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Triglycerides and disease

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Introduction

The human body is an engine converting chemical energy from food into mechanical work and heat, whilst also storing reserves for replacement and repair of cells, tissues and organs. When studying the numerous pathways and enzymes of intermediary metabolism, one little thinks how useful they are in clarifying clinical events.

With increasing specialization in medicine, the metabolic physician is fast taking the place of the old style general internal medicine physician. Metabolic medicine is concerned with understanding and treating abnormalities or changes in biochemical reactions in any part or system of the body which result in malfunction or disease in patients of any age, from the neonate to the pensioner.

Although many metabolic diseases arise from genetic causes, environmental changes also affect intermediary metabolism. Thus the metabolic physician must understand the link between cellular and biochemical events, genetics and molecular biology. The field of metabolic medicine covers the whole spectrum of disease and, unless all physicians and paediatricians attempt to understand abnormal metabolism and its relationship with cell and molecular biology, modern medicine may pass them by.

The recent 'Health of the Nation' document has emphasized one of the many metabolic problems in the community, namely the causation and prediction of coronary artery disease. Recent advances in our understanding of lipid metabolism have established a link between cholesterol and coronary artery disease. The subject of this review is concerned with a less well-defined area, namely the importance and role of triacylglycerols (triglycerides (Tg)) in vascular disease. Why they are important and when should they be measured?

During the last 30 years there has been much progress in identifying risk factors for ischaemic heart disease (IHD). In the 1960s physicians began endorsing the cessation of smoking as a preventative measure against both IHD and lung cancer. In the 1970s the United States National High Blood Pressure Education Program led a campaign against hypertension, setting guidelines for diagnosis and management. In the 1980s the focus shifted to the diagnosis and management of hypercholesterolaemia. The identification and appropriate management of these three risk factors are widely believed to have contributed to the declining cardiovascular mortality in the Western world (for example, in the United States the annual rate of cardiovascular mortality dropped by approximately 25% from 1968 to 1987). However, cardiovascular disease is still the largest cause of death in many of the industrialized nations and the US Department of Health Statistics predicts that one million US citizens will have died of IHD in 1991.[1]

Among other factors now being recognized as contributing to cardiovascular risk and vascular disease is hypertriglyceridaemia. However, the association between triacylglycerol (Tg) levels and IHD is not straightforward and controversy still exists as to the significance of this association. The evidence for a link between increased plasma Tg and IHD is not as strong as that linking increased low density lipoprotein (LDL) concentrations and IHD; There is no direct relationship between plasma Tg concentration and cardiovascular risk, even in the pure genetic trait (familial triglyceridaemia and very high plasma Tg levels).

Epidemiology

1. Ischaemic heart disease and triglycerides

Since the 1950s, an association between increased plasma Tg and myocardial infarction has been noted in both case control and cross-sectional studies.[2–17] However, in only three of these studies[12,15,17] did the association between Tg and IHD remain significant after controlling for all

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other lipid variables. Many cross-sectional studies have studied hypertriglyceridaemia as a risk factor using angiographic determination of IHD[18-32] and all but three showed an association between the hypertriglyceridaemia and disease severity.[29-31] However, these types of studies do not determine whether an increased plasma Tg precedes development of IHD. This can only be ascertained by prospective studies.

2. Thrombogenesis and hypertriglyceridaemia

i. Factor VII coagulant activity Epidemiological, clinical and experimental studies suggest that hypertriglyceridaemia predisposes an individual to thrombosis. How do Tg-rich lipoproteins relate to thrombogenesis? Do they reduce fibrinolysis? In middle-aged men, factor VII coagulant activity (FVIIc) is associated with an increased risk of development of IHD and both serum cholesterol and Tg concentrations correlate positively with FVIIc.[33,34] FVIIc activity increases in subjects fed diets enriched with fat, the character of the rapid response suggests that the post-prandial lipaemia[35] attributes factor VII.[36,37]

Factor VII can exist in several forms and interacts with phospholipid complexes in the plasma.[38,39] However, the precise molecular mechanism of its association with Tg-rich lipoproteins is still unclear, although it has been suggested that negatively charged Tg-rich lipoproteins may activate the intrinsic coagulation pathway, and thereby factor VII, by activating factor XII.[40]

ii. Fibrinolysis Circulating plasminogen, the inactive precursor of plasmin, is activated by the resultant biological activity of a complex formed by tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI). The former, tPA, is a serine protease produced by endothelial cells which converts plasminogen to the highly active fibrinolytic protease, plasmin. The biological activity of tPA is modulated by PAI in the formation of a tPA/PAI complex. An imbalance in the relative amounts of tPA or PAI can affect the formation of this complex which has recently been shown to bind to a lipoprotein-related receptor[41,42] and may lead to a procoagulant (thrombogenic) state.

Decreased fibrinolysis has also been observed in association with smoking, hyperinsulinaemia, diabetes and obesity. This decrease is attributable to an increase in PAI-I activity.[15] The molecular mechanism responsible for the correlation between decreased fibrinolysis and plasma Tg concentrations[15,43] is as yet unknown although Tg-rich very low density lipoprotein (VLDL) increases PAI release from both endothelial and Hep G2 cells.[40]

Physiology and pathophysiology of triglycerol-rich lipoprotein metabolism

Plasma Tg measurements are the sum of Tg contained within the various lipoprotein particles in varying amounts. After absorption from the intestine, dietary fat is packaged into large Tg-rich chylomicron particles which contain a number of surface apolipoproteins A-I, A-IV and B-48. After secretion to blood, chylomicrons acquire apo-Cs and apo-E from high density lipoprotein (HDL). Lipoprotein lipase, an enzyme located on the endothelium of capillaries and adipocytes, acts on the chylomicron particle and hydrolyses Tg to nonesterified fatty acids (NEFA) and glycerol. Lipoprotein lipase is synthesized by a variety of cell types, mainly adipocytes and skeletal muscle cells but also by cells of the lungs, spleen and liver[44] and is then transferred to the endothelium where it binds to glycan chains of heparan sulphate proteoglycans. This locates the enzyme in the outer part of the glycocalyx where it can interact with the large lipoprotein particles. The action of lipoprotein lipase progressively shrinks the chylomicron particles in size and the remnant particle is often then cleared by the liver.

A new lipoprotein particle is then synthesized, assembled and secreted by the liver. This particle is a VLDL particle, containing cholesterol, Tg and apoprotein.[45,46] VLDL particles are hydrolysed by lipoprotein lipase in the same way as the chylomicron particles. Studies of human lipase deficiency syndromes have confirmed that the lipolysis of large triglyceride-rich VLDL to smaller remnants depends on this activity of lipoprotein lipase.

In contrast, the conversion of intermediate density lipoprotein (IDL) to LDL involves the action of hepatic lipase, located on the plasma membrane of liver parenchymal cells. Individuals with increased lipoprotein lipase activity (for example, young women or athletes) tend to have low VLDL-Tg concentrations and high HDL (particularly HDL_2). Conversely, individuals with greater hepatic lipase activity (men and individuals taking anabolic steroids) have low HDL levels.[47,48] In individuals deficient in hepatic lipase,[49] accumulate IDL and HDL_2 particles. The HDL_2 particles are large and Tg-rich, reflecting their continuous acquisition of Tg from VLDL in exchange for cholesteryl ester. This exchange is mediated by cholesteryl ester transfer protein (CETP) whose effects are normally balanced by the action of hepatic lipase which catalyses the hydrolysis of HDL_2 to the smaller and denser lipid-poor HDL_3 particle.[50] Individuals who lack either the CETP gene or are completely deficient in Tg-rich particles (that is, abetalipoproteinaemia) accumulate cholesteryl ester-enriched HDL_2 and larger apo-E rich HDL_1 particles. These HDL_1 particles have a

much higher affinity than LDL for binding to the LDL receptor and are likely to contribute to atherogenesis.

VLDL metabolized by the liver varies in size and composition depending on the relative proportions of lipid and lipoproteins contained within it. Large Tg-rich VLDL are converted to remnant particles and cleared directly from the circulation without contributing to the LDL pool. However, smaller (denser) cholesterol-enriched VLDL are rapidly converted to LDL and are main contributors to the circulating LDL pool.[47] In familial hypercholesterolaemia and in type III (remnant) hyperlipidaemia, abnormalities of metabolism of Tg-rich lipoproteins have been identified. In classical familial hypercholesterolaemia the LDL receptor is abnormal, whereas in the latter the apo-E lipoprotein is abnormal resulting in defective binding to its receptor. In both these conditions there is an accumulation of remnants rich is cholesteryl ester and the conversion of IDL to LDL is inhibited. Thus both conditions are associated with atherogenesis and an increased risk of development of IHD.

Role of Tg-rich lipoproteins in post-prandial hypertriglyceridaemia and pathogenesis of IHD

The mechanism explaining the atherogenic potential of Tg-rich lipoproteins is still not entirely clear, although the link between Tg-rich and HDL concentrations has been clearly documented in the post-prandial state. Plasma Tg concentrations are strongly influenced by plasma HDL concentrations (see below)[51] and patients with low HDL concentrations may have an exaggerated post-prandial lipaemia.[52]

Tg-rich lipoproteins may contribute to the increased risk of IHD in several indirect ways. Tg-rich particles are the only naturally occurring plasma lipoproteins that can induce cholesteryl ester accumulation in cultured macrophages,[53] being able to bind to macrophage receptors which recognize and bind peroxidized lipoproteins.[54] Stimulated macrophages from hypertriglyceridaemic subjects release greater quantities of superoxide than stimulated macrophages from control subjects.[55] In addition, the CETP is more active in individuals with sustained hypertriglyceridaemia. When Tg-rich lipoproteins are present in excess. CETP promotes the exchange of Tg to HDL and cholesteryl ester to VLDL.[56,57] These VLDL particles enriched with cholesteryl ester may then promote the delivery of cholesteryl ester to macrophages, while Tg-enriched HDL is a good substrate for hepatic lipase. Hydrolysis of Tg produces smaller HDL particles which are more rapidly cleared from circulation. The overall effect is an increase in cholesteryl ester in macrophages and a lower level of circulating HDL.[47] Hypertriglyceridaemia also results in smaller dense LDL particles and studies have indicated that these particles are also associated with an increased risk of IHD.[48] Furthermore, the combination of moderate hypertriglyceridaemia, low HDL and small dense LDL particles appears to increase the risk of the development of IHD.[58]

The importance of the generation of post-prandial Tg-rich lipoproteins and their relevance to the development of premature atherosclerosis is still unresolved. Large Tg-rich chylomicrons are unable to penetrate the healthy arterial cell wall, however, chylomicron and VLDL remnants, smaller and containing a higher proportion of cholesteryl esters, are therefore more likely to participate in the atherogenic process. Furthermore, it has been suggested that an exaggerated post-prandial lipaemia may induce uptake of Tg-rich lipoprotein remnants[59] and also cholesterol esters by arterial cells.[60-63]

HDL particles are secreted as disc-shaped precursors devoid of cholesteryl esters. The action of an enzyme, lecithin cholesterol acyltransferase (LCAT) esterifies cholesterol and transforms the HDL into a spherical-shaped particle. There are two major subfractions of mature HDL; a smaller denser HDL_3 and a larger more lipid-rich HDL_2 particle. The concentration of HDL_3 remains fairly constant whilst the concentration of HDL_2 varies considerably between individuals, and inversely with the level of post-prandial hyperlipidaemia.[51] Individuals with high lipoprotein lipase activity consequently have more HDL_2 because of the transfer of Tg from VLDL. Individuals with high levels of HDL_2 may be less at risk of developing IHD because they can rapidly clear Tg-rich lipoproteins from their circulation.[64] Conversely, individuals with low lipoprotein lipase activity have less HDL_2 and more HDL_3.

Fish oils, non-esterified fatty acids (NEFA) and their relationship to plasma Tg abnormalities

Increased concentrations of dietary saturated NEFA have been positively correlated with the development of IHD.[65-67] Populations whose diet is rich in polyunsaturated fatty acid (PUFA) are less prone to IHD[68-70] and have lower concentrations of LDL cholesterol. Increased plasma LDL cholesterol and more recently Tg[71,72] have been suggested as independent risk factors in the development of IHD. *In vivo* studies have shown that NEFAs are important regulators of plasma lipids[73] and *in vitro* studies have indicated that they regulate lipid and lipoprotein synthesis and secretion.[74-79] But so far there has not been a definitive prospective trial examining their effects on the development and progression of IHD.

Dietary fat contains NEFAs of different acyl chain length and degree of branching. They vary in number and position of unsaturated double bonds (in *cis* or *trans* conformation) and the *cis* form is more frequently found in the diet. The nomenclature for unsaturated fatty acids is confusing and unfortunately there are several systems. The position of the first double bond from the terminal methyl carbon (*n* or omega carbon atom) is often used to specify the type of fatty acid. Oleic acid, which is present in high concentrations in avocado pears and olive oil is termed an *n*-9 or omega-9 fatty acid, with a total of 18 carbon atoms and a single double bond between carbon atom number 9 and number 10.

Fish oils are unique fats in the human diet because they are a rich source of *n*-3 NEFAs characterized by the presence of a double bond three carbon atoms from the terminal methyl group. Two of these NEFAs, eicosapentanoic acid (EPA, C20:5) and decosahexanoic acid (DHA, C22:6) are present in high concentrations in fish, which eat the algae and phytoplankton that synthesize them.[80] EPA can serve as a substrate for cyclo-oxygenase and lipoxygenases, the enzymes that initiate the synthesis of prostaglandins, thromboxanes, prostacyclins and leukotrienes. It can also inhibit synthesis of leukotriene and thromboxane and a different series of prostaglandins by the vascular endothelium. The overall effect of these changes is to reduce platelet aggregation and to promote the vasodilator effect of prostacyclin.[80,81] All *n*-3 NEFAs (from linolenic C 18:3) to DHA (C22:6)) can be elongated and desaturated or converted back to EPA[82] but this pathway is thought to be of minor biological significance in humans.[81]

Relevance to diet

Diets rich in saturated fat increase plasma LDL cholesterol concentrations,[83] but the effects of dietary monounsaturated fatty acids are less well understood. Thus increase in the concentrations of Tgs, (VLDL)-TG, (LDL)-TG, and LDL cholesterol[84] occur but further clinical studies are needed to define the mechanism by which they do so. Both high carbohydrate and modified fat diets may lower LDL cholesterol, but high carbohydrate diets have a number of disadvantages: they are less palatable, lower the plasma HDL concentration and may impair glycaemic control in certain individuals.[85] Since high carbohydrate diets are no more effective at lowering LDL cholesterol than a modified fat diet,[86] the latter is preferred and has fewer potential side effects.

Polyunsaturated oils (such as corn and sunflower oils) have traditionally been advocated as preferable alteranatives to saturated fat, because studies have shown that increased dietary intake of linoleic acid at the expense of saturated fat will lower LDL cholesterol, but some studies also show a reduction of HDL cholesterol.[85]

The *n*-3 polyunsaturated fatty acids differ from linoleic acid in their effects on plasma lipids. There is widespread agreement that EPA and DHA reduce plasma concentrations of Tg and VLDL-Tg, with the most marked effect in subjects with high initial concentrations.[82] Studies of healthy volunteers did not show a significant change in total or LDL cholesterol with a moderate consumption of fish oil but a large consumption did reduce both synthesis of LDL cholesterol and apo-B.[87] Fish oil supplementation in patients with non-insulin-dependent diabetes mellitus[88] and impaired glucose tolerance (IGT)[89] has reduced Tg in both groups but increased apo-B in the diabetic subjects. In other studies there have been no changes in apo-B concentrations.[81]

Several animal and *in vitro* studies of perfused rat livers, rat hepatocytes, Hep G2 and CaCo-2 cells have investigated potential mechanisms of the role of unsaturated fatty acid. The results are confusing with some studies showing an inhibition of Tg synthesis and secretion,[90-93] others an inhibition of secretion alone[94,95] and yet others a stimulation of synthesis and secretion.[96]

It is difficult to reconcile all these data. A particular inconsistency is in rationalizing a reduced rate of synthesis of VLDL with an unchanged or even increased LDL concentration. The latter may be due to either a faster synthesis of LDL or its slower removal. Decreased LDL fractional clearance rates and decreased numbers of LDL receptors have been reported in animals treated with EPA.[82] In addition, LDL binding to Hep G2 cells was inhibited by preincubation with EPA,[97] however, preincubation with linoleic acid also reduced binding and is known to lower LDL concentrations. Thus the significance of reduced LDL binding by EPA is questionable. Further research is obviously needed to evaluate the mechanisms by which EPA regulates lipid and lipoprotein metabolism.

Can EPA modulate the atherogenic potential of the LDL particle? When a diet supplemented with fish oil was fed to pigs for 6 months, the total cholesterol was reduced by 30%, as was atherogenesis (measured by morphometric criteria, including size and number of the lesions and number of monocytes attached to a lesion in the endothelium) in comparison with those pigs given corn oil.[98] Greater fluidity of the lipid contained within the lipoprotein particle has also been associated with reduced atherogenesis.[82,99] Therefore, evidence is emerging that fish oil may change the properties of the LDL particle itself and

thus prevent atherogenesis. A typical western diet is rich in linolenic acid [18:2 (*n*-6)] and this competes for the desaturase that catalyses the transformation from linolenic acid to EPA.[100] Studies of the effects of linolenic acid on plasma lipids have shown that it did not lower Tg but increased phospholipid EPA and DHA levels.[82]

Very little is known about the effects of *n*-3 NEFAs on HDL metabolism. HDL cholesterol concentrations have tended to rise with fish oil supplementation.[82] The mechanism is as yet unexplained, which is not surprising given the inverse relationship between plasma Tg and HDL concentrations.[101]

Aetiology of hypertriglyceridaemia

Hypertriglyceridaemia is present when plasma Tg concentrations exceed 2.3 mmol/l in subjects below the age of 20 years. Hypertriglyceridaemia may arise from a primary genetic abnormality or a number of polygenic interactions, or is acquired secondary to another metabolic disorder (for example, diabetes mellitus, obesity, alcoholism). This is an excellent example of metabolic abnormalities causing common problems in clinical medicine.

1. Genetic

Genetic abnormalities in the metabolism of Tg may affect chylomicron and VLDL assembly, hepatic lipase, lipoprotein lipase and its co-factor apolipoprotein C-II, and apolipoprotein E (apo-E).[102] Apo-E is the ligand for the LDL receptor-related protein (LRP). The apo-E gene is present in three common alleles termed E2, E3 and E4 (Table I). Genetic abnormalities of hepatic lipase result in an accumulation of remnant particles and large Tg-rich HDL particles, and abnormalities of lipoprotein lipase or apolipoprotein C-II result in defective hydrolysis of Tg-rich lipoproteins, severe hypertriglyceridaemia and an increased risk of pancreatitis. It has also been shown that subjects with endogenous hypertriglyceridaemia of unknown aetiology have increased peripheral insulin resistance and normal hepatic insulin sensitivity.[103]

2. Acquired (secondary hypertriglyceridaemia)

A wide range of metabolic, hormonal and nutritional factors, together with drugs and disease states may secondarily affect Tg metabolism (Table II).

Obesity In subjects with obesity there is increased VLDL and apo-B production[104] and hypertriglyceridaemia. The increased risk of IHD appears to be greater with abdominal than with gluteo-femoral obesity.[105] A number of specific nutrients (such as fructose and alcohol) may affect Tg metabolism.[104] Fructose may induce hypertriglyceridaemia due to insulin resistance which develops after fructose feeding and the alteration of the composition of VLDL which occurs reducing removal of VLDL remnants. Alcohol may increase the rate of lipogenesis, decrease NEFA oxidation, decrease lipoprotein lipase and increase fatty acid esterification. It has been suggested that alcohol consumption in women may affect cardiovascular risk factors less than in men.[106]

Lipoatrophy This is a rare metabolic disorder characterized by absence of adipose tissue. It frequently affects a specific area of the body, usually along a dermatomal distribution; when generalized, it is frequently accompanied by hypertriglyceridaemia. The basis for this is not clear and may reflect a loss of adipose tissue lipoprotein lipase and an increase in VLDL production associated with increased NEFAs. Many patients with hypertriglyceridaemia have gout and vice versa, but the precise explanation for this association is uncertain.

Other common diseases In diabetes the risk of IHD is increased 2–3-fold over age- and sex-matched controls[107] and major contributions to this increased risk are the associated lipid and lipoprotein abnormalities.[108] The most frequent lipid abnormality in both insulin-dependent (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) is a raised plasma Tg.[109] IDDM is primarily a condition of insulin deficiency whereas the insulin

Table I Apo E alleles

1.	Apo E_2 – defective in binding to LDL-receptor-related protein, with subsequent impairment of removal of remnant particles and increased risk of IHD
2.	Apo E_2 heterozygotes (E_2/E_3) – increase in atherogenic remnants is balanced by decrease in LDL. Thus no increased IHD risk
3.	Apo E_2/E_2 phenotype acting with a secondary disorder to produce a remnant hyperlipidaemia (for example, as in diabetes mellitus, hypothyroidism, renal disease)

Table II Secondary causes of hypertriglyceridaemia

Diabetes
Obesity
Lipoatrophy
Hyperuricaemia
Type I glycogen storage disease

status of NIDDM remains controversial.[110,111] In NIDDM there are also elevations of plasma glucose and NEFA[112-114] both of which are important regulators of plasma Tg in diabetes mellitus.[115]

Renal disease Different forms may also cause hypertriglyceridaemia[104] or hypercholesterolaemia (for example, nephrotic syndrome). The precise mechanism for these different lipid changes is uncertain but production of VLDL is increased and catabolism may be decreased. In renal failure, the commonest lipid abnormality is hypertriglyceridaemia due to an accumulation of remnant particles associated with decreased catabolism. The reason for this is again unclear but it may be associated with a circulating inhibitor of lipoprotein lipase present in uraemic patients.

Paraproteinaemia Hypertriglyceridaemia may be associated with systemic lupus erythematosus (SLE) and multiple myeloma, where there is reduced removal of remnant particles, perhaps due to a herapin-binding protein affecting lipoprotein lipase activity.

Hormones Thyroxine and triiodothyronine exert effects at a variety of levels,[73] by increasing the rate of VLDL catabolism through an effect on lipoprotein lipase and on the LDL receptor-mediated uptake of lipoproteins. In hypothyroidism, the removal of VLDL remnants may be impaired because of a reduction in hepatic lipase, and subjects with apo E2/E2 phenotype and hypothyroidism may develop remnant (type III) hyperlipidaemia (see p. 683) (Table I). The effects of thyroxine on hepatic lipid and lipoprotein metabolism are not clear. It is known that VLDL secretion from hepatocytes derived from hyperthyroid animals *in vitro* is decreased but this has not been confirmed *in vivo*. This discrepancy has been attributed to an increased utilization of plasma NEFAs *in vivo*.[116]

Sex hormones including oestrogen, progesterone and testosterone may also affect Tg metabolism. Oestrogens reduce the effect of lipoprotein lipase and increase the synthesis of VLDL-Tg and may also induce Type III hyperlipidaemia in association with the apo E2/E2 phenotype similar to hypothyroidism. Progesterone decreases plasma Tg and testosterone increases the activity of hepatic lipase.

Dexamethasone increases Tg secretion and does not alter Tg synthesis.[117,118] Glucagon inhibits VLDL-Tg secretion without affecting synthesis and parallels the rapid inhibitory effect of glucagon on *de novo* fatty acid synthesis.[116] Short-term treatment of an isolated rat hepatocyte preparation with adrenaline and noradrenaline inhibits Tg secretion.[119]

Insulin

The concentration of Tg in plasma from fasted non-diabetic subjects correlates positively with that of insulin[43,120-122] which is a potent stimulator of both fatty acid[73] and Tg synthesis.[74,123,124]

The net effect of insulin on Tg appears to be governed by opposing forces. Thus, insulin inhibits adipose tissue lipolysis, decreases the NEFA flux into the plasma and also activates adipose tissue lipoprotein lipase: all these actions favour a decline in plasma Tg. Opposing this is the stimulatory effect of insulin on lipogenesis, esterification of fatty acid to form Tg and coupling of Tg to apolipoprotein-B.[73]

In vitro studies to date have not greatly clarified the metabolic interactions by which insulin, glucose and NEFAs control plasma Tg concentration. Short-term incubation (<24 hour) with rat hepatocytes and Hep G2 cells show that insulin inhibits Tg secretion,[76,117,123,125-128] stimulates Tg synthesis[74,123,124] and increases hepatocellular storage in a cytosolic pool.[129] Hepatocytes from streptozotocin-diabetic rats maintained in culture for up to 3 days show decreased VLDL secretion compared with hepatocytes from control rats. Addition of insulin further decreased VLDL secretion.[130] The effect of insulin on Tg secretion in rat hepatocytes has been thought to be biphasic during a 72 hour incubation, with inhibition of Tg secretion during the first 48 hours and stimulation during the remaining 24 hours.[131,132] The effect of insulin on Hep G2 cells is not biphasic and inhibition of triglyceride and apo-B secretion could only be demonstrated over 72 hours.[133,134]

There is increasing evidence that the rate of Tg synthesis is controlled by coordinate regulation of the activities of phosphatidate phosphohydrolase (PAP) and diacylglycerol acyltransferase (DGAT) and by the availability of their substrates. PAP and DGAT are key regulatory enzymes in Tg production and the activity of both enzymes has been shown to increase in the presence of oleate.[135,136] Furthermore the activity of PAP is increased in diabetes.[137] Tg is stored within the hepatocyte and that required for VLDL assembly and secretion is derived from the intracellular pool rather than a change in the rate of *de novo* fatty acid synthesis.[129,138]

Inborn errors of metabolism

Type I glycogen storage disease is characterized by a deficiency of glucose-6-phosphatase. Patients with this condition are prone to attacks of hypoglycaemia with a compensatory increase in NEFAs and subsequently VLDL synthesis. This is due to an increase in glycolysis (due to the deficiency of glucose-6-phosphatase) with a subsequent increase in malonyl CoA and increased fatty acid synthesis.

Malonyl CoA also inhibits palmitoyl carnitine transferase and therefore fatty acid oxidation is reduced and esterification is increased with subsequent increased VLDL synthesis.[104]

Drugs

A variety of drugs may induce or affect circulating Tgs.[104] Oestrogens and anti-hypertensives are the common offenders. Thiazide diuretics and non-selective β blockers commonly cause hypertriglyceridaemia.

Chloroquine and the calcium antagonist, verapamil, an inhibitor of voltage-dependent calcium channels; and trace metals such as lanthanum, nickel, cobalt and manganese also inhibit VLDL-Tg by 55–95%.[139,140] However, selenium has been shown to increase VLDL secretion in the isolated perfused rat liver by decreasing fatty acid oxidation and increasing fatty acid esterification.[141]

Hypertriglyceridaemia associated with diabetes mellitus

Of all the diseases associated with hypertriglyceridaemia, the risk of developing ischaemic heart disease (IHD) is increased 2–3-fold in subjects with diabetes mellitus.[71,107] IHD is the principal cause of morbidity and mortality in NIDDM.[142,143] Dyslipidaemia is also one of the main risk factors for the macrovascular complications of diabetes.

Hyperglycaemia is a risk factor for microvascular complications but not for macrovascular complications of diabetes. The WHO multinational study found no relationship between either plasma glucose concentration or diabetes duration and major Q wave abnormalities on electrocardiography. The prospective Whitehall study also failed to find a significant relationship between glucose intolerance and macrovascular complications.[144]

Hypertriglyceridaemia and non-insulin-dependent diabetes

1. NIDDM

The most frequently observed lipid abnormality in NIDDM is a raised plasma Tg which occurs in 25–30% of subjects.[145] The Framingham and Paris prospective studies[71] have suggested that Tg might be an independent cardiovascular risk factor in the normal population and in diabetic subjects (although the plasma HDL values were not reported from the Paris study[11a]).

Impaired glucose tolerance (IGT), a major risk factor for diabetes mellitus, may be associated with an increased risk of IHD.[146] In the US 11.2% of citizens between the ages of 20 and 74 years have IGT compared with 6.6% with diabetes.[82] The Second National Health and Nutrition Examination Survey (NHANES II) has examined the prevalence of age-standardized risk factors in subjects with IGT, diagnosed and undiagnosed diabetes compared with matched controls.[82] Of the cardiovascular risk factors studied, there was a higher percentage with angina (identified by clinical history) in subjects with IGT compared with normal controls and diabetics.

A recent study has suggested that there is a genetically inherited predisposition to abnormal plasma lipids and insulin in the offspring of subjects with NIDDM.[147] Furthermore, the normal offspring of an hypertensive parent tend to have impaired insulin-mediated glucose disposal, hyperinsulinaemia and abnormal lipids[148] and it has been suggested that the offspring of subjects with IGT have increased plasma insulin and Tg concentrations.[149] Genetic variation at the apo-B and apo-AI-CII-AIV loci has also been shown to contribute to the development of NIDDM in individuals who are overweight.[150] This is an area where there has been little research to date and more is needed to determine whether there is genetic linkage between an inherited predisposition towards NIDDM and abnormalities of plasma lipids.

In NIDDM there may be a 50–100% increase in plasma Tg; measurements greater than this are usually attributable to inherited genetic defects of lipoprotein metabolism which have been exacerbated by hyperglycaemia.[109,145,151,152] There is general agreement that VLDL production is increased[153–155] but there is disagreement as to the mechanism of this increase. The major problems which need to be resolved are whether subjects with NIDDM are hyperinsulinaemic (which has been the consensus view until recently) or whether they are insulin deficient.[110,111,156] Since commercially available insulin assays cross-react with proinsulin, they measure both insulin and proinsulin as insulin. Further developments in insulin assay technology now allow the detection of insulin in the presence of proinsulin and proinsulin-like molecules.[157] This may not be important in normal individuals but is important in NIDDM where plasma proinsulin and 32–33 proinsulin are increased and contribute significantly to the insulin measurement.[110] Recently it has been shown that subjects with IGT have increased plasma proinsulins and it would be important to determine whether they may also be insulin deficient.[158]

2. Mechanism of triglyceridaemia in NIDDM

Increased plasma NEFA concentrations are recognized as important in the development of lipoprotein abnormalities in diabetes[115] and may have a

central role in causing the increase in plasma Tg. In diabetes, plasma NEFA concentrations are increased due to the release from inhibition of hormone-sensitive Tg lipase in adipose tissue secondary to relative insulin deficiency.[159] In subjects with NIDDM there is impairment of suppression of hepatic glucose production by insulin. This has been attributed to impairment of glucose utilization and increased NEFA oxidation and it has been suggested that this may be a mechanism to explain the pathogenesis of insulin resistance.[160]

Clofibrate improves glucose tolerance without affecting insulin concentration suggesting that it acts by reducing plasma NEFA concentration, with a subsequent improvement in insulin sensitivity.[161] Similarly, a different lipid lowering agent, nicotinic acid, may lower plasma Tg by reducing plasma NEFA.[162,163] To date clofibrate is the only therapeutic agent effective in lowering plasma Lp(a) concentrations,[164] although whether it does this by plasma NEFA concentrations or through an effect on apo-B synthesis, is not known. The hypoglycaemic agent metformin also lowers VLDL-Tg and VLDL-apo-B but again the mechanism is uncertain.[165]

It has therefore been suggested that two pathways may lead to hypertriglyceridaemia in subjects with NIDDM. In both, the role of insulin in increasing glucose uptake is central to the hypothesis. Subjects with severe fasting hyperglycaemia attributable to an inadequate insulin response have increased plasma NEFA concentrations due to inadequate suppression of plasma NEFA by insulin.[115] It is also possible that it is the increased plasma NEFA concentration in conjunction with a normal plasma insulin concentration which produces the increase in plasma Tg. Conversely, subjects with mild or moderate hyperglycaemia and an increased insulin response have normal plasma NEFA concentrations in the presence of hyperinsulinaemia.[166] In the latter, the hyperinsulinaemia *per se* is responsible for the increase in plasma Tg. It is clear that there are still a number of questions concerning the role of insulin in regulation of plasma Tg concentrations which still need to be answered.

The composition of the VLDL particle is altered in NIDDM; VLDL apo-B production is increased, but it is also possible that this is due to obesity[167] which may be an important factor in NIDDM.[168]

To date the consensus view is that lipoprotein lipase activity is decreased in NIDDM,[109] which would concur with the decreased clearance of VLDL remnants in NIDDM. However, inconsistencies remain to be explained, since hyperinsulinaemia occurs in NIDDM, and insulin activates rather than reduces lipoprotein lipase.

The recent European Consensus on NIDDM stated that in view of the importance of hyperlipidaemia as risk factors in the development of macrovascular disease, total cholesterol, HDL cholesterol and triglyceride should be checked annually and if the results were abnormal and therapeutic intervention appropriate, values should be checked every 3 months.[169]

3. *Role of hyperinsulinaemia and IHD*

Resolution of these problems is essential when considering the effects of insulin on the genesis of lipid abnormalities in NIDDM. Is the hyperinsulinaemia a genuine finding in NIDDM or is it an artefact arising from measurements obtained with the insulin assay?

In vitro studies suggest that insulin inhibits Tg secretion in short-term hepatocyte incubations (<24 hours) and it is therefore unlikely that insulin *per se* causes the increase in plasma Tg observed in NIDDM. However, it is possible that there is a biphasic response, with insulin inhibiting hepatic Tg secretion in short-term incubations but stimulating Tg secretion in the final 24 hours of a 72 hour incubation.[131,132] Obviously the role of insulin in relation to plasma Tg abnormalities still needs to be defined, an important fact for clinicians looking after diabetic patients.

There is some evidence for a role of hyperinsulinaemia *per se* as a cardiovascular risk factor.[170–173] Increased plasma insulin concentrations after myocardial infarction were described as early as 1965[174] and subsequent prospective studies have suggested that serum insulin may be an independent cardiovascular risk factor.[143] Although the recent 15-year follow-up of the Paris prospective study reported that plasma insulin and glucose were not predictors of coronary heart disease mortality,[175] it is suggested that insulin sensitivity, glucose intolerance, blood pressure, body fat mass and its distribution, and serum lipids are a network of interrelated functions and all are associated with an increased incidence of IHD.[176] These authors suggest it is the increased plasma insulin occurring as part of this insulin insensitivity that is primarily responsible for the increased plasma Tg. Subjects with NIDDM treated with insulin show a decrease in plasma Tg[177–180] and the effect of a single insulin injection is to decrease hepatic Tg secretion.[181] An increase in central adiposity is associated with a deterioration of insulin sensitivity and adverse changes in blood pressure and plasma lipids.[182–184] Thus it has been suggested that obesity influences plasma lipids more in diabetic than non-diabetic obese controls.[185]

4. *Hypertriglyceridaemia and Syndrome X*

Insulin may have direct effects on the arterial cell wall which may initiate the atherogenic process for

it has been shown that insulin increases LDL binding to bovine smooth muscle cells.[186] Hyperinsulinaemia has also been described in association with microvascular angina where there was no evidence of macroscopic coronary artery disease at angiography.[187] Furthermore, hyperinsulinaemia along with other risk factors and IHD is one of the major features of Syndrome X, first described by Reaven.[115]

With the recent development of sensitive and specific assays for the measurement of proinsulin and intermediates in proinsulin processing, interest has arisen as to whether these molecules may be involved in the atherogenic process. 32–33 split proinsulin (an intermediate in proinsulin conversion to insulin) is known to be increased in subjects with NIDDM.[110] It has also been suggested that this proinsulin form may be an independent cardiovascular risk factor.[188]

Hypertriglyceridaemia and insulin-dependent diabetes

It is well established that increased plasma Tg and VLDL metabolism in IDDM depends on the degree of diabetic control and therefore on insulin levels. In IDDM, acute insulin deficiency (for example, diabetic ketoacidosis) produces several changes in VLDL metabolism.[145] Initially there is a rapid increase in mobilization of NEFAs from adipose tissue resulting in increased VLDL-Tg secretion by the liver. With continuing insulin deficiency, the liver converts NEFAs into ketone bodies and consequently VLDL-Tg secretion is reduced. Lipoprotein lipase activity is reduced due to the insulin deficiency and results in decreased clearance of VLDL remnants by the liver. Treatment of diabetic ketoacidosis with insulin corrects these metabolic abnormalities and reverses the dyslipidaemia.

Changes in other plasma lipoproteins also vary with the extent of hyperglycaemia. LDL is increased and plasma HDL is low in poorly controlled IDDM and corrects to normal with intensive insulin therapy. Hepatic lipase activity is lower and, as with NIDDM, there is a higher HDL_2:HDL_3 ratio.[109]

It seems unlikely that there is a genetically inherited lipid abnormality associated with the development of IDDM. Plasma lipids and lipoproteins in children with IDDM measured during the first 2 years of the disease were abnormal (except for the cholesterol content in HDL and LDL) but all values normalized with treatment.[189]

Subjects with IDDM and albuminuria but otherwise normal renal function have several abnormalities of their plasma lipids and lipoproteins which may increase their risk of developing IHD.[190] Plasma VLDL-Tg, LDL cholesterol and apo-B are increased whereas HDL cholesterol and apo-A1 are reduced compared with IDDM patients without albuminuria.[191–193] The fall in HDL cholesterol is attributed to a reduction in HDL_2[194] and are also present with microalbuminuria (albumin excretion rate 20–200 μg/minute). Recently it has been shown that plasma Lp(a) concentrations are increased.[195,196] It is therefore possible that these adverse changes in plasma lipids and lipoproteins associated with albuminuria may contribute to the increased incidence of macrovascular disease observed with diabetic renal disease. The overall picture is less well understood with NIDDM and microalbuminuria.[197]

Management of hypertriglyceridaemia

Diet and lifestyle

Tg concentrations should always be analysed after an overnight fast and on at least two different occasions prior to diagnosis and treatment.[198] Patients with triglyceride values between 2.3 mmol/l and 4.5 mmol/l are defined as having moderate hypertriglyceridaemia[198–201] and the underlying cause for the hyperlipidaemia should be determined. Primary causes (for example, familial hypertriglyceridaemia or familial combined hyperlipidaemia) or secondary causes (for example, obesity, alcohol consumption, diabetes, renal failure and treatment with β blockers, thiazide diuretics or oestrogen) may be identified. The basis of therapy is mainly by dietary and lifestyle modifications.

If the patient is overweight (increased body mass index), the calorie intake should be reduced to lose weight. The daily fat in the diet should be restricted to no more than 30% of the total calorie intake, with an approximate equal distribution between monosaturated and polyunsaturated fat, and cholesterol intake should be less than 7.75 mmol (300 mg) per day.[198–200]

In familial lipoprotein lipase and apo-B CII deficiency (type 1 hyperlipidaemia), chylomicronaemia is aggravated by dietary fat and is best treated by careful reduction of daily fat intake (15–20% of total calorie intake). This can be supplemented by medium chain triglycerides which are absorbed directly into the portal circulation and do not increase chylomicronaemia. There is controversy concerning the possible beneficial effect of monounsaturated fatty acids (see section on non-esterified fatty acids and their relation to plasma Tg abnormalities, pp. 681–682). Polyunsaturated fatty acids are of benefit in the treatment of hypertriglyceridaemia in patients with and without diabetes and omega-3 fatty acids may be of benefit

since they also reduce platelet aggregation and promote the vasodilatory effect of prostacyclin (see p. 682).[80,81]

Alcohol stimulates the synthesis of Tg in the liver.[202] In patients with defective clearance of Tg-rich lipoproteins, alcohol causes a marked increase in the degree of hypertriglyceridaemia, particularly in obese subjects, and thus alcohol consumption should be reduced (to <25 g/day) or avoided.[198,200]

Drug therapy

Medical treatment of isolated moderate hypertriglyceridaemia may be controversial, although there is clear evidence that Tg-rich lipoproteins are atherogenic and that hypertriglyceridaemia frequently occurs in patients with IHD particularly in association with low HDL concentrations. Attempts to treat the hypertriglyceridaemia with specific drugs should be made in the context of assessment of the increased risk of IHD, and where modifications of diet and lifestyle have failed to correct the abnormal lipid pattern.

To date there have been no primary prevention trials specifically evaluating the effects of reduction of Tg-rich lipoprotein concentrations on the development of IHD. However, cholesterol studies have demonstrated the combined benefit of reductions in cholesterol and Tg concentrations. In the Helsinki Heart Study patients with type IIb hyperlipidaemia treated with gemfibrozil showed the largest reduction in coronary events.[203] Furthermore in two secondary prevention studies, patients treated with nicotinic acid had fewer coronary events and a lower overall mortality than did controls.[204,205] The results reported from the Cholesterol Lowering Atherosclerosis Study (CLAS)[206,207] and the Familial Arteriosclerosis Treatment Study (FATS)[208] show stabilization and/or regression of coronary plaques in patients treated with drugs which lowered LDL cholesterol and Tg and increased HDL cholesterol. Two other studies using coronary angiography, Leiden Diet Trial[209] and the NIH Type II Coronary Intervention Trial[210] showed that the best predictor of atherosclerosis progression was the ratio of total cholesterol to HDL.

There is incomplete information concerning treatment of Tg-rich lipoproteins in the regression or prevention of atheroma and at present the major indication for drug therapy of severe hypertriglyceridaemia to eliminate the risk of pancreatitis.

Fibrates, characterized by an aryloxy group with different substituents, derive from clofibrate. These, despite chemical similarities, differ in some pharmacokinetic behaviour, but all are effective in reducing circulating Tg and VLDL concentrations, and may correct many of the other lipoprotein abnormalities.[201]

The main mechanism of action of fibrates is through increasing catabolism of Tg-rich lipoproteins, usually through an increase in lipoprotein lipase synthesis. Fibrates have also been shown to increase the activities of other enzymes, LCAT (which catalyses the esterification of cholesterol in plasma) and hepatic lipase (which hydrolyses Tg in HDL_2 and IDL particles). The precise mechanism by which fibrates act is uncertain and it has also been shown that gemfibrozil decreased hepatic apo-B mRNA level *in vitro*.[211] Most patients regardless of their lipoprotein phenotype respond to treatment with fibrates with reduction in plasma Tg and an increase in HDL cholesterol.[203] Fibrates are therefore the treatment of choice for primary hypertriglyceridaemia; patients with dyslipoproteinaemia where specific drug therapy (for example, diabetics), diet and lifestyle changes have not succeeded.

Nicotinic acid derivatives are also effective in reducing plasma Tg by decreasing mobilization of NEFA from adipose tissue, but at the cost of unpleasant side effects,[198] which include cutaneous vasodilation, skin rashes, gastrointestinal upsets, hepatic dysfunction, glucose intolerance and hyperuricaemia. Cutaneous vasodilatation is maximal during the first weeks of therapy but thereafter a degree of tolerance develops, especially if the dose is gradually increased. Aspirin given 30 minutes before dosage may help to minimize symptoms of flushing. Analogues, such as Acipimox, have been more effective, particularly in diabetic subjects. Reportedly the associated increase in plasma HDL concentration also leads to a significant reduction in mortality from all causes, including IHD. Nicotinic acid analogues are useful in patients with combined hyperlipidaemia as well as for severe hypertriglyceridaemia.

Omega-3 fatty acids (fish oils)

There is considerable debate as to the therapeutic usage of fish oils in the treatment of hyperlipidaemia. They are most effective at lowering plasma Tg. Plasma LDL and HDL cholesterol may slightly increase, although the effect of LDL does appear to change with the different hyperlipidaemic phenotypes. Subjects with the higher initial plasma Tg concentrations respond with a greater increase of plasma LDL concentration but may alter the composition of the particle so that it is less atherogenic.[99] Thus there are both advantages and disadvantages to their use in patients. The importance of an increased plasma Tg concentration in exacerbating atherosclerosis has been recognized[71,102] but the mechanism by which Tg-rich lipoproteins do so is unknown. One possibility is

that VLDL from hypertriglyceridaemic subjects interacts with LDL receptors, and is known to be toxic to cultured endothelial cells converting murine peritoneal macrophages into foam cells *in vitro*.[82] As fish oil is particularly effective at lowering plasma Tg this is perhaps a significant reason for its use in the treatment of hypertriglyceridaemia. A further group of patients who may benefit from fish oils are those with type V hyperlipidaemia and extremely high plasma Tg concentrations. Untreated, these individuals are at risk of acute pancreatitis.[212] Fish oils are also beneficial in the treatment of secondary hypertriglyceridaemia such as diabetic hypertriglyceridaemia.[81,213]

It has been suggested that fish oils could be given to all patients at increased risk of IHD with increased plasma Tg. There may be other potential benefits, by effects on platelet function, blood pressure, blood flow, inflammatory processes and atherogenesis. This may explain the lower incidence of IHD in Mediterranean populations with an increased dietary fish oil consumption. However, for most patients, added fish oils, other than those taken in a fish diet, should be reserved for very high plasma Tg levels (in excess of 20 mmol/l), since some patients may develop hepatic dysfunction.

HMG CoA reductase inhibitors

Statins act by competitively inhibiting HMG CoA reductase and thereby interfere with the conversion of HMG CoA to mevalonic acid. They are very potent inhibitors of the reductase which is a rate-limiting enzyme in the synthesis of cholesterol. The hepatocyte responds to the decrease in intracellular cholesterol by increasing expression of LDL receptors and therefore promotes internalization of cholesterol from the plasma. Although statins are the treatment of choice in hypercholesterolaemia and may be useful in genetic conditions, high doses may also reduce plasma Tg by approximately 10%[214] but are not the treatment of choice for severe hypertriglyceridaemia. Side effects include mild hepatic dysfunction with rise in plasma transaminases and more rarely, a reversible myositic syndrome in approximately 0.5% of patients. The risk of myositis is greater when these drugs are used in conjunction with either fibrates or nicotinic acid.

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