INFLUENCE OF DIETARY FATTY ACIDS ON THE ATHEROGENIC LIPOPROTEIN PHENOTYPE

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INTRODUCTION

The atherogenic lipoprotein phenotype (ALP) describes a common collection of abnormalities in plasma lipoproteins which confer increased risk of coronary heart disease (CHD) upon normal, healthy individuals. Although its prevalence in Europe is at present unknown, it is predicted that northern Europe will share a similarly high population frequency of the ALP to that seen in north America, where between 30–35% of middle aged men may be affected (Austin *et al.* 1990). As defined in the post-absorptive or fasting state, the ALP is characterized by a moderately raised level of serum triacylglycerol (TG), a low level of high density lipoprotein (HDL) and a predominance of abnormally small, dense low density lipoprotein (LDL) and HDL particles. Importantly, levels of total and LDL cholesterol are typically 'normal' or only moderately raised. With the exception of this latter finding, all these features have been associated with an increased atherogenicity of circulating lipoproteins and a predisposition to increased CHD risk. Despite the predicted prevalence of the ALP in European populations, this dyslipidaemia is not currently recognized by routine clinical procedures, largely because of technical difficulties in the measurement of lipoprotein subclasses and the fact that values for serum TG and cholesterol that are characteristic of an ALP usually fall below clinically defined action limits. Heritability studies reveal that up to 50% of the variability in the expression of an ALP is due to genetic factors, whilst the remaining variability is ascribed to environmental influences, principally diet, smoking and physical inactivity. The relevance of assigning relative contributions to genetic and environmental factors is academic given that in reality the ALP is likely to develop through a complex interaction between dietary factors and specific lipoprotein genes, the expression of which is susceptible to certain dietary stimuli. The atherogenicity of the ALP may well arise from an impaired metabolic capacity to remove TG-rich lipoproteins of dietary origin (chylomicrons (CM) and CM remnants) and very low density lipoprotein (VLDL) of hepatic origin from the circulation, leading to their conversion into small, atherogenic cholesterol-enriched remnants and LDL. An exciting development in this area, that links the metabolism of TG-rich lipoproteins of dietary origin with the ALP, is the recognition of a significant positive relationship between the extent of postprandial lipaemia and fasting TG status. This finding, together with the fact

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that most individuals are in a postprandial state for at least 16 h a day, may provide a coherent mechanistic basis to explain how diet, especially dietary fatty acids, exert through postprandial events a significant influence on an ALP, and thus on lipoprotein mediated CHD risk.

DEFINITION OF THE ALP AND ITS ASSOCIATED CORONARY HEART DISEASE RISK

The consistent finding of an inverse association between serum TG and HDL cholesterol in epidemiological studies (Castelli *et al.* 1977), and the appearance of multiple subpopulations of LDL in hypertriglyceridaemia (Fisher *et al.* 1980) provided an early indication of the interrelationship between TG-rich lipoproteins and cholesterol. Later studies conducted in individuals with combined hyperlipidaemia led to the identification of a clustering of metabolically related lipoprotein abnormalities in TG-rich lipoproteins, LDL and HDL (Austin & Krauss, 1986). The same workers extended these observations to a normal, community based population, within which the 'ALP' was first defined and shown to be related to an increase in risk of CHD (Austin *et al.* 1990). The original characteristics of an ALP are listed in Table 1. More recently, postprandial studies have linked the expression of this phenotype with an exaggerated postprandial lipaemic response, that is, subjects who express an ALP also show a significant increase in the postprandial level of plasma TG and thus TG-rich lipoproteins in response to the ingestion of a fat-containing meal. For this reason many believe that the definition of an ALP should include indices of enhanced postprandial lipaemia (Table 1).

TRIACYLGLYCEROLS AND CORONARY HEART DISEASE RISK

The downgrading of TG as a coronary risk factor in multivariate analyses by correction for its covariates such as HDL has received serious criticism, since the abnormalities in TG and HDL may have a common metabolic basis and cannot therefore be treated as covariates. The controversy surrounding TG and CHD risk has largely been resolved by the demonstration of the value of TG as a 'conditional marker' of CHD risk in both primary and secondary trials of CHD prevention (Manninen *et al.* 1991). Additional support has come from an improved understanding of the pathophysiology of TG. The original hypothesis of Zilversmit (1979) which suggested that atherosclerosis was a postprandial phenomenon has been corroborated by the consistent finding of enhanced postprandial lipaemia in patients with CHD (Patsch *et al.* 1992; Groot *et al.* 1991) and in those at increased risk of disease such as non-insulin dependent diabetics (Lewis *et al.* 1991). Cell culture studies have confirmed that TG-rich lipoproteins and especially CM remnants that are found in abundance in the postprandial phase promote the rapid accumulation of cholesterol within arterial cells (Ellsworth *et al.* 1986). More important within the context of this review is the capacity of moderate increases in fasting TG to exert an indirect influence on atherogenesis through the promotion of structural changes in LDL and HDL that are typically found in an ALP.

LOW DENSITY LIPOPROTEIN SUBCLASSES

The predominance of small dense LDL, described by electrophoretic separation as 'LDL subclass pattern B' or as LDL-III on the basis of hydrated density (Fig. 1), has been associated with a three-fold increase in risk of acute myocardial infarction in young men (Austin *et al.* 1988). More recently, an even greater relative risk of CHD has been

Table 1. *Principal characteristics of the atherogenic lipoprotein phenotype*

Parameter	Fasting Plasma Concentrations
Triglycerides	1·5–2·3 mmol/l
Small and dense LDL-III (density 1·044–1·060 g/ml)	> 100 mg total lipoprotein mass/100 ml plasma
HDL-cholesterol	< 1 mmol/l
HDL_2 (large HDL, particle diameter 9·7–12·9 nm)	< 10% total HDL
Enhanced postprandial lipaemia	
Cholesterol ester enriched VLDL- and CM-remnants	
Insulin resistance	

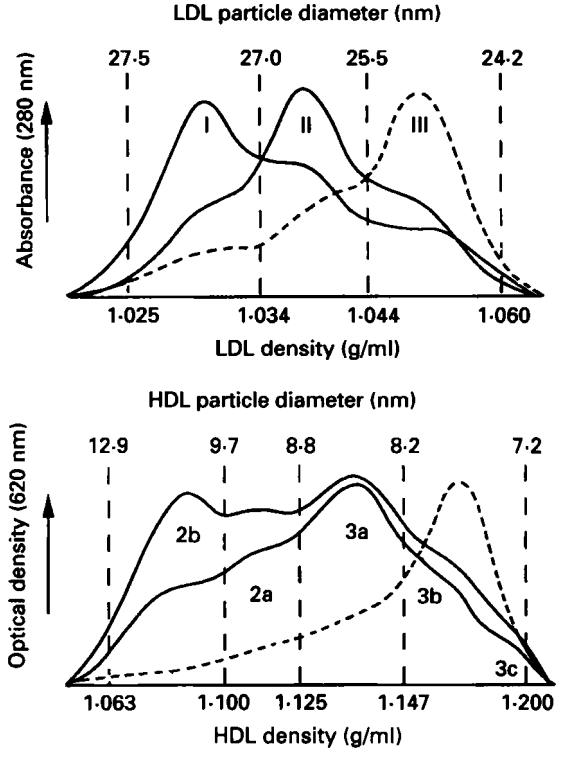

Fig. 1. Plasma LDL and HDL subclasses. Distribution profiles of plasma LDL and HDL subclasses representative of typical normal (non-ALP) —— and ALP ----- subjects, as measured by density gradient ultracentrifugation (particle density) and non-denaturing, polyacrylamide, gradient gel electrophoresis (particle size).

associated with a predominance of small dense LDL (Griffin *et al.* 1994). Several theories have been put forward to explain the increased atherogenicity of small dense LDL. It may be that this LDL subclass has prolonged residence time in the circulation, owing to its slow removal by cellular LDL receptors, thus increasing the time available for its infiltration into the arterial wall. Having crossed into the arterial wall, small dense LDL is believed to be selectively sequestered or 'trapped' in the subendothelial space by components of the extracellular tissue matrix (Camejo *et al.* 1990). Finally, small dense LDL shows an increased susceptibility to oxidative modification *in vitro* (de Graaf *et al.* 1991). If expressed *in vivo*, this property would promote the internalization of LDL into cells associated with

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the atherosclerotic plaque. A predominance of small dense LDL found in clinically defined hypertriglyceridaemia, as opposed to that found in an ALP, is frequently accompanied by a raised concentration of apoprotein B (Durrington, 1989). As each LDL particle is known to contain a single unit of apo B, the concentration of apo B corresponds to the number of LDL particles. A serum level of apo B $> 1{\cdot}3$ mg/ml has been clinically defined as hyperapo-β-lipoproteinaemia (LDL has 'β' migration on agarose electrophoresis) and is associated with increased CHD risk. However, while this condition is associated with small dense LDL it is not a term that should be used synonymously with the ALP. A predominance of small dense LDL-III is not always associated with raised apo B and the ALP may be expressed at much lower levels of TG than that usually found in patients within hyperapo-β-lipoproteinaemia.

HIGH DENSITY LIPOPROTEIN SUBCLASSES

The excretion of cholesterol from the body, whether from peripheral tissues or arterial lesions, can only be achieved by a process of reverse cholesterol transport that is mediated through HDL. This lipoprotein is responsible for the efflux of cholesterol from cells and its transportation back to the liver, either directly or indirectly *via* its transfer to other lipoproteins. Raised levels of large HDL (HDL_{2b+2a}; Fig. 1) have been inversely correlated with CHD risk in a number of case-control studies (Ballantyne *et al.* 1982; Hamsten *et al.* 1986). While this is often mistakenly assumed to reflect the protective role of this subfraction, HDL_2 is probably not the reverse transport vehicle *per se* but represents a cholesterol enriched product of an efficient reverse transport system which utilizes very small HDL particles (pre-β HDL) or even dissociated HDL protein (apo A–I) for cholesterol efflux from cells (Schmitz & Lackner, 1993). Whatever the transport vehicle, it is generally assumed that a low level of HDL, such as that found within an ALP (HDL cholesterol < 1 mmol/l or 380 mg/l) is detrimental in terms of CHD risk, as it represents a compromised pathway for the excretion of cholesterol. It is worth noting that there is, as yet, no firm experimental evidence upon which to base this assumption or the commonly held view that raised HDL levels are cardioprotective.

METABOLIC DETERMINANTS OF AN ALP

INSULIN RESISTANCE AND THE ALP

Our knowledge of the relationship between insulin resistance, lipoproteins and CHD has evolved to the extent that the ALP is now generally considered to be the dyslipidaemia associated with the insulin resistance syndrome. This is in keeping with the role of TG as the major determinant of the ALP, and the findings of several recent studies that have shown direct linkage between indices of insulin resistance and key features of the ALP such as small dense LDL (Reaven *et al.* 1993*a*; Selby *et al.* 1993). Furthermore, about 25% of the normal, non-diabetic population are reported to be affected by insulin resistance (Reaven, 1988). This figure is similar to that reported for the ALP and provides further support that the increased risk of CHD in insulin resistance syndrome is mediated through this dyslipidaemia (Despres & Marette, 1994). A critical review of the effects of insulin on lipid metabolism reveals a major impact of insulin resistance on TG-rich lipoproteins through the failure of this hormone to suppress the activity of hormone sensitive lipase (HSL) and activate lipoprotein lipase (LPL) in adipose tissue (Frayn, 1993). The rate of VLDL production in the liver is partly controlled by the supply of non-esterified fatty acids (NEFA) for TG synthesis. Hence, failure of insulin to perform its normal

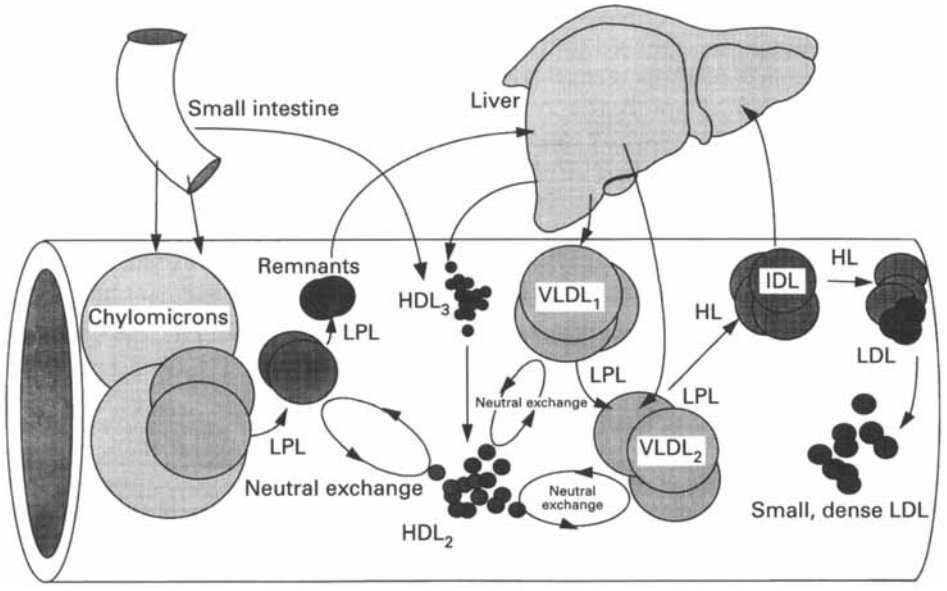

Fig. 2. Lipoprotein transport pathways. Schema showing the inter-relationships between transport pathways of exogenous (dietary), endogenous (hepatic) TG-rich lipoproteins and HDL, and the site of action of lipoprotein lipase (LPL), hepatic lipase (HL) and neutral lipid exchange.

function of suppressing the action of HSL and release of NEFA into the circulation during the postprandial phase will lead to increased synthesis and secretion of VLDL. Insulin also activates LPL during the postprandial phase. As LPL is the rate limiting enzyme for the hydrolysis of TG in CM and VLDL, its impaired activation will result in a reduced capacity to clear TG-rich lipoproteins from the circulation, leading to enhanced postprandial lipaemia and raised fasting TG levels. Within this scenario it is clear that the frequency, rate and extent of entry of exogenous dietary triacylglycerols (as CM) will act as a major perturbation in an already compromised lipid transport system.

TRIACYLGLYCEROLS AND VLDL HETEROGENEITY

Recent studies indicate that even mild elevations in fasting serum TG such as that found in an ALP (TG 1·5–2·3 mmol/l) are significantly associated with a raised concentration of small dense LDL (Griffin *et al.* 1994) and reduced levels of HDL (Castelli, 1994). Serum TG levels in the postabsorptive fasting state reflect levels of circulating VLDL which represent the principal transporter of endogenous TG synthesized in the liver (Fig. 2). The liver produces different forms of VLDL that vary in size and density as a result of their varying TG content. Evidence from cell culture studies suggests that the relative distribution of large and small VLDL particles in serum depends upon the supply and nature of lipid substrates (NEFA) that are made available for TG synthesis in the liver (Patsch *et al.* 1983; Dixon & Ginsberg, 1993). If this were the case, when large amounts of NEFA became available, either from the diet or lipid storage sites, the production of larger TG-rich VLDL ($VLDL_1$ S_f60-400) would be favoured. Conversely, lower NEFA levels would tend to favour the production and secretion of smaller VLDL particles ($VLDL_2$, S_f20-60). The majority of LDL in the circulation is derived from VLDL *via* a delipidation cascade produced by the action of endothelial lipases in the lining of blood vessel walls of the peripheral (LPL) and hepatic circulations (hepatic lipase; HL). Kinetic experiments with

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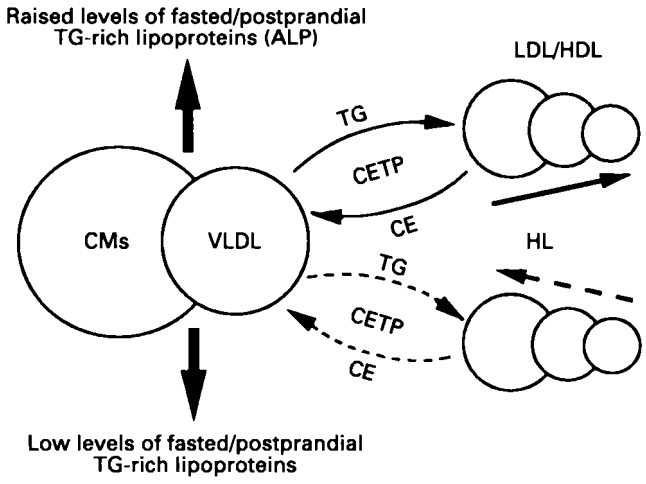

Fig. 3. Neutral lipid exchange as a mechanism for the reduction in the particle size of LDL and HDL. Under normal circumstances when fasting TG levels are low the rate of transfer of TG from TG-rich lipoproteins to LDL and HDL in exchange for cholesteryl esters (CE) is low. An increase in the concentration of TG-rich lipoproteins, either in the form of chylomicrons and their remnants or large VLDL, accelerates this process leading to transient enrichment of LDL and HDL with TG. This additional TG is then hydrolysed by hepatic lipase (HL) which generates small and dense particles.

tracers have shown that the precursor–product relationship between VLDL and LDL is affected not only by the amount of VLDL but also by the type of VLDL (Shepherd & Packard, 1987), the latter determining the quantity and quality of LDL. Under normal circumstances when fasting TG levels are low, large VLDL, after rapid delipidation, become VLDL remnants that are removed directly from the circulation without being converted into IDL and then LDL (Fig. 2). As VLDL remnants are removed by membrane receptors in a manner similar to that described for CM remnants, large VLDL are often referred to as the 'liver's chylomicrons'. On the other hand, the smaller $VLDL_2$ are progressively delipidated into IDL and then LDL and are often referred to as 'pre-LDL'. Although a relationship between VLDL heterogeneity and the ALP is not yet established, the consistent finding of small dense LDL and HDL in conditions characterized by the presence of large TG-rich $VLDL_1$ such as diabetes and obesity (James & Pometta, 1991; Fisher *et al.* 1993), suggests that $VLDL_1$ is a major determinant of the remodelling of LDL and HDL into smaller and denser particles by mechanisms that involve the donation of TG in exchange for cholesterol esters (see neutral lipid exchange and Fig. 3). In contrast, the overproduction of $VLDL_2$ is associated with increases in LDL mass as found in common hypercholesterolaemia (Gaw *et al.* 1995). It is important to appreciate that levels and subclass distribution of HDL are also affected by serum TG levels and VLDL heterogeneity.

ROLE OF LPL AND HL, CHOLESTERYL ESTER TRANSFER PROTEIN AND NEUTRAL LIPID TRANSFERS

As the rate limiting enzyme for the hydrolysis of TG carried in CM and VLDL, LPL is regarded as one of the major determinants of postprandial and fasting TG levels. Its activity is regulated by apoproteins associated with TG-rich particles and HDL, such as apo C-II which activates LPL or apo C-III which inhibits its action. Endothelial lipases show a preference for lipoproteins of different sizes (Nicoll & Lewis, 1980; Jackson *et al.* 1987). LPL hydrolyses the TG within larger lipoproteins such as CM and VLDL *in vitro*

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and is even reported to express a preference for TG contained in CM rather than in VLDL through mechanisms that are incompletely understood. In contrast, HL acts primarily on smaller lipoproteins in the range of IDL, LDL and HDL and small CM remnants. The reason why these enzymes show a preference for lipoproteins of different sizes is not clear but may relate to the exposed surface area of the particle, the packing pressure of its lipid bilayer or relative exposure of lipid substrate. As an enzyme that is activated by insulin, adipose tissue LPL may be a key determinant of the ALP, through its role in the regulation of TG levels, particularly during the postprandial phase. Physical exercise stimulates the activity of LPL in skeletal muscle. The resulting lower fasting TG levels may explain changes in lipoproteins that follow acute bouts of exercise and differences in lipoprotein profile between athletes and non-athletes (Dufaux *et al.* 1982). The activity of HL has been implicated in the generation of small dense LDL and HDL (Zambon *et al.* 1993; Watson *et al.* 1994), though evidence for regulation of this enzyme by dietary or hormonal factors is lacking.

Cholesteryl ester transfer protein and neutral lipid exchange

The transfer of TG from TG-rich lipoproteins to smaller lipoproteins in exchange for cholesteryl esters has been known for some time to occur by a mechanism of neutral lipid exchange (Nichols & Smith, 1965). This process occurs under the influence of lipid transfer proteins the best characterized of which is cholesteryl ester transfer protein (CETP). The net mass transfer of TG from large TG-rich lipoproteins to smaller lipoproteins in exchange for cholesterol results in the transient enlargement of the smaller lipoproteins which are then reduced in size by the action of lipases such as HL (Fig. 3). The extent to which this reaction occurs is a direct function of the concentration of donor TG-rich lipoproteins (large $VLDL_1$, CM and CM remnants) and thus serum TG levels. Raised TG levels can therefore promote shifts in the size and density distribution of both LDL and HDL towards small dense particles *via* excessive neutral lipid exchange as illustrated in Fig. 3. In addition, turnover studies have shown that small, dense HDL are removed more rapidly from the circulation and thus are unavailable for reverse cholesterol transport. In this way, neutral lipid exchange may promote low levels of HDL_2 that characterize an ALP. The work of Lagrost *et al.* (1990, 1993) and many others would suggest that the transfer capacity of CETP may be rate limiting in this reaction, though this theory is controversial as the kinetics of the CETP reaction have been defined *in vitro*. With the possible exception of the rate condition of inherited CETP deficiency, there is usually little correspondence between the activity or mass of plasma CETP and lipoprotein subclasses and these *in vitro* findings. This would imply that whilst the activity of CETP is required for the exchange of neutral lipids *in vivo*, it is not necessarily rate limiting. Whatever the impact of CETP on lipoprotein subclasses, a number of reports have claimed that its activity can be modified by dietary fatty acids, and these will be reviewed.

POSTPRANDIAL LIPAEMIA AND THE ALP

The impaired removal of dietary TG in the form of CM and partly hydrolysed CM remnants following the ingestion of a fat-containing meal is likely to enhance neutral lipid exchange through the persistence in the circulation of TG-rich donor lipoproteins and thus increased substrate availability (Figs 2, 3). There is also the possibility of competition between large $VLDL_1$ and CM and large CM remnants for a common saturable lipolytic pathway during the postprandial phase (Brunzell *et al.* 1973) when TG levels can be increased as much as three-fold over baseline values for a period of several hours. The idea that the degree of postprandial lipaemia could be determined by fasting TG level, that is

by levels of large TG-rich VLDL, has been suggested by Weintraub *et al.* (1988) and more recently by others (O'Meara *et al.* 1992; Chen *et al.* 1993). The postprandial shift in HDL subclass distribution away from the cholesterol-enriched HDL_2 towards the smaller HDL_3 and the significant inverse relationship between the extent of postprandial lipaemia and HDL cholesterol provide convincing evidence for the impact of postprandial events on neutral lipid exchange (Patsch *et al.* 1987). The relationship between postprandial lipaemia and LDL subclasses is less well established, probably because the remodelling of LDL subclasses is mediated through longer term, indirect influences of postprandial events on fasting VLDL levels. There is, however, recent evidence for a significant inverse relationship between LDL size and the magnitude of postprandial lipaemia (Nikkila *et al.* 1994).

THE LDL RECEPTOR PATHWAY

Although the role of the LDL receptor pathway in the regulation of total and LDL cholesterol levels is well established, it is not central to the understanding or development of the ALP, as this dyslipidaemia is not usually associated with raised levels of LDL. Nevertheless, the existence of structural heterogeneity in LDL raises the possibility that different LDL subclasses will be metabolically distinct with respect to their interaction with the LDL receptor and thus express differences in binding affinity. In this way, diet-induced changes in the activity of the LDL receptors could influence the distribution of LDL subclasses. Cell culture studies indicate that large and small LDL species (LDL-I, III) bind with lower affinity to the receptor compared to the LDL of intermediate size and density (LDL-II) (Nigon *et al.* 1991). Human and animal studies show that LDL of large and intermediate size are preferentially removed in response to drug induced stimulation of LDL receptors, leaving plasma relatively enriched in small dense LDL (Beltz *et al.* 1987; Franceschini *et al.* 1990). The discrepancy in the uptake of large LDL-I between these different studies may be explained by the recent finding that this LDL subclass is rapidly converted *in vivo* (Caslake *et al.* 1994) into what has been shown to be 'receptor active' LDL-II in cell culture.

GENETIC BASIS OF AN ALP

The ALP is believed to develop through genetic–environmental interactions (Austin, 1991). A number of common mutations in the LPL and functionally related apoprotein genetic loci have been identified as possible susceptibility genes that interact with diet to produce changes in postprandial and fasting TG levels. In addition to the control of peripheral lipolysis, the genetic regulation of the enzymes of intracellular lipolysis in adipose tissue (hormone sensitive lipase, HSL) and hepatic lipogenesis may be equally important as mechanisms that are genetically susceptible to diet. The role of diet inducible gene transcription factors in the liver may be particularly important in this context as potential determinants of the regulatory influence of specific fatty acids on energy metabolism.

The apo E polymorphism (apo $\epsilon 2$ allele) has also been implicated in the impaired removal of dietary CM and CM remnants *via* its influence as a ligand for the CM remnant receptor and may contribute to variation in the postprandial response. However, despite the well known relationship between the E2 polymorphism and low LDL levels that results from an upregulation of LDL receptors (Uterman, 1987), no relationship could be found between E phenotype and the distribution of LDL particle size in a postprandial study by Nikkila *et al.* (1994). More recently, apo E phenotype has been related to the magnitude of response of LDL subclasses to a reduced fat diet, with the greatest reduction in large LDL occurring in E4/E4 subjects (Dreon *et al.* 1995). Interestingly, the apo $\epsilon 4$ allele has also been

associated with increased CHD risk in non-insulin dependent diabetics and seems to disturb the normal relationship between insulin and TG (Despres & Marette, 1994).

The predominance of small dense LDL in an ALP in various population groups has been ascribed in complex segregation analysis to the effects of a single major gene with a polygenic mode of inheritance (Austin, 1993). The nature of this single gene has yet to be elucidated, though the LDL receptor gene on the short arm of chromosome 19, also the site of the insulin receptor gene, is a favoured candidate (Nishina *et al.* 1992).

INFLUENCE OF DIETARY FATTY ACIDS ON ALP STATUS

A recent government report from the Committee on Medical Aspects of Food Policy (Department of Health, 1994) recognized that "the quality and quantity of fat are fundamental and modifiable determinants of plasma lipid levels". 'Plasma lipids' in this context refers to traditional, cholesterol based CHD risk factors. The report continues to promote the inflated perception of the efficacy of cholesterol lowering diets in a free living population as reviewed by Ramsay *et al.* (1991). It also gives little consideration to TG and dismisses the impact of n-3 polyunsaturated fatty acids (PUFA) on TG because of their 'minimal' effects on HDL and LDL cholesterol levels and their potentially adverse effects in raising LDL cholesterol in hypertriglyceridaemic patients. The ALP is a common, high risk phenomenon associated with an intolerance to dietary fat with a resultant proliferation of remnant lipoproteins of postprandial origin and modifications in LDL structure, neither of which is reflected in the cholesterol content of lipoproteins. As such, there is a clear need for a shift in emphasis away from cholesterol so that, in future, dietary studies can take full advantage of the most recent developments in our understanding of TG metabolism in relation to CHD.

The identification of TG as the major determinant of the ALP establishes this lipid as the primary target for dietary fatty acid modifications as a means of correcting the lipoprotein abnormalities of an ALP, with emphasis on the attenuation of enhanced postprandial lipaemia. The effects of TG on LDL and HDL subclasses are modulated through the combined actions of endothelial lipases (LPL, HL), lipid transfer proteins (CETP) and lipoprotein receptors, all of which may be subject to dietary influence. Finally, weakness in the inter-relationships between insulin resistance, serum lipids and lipoproteins and diet has been ascribed to underlying genetic variation. The ALP with its strong genetic basis and metabolic links with insulin resistance offers the most relevant phenotypic classification with which to address the influence of genetic susceptibility to diet in normal, free-living populations.

DIETARY EFFECTS ON PLASMA TRIACYLGLYCEROLS

SATURATED FATTY ACIDS

The replacement of dietary saturated fatty acids (SFA) with unsaturated fatty acids is frequently accompanied by a lowering of fasting serum TG (Durrington, 1989). The question arises, can these effects be attributed to the various properties of the unsaturated fatty acids themselves or are they simply permissive effects produced by the removal of the SFA. The established cholesterol raising properties of dietary SFA have overshadowed their effects on TG, with the result that greater importance has been attached to the nature of the substitute, unsaturated fatty acids.

The chronic and acute ingestion of foods enriched in saturated fats produces enhanced postprandial lipaemia (Demacker *et al.* 1991; Zampelas *et al.* 1994). The reason for this effect is not clear but may relate to saturated TG producing smaller CM which show a

delayed clearance from the circulation *via* LPL (Levy, 1991; Murphy *et al.* 1993). In contrast to the potentially beneficial impact of these postprandial effects on the ALP, a recent cross-sectional study which compared rural and urban populations found that a lower intake of dietary SFA, but not total fat, in the rural population was associated with raised triacylglycerols, small LDL size and low HDL levels (Campos *et al.* 1991). The findings of this particular study were, however, confounded by high intakes of simple carbohydrates in the rural population that led to the phenomenon of carbohydrate-induced hypertriglyceridaemia.

POLYUNSATURATED FATTY ACIDS OF THE N-3 AND N-6 SERIES

Linoleic acid (C18:2, n-6, found in many vegetable oils) is normally the principal dietary PUFA contributing to the commonly used polyunsaturated:saturated fatty acid ratio. In most studies, the replacement of saturates with linoleic acid reverses nearly all the effects on lipids and lipoproteins produced by long chain SFA.

The TG lowering action of linoleate-rich diets has been described as an inconsistent phenomenon (Grundy & Denke, 1990). The TG lowering effects of n-6 PUFA in particular may be restricted to individuals with an overproduction of VLDL. These effects could result from the inhibitory effects of linoleic acid on lipogenic enzymes involved in the synthesis of hepatic fatty acids (fatty acid synthetase) and TG (phosphatidate phosphohydrolase and diacylglycerol acyl transferase (Herzberg, 1991; Schmidt *et al.* 1993). Although an inhibition of fatty acid synthesis has been shown to limit the production of VLDL in rats, the quantitative significance of this mechanism in western man is negligible, given that fatty acid requirements are satisfied from dietary intake. Dietary long chain n-3 PUFA from fish oils, which includes as the biologically active species eicosapentaenoic acid (20:5, n-3) and docosahexaenoic acid (22:6, n-3), have similar inhibitory effects on these enzymes and VLDL production, but in contrast to n-6 PUFA they markedly reduce postprandial and fasting TG. The incorporation of PUFA into VLDL should, through their relatively greater molecular volume, lead to the production of larger VLDL particles ($VLDL_1$) which, according to kinetic turnover studies, are cleared rapidly from the circulation without being converted into LDL (Shepherd & Packard, 1987). This is supported by the findings of several groups who have shown that n-6 enriched diets are followed by a decrease in the rate of synthesis of VLDL and a fall in LDL, that is presumably derived from smaller $VLDL_2$ (Illingworth *et al.* 1981; Turner *et al.* 1981; Cortese *et al.* 1983). Although this qualitative theory is attractive, a decrease in the absolute number of VLDL particles that are synthesized is likely to make a greater contribution to the decrease in LDL.

In common with n-6 series, dietary n-3 PUFA also suppress the production of VLDL in man (Nestel *et al.* 1984) but may produce different qualitative effects upon VLDL from those produced by n-6 PUFA by inducing a shift in the distribution of VLDL subclasses towards predominantly small VLDL ($VLDL_2$ S_f20-60) (Harris, 1989). While this could be viewed as a secondary effect resulting from the efficient removal of larger $VLDL_1$ particles, the precursor–product relationship between $VLDL_2$ and LDL would suggest that it contributes to the increases in LDL that can occur following n-3 PUFA supplementation in normal and hypertriglyceridaemic groups (see LDL subclasses).

Effects of PUFA on postprandial lipaemia

In addition to their effects on VLDL metabolism, dietary PUFA may exert a significant effect on the ALP by attenuating both the duration and magnitude of postprandial lipaemia compared with highly saturated diets (Harris *et al.* 1988*a*; Weintraub *et al.* 1988;

Demacker *et al.* 1991). This effect is markedly greater for the n-3 PUFA which can reduce postprandial TG levels by up to 60%.

Both acute and chronic consumption of n-3 PUFA have been associated with reductions in postprandial response, characterized by reductions in CM and CM remnants. There are a number of explanations for these effects. First, the size and fatty acid composition of CM may increase their ability to compete with large VLDL as substrates for LPL (Levy *et al.* 1991). Secondly, an increase in post-heparin LPL activity has been observed following a fish oil acute test meal, compared with a SFA-rich meal (Zampelas *et al.* 1994), though several other studies which examined long term intervention with fish oils refute these findings (Weintraub *et al.* 1988; Nozaki *et al.* 1991). While these mechanisms may all be operative in the short term, the greater impact of a habitual intake of n-3 PUFA on the attenuation of postprandial lipaemia would favour longer term effects, possibly mediated through the upregulation of LPL gene expression in adipose tissue as demonstrated in the rat (Murphy *et al.* 1993). This theory of increased catabolism *via* LPL is somewhat controversial, not only because of evidence to suggest that n-3 PUFA decrease the rate at which CM are produced and secreted rather than increasing their catabolism (Harris & Muzio, 1993), but also because n-3 PUFA are not usually associated with changes in the activities of LPL and HL in post-heparin plasma, despite significant decreases in TG (Nozaki *et al.* 1991). This finding highlights other possible explanations for the TG lowering property of n-3 PUFA, which is to inhibit lipogenic enzymes responsible for TG synthesis in the liver and enterocyte (Marsh *et al.* 1987; Rustan *et al.* 1988) and decrease the synthesis and secretion of newly synthesized TG in cells of the human intestine (Murthy *et al.* 1990).

Effects of dietary n-3 PUFA on gene expression

Dietary n-3 PUFA are among a number of substances, such as the fibric acid derivatives, that may activate nuclear receptors in the liver that function as gene transcription factors. These receptors are part of the steroid hormone receptor superfamily, two of which include the peroxisome proliferator-activated receptor and the apo A-I regulatory protein. The induction of the former by dietary n-3 PUFA in rats promotes the β-oxidation of fatty acids in peroxisomes, whereas activation of the latter may repress transcription of the apo C-III gene, removing the inhibitory effects of this peptide on the activity of LPL. The recent detection of peroxisome proliferator-activated receptor in adipose tissue (Tontonoz *et al.* 1994) has major implications for the impact of dietary n-3 PUFA on gene expression in the postprandial phase.

MONOUNSATURATED FATTY ACIDS (MUFA)

Compared to a typical American diet (10% total calories MUFA), a modified 'Step I' diet of the American Heart Association enriched with olive oil (18% total calories MUFA) produced no significant effects on fasting TG levels in healthy, normolipaemic young men (Ginsberg *et al.* 1990). MUFA-rich diets were also shown to have no significant effects on TG levels when compared with PUFA-rich diets (Berry *et al.* 1991; Wardlaw *et al.* 1991; Nydahl *et al.* 1994*a*). A significant decrease in TG has been reported in similar subjects given diets rich in oleic acid (54% total calories MUFA) compared with a high SFA diet, but in the same study, the oleic acid-rich diet was not as effective in lowering TG as diets enriched with mixtures of high PUFA and MUFA (Chan *et al.* 1991). In contrast, Wardlaw & Snook (1990) compared SFA, n-6 PUFA and MUFA-rich diets and found that the PUFA and MUFA diets significantly reduced TG levels relative to the SFA diet. Thus, current evidence would indicate that MUFA enriched diets may have some effects on TG

levels in normolipaemic men when compared with experimental diets enriched with SFA. Interestingly, MUFA enriched diets have also been associated with either no change or increases in TG levels when compared with a high PUFA diet (Mata *et al.* 1992*a*, *b* respectively), or a high SFA diet (Mata *et al.* 1992*b*), though these latter changes were confined to subjects with the highest body weight.

Effects of MUFA on postprandial lipaemia

Literature on the postprandial effects of MUFA on the ALP and on lipoprotein metabolism in general is limited. It is difficult to reach definite conclusions from the information that is available on postprandial changes in plasma TG, following either acute test meals or a standard test meal after a dietary intervention period. Studies which compared various acute test meals enriched with either MUFA or PUFA showed no significant effects on postprandial triglyceride levels (de Bruin *et al.* 1993). Lichtenstein *et al.* (1993) showed a similar lack of response to acute test meals following intervention periods with n-6 PUFA and MUFA enriched diets. In contrast, when periods of olive oil and fish oil supplementation were compared, the former increased postprandial TG levels in the CM fraction following a standard test meal (Brown & Roberts 1991).

It has become apparent that metabolic events in the postprandial phase cannot be adequately described by following changes in TG levels or even lipid markers of CM metabolism such as retinyl palmitate. A recent advance in this area has been the measurement of apoprotein B-48, the only form of apo B found in human CM that can be used as a specific marker of CM and CM remnant metabolism. This assay, when used in conjunction with the retinyl palmitate loading technique, has provided new insight into the effects of different dietary fatty acids on the magnitude and the extent of postprandial lipaemia. In the study of de Bruin and co-workers, even though postprandial TG levels were not different following soybean (n-6 PUFA) or the olive oil test meal, the postprandial decrease in HDL was less with the olive oil test meal, whilst both retinyl palmitate concentration (area under the curve) in the CM and apo B-48 in the CM remnant fractions were higher following the olive oil *v.* the soybean oil meal. In addition, a correlation between the slow removal of CM remnant on the olive oil diet with HL activity led the authors to postulate that the CM remnants produced by the acute ingestion of olive oil were smaller and compete with HDL for a receptor pathway that is dependent on HL, whereas the n-6 PUFA-rich CM and CM remnants were possibly removed by an additional non-enzymic pathway (Brouwer *et al.* 1993). The 'HL hypothesis' was suggested by these authors to explain the HDL 'sparing effect' associated with MUFA, though an alternative hypothesis could be that olive oil CM-TG are not hydrolysed as efficiently as those produced by n-6 PUFA so that HL has to act on these particles for longer in order to make them recognizable by hepatic receptors. Although these studies are inconclusive with respect to the effects of MUFA meals or diets on postprandial lipaemic responses, they highlight the metabolic importance of small CM remnants that have been recently detected by the analysis of apo B-48 within IDL and LDL density intervals (Isherwood *et al.* 1995).

TRANS FATTY ACIDS

Recent interest in dietary *trans* fatty acids has grown from their similarity to SFA and potentially adverse effects on plasma lipoproteins. While more than half the *trans* fatty acids in the diet originate from vegetable or fish oils rich in n-6 or n-3 PUFA respectively that have undergone partial hydrogenation during food manufacturing processes, they also occur naturally in dairy products. Although consumption of *trans* fatty acids, is still relatively low in westernized countries, it has increased from 3–4% of the daily energy

intake in the USA (Senti, 1985) to 5–7% (Mensink & Katan, 1993), and speculations are that the figure may be even higher. While *trans* fatty acids are reported to increase the concentration of LDL and decrease HDL relative to their *cis* isomers (Mensink & Katan, 1993; Judd *et al.* 1994), some studies have shown TG levels to be unaffected (Abbey & Nestel, 1994).

DIETARY EFFECTS ON LDL AND LDL SUBCLASSES

SATURATED FATTY ACIDS

In common with dietary cholesterol, the capacity of the long chain SFA C:12, C:14 and C:16 to raise LDL cholesterol are well founded and thought to arise through the suppression of LDL receptor synthesis in the liver (Grundy & Denke, 1990). Hence, the replacement of dietary saturates with unsaturates is accompanied by predictable quantitative effects resulting from the enhanced clearance of LDL from the circulation. These include a fall in the number of circulating LDL particles, apoprotein B and serum cholesterol. The effects of saturated 'atherogenic' diets on LDL structure of non-human primates is well documented but will not be considered within the context of the ALP because of significant phylogenetic differences in lipoprotein heterogeneity between species.

Qualitative effects of SFA and the LDL receptor

Theoretically, a stimulation of LDL receptors in response to the removal of dietary SFA in humans can induce a preferential clearance of larger, relatively cholesterol-rich LDL species, leaving the plasma relatively enriched in small dense LDL. This effect has been demonstrated in guineapigs (Witztum *et al.* 1985) and is a common finding in humans on cholesterol lowering drugs that stimulate this receptor pathway (Young *et al.* 1989; Franceschini *et al.* 1990; Griffin *et al.* 1992). While the short term outcome of this effect on the distribution of LDL subclasses appears to be unfavourable in terms of CHD risk, this is probably outweighed by the reduction in total circulating LDL mass that is likely to accompany a reduction in SFA. The effect of dietary lipid saturation on the metabolism of two LDL subfractions of the guineapig has been recently examined by Fernandez *et al.* (1993). In this study, animals fed a diet enriched in SFA showed a slower clearance of LDL and a predominance of larger LDL as compared to animals fed a PUFA enriched diet. The latter animals also showed a shift in LDL towards smaller and dense LDL. Although these observations would be in accord with potentiation of receptor activity on the PUFA diet leading to the uptake of large 'receptor active' LDL, with the opposite finding on the SFA diet, the study showed that, in the guineapig, small dense LDL was catabolized more rapidly than its larger and lighter counterparts, a finding confirmed for the catabolism of human LDL in the same animal by Swinkels *et al.* (1990), but not by Witztum *et al.* (1985). These inconsistencies are difficult to resolve and their relevance to humans questionable in view of the limited amount of data available on the effects of SFA on LDL subclasses and the inevitable differences between human and animal models. For example, serum TG levels in the guineapig are low compared to humans and as such are not a major determinant of LDL subclass distribution.

Dietary response to fat intake as determined by LDL subclass pattern

A recent dietary intervention trial examined the response of men subdivided on the basis of their LDL subclass pattern (pattern A (normal) or pattern B (small dense)) to a high fat (46% total calories) followed by a low fat (24% total calories) diet (Dreon *et al.* 1994). LDL cholesterol was shown to fall in both groups on moving from the high to low fat diet.

However, pattern B subjects showed greater decreases in apo B and LDL cholesterol than pattern A subjects. Furthermore, over 40% of the pattern A subjects converted to pattern B on the low fat diet. These findings indicate that individuals with the abnormal, LDL pattern B may achieve greater benefits from dietary modification through reductions in the number of LDL particles as compared with individuals with normal, LDL pattern A, within whom the cholesterol lowering arises from a shift from a predominantly cholesterol-rich LDL to a relatively cholesterol-poor, small dense LDL subclass. This latter finding has been recently confirmed by the same workers who showed that the magnitude of the apparent shift in LDL subclasses in response to a reduced fat diet (SFA replaced by carbohydrate representing 60% total energy content) was related to E phenotype in the increasing order of E3/2 to E3/3 to E4/3, E4/4 (Dreon *et al.* 1995). As already mentioned, the impact of this change in LDL subclasses in terms of increased CHD risk is likely to be offset by a fall in total LDL mass.

PUFA OF THE N-3 AND N-6 SERIES

It can be speculated that the influence of dietary n-6 and n-3 PUFA on the distribution of LDL subclasses will reflect their capacity to reduce the concentration of TG-rich lipoproteins (Fig. 3). This includes VLDL in the fasted state and CM and large CM remnants in postprandial plasma. However, while the direct influence of dietary n-6 PUFA on discrete LDL subclasses has yet to be elucidated, data relating to the lipid and protein composition of total LDL suggest that n-6 PUFA have minimal effects on LDL heterogeneity.

Effects of n-3 PUFA on LDL size distribution

In contrast to the expected effects of dietary n-6 PUFA, failure to demonstrate qualitative effects of n-3 PUFA on LDL particle size in normal and hypertriglyceridaemic groups is surprising given their potent TG-lowering action (Sullivan *et al.* 1986; Homma *et al.* 1991; Nenseter *et al.* 1992). A significant redistribution of LDL particle size can be inferred from alterations in LDL composition, such as a change in the cholesterol or lipid to protein ratio. However, data from Harris (1989) and others (Nestel, 1986; Sullivan *et al.* 1986) indicate that n-3 PUFA have no significant effects on LDL composition. In this respect, there are two points worth noting. First, the effects of dietary n-3 PUFA on the quantitative distribution of visually discrete LDL subclasses, as resolved by density gradient centrifugation procedures, has not been reported. Secondly, it is quite possible that quantitative changes could occur in LDL subclasses without significantly altering the lipid or protein composition of total plasma LDL. A recent study reported significant increases in LDL particle diameter accompanied by a 30% fall in TG in response to n-3 PUFA supplementation in patients with combined hyperlipidaemia (Contacos *et al.* 1993). The finding of an increase in LDL particle size in response to n-3 PUFA was corroborated by Suzukawa *et al.* (1994) but not by Homma *et al.* (1991) who actually found decreases in LDL particle size.

Increases in LDL induced by n-3 PUFA

The most consistent finding in n-3 PUFA studies regarding lipoprotein heterogeneity is an increase in small VLDL (S_f20-60) (Sullivan *et al.* 1986; Harris, 1989). As the main progenitor of LDL this is believed to account for the n-3 PUFA induced increases in LDL mass that occur in normolipidaemic and hypertriglyceridaemic subjects (Sullivan *et al.* 1986; Harris *et al.* 1988*b*; Levine *et al.* 1989; Deck & Radack, 1989). This LDL raising property, which occurs either through increased conversion of VLDL (Huff & Telford, 1989) or competition between VLDL and LDL for the LDL receptor (Gianturco &

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Bradley, 1991), may be more frequently associated with the use of n-3 PUFA capsule supplements. The substitution of SFA for n-3 PUFA derived from dietary fish oils is reported to produce either varying effects, no effects or even significant decreases in LDL-cholesterol and apo B (Nestel *et al.* 1984; Hänninen *et al.* 1989; Childs *et al.* 1990; Friday *et al.* 1991; Connor *et al.* 1993; Illingworth & Schmidt, 1993; Jiang & Sim, 1993). Different species of fish have also been shown to produce variable effects on LDL levels (Gerhard *et al.* 1991). These findings might suggest that it is the increase in total fat intake associated with capsule supplements and not the n-3 PUFA *per se* which causes the increase in LDL cholesterol levels. However, differences in energy intake over a short period of oil supplementation are usually negligible and subjects taking capsule supplements have also been shown to compensate and thus maintain an energy balance.

Effects of PUFA on the susceptibility of LDL subclasses to oxidative modification

The oxidative modification of LDL is thought to be a prerequisite for the deposition of LDL cholesterol in the artery wall. A recent examination of the effects of linoleate and oleate enriched diets on the susceptibility of LDL subfractions to oxidative modification suggested that the increased susceptibility of small dense LDL (LDL-III) to oxidation was linked to the preferential enrichment of this subfraction with linoleic acid (Reaven *et al.* 1994). This finding was consistent with earlier work that ascribed at least part of the increase in oxidative susceptibility of small dense LDL to its higher ratios of 18:2 and 20:4 (arachidonic acid) to vitamin E levels (de Graaf *et al.* 1991). The reason why small dense LDL should contain higher levels of PUFA is not clear. The increased oxidative susceptibility of PUFA enriched LDL and its rapid removal by scavenger receptors has been offered as another possible but unlikely explanation for the LDL lowering effects of dietary PUFA (Norum, 1992). Far from being a favourable effect, this could be regarded as a potentially pro-atherogenic influence of PUFA. Furthermore, there is considerable disparity between the effects of n-6 and n-3 PUFA on LDL levels despite the fact that highly unsaturated n-3 PUFA would be expected to confer even greater susceptibility to oxidation on LDL, thus enhancing its removal.

MONOUNSATURATED FATTY ACIDS OF THE N-9 SERIES

While information on the effects of dietary MUFA on LDL subclasses is sparse, minimal effects on LDL heterogeneity could be predicted, given the apparent lack of evidence for a TG lowering influence of MUFA. A comparative study of the effects of n-6 PUFA *v.* MUFA showed there to be no differences in the concentrations of either large or small LDL between these two diets (Dreon *et al.* 1990). This finding is in contrast to the moderate LDL cholesterol lowering property of MUFA, the degree of which is often compared to that of n-6 PUFA (Ginsberg *et al.* 1990; Wardlaw & Snook, 1990; Chan *et al.* 1991; Wahrburg *et al.* 1992; Nydahl *et al.* 1994*a*, *b*). The concomitant reduction in apo B levels after the consumption of a MUFA-enriched diet suggests that the decrease in LDL cholesterol may arise from either an increased clearance or decreased synthesis of LDL particles rather than changes in their cholesterol content (Chan *et al.* 1991). Grundy & Denke (1990) suggested that MUFA and PUFA may allow the natural expression of LDL receptor activity, if this is the main variant, whereas long chain SFA have a suppressive effect.

MUFA and susceptibility of LDL to oxidative modification

One of the protective effects of dietary MUFA on an ALP is thought to be the relatively lower susceptibility of oleic acid-rich cholesteryl esters, phospholipids and TG to lipid peroxidation and thus the oxidative modification of LDL as compared to n-3 and n-6

PUFA. As discussed, this protective effect seems to be confined to small dense LDL (Reaven *et al.* 1994) which is the LDL subclass that expresses increased susceptibility to oxidation *in vitro* (de Graaf *et al.* 1991; Tribble *et al.* 1992). When oxidative stress was assessed in the Jerusalem Nutrition Study (Berry *et al.* 1991) it was found that LDL isolated after a PUFA enriched diet contained significantly increased levels of lipid peroxides as compared to LDL isolated after a MUFA enriched diet. In addition, LDL isolated from mildly hypercholesterolaemic subjects on a MUFA-rich diet was found to be less susceptible to copper mediated oxidation and lipid peroxide formation, and less susceptible to LDL-protein modification than the LDL isolated from the same subjects on a n-6 PUFA diet (Reaven *et al.* 1993*b*). Although compositional changes occurred in the HDL, this lipoprotein retained its powerful capacity to inhibit the oxidative modification of LDL *in vitro*.

DIETARY EFFECTS ON HDL AND HDL SUBCLASSES

SATURATED FATTY ACIDS

The relative cholesterol raising potencies of the different saturates and the mechanisms by which they are controlled lie beyond the scope of this review. However, dietary saturates do seem to exert differential effects on HDL. Myristic acid (C:14) has been shown to raise HDL (as large cholesteryl ester-rich HDL_2) to a greater extent than either lauric (C:12) or palmitic acid (C:16) (Zock *et al.* 1994). High fat diets in general are known to stimulate the synthesis of apoprotein A-I in the gut (Shepherd *et al.* 1978). This effect in the presence of efficient LPL mediated lipolysis and sufficient esterification capacity would facilitate the synthesis of nascent HDL and conversion of HDL_3 to cholesterol-rich HDL_2, processes which, through association with reverse cholesterol transport, are considered beneficial.

Effects on CETP activity

High fat diets are reported to increase the activity of CETP and CETP mRNA levels (Tall, 1993). Since CETP is intimately involved in the remodelling of LDL and HDL subclasses, it has been proposed that the effects of dietary SFA on these lipoprotein subclasses are mediated through this lipid transfer protein. The stimulatory effect of a high fat diet on CETP activity has been ascribed to the dietary cholesterol component rather than the SFA (Tall, 1993), though this is not the view held by others who report a stimulation of CETP mediated redistribution of HDL particle size and transfer of cholesteryl esters between HDL_3 and LDL by medium and long chain SFA (Lagrost & Barter, 1992). Whatever the mechanism, the increases in VLDL and LDL associated with dietary SFA will almost certainly negate the benefits of raising HDL levels through a stimulation of CETP.

PUFA OF THE N-3 AND N-6 SERIES

Decreases in the concentration of HDL in response to dietary n-6 PUFA in excess of about 10% of total calories is a reasonably well established finding that may be attributed to a fall in the synthesis of apo A–I (Shepherd *et al.* 1978). Intakes below this level seem to have no significant effects. In reviewing the impact of dietary n-3 PUFA on HDL, Schmitz & Lackner (1993) suggest that the outcome depends upon the lipoprotein phenotype and the amount of n-3 PUFA ingested. If this is the case, it is predictable that subjects with raised TG levels may be more responsive to n-3 PUFA in terms of reciprocal changes in HDL and TG in both fasting and postprandial plasma. A decrease in the transfer of cholesteryl esters

from HDL to VLDL and LDL, as reported by Bagdade *et al.* (1992), would favour an accumulation of large HDL_2 and thus HDL cholesterol, as previously found in healthy volunteers on fish oil supplements (Blonk *et al.* 1990; Childs *et al.* 1990). Similar diets in hypertriglyceridaemic subjects are reported to increase the smaller HDL_3 subclass (Deck & Radack, 1989), which is in agreement with Schmidt *et al.* (1993). While the origin of these effects is not clear, decreases in the activity of lecithin–cholesterol acyl transferase (*EC* 2.3.1.43), due to polyunsaturation of phosphatidylcholine, and CETP have been implicated.

MONOUNSATURATED FATTY ACIDS OF THE N-9 SERIES

Views concerning the influence of MUFA on HDL cholesterol levels are controversial. A commonly held view is that the isocaloric replacement of SFA with MUFA does not decrease HDL and may even increase its levels (Grundy, 1989; Grundy & Denke 1990; Spiller *et al.* 1992; Lichtenstein *et al.* 1993). This is also supported by the recent findings of Mata and co-workers (1992*a*, *b*) who observed higher HDL cholesterol and apo A–I levels after MUFA-rich diets compared to PUFA-rich diets in both men and women. Nevertheless, numerous studies have reported either no effects of dietary MUFA on HDL cholesterol and/or apo A–I (Wardlaw & Snook, 1990; Kris-Etherton *et al.* 1993), or even decreases in this lipoprotein relative to SFA-rich diets (Foley *et al.* 1992; Valsta *et al.* 1992; Nydahl *et al.* 1994*b*). It is interesting to note that elevations in apo A–I have been accompanied by reductions in HDL cholesterol (Wahrburg *et al.* 1992). This finding might suggest that under certain circumstances dietary MUFA promotes the synthesis of new HDL particles. Valsta *et al.* (1992) suggested that dietary MUFA, provided in the form of rapeseed oil, may result in a more favourable ratio of HDL_2-C to LDL-C and apo A–I to apo B than n-6 PUFA. This effect was, however, attributed to greater decreases in LDL concentrations rather than HDL. In cell culture studies, HDL_3 obtained from women on a MUFA-rich diet induced the greatest efflux of cholesterol from fibroblasts, reduced the content of intracellular cholesterol and enhanced LDL degradation, when compared with HDL isolated from subjects fed SFA, n-6 and n-3 PUFA-rich diets (Sola *et al.* 1993).

Incubations of HDL *in vitro* with either VLDL or LDL that included sodium oleate in the media as the sole source of fatty acid did not affect HDL particle size. When CETP was included in the media, HDL particle size was reduced (Barter *et al.* 1990). However, when diet induced alterations of CETP activity were investigated *in vivo*, a significant decrease following a MUFA-rich and a non-significant decrease following a PUFA-rich diet was observed in CETP activity when compared to a control SFA-rich diet (Groener *et al.* 1991). It could be concluded from these studies that while a MUFA-rich diet may reduce CETP activity *in vivo* this is inconsistent with the reduction of HDL particle size found *in vitro* and the postulated role of CETP in neutral liquid exchange (Fig. 3). Further research is clearly necessary to verify the effects of dietary MUFA on HDL subclasses and to elucidate its underlying mechanisms of action.

TRANS FATTY ACIDS

Although *trans* fatty acids are considered hypercholesterolaemic compared to their *cis* isomers and produce equivalent effects on LDL levels to that of SFA, they are reported to decrease HDL levels and, as such, behave differently to the long chain SFA. These effects were found to be either as pronounced as those of long chain SFA (Judd *et al.* 1994) or even greater than them (Mensink & Katan, 1990). Raised CETP activity has been reported in response to the *trans* isomer of oleic acid, namely elaidic acid *v.* its *cis* isomer (Abbey & Nestel, 1994). While CETP activity was significantly correlated with HDL levels in subjects

consuming the diet rich in elaidic acid, there was no significant difference in mean HDL cholesterol concentrations between diets rich in oleic and elaidic acids. Lagrost (1992) also found that elaidic acid increased CETP activity, the CETP mediated redistribution of cholesteryl esters and net mass transfer of cholesterol from HDL_3 to LDL. Overall, these findings imply that *trans* fatty acids produce a shift in the distribution of HDL particle size towards smaller particles through increased lipid transfer that is independent of TG levels.

DIET–GENE INTERACTIONS AND INSULIN RESISTANCE

DIET–GENE INTERACTIONS

Diet–gene interactions are believed to underlie not only the significant variations in the response of plasma lipids and lipoproteins to dietary fatty acids but also the development of an ALP. One of the best examples of a common, diet–gene interaction is provided by the apoprotein E4 polymorphism. Several groups have shown that PUFA enriched diets induced significantly greater reductions in total plasma and LDL cholesterol in individuals who possess the apo ϵ4 allele (Tikkanen *et al.* 1990; Manttari *et al.* 1991). Although these studies showed no associations between apo E phenotype and changes in either TG or HDL, the response of LDL cholesterol to the substitution of saturated fat with carbohydrate has been linked to a more marked reduction of larger, relatively cholesterol-rich LDL within subjects expressing the ϵ4 allele (Dreon *et al.* 1995). While a review of the interactions between diet and genes which encode the metabolic determinants of the ALP is urgently required, the nature of these genes and how they interact with dietary factors is still unclear. Molecular studies on familial combined hyperlipidaemia, a condition characterized by patterns of lipoprotein heterogeneity similar to that of an ALP, have provided a significant insight into the nature of candidate, ALP genes. Examples of such genes include: (i) *LPL gene*. The issue of whether dietary fatty acids, and in particular, long chain n-3 PUFA, can upregulate LPL gene expression is contentious and is likely to remain so until the activity and gene expression of LPL is measured accurately in specific human tissues; (ii) *apo AI-CIII-AIV gene cluster*. Dietary induced variation in apo A–I levels have been attributed to variation in this gene cluster by Xu *et al.* (1990). In addition, apo A–IV levels are positively associated with dietary fat intake (Weinberg *et al.* 1990); (iii) *ATHS gene*. Linkage of small dense LDL (pattern B) to the LDL receptor gene has been recently confirmed (Rotter *et al.* 1994). There were earlier but as yet unconfirmed reports that the expression of this genetic linkage was modified by levels of dietary fat and carbohydrate (Nishina *et al.* 1992). Future knowledge of the way in which these and other genetic loci such as HL and CETP influence dietary responsiveness and the development of the ALP will assist greatly in the formulation and targeting of dietary recommendations towards genetically susceptible groups.

INSULIN RESISTANCE

The effects of gene–diet interactions on the ALP might well be mediated through mechanisms of insulin resistance. The resistance of peripheral tissues to the action of insulin has been attributed to the degree of saturation of fatty acids in the phospholipids of muscle membranes (Storlien *et al.* 1991). This is thought to be regulated by interactions between genetically controlled enzyme systems responsible for the elongation and desaturation of fatty acids, and dietary fatty acids. In rats, dietary SFA, n-6 PUFA and MUFA have all been shown to promote insulin resistance *via* this mechanism, whereas long chain n-3 PUFA prevent its development. Moreover, in human skeletal muscle these latter fatty acids

were shown to improve insulin action (Borkman *et al.* 1993). In view of the widespread effects of improved insulin sensitivity on lipid metabolism and possibly independent effects of n-3 PUFA on LPL gene expression, it is reasonable to speculate that in addition to inappropriate high intakes of fat and physical inactivity, the prevalence of the ALP is a result of a dietary deficiency in long chain n-3 PUFA derived from fish oils that has developed over the past forty years. This idea would offer the most rational approach to future intervention studies designed to address the influence of dietary fatty acids on CHD risk mediated through the ALP.

CONCLUSIONS

The ALP is a relatively new concept which describes a common collection of abnormalities in LDL and HDL that predispose apparently healthy individuals to increased CHD risk. As a likely product of insulin resistance, the ALP represents a dyslipidaemia of TG metabolism. It has a genetic origin but can be influenced, possibly through gene expression, by dietary factors and should therefore be regarded as a modifiable risk factor for CHD. Despite significant advance in our understanding of the genetic and metabolic basis of an ALP, few studies have addressed the influence of dietary fatty acids on the ALP as a distinct dyslipidaemia. In the absence of specific research material, much can be learnt from the effects of dietary fatty acids on determinants of lipoprotein heterogeneity, the most important of which are the TG-rich lipoproteins of hepatic and dietary origin. In this respect, dietary SFA and n-6 PUFA have minimal effects relative to the long chain n-3 PUFA which clearly harbour the greatest potential to correct the abnormalities of an ALP and may even offer longer term benefits by increasing insulin sensitivity through gene–diet interactions. In addition, the ability of dietary MUFA to confer protection against the oxidative modification of LDL is particularly relevant in the case of the ALP, given its low levels of HDL and predominance of oxidatively susceptible small dense LDL.

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