Lipoprotein transport in the metabolic syndrome: pathophysiological and interventional studies employing stable isotopy and modelling methods

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ABSTRACT

The accompanying review in this issue of Clinical Science [Chan, Barrett and Watts (2004) Clin. Sci. 107, 221–232] presented an overview of lipoprotein physiology and the methodologies for stable isotope kinetic studies. The present review focuses on our understanding of the dysregulation and therapeutic regulation of lipoprotein transport in the metabolic syndrome based on the application of stable isotope and modelling methods. Dysregulation of lipoprotein metabolism in metabolic syndrome may be due to a combination of overproduction of VLDL [very-LDL (low-density lipoprotein)]-apo (apolipoprotein) B-100, decreased catabolism of apoB-containing particles and increased catabolism of HDL (high-density lipoprotein)-apoA-I particles. These abnormalities may be consequent on a global metabolic effect of insulin resistance, partly mediated by depressed plasma adiponectin levels, that collectively increases the flux of fatty acids from adipose tissue to the liver, the accumulation of fat in the liver and skeletal muscle, the hepatic secretion of VLDL-triaclylglycerols and the remodelling of both LDL (low-density lipoprotein) and HDL particles in the circulation. These lipoprotein defects are also related to perturbations in both lipolytic enzymes and lipid transfer proteins. Our knowledge of the pathophysiology of lipoprotein metabolism in the metabolic syndrome is well complemented by extensive cell biological data. Nutritional modifications may favourably alter lipoprotein transport in the metabolic syndrome by collectively decreasing the hepatic secretion of VLDL-apoB and the catabolism of HDL-apoA-I, as well as by potentially increasing the clearance of LDL-apoB. Several pharmacological treatments, such as statins, fibrates or fish oils, can also correct the dyslipidaemia by diverse kinetic mechanisms of action, including decreased secretion and increased catabolism of apoB, as well as increased secretion and decreased catabolism of apoA-I. The complementary mechanisms of action of lifestyle and drug therapies support the use of combination regimens in treating dyslipoproteinaemia in subjects with the metabolic syndrome.

Key words: interventional study, lipoprotein transport, metabolic syndrome, modelling, pathophysiological, stable isotopy.

Abbreviations: ABCA1, ATP-binding cassette protein A1; apo, apolipoprotein; CETP, cholesteryl ester transfer protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FCR, fractional catabolic rate; HDL, high-density lipoprotein; HL, hepatic lipase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; LpA, lipoprotein A; LPL, lipoprotein lipase; LRP, LDL-related protein; LXR, liver X receptor; NEFA, non-esterified fatty acid; MTP, microsomal triacylglycerol transfer protein; PLTP, phospholipid transfer protein; PPAR, peroxisome-proliferator-activated receptor; RCT, reverse cholesterol transport; RLP, remnant-like particles; SNP, single nucleotide polymorphism; SR-B1, scavenger receptor B1; SREBP, sterol regulatory element-binding protein; VLDL, very-LDL.

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INTRODUCTION

The metabolic syndrome encapsulates visceral obesity, insulin resistance, diabetes, hypertension and dyslipidaemia [1,2]. There is good evidence that dyslipidaemia is a central mediator of atherogenicity in this condition [3,4]. Dyslipidaemia is an almost invariant feature of the metabolic syndrome and is characterized by high plasma triacylglycerols, low HDL (high-density lipoprotein)-cholesterol and high concentrations of apo (apolipoprotein) B-containing lipoproteins [3]. Knowledge of the pathophysiology of dyslipoproteinaemia in the metabolic syndrome provides a more rational strategy for managing at risk individuals. It may also lead to the discovery and development of new therapeutic agents. While much has been learned from epidemiological, clinical and cellular studies, key mechanistic advances in our understanding of this type of dyslipidaemia and its response to therapy have recently been made with the use of stable isotope tracers and mathematical modelling. In this review, we focus on the application of these methods to elucidating the dysregulation and therapeutic regulation of lipoprotein transport in subjects with metabolic syndrome from studies chiefly carried out by our group. For a contemporary account of lipoprotein metabolism and the principles of stable isotope of lipoprotein system the reader is referred to the accompanying review [4a]. The effects of the metabolic syndrome on plasma lipoprotein concentrations and kinetics are shown in Table 1 and discussed in detail below.

OBSERVATIONAL STUDIES: PATHOPHYSIOLOGY OF DYSLIPROTEINAEMIA

VLDL (very-low-density lipoproteins)

We have consistently demonstrated in stable isotope studies that centrally obese subjects have elevated hepatic VLDL-apoB secretion compared with non-obese individuals [5–7]. This abnormality was also associated with delayed clearance of IDL (intermediate-density lipoprotein), LDL (low-density lipoprotein) and chylomicron remnant particles [5,8,9]. We also showed that changes in the hepatic secretion of VLDL-apoB were positively and significantly correlated with changes in visceral adipose tissue area measured by magnetic resonance imaging [10]. This correlation may, in part, reflect the impact of visceral fat or hepatic fat content on hepatic insulin resistance and lipoprotein metabolism [11–13].

The dysregulation of VLDL metabolism in the metabolic syndrome is a critical event that, as we show later, has a major qualitative and quantitative impact on the metabolism of LDL and HDL. The mechanisms whereby visceral obesity increases plasma VLDL concentration are complex, but can be best understood in relation to increased hepatic lipid supply and availability [14–17], and the intrinsic effects of insulin resistance on hepatic output of VLDL and catabolism of VLDL in peripheral tissue [18,19]. Since intra-abdominal adipocytes are very lipolytically active [20], visceral fat accumulation in the metabolic syndrome results in markedly increased flux of free fatty acids in the portal vein to the liver. The increased portal flux of fatty acids to the liver stimulates hepatic secretion of apoB by increasing synthesis of cholesterol esters and triacylglycerols [15]. The net effect of these processes is increased production of larger, triacylglycerol-rich VLDL particles relative to smaller VLDL particles. Malmstrom et al. [21] reported that, in healthy individuals, insulin decreases the hepatic production of VLDL1 particles without affecting VLDL2, and that lowering of plasma fatty acids levels also results in a shift toward hepatic production of VLDL3 particles. Increased production of VLDL3-apoB in diabetes and insulin resistance is, however, important for these particles and are probably the source of small dense LDLs [22].

Consistent with this, Type II diabetics were shown to have a specific increase in hepatic secretion of VLDL1 that was not suppressible with an acute insulin infusion [23]. Coupling of both triacylglycerol and apoB production in the setting of increased VLDL1 particle secretion has recently been reported by Taskinen [24] employing dual tracers. This suggests increased VLDL1 particle secretion and not increased particle size. As discussed later, increased hepatic secretion of VLDL1 particles may be particularly important for postprandial dyslipidaemia, as well as the generation of small dense LDL particles and increased catabolism of HDL-apoA-I. However, unpublished observations from our group show that, in subjects with the metabolic syndrome with plasma triacylglycerols between 1 and 2.5 mmol/l, VLDL-apoB concentration is chiefly determined by apoB production and VLDL-triacylglycerol concentration by catabolism, with no apparent coupling between apoB and

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Table 1: Lipoprotein concentrations and their determinants in the metabolic syndrome

↑, Mild increase; ↑↑, marked increase; ↓↓, marked decrease; ↔, no change; ?, not investigated. For further explanations, see text.
triaclyglycerol secretion rates (G. F. Watts and D. C. Chan, unpublished work).

**Cell biological perspectives**

Clinical and cell biological studies have clearly shown that insulin resistance increases hepatic VLDL secretion by several mechanisms [25–28]: increased fatty acid flux to the liver, resistance to a direct inhibitory effect of insulin on apoB secretion, decreased post-translational degradation of apoB, increased expression of MTP (microsomal triacylglycerol transfer protein), increased de novo lipogenesis related to increased expression of SREBP (sterol regulatory element-binding protein)-1c and decreased expression of PPARs (peroxisome-proliferator-activated receptors). Chronic hyperinsulinaemia also increases expression of SREBP-1c [29], thereby activating hepatic lipogenic enzymes (fatty acid synthase and acetyl CoA carboxylase) and channelling hepatic fatty acids from storage triacylglycerol pools into a secretory pool [11,30]. Increased de novo lipogenesis increases the availability of triacylglycerols for assembly and secretion of VLDL particles. In skeletal muscle and adipose tissue, insulin resistance also impairs triacylglycerol-rich lipoprotein catabolism by lipoprotein lipase activity [31]. Defective clearance of exogenously derived lipoproteins in insulin resistance also exacerbates hypertriglyceridaemia by increasing the competition between chylomicrons and VLDL for lipolysis by LPL (lipoprotein lipase) and receptor-mediated clearance [32–35]. Hence both hepatic oversecretion of VLDL particles and triacylglycerols, as well as intrinsic and competitive clearance defects, account for hypertriglyceridaemia in metabolic syndrome.

**Genetic associations**

Genetic factors evidently also determine the kinetics of VLDL-apoB and triacylglycerol metabolism in the metabolic syndrome [36,37]. These include genetic polymorphisms of transport and/or enzymic proteins involved in the regulation of lipid substrate availability and the processing of apoB in the liver. Accordingly, we have reported previously [35,36] that, in subjects with visceral obesity, the hepatic secretion of apoB was dependent on apoB signal peptide, apoE, CETP (cholesteryl ester transfer protein) and MTP gene polymorphisms. Our results underline the potential roles of genes that regulate intrahepatic processing of apoB and lipid substrates supply to the liver in determining apoB metabolism in metabolic syndrome. This important area of gene–environment risk factor interaction warrants intense investigation. Given the recent demonstration that SNPs (single nucleotide polymorphisms) across the apoAV locus are associated with plasma triacylglycerol levels [38], future studies should also examine the kinetic abnormalities of this apolipoprotein and its relationship with VLDL kinetics.

**IDLs and chylomicron remnants**

Using a bolus injection of [2H3]leucine and compartment modelling, we have found [5] that, in the postabsorptive state, obese men with metabolic syndrome on average have a 30% lower fractional catabolic rate (FCR) of IDL-apoB compared with non-obese controls. This is supported by our data [39] in similar subjects and in obese post-menopausal women with Type II diabetes obtained using an intravenous injection of a labelled chylomicron remnant-like emulsion that is cleared by the same receptor as IDL-apoB. In both of these groups, we found that the catabolic rate of chylomicron remnants was decreased by 40% compared with control subjects. We also reported recently [8] that viscerally obese men had a 4-fold increase in plasma concentrations of RLP (remnant-like particles)-cholesterol and a 2-fold increase in apoB-48 concentrations, both recognized markers of triacylglycerol-rich lipoproteins of intestinal origin. In the study of obese diabetic women, we found a significant positive association between delayed fractional clearance of the labelled chylomicron remnant-like emulsion, measured as rate of appearance of 13CO2 and plasma apoB-48 concentrations (r = 0.641, P = 0.007) [39]. As suggested earlier, the kinetic abnormalities in remnant lipoprotein reflect delayed clearance of triacylglycerol-rich lipoprotein related to an effect of insulin resistance that decreases LPL activity, hepatic remnant receptor and the synthesis of heparan sulphate proteoglycans [40–43]. In the same study [5], we also found that plasma concentration of apoC-III was significantly higher in the obese men compared with non-obese controls (162 ± 34 mg/l compared with 118 ± 24 mg/l). More importantly, plasma apoC-III concentration was positively associated with plasma triacylglycerol, RLP-cholesterol and apoB-48 concentrations and inversely associated with the percentage rate of conversion of VLDL into IDL particles [5]. Furthermore, in hypertriglyceridaemic subjects with normal rates of VLDL secretion, increased rates of apoC-III secretion were inversely associated with VLDL-apoB catabolism [44]. This metabolic abnormality may relate to the inhibitory effect of apoC-III on lipolysis and hepatic clearance of triacylglycerol-rich lipoproteins [45,46]. The reason for enhanced synthesis of apoC-III in the metabolic syndrome may involve an effect of insulin resistance in decreasing the expression of PPAR-α in the liver [47], but this requires further study. The apoC-III gene has an insulin-response element and a direct effect of insulin resistance on expression and synthesis of apoC-III is also possible [48].

The aforementioned stable isotope studies of lipoprotein remnant kinetics have been carried out in the postabsorptive state, but the findings are compatible with the consistent demonstration, based on retinol labelling and time-dependent apoB-48 responses, of excessive postprandial lipidaemia in both obese and
diabetic subjects [49–51]. The removal of chylomicron remnants and IDL by the liver involves receptors, such as LDL and LRP receptors, which bind the particle and interact with heparan sulphate proteoglycans, as well as the enzymes LPL and HL (hepatic lipase) [52]. Our observation that LDL receptor activity is decreased in obesity supports our isotopic findings in delayed fractional catabolism of IDL-apoB and a chylomicron remnant-like emulsion [5,8,51]. That synthesis of perlecans, a core protein of heparan sulphate proteoglycans, is decreased in experimental diabetes also points to an additional mechanism for delayed remnant clearance by the liver involving decreased binding to proteoglycans [43]. HL is also involved in hepatic uptake of triacylglycerol-rich lipoproteins [52]. Since the activity of this enzyme is increased in insulin resistance [53], an alternative mechanism must account for delayed clearance of remnants by the liver. Whether increased production of apoB-48 occurs in human insulin resistance and Type II diabetes is unclear. Experimental data have recently suggested a mechanism operating via increased mass and/or activity of MTP [54], but direct human evidence is required. The impact of insulin resistance on the transport of apoE from HDL to chylomicron remnant also warrants examination, for apoE recycling via HDL may be a critical determinant of postprandial lipidaemia, with implications for the metabolic syndrome [55].

### LDLs

In a recent stable isotope study [5], we found that the fractional catabolism of LDL-apoB was on average diminished by 40% in men with the metabolic syndrome compared with non-obese controls (0.35 ± 0.02 compared with 0.56 ± 0.10 pools/day; P < 0.05). Using plasma ratios of lathosterol/cholesterol and campesterol/cholesterol as markers of cholesterol synthesis and absorption respectively, we have also suggested that subjects with the metabolic syndrome have an increased rate of de novo cholesterol synthesis with reciprocal depression in the fractional rate of cholesterol absorption [56]. These changes in cholesterol homeostasis may be causally related to the reduced FCR of LDL-apoB. As suggested by radiokinetic data, the increase in pool size of VLDL in insulin resistance results in an increased production rate of small dense LDL particles that are catabolized more slowly by the liver [57,58]. This mechanism has not, however, been formally demonstrated in the metabolic syndrome with stable isotopes, although there is evidence that subjects who accumulate small dense LDL have increased hepatic production of VLDL2-apoB [22].

Insulin resistance down-regulates LDL receptor expression and activity via a direct mechanism and indirectly by altering hepatic cholesterol content and metabolism. SREBP-2 has been shown recently [27] to regulate cholesterol metabolism by activating the expression of genes required for synthesis and transport of cholesterol. Hyperinsulinaemia in the metabolic syndrome could stimulate LXRs (liver X receptors) and this, in turn, could stimulate hepatic cholesterol synthesis via SREBP-2 [27,59]. This suggests that, in the metabolic syndrome, delayed clearance of LDL particles by the liver may be governed partly by an increase in the pool size of free cholesterol in the liver. As discussed later, delayed catabolism of LDL in insulin resistance may also reflect re-modelling of LDL particles consequent on hypertriglyceridaemia, increased lipid transfer via CETP [60] and increased lipolysis by HL [53]. Glycation of the lysine and arginine residues of LDL-apoB decreases receptor-mediated catabolism of LDL [61]. This mechanism may, therefore, also contribute to expansion in the plasma pool of small dense LDL particles in Type II diabetes with poor long-term glycaemic control.

Plasma concentrations of LDL-cholesterol are, however, usually normal or marginally elevated in individuals with visceral obesity and insulin resistance [62]. This is because the LDL particles have been remodelled to the small dense subclass, or so called ‘LDL-phenotype Pattern B’. These LDL particles are rich in apoB relative to cholesterol and are generated by the concerted action of CETP and HL. The primary metabolic event that allows the remodelling of LDL is considered to be the increase in hepatic VLDL1 production with expansion of plasma VLDL1 pool size, although the contribution of delayed catabolism of VLDL1 must also be substantial [22,63]. Hypertriglyceridaemia, which in insulin resistance and diabetes chiefly reflects increased plasma VLDL1-triacylglycerol concentrations, enhances the CETP-mediated exchange of VLDL-triacylglycerols for LDL cholesteryl esters. Under the action of HL, which is up-regulated in insulin resistance, the triacylglycerol-rich LDL particles are hydrolysed into small dense LDL. Hence the accumulation of small dense LDL in the metabolic syndrome involves re-modelling of LDL particles as a consequence of hypertriglyceridaemia [52]. As reviewed later, the same metabolic process is involved in the remodelling of HDL particles. The accumulation of small dense LDLs in the metabolic syndrome is particularly significant for these particles are catabolized slowly by the liver, interact avidly with arterial wall matrix proteoglycans and are highly susceptible to chemical modification such as oxidation, all of which increase their atherogeneity [64,65]. The importance of these LDL particles for progression of CAD (coronary artery disease) in the metabolic syndrome has been underlined recently in an angiographic analysis of the DAIS (The Diabetes Atherosclerosis Intervention Study) trial and by demonstration that they independently impair endothelial function in Type II diabetes [66,67].
**HDLs**

The kinetics of HDL-apoA-I and -apoA-II have been studied with stable isotopes in both insulin-resistant and diabetic patients. Two groups have reported independently that in overweight/obese subjects with IGT (impaired glucose tolerance) and insulin resistance, low plasma levels of HDL-cholesterol and -apoA-I were associated with enhanced HDL-apoA-I catabolism with no relationship found with HDL-apoAII kinetics, which was in fact found to be unimpaired in these subjects [68,69]. Similar findings were reported in Type II diabetes mellitus by Duvillard et al. [70]. Frenais et al. suggested that, in Type II diabetes mellitus, increased HDL-apoA-I catabolism was probably a consequence of increased HDL-triacylglycerol concentration, insulin resistance and a decrease in the ratio of apoA-I to apoA-II. In a study of dyslipidaemic subjects with the metabolic syndrome [6], we have confirmed the foregoing observations that low plasma levels of HDL-apoA-I are related to an increase in HDL-apoA-I catabolism (0.30 ± 0.01 compared with 0.20 ± 0.03 pools/day) compared with controls. However, we also found that the subjects with metabolic syndrome had an increased rate of HDL-apoA-I production (15.5 ± 0.8 compared with 12.0 ± 0.0 mg·kg⁻¹ of body weight·day⁻¹) and a trend to a significant increase in the catabolism of apoA-II relative to controls (0.23 ± 0.02 compared with 0.17 ± 0.03 pools/day; P = 0.089). We have also investigated recently the kinetics of LpA (lipoprotein A)-I and LpA-I/A-II particles in similar subjects. Compared with controls, we found that subjects with the metabolic syndrome had a significant decrease in plasma LpA-I and LpAI/AII concentrations due to accelerated catabolism of both of these particles (G. F. Watts and D. C. Chan, unpublished work). These new findings require confirmation.

The results of stable isotope studies showing that the FCR of HDL-apoA-I is increased in the metabolic syndrome are compatible with earlier radiokinetic data. Brinton et al. [72] first demonstrated that subjects with hypertriglyceridaemia and low HDL-cholesterol, who did not necessarily have metabolic syndrome, had higher fractional catabolism of both HDL-apoA-I and -apoA-II. Smaller studies, however, suggested that, in hypertriglyceridaemic subjects, the transport rate of HDL-apoA-I and the fractional catabolism of HDL-apoA-II were both increased [73]. The methods employed in these early radiokinetic studies were quite different and perhaps not as consistent and rigid as the more recent stable isotope studies.

**Experimental studies**

Experimental radiokinetic studies in the New Zealand White rabbit clearly show that enhanced HDL-apoA-I clearance is directly dependent on the triacylglycerol enrichment of HDL and on the activity of HL [74,75]. Increased triacylglycerol content of HDL is due to increased exchange of neutral sterols with large VLDL₃ via the action of CETP, which can also be up-regulated by an increased plasma concentration of NEFAs (non-esterified fatty acids) [76,77]. Clinical and cell biological studies have shown that plasma NEFAs plays an important role in modulating neutral lipid transfers through a dual effect on both the level and activity of CETP. More specifically, NEFAs could up-regulate both the expression of the CETP gene and the secretion of the protein in cultured cells [76]. Also, it could dissociate the heteroexchange of cholesteryl esters and triacylglycerols and favour the redistribution of neutral lipids from HDL towards plasma apoB-100-containing lipoproteins [77]. In healthy individuals, an intra-lipid infusion (an effect equivalent to significant expansion with VLDL₁ pool size) that increased the triacylglycerol content of HDL resulted in a 26 % increased in the FCR of apoA-I, chiefly due to enhanced clearance of LpA-I (HDL₂ subpopulation), but not LpA-I/A-II (HDL₃ subpopulation) particles [75]. Several sources of experimental and clinical evidence show that HL activity is increased in insulin resistance and correlates with the central adiposity [78–80]. Despres et al. [79] were the first to show a positive correlation between intra-abdominal fat and post-heparin lipase activity which, in turn, correlated inversely with plasma HDL₂-cholesterol concentrations. Triacylglycerol-enriched HDL, generated by increased neutral lipid exchange with VLDL₁, is a preferred substrate for HL, which accelerates the catabolism of these thermodynamically unstable particles [80]. The apparent increase in HDL-apoA-I catabolism from in vivo studies concurs with in vitro data showing that apoA-I enhances HL-mediated phospholipid hydrolysis of reconstituted HDL particles, whereas HDL-apoA-II inhibits this process [81,82]. The precise mechanism of the increased HL activity in insulin resistance and diabetes is unknown, but is considered to be due to a long-term effect of hyperinsulinaemia [83]. In summary, compelling data suggest that, in insulin resistance, hepatic oversecretion of VLDL-triacylglycerol, increased CETP activity and increased HL activity in insulin resistance is critical not only to the increased production of small dense LDL, but also to increased catabolism of HDL₂ particles.

**New areas for research**

There are several new areas of research concerning the effect of the metabolic syndrome on HDL metabolism. In vitro studies have recently pointed to key roles of several new proteins in RCT (reverse cholesterol transport), including ABCA1 (ATP-binding cassette transporter A1), SR-B1 (scavenger receptor class B1) and PLTP (phospholipid transfer protein) [84–87]. Recent evidence also supports the important role of pre-β HDL particles in HDL metabolism and RCT [88]. Hence further studies should also examine pre-β HDL kinetics.
and the relationship with cellular cholesterol efflux and RCT. The effect of insulin resistance on the transport of LpA-I and Lp-A/A-II particles also needs further investigation in vivo. The impact of genetic mutations or SNPs involved in RCT [e.g. apoA-I, ABCA-I, LCAT (lecithin: cholesterol acyltransferase), CETP, PLTP and HL] on HDL kinetics warrants further investigation [89].

**INTERVENTIONAL STUDIES: REGULATION OF DYSLIPOPROTEINAEMIA**

**Lifestyle modifications**

The phenotypic expression of the metabolic syndrome results primarily from the interaction of genetic and environmental factors, chiefly excess caloric intake and decreased physical exercise. Lifestyle modifications are, hence, the first approach to correct the dyslipidaemia in the metabolic syndrome [2,90]. Studies of lifestyle changes on lipoprotein metabolism in obesity that have employed stable isotope only are reviewed here.

**Weight loss**

Weight reduction consistently improves dyslipidaemia and insulin resistance in obese subject [91,92]. An early radiokinetic study by Ginsberg et al. [93] showed that weight loss decreased hepatic secretion of VLDL-apoB by 60%; however, this investigation was uncontrolled, only six subjects were studied and no data were provided on changes in the adipose tissue compartment. In a randomized controlled trial employing a traditional low-fat low-energy diet, we investigated [10] the effect of weight loss in 26 viscerally obese men on apoB-100 kinetics using a primed infusion of [13C]leucine and MRI to measure changes in adipose tissue compartments. Kinetic studies were carried out before and after weight loss at isoenergetic steady state. Weight reduction of approx. 10 kg decreased hepatic apoB-100 secretion and reciprocally up-regulated the LDL catabolism by 50% and 125% respectively. These changes were predominantly related to loss of intraperitoneal adipose tissue mass as opposed to changes in subcutaneous and retroperitoneal fat, as well as to improvements in hepatic insulin sensitivity, measured by HOMA (homoeostasis model assessment) score. Reduction in intraperitoneal adipose tissue mass remained a significant predictor of the conversion of pre-β-HDL into α-HDLs, suggesting stimulation of RCT irrespective of an increase in the HDL-cholesterol concentrations. This notion and other aspects of HDL kinetics require to be investigated in the metabolic syndrome in relation to weight loss using different dietary regimens.

**Dietary factors and physical activity**

In a meta-analysis of stable isotope studies, we have shown [96] that total dietary fat (mainly as saturated fatty acids) exerts a significant effect in increasing hepatic secretion of VLDL-apoB. Other dietary factors, such as fatty acid composition and plant sterols, can also play an important role in regulating lipoprotein metabolism [97]. Gill et al. [98] recently found that, in non-obese hypercholesterolaemic subjects, substituting mono-unsaturated for saturated fatty acids increased the clearance of LDL-apoB, but did not alter the production and catabolic rates of VLDL1 and VLDL2. Two uncontrolled radiokinetic studies have reported that dietary plant sterol supplementation reduces hepatic secretion of VLDL-apoB in hypercholesterolaemic patients with Type II diabetes [99,100]. However, the effects of these dietary factors on apoB and apoA kinetics in the metabolic syndrome have not been investigated.

Physical inactivity is a risk factor for CHD (coronary heart disease) [101,102] and potentially contributes to the dyslipoproteinaemia of the metabolic syndrome. In a recent controlled study of Type II diabetic patients, supervised aerobic exercise [60–85 % \(\dot{V}O_2\max\) (maximal oxygen uptake) four times/week for 6 months] decreased hepatic secretion of VLDL-apoB with parallel improvements in insulin sensitivity and reduction in total body fat [103].

**Pharmacotherapies**

In addition to lifestyle changes, several lipid-regulating agents may be used to improve dyslipidaemia in the metabolic syndrome [104,105]. The commonly used agents that have been employed in stable isotope studies include HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase inhibitors, fibrate derivatives and fish oils, so that only these are reviewed in detail here. The putative mechanisms of action of these therapies on lipoprotein metabolism in the metabolic syndrome are summarized in Table 2, and are discussed further below.

**HMG-CoA reductase inhibitors**

The currently available HMG-CoA reductase inhibitors (or statins) include lovastatin, pravastatin, simvastatin, fluvastatin, atorvastatin and rosuvastatin. Statins are most effective in treating hypercholesterolaemia due to increased plasma concentrations of LDL particles [106,107]. However, they are significantly less effective in lowering plasma triacylglycerol and raising HDL-cholesterol levels. Recent clinical end-point trials have
demonstrated that statins can decrease cardiovascular events in patients with impaired glucose tolerance, Type II diabetes mellitus, hypertension and the metabolic syndrome, irrespective of the initial level of cholesterol [108–118].

The fundamental mechanism of action of statins on cholesterol metabolism involves decreased conversion of HMG-CoA into mevalonate by competitive inhibition of the rate-limiting enzyme HMG-CoA reductase [119]. This decreases the flux of mevalonate to sterol precursors and cholesterol. At the molecular level, the reduction in intracellular cholesterol content up-regulates the activity of SREBPs, which, in turn, induces the expression and synthesis of LDL-receptors [27]. As a consequence, the hepatic clearance of LDL and chylomicron remnant from plasma is increased. In addition to an inhibitory effect on de novo cholesterol synthesis, statins may also decrease cholesterol esterification rates, triacylglycerol substrate availability, the expression of apoB and possibly other related gene products, such as MTP [120–122].

**ApoB kinetics**

Consistent with the aforementioned mechanisms, we have consistently demonstrated that, in centrally obese subjects, atorvastatin (40 mg/day for 6 weeks) significantly increases the catabolism of all apoB-100-containing lipoproteins, including IDL and LDL-apoB [123]. However, in these subjects, atorvastatin did not decrease VLDL-apoB secretion, and we have interpreted this as being partly due to a direct or indirect effect of persistent insulin resistance on the hepatic processing of lipids and apoB. Forster et al. [124] and Bilz et al. [125] have also independently reported that, in combined hyperlipidaemic subjects, atorvastatin increases the conversion rate of VLDL into LDL and the catabolic rate of LDL without altering apoB production, but their subjects were not selected for having the metabolic syndrome. In a recent kinetic study by Caslake et al. [126] in subjects with moderate hypercholesterolaemia, rosuvastatin (40 mg/day for 8 weeks) was shown to improve dyslipidaemia, chiefly by increasing the clearance of LDL with a small effect in decreasing the synthesis of VLDL1. These kinetic studies evidently question whether persistent insulin resistance plays a major role in blunting the effect of statins on hepatic secretion of apoB in the metabolic syndrome. However, the insulin-resistant status of the subjects employed in these studies was not documented. We have also reported that atorvastatin can improve triacylglycerol-rich lipoprotein metabolism in insulin resistance by decreasing plasma concentrations of apoB-48, apoC-III and RLP-cholesterol, as well as by increasing the fractional catabolism of IDL-apoB and chylomicron remnants [127,128]. The efficacy of statins in improving the catabolism of chylomicron remnants may depend on their potency in inhibiting HMG-CoA reductase; we have found that, in contrast with atorvastatin, pravastatin (a weaker statin) does not significantly alter plasma apoB-48 levels or the fractional catabolism of a chylomicron remnant-like emulsion in Type II diabetes (G. F. Watts and P. H. R. Barrett, unpublished work). In another recent study, we reported that inhibition of HMG-CoA reductase with atorvastatin promoted intestinal absorption of dietary cholesterol, as measured by the plasma campesterol/cholesterol ratio [129]. This counter-regulatory mechanism could increase cholesterol availability in the liver and stimulate apoB secretion, thereby diminishing the effect of statins on hepatic secretion of apoB and the triacylglycerol-lowering potency of these drugs. The foregoing notions suggest that consideration should be given to combining a statin with agents that improve triacylglycerol metabolism and/or cholesterol absorption in subjects with the metabolic syndrome. Figure 1 shows isotopic enrichments for VLDL-, IDL- and LDL-apoB following an intravenous injection of [13C]cholesteryl oleate in subjects with the metabolic syndrome before and after treatment for 6 weeks with atorvastatin. The changes in the contour of the enrichment curves are suggestive of enhanced catabolism of these lipoproteins with atorvastatin [123,127].

**ApoA-I kinetics**

Generally, statins do not appreciably elevate plasma HDL-cholesterol concentrations. The significant increases reported are probably consequent on a
Figure 1  Isotopic enrichments for (a) VLDL-, IDL- and LDL-apoB following an intravenous injection of $^{[3]H}_3$leucine and
(b) breath CO$_2$ for intravenous injection of $^{[13]C}$cholesteryl oleate in subjects with the metabolic syndrome before
and after treatment for 6 weeks with atorvastatin

Fibrate derivatives
Fibrate derivatives are pharmacological agonists of the nuclear hormone receptor PPAR-α [136,137]. Fenofibrate, gemfibrozil, cipofibrate and bezafibrate are

triaclyglycerol-lowering effect. Asztalos et al. [130] have also suggested that statins that are more potent
in inhibiting CETP activity, such as atorvastatin, are
more likely to increase the plasma concentrations of
apoA-I and pre-α1 HDL subspecies. As reported recently
with rosuvastatin [131], the efficiency of a statin in
decreasing plasma triacylglycerol and LDL$_{3}$-cholesterol
and in increasing HDL-cholesterol and -apoA-I levels
depends on the pretreatment plasma lipid profile. In
this study [131], the most impressive results were seen
in subjects with atherogenic lipid phenotype typical
of the metabolic syndrome. In vitro data suggest
that inhibition of cholesterol biosynthesis with statins
increases the production of apoA-I by decreasing a
Rho signalling pathway which, in turn, activates PPAR-
α [132]. Whether this translates into an appreciable
effect on HDL-apoA-I transport in vivo remains to be
demonstrated. In a recent isotope study, however, we
found that atorvastatin did not significantly alter HDL-
apoA-I production or catabolism in subjects with the
metabolic syndrome [6]. Recent clinical data also suggest
that high dose atorvastatin lowers HDL-cholesterol
concentrations [133], but this is not seen with simvastatin,
pravastatin or rosuvastatin. A radiokinetic study in New
Zealand White rabbits has suggested that this effect of
atorvastatin results from enhanced catabolism in HDL-
apoA-I, without a concurrent increase in production
rate [134]. This observation is, however, not wholly
compatible with human data showing that atorvastatin
increases the plasma concentrations of large cholesterol-
rich α1 HDL particles [130], since this should slow
the fractional catabolism of HDL-apoAI. Rosuvastatin
is the most potent statin available to date [107] and,
as mentioned earlier, can have a significant effect in
raising plasma HDL-cholesterol and HDL-apoA-I in the
metabolic syndrome across a wide dose range [107,135].
However, the mechanism of action of rosuvastatin on
HDL metabolism in the metabolic syndrome and Type II
diabetes requires further investigation.
the common ones used to treat high plasma levels of triacylglycerol-rich lipoproteins and low HDL-cholesterol. Clinical trials have shown that fibrates can reduce cardiovascular events in high-risk subjects with Type II diabetes and the metabolic syndrome [138–141].

The mechanisms of action of fibrates on lipoprotein metabolism and atherogenesis have been elucidated consistently in animal and in vitro studies [142]. Briefly, PPAR-α activation by fibrates can regulate a variety of genes involved in lipid metabolism, thrombosis and inflammation [137,142,143]. Briefly, PPAR-α agonists reduce triacylglycerol substrate availability in the liver by stimulation of peroxisomal and mitochondrial β-oxidation, thereby decreasing hepatic VLDL secretion. The stimulation of fatty acid catabolism results from an effect of PPAR-α activation that increases the expression of key proteins, including FABP (fatty acid binding protein), acyl-CoA synthase and carnitine palmitoyl transferase-I [144]. Fibrates also promote intravascular VLDL lipolysis by inducing and repressing the genetic expression of LPL and apoC-III respectively, via the corresponding PPREs (peroxisome proliferator responsive elements) [145]. ApoA-V has recently been implicated as a facilitator of VLDL lipolysis [146], and fenofibrate recently shown to increase expression of this apolipoprotein [147]. Fibrates also increase the expression of apoA-I, apoA-II, ABCA1 transporters and SR-B1 by activating the LXR-α pathway, thereby promoting cholesterol transport from the periphery to the liver via HDL [142,148–150]. Recent evidence also suggests that fibrates stimulate the receptor-mediated uptake of LDL by the liver by inhibiting hepatic cholesterol synthesis as a consequence of the regulation of SREBPs [151]. The kinetic effects of fibrates on lipid and lipoprotein metabolism, in particular HDL metabolism, potentially provide a major mechanism for the benefit of fibrates in the clinical end-point trials referred to above.

**ApoB and apoA-I kinetics**

In a recent stable isotope study [6], we showed that fenofibrate significantly increased the catabolism of VLDL-apoB (3.77 ± 0.30 compared with 5.00 ± 0.49 pool/day; P < 0.01), IDL-apoB (2.86 ± 0.21 compared with 3.84 ± 0.40 pools/day, P < 0.01) and LDL-apoB (3.5 ± 0.02 compared with 4.4 ± 0.02 pools/day, P < 0.01) in subjects with the metabolic syndrome (Figure 2). The latter is consistent with the work of Caslake et al. [152] showing that, in mixed hyperlipidemia, fenofibrate increases the production of large LDL particles that have a higher affinity for and are catabolized more rapidly by hepatic LDL-receptors. Relative to atorvastatin, we also found [6] that fenofibrate also increased the production of apoA-I (15.4 ± 0.70 compared with 18.1 ± 1.6 mg·kg⁻¹·day⁻¹; P < 0.01), despite the fact that plasma CETP activity was inhibited less with fenofibrate than with atorvastatin [6]. These kinetic changes with fenofibrate treatment were coupled with a significant increase in plasma concentrations of apoA-I and apoA-II, as well as a decrease in plasma apoB, apoC-III and lathosterol concentrations. Our recent unpublished observations also suggest that fenofibrate did not change LpA-I concentration, but increased LpA-I/A-II concentration (11 %, P = 0.018) as a result of increased production of LpA-I/A-II particles (18 %, P = 0.022; G. F. Watts and P. H. R. Barrett, unpublished work). Fenofibrate treatment in this study did not decrease the hepatic secretion of VLDL-apoB, which could again be due to an effect of persistent insulin resistance. Our kinetic findings of the effects of fenofibrate are exactly similar to those recently reported in subjects with frank mixed hyperlipidaemia in the absence of apparent insulin resistance [125]. These results are also compatible with an earlier radiokinetic study employing gemfibrozil [153]. The significance of increasing the production of HDL-apoA-I for preventing and reducing atherosclerosis has been well documented by experimental studies in several animals [154,155] and, recently, in a human
study of patients with acute coronary syndrome infused with apoA-I–Milano phospholipid complexes [156].

**Fish oil supplementation**

Fish oils are a rich source of \( n-3 \) fatty acids EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) [157]. Increasing evidence suggests that fish oil consumption protects against coronary disease and sudden death [158,159]. Regulation of lipid and lipoprotein metabolism by fish oils may be a significant mechanism underlying their anti-atherogenic properties, which also include anti-inflammatory, anti-thrombotic and hypertensive effects. Human studies have consistently shown that fish oil supplementation decreases plasma triacylglycerol concentrations by up to 40% [160]. The mechanism of action of fish oils on plasma triacylglycerol level could result in a decrease in hepatic triacylglycerol synthesis as a consequence of inhibition of diacylglycerol acyltransferase, fatty acid synthase and acetyl-CoA carboxylase enzyme activities [161]. Fish oils also enhance fatty acid \( \beta \)-oxidation by stimulating PPAR-\( \alpha \), although their effects on this transcription factor are much weaker than fibrates [162]. Fish oils additionally decrease the hepatic pool of triacylglycerols by suppressing the expression of SREBP-1c gene, thereby inhibiting \textit{de novo} synthesis of both fatty acids and triacylglycerol. \( n-3 \) Fatty acids apparently decrease the expression of the SREBP-1 gene by accelerating the catabolic rate of SREBP-1 mRNA [163,164].

We have reported that, in subjects with metabolic syndrome, fish oils (4 g/day for 6 weeks) diminish VLDL-apoB production (14.8 ± 2.3 compared with 10.1 ± 1.2 mg·kg\(^{-1}\)·day\(^{-1}\); \( P < 0.05 \)) and enhance conversion of VLDL into LDL-apoB (22.7 ± 3.9 compared with 39.2 ± 4.5%; \( P < 0.05 \)) [165]. Fish oil supplementation did not alter the FCRs of apoB in VLDL, IDL and LDL nor the catabolism of the chyomicron remnant. In a recent uncontrolled study, Frenais et al. [166] found that, in five diabetic patients, the FCR and absolute production rate of HDL-apoA-I were significantly decreased after treatment with fish oils. The authors [166] concluded that this was probably, in part, related to a decrease in plasma triacylglycerol levels which, as reviewed above, results in a more thermodynamically stable HDL particle in the plasma. In another study of overweight or Type II diabetic patients by our group [167], fish oils also significantly increased plasma concentrations of HDL-cholesterol, in particular HDL2-cholesterol, consistent with a decrease in catabolism of HDL-apoA-I. Moreover, fish oil supplementation has the potential of reducing postprandial triacylglycerol-rich lipoprotein concentrations in both lean and obese individuals [168,169]. In a recent study of obese subjects, we have also found [170,171] that EPA and DHA have differential effects on plasma lipid and lipoproteins. EPA significantly decreased HDL\(_3\)-cholesterol by 7%, whereas DHA increased HDL\(_2\)-cholesterol and LDL-cholesterol by 29% and 8% respectively [170,171]. Further investigations should also explore the effects of fish oils, as well as the differential effects of EPA and DHA, on NEFA, triacylglycerol, apoB and apoA-I kinetics in the metabolic syndrome.

**Combination therapy**

In many patients with the metabolic syndrome, lipid-regulating monotherapy (e.g. statins or fibrates) may not provide adequate improvement in dyslipidaemia. More aggressive treatment strategies involve use of dual or multiple lipid-regulating agents to treat the lipid and lipoprotein abnormalities. This approach harnesses the complementary mechanisms of action of the different agents [172]. Possible combinations include statin–fibrate, statin–niacin and statin–fish oils regimens. While effective in correcting dyslipidaemia, increased risk of myopathy has been shown in patients concomitantly treated with statin and fibrates [173].

Combination of a statin with fish oils is a safe and effective treatment of mixed hyperlipidaemia that, in our view, is undervalued and not widely used in the metabolic syndrome [128,174]. We have reported recently [128] that, in insulin-resistant obese men, combination treatment with atorvastatin and fish oils resulted in additive effects on plasma triacylglycerol (−40%) and HDL-cholesterol (+15%) greater than either of atorvastatin or fish oil alone. Kinetic data revealed that atorvastatin + fish oils decreased VLDL-apoB secretion and increased the FCRs of VLDL-, IDL- and LDL-apoB and the percentage conversion of VLDL into LDL (Figure 3). These improvements were not achieved by either atorvastatin or fish oil monotherapy [175]. Unpublished data also show that adding fish oils to atorvastatin raised HDL-cholesterol in these patients by decreasing the fractional catabolism of HDL-apoA-I (G. F. Watts and D. C. Chan, unpublished work). The cardiovascular benefit of the combination of rosuvastatin and fish oils (Omocor) is at present being trialled in patients with heart failure.

Therefore combination lipid-regulating therapy is an important advance in managing dyslipidaemia in the metabolic syndrome. When accompanied by hypertriglyceridaemia and low HDL-cholesterol, LDL elevations can be managed using a statin in combination with niacins, fibrates or fish oils. Statins should be used as first-line agents to lower LDL-cholesterol [2,90] and additions of fibrates, niacins or fish oils to decrease triacylglycerols, elevate HDL-cholesterol and reduce formation of atherogenic small dense LDL particles. However, the combinations of statins with fibrates or niacins have the potential for interactions that increases the risks of adverse effects, such as myositis and hepatotoxicity [173]. The precise mechanism of action of these and other drug
CONCLUSIONS

Dyslipoproteinaemia is a cardinal feature of the metabolic syndrome characterized by elevated plasma triacylglycerols, reduced HDL-cholesterol, elevated apoB concentrations and a predominance of small dense LDL particles. The mechanisms for dyslipidaemia in the metabolic syndrome have been elucidated by the use of tracer kinetic studies. The abnormalities in lipoprotein metabolism arise from dysregulation of both apoB and apoA metabolism, including overproduction of VLDL-apoB and decreased catabolism of chylomicron remnants, VLDL- and LDL-apoB, as well as an increased catabolism of HDL-apoA-I particles. These mechanisms are compatible with a wide body of experimental and cell biological studies indicating that increased lipid substrate availability in the liver is a critical abnormality that dysregulates lipoprotein transport in plasma. Abnormal skeletal muscle lipid metabolism may play a critical role in regulating fatty acid supply to the liver, but its precise causal relationship with defective lipoprotein transport remains to be established. Stable isotope kinetic studies have also elucidated the mechanism of action of several therapeutic approaches for regulating dyslipidaemia in metabolic syndrome, including weight loss, statins, fibrates and fish oils. The differential effects of many of these therapies on lipoprotein kinetics support the use of complementary treatments; a good example of this is statin–fibrate combination that enhances the catabolism of apoB-containing lipoproteins and increases the production of apoA-I. Development of new tracer methodologies are required for investigating in vivo cholesterol efflux from cells, cholesterol transport in body and the turnover of the HDL subpopulation of particles.
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REFERENCES

Lipoprotein metabolism in the metabolic syndrome: pathophysiological and interventional studies


Lipoprotein metabolism in the metabolic syndrome: pathophysiological and interventional studies


125 Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. N. Engl. J. Med. 345, 1583–1592


146 Frenais, R., Ogussргrnm, K., Maugеais, C. et al. (2001) Induction of the fatty acid transport protein 1 and acyl-CoA synthase genes by dimer-selective rexinoids suggests that the peroxisome proliferator-activated receptor-retinoid X receptor heterodimer is their molecular target. J. Biol. Chem. 275, 12612–12618


150 Repa, J. J., Liang, G., Ou, J. et al. (2002) Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXr and LXRb. Genes Dev. 16, 2819–2830


