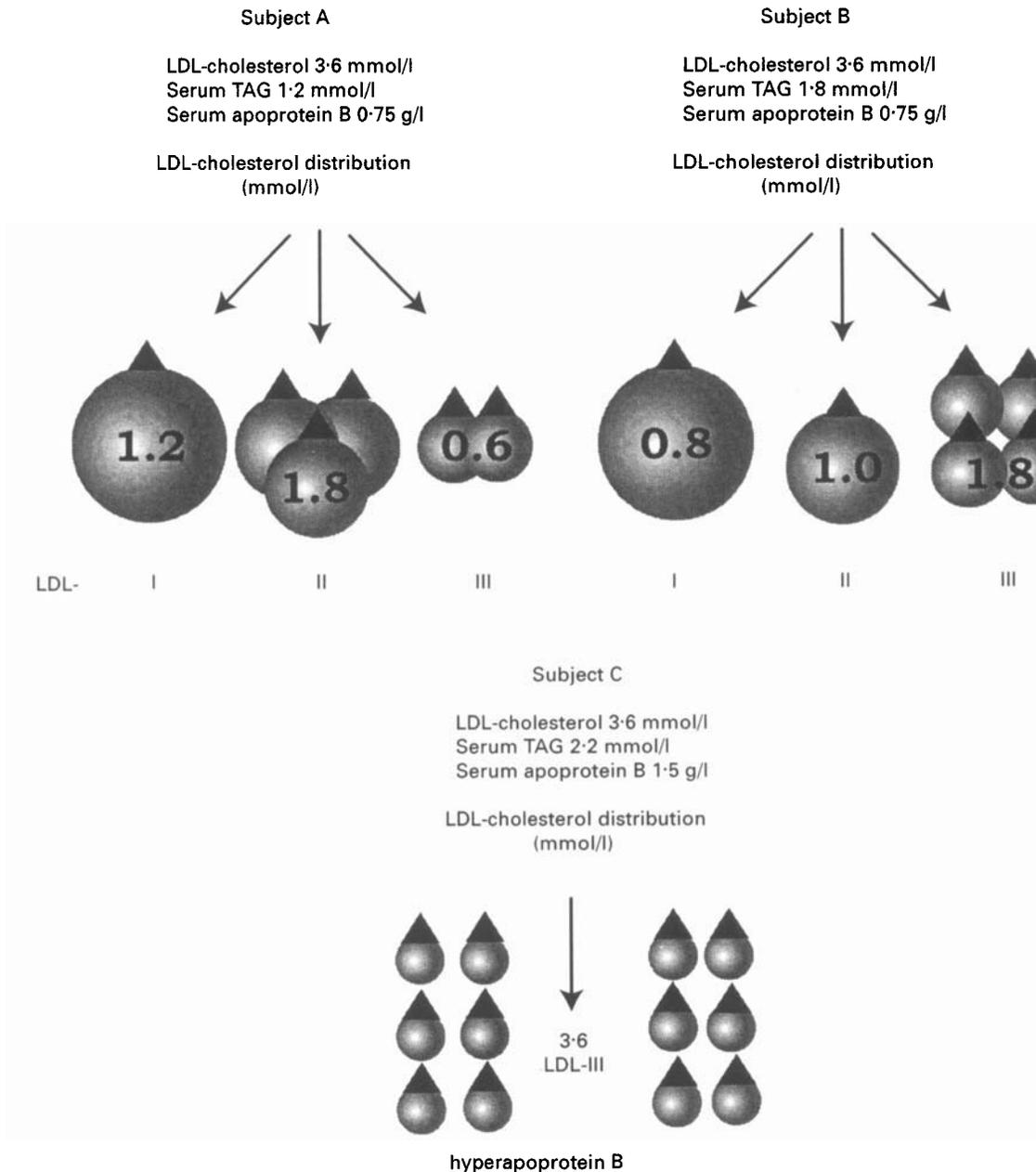


Fig. 2. Variable distribution of serum cholesterol between the LDL subclasses of three different subjects (A–C). Subject A, a normal individual with the majority of cholesterol transported in large light LDL-I (density 1.025–1.034 g/ml, size 26.0–27.5 nm) and -II (density 1.034–1.044 g/ml, size 25.5–26.4 nm). Subject B, a raised serum triacylglycerol (TAG) associated with a shift in the distribution of cholesterol into small dense LDL-III (density 1.044–1.060 g/ml, size 24.2–25.5 nm). Subject C, a raised serum apoprotein B (▲) in combination with a raised serum TAG is associated with an increase in the number of small dense LDL particles (hyperapoprotein B; apoprotein B > 1.3 g/l). (Data modified from Packard, 1995.)

(Millar & Packard, 1998). This is relevant because of the precursor–product relationship between VLDL and LDL, and since the fate of a VLDL particle is largely dependent on its pedigree (Shepherd & Packard, 1987). It is well established that the rate at which VLDL is synthesized and secreted into the circulation is determined by the availability of lipid substrates (non-esterified fatty acids), and lipoproteins arriving from the periphery. The packaging of TAG into a VLDL particle and its association

with apoB involves the activity of a microsomal triacylglycerol transfer protein, and is regulated, certainly in the latter stages, by the action of insulin. In normal healthy individuals with serum TAG level below 1.5–1.6 mmol/l, the liver produces a small VLDL particle, known in operational terms as VLDL₂ (particle size 30–35 nm, density 1.006–1.010 g/ml). At serum TAG values above 1.5–1.6 mmol/l, VLDL becomes enriched with TAG and secreted into the circulation as a larger and lighter particle

Table 1. Atherogenic properties of small dense LDL: a sequential series of mechanisms confer increased atherogenic potential on small dense LDL

Reduced binding to LDL receptor	Residence time ↑
	↓
Increased penetrance of arterial wall	Infiltration ↑
	↓
Increased affinity for arterial proteoglycans	Sequestration ↑
	↓
Increased susceptibility to oxidation	Oxidation ↑
	↓
Increased cholesterol deposition	Atherosclerosis

↓ Sequence of processes; ↑, increase in the individual process.

known as VLDL₁ (particle size >35 nm, density <1.006 g/ml; Tan *et al.* 1995). Large VLDL is typically overproduced in insulin-resistant states such as obesity and non-insulin-dependent diabetes which are characterized by a predominance of small dense LDL (James & Pometta, 1991; Fisher *et al.* 1993). It is possible that insulin resistance affects both ends of the VLDL synthetic pathway through promoting an oversupply of lipid from the periphery (insulin-resistant individuals commonly express a delayed clearance of postprandial fat) and failure to suppress the overproduction of large TAG-rich VLDL. This overproduction, although typically described in kinetic studies performed in the post-absorptive state, almost certainly impinges on postprandial lipaemia. Infusion studies which reproduced the metabolic effects of dietary fat by the intravenous administration of an artificial lipid emulsion 'Intralipid' have shown that large VLDL also increases rapidly, early in the postprandial phase (Bjorkegren *et al.* 1996). This phase is typically characterized by high levels of TAG associated with intestinally-derived chylomicrons and chylomicron remnants. The interpretation of this rapid increase in VLDL₁ is based on competitive interactions between large VLDL₁ and chylomicrons and chylomicron remnants for the enzyme lipoprotein lipase (EC 3.1.1.34). The VLDL₁ competes less effectively for lipoprotein lipase,

extending its residence time in the circulation. The consequences of this are several fold, but in essence will result in an increase in the net exchange of TAG from VLDL₁ into LDL via cholesteryl ester transfer protein, which ultimately leads to the production of small dense LDL through the action of hepatic lipase (EC 3.1.1.3; for review, see Griffin, 1997). A summary of some causes and effects of large TAG-rich VLDL is shown in Fig. 3.

The genesis of hyperapo-B and raised LDL particle number also involves the overproduction of VLDL in the liver, but in this case the VLDL is small and not TAG-rich (Sniderman *et al.* 1998). The fact that small dense LDL is always associated with hyperapo-B would suggest that the production of large TAG-rich VLDL₁ is not the only mechanism by which small dense LDL can be formed, and that increased numbers of small VLDL₂ particles have the same effects on the neutral-lipid exchange reactions as fewer VLDL₁ particles.

Summary

Despite a considerable weight of evidence to implicate serum cholesterol in atherosclerosis, it is a general misconception that raised levels of LDL-cholesterol represent the most common atherogenic stimulus. The greatest source of lipid-mediated cardiovascular risk still arises from LDL, but as a result of its small size, and increased density and particle number rather than its cholesterol content. These abnormalities in LDL develop through interactions with TAG-rich lipoproteins, of which large VLDL is probably the most interactive, notably in the postprandial phase. The direct atherogenicity of TAG-rich lipoproteins, including chylomicron remnants, has not been considered here but should not be ruled out. The influence of diet on mechanisms of lipoprotein atherogenicity, whilst also beyond the scope of the present review, offers considerable insight into the primary prevention of CHD, and as such represents the most exciting aspect of this topic.

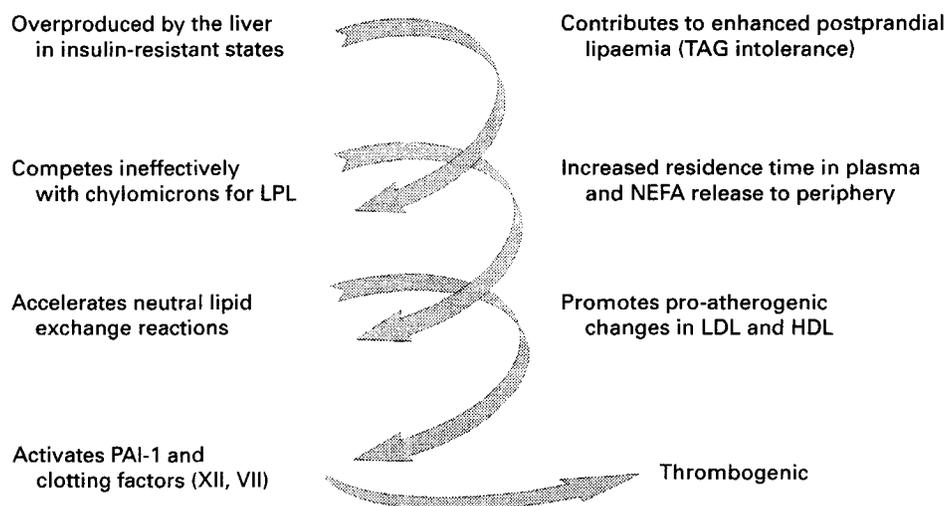


Fig. 3. Causes and effects of large triacylglycerol (TAG)-rich VLDL. Potential atherogenic properties of large TAG-rich VLDL; LPL, lipoprotein lipase (EC 3.1.1.34; PAI-1, plasminogen-activator inhibitor; NEFA, non-esterified fatty acids.

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