SIALIC ACID IN LIPOPROTEINS

WITH A SPECIAL REFERENCE TO LOW DENSITY LIPOPROTEINS

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Academic dissertation

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Helsingin yliopiston verkkojulkaisut
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to my family

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles, which are referred to in the text by their Roman numerals.

I Melajärvi (Lindbohm) N, Gylling H, Miettinen TA. Sialic acids and the metabolism of low density lipoprotein. J Lipid Res 1996; 37: 1625-1631

II Lindbohm N, Gylling H, Miettinen TE, Miettinen TA. Sialic acid content of LDL and lipoprotein metabolism in combined hyperlipidemia and primary moderate hypercholesterolemia. Clin Chim Acta 1999; 285: 69-84

III Lindbohm N, Gylling H, Miettinen TA. Sialic acid content of LDL and its relation to lipid concentrations and metabolism of LDL and cholesterol. Submitted.

IV Lindbohm N, Gylling H, Miettinen TE, Miettinen TA. Statin treatment increases the sialic acid content of LDL in hypercholesterolemic patients. Atherosclerosis. In press.

ABBREVIATIONS

AMI	acute myocardial infarction
ANOVA	analysis of variance
apo	apolipoprotein
Asn	asparagine
BMI	body mass index
BMDP	BioMedical Data Program
CAD	coronary artery disease
CE	cholesterol ester
CETP	cholesterol ester transfer protein
CM	chylomicron
CMR	chylomicron remnant
d	density
DM	diabetes mellitus
FCHL	familial combined hyperlipidemia
FCR	fractional catabolic rate
FFA	free fatty acids
FH	familial hypercholesterolemia
HDL	high density lipoprotein
HL	hepatic lipase
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
IDL	intermediate density lipoprotein
LCAT	lecithin:cholesterol acyltransferase
LDL	low density lipoprotein
Lp(a)	lipoprotein(a)
LPL	lipoprotein lipase
PLTP	phospholipid transfer protein
TG	triglyceride
TR	transport rate
TSA	total sialic acid
VLDL	very low density lipoprotein

1 INTRODUCTION

Atherosclerosis is a major cause of morbidity and mortality all over the world, and its most common manifestation is coronary artery disease (CAD). The pathology of atherosclerosis is due to deposition of lipids, especially cholesterol, in the arterial wall intimal layer both intracellularly and extracellularly. Cholesterol in the atherosclerotic plaques is mainly derived from circulating lipoproteins, especially low density lipoprotein (LDL), and high levels of LDL cholesterol in blood are strongly associated with CAD (Keys 1970, Martin et al 1986). Furthermore, increased atherogenicity is associated with small dense LDL particles (Austin et al 1988, Griffin et al 1994) and minimally modified LDL generated by various physical and chemical modifications (Steinberg et al 1989).

Sialic acids are aminosaccharides characteristically located in the terminal ends of carbohydrate chains of glycoproteins, and their removal exposes galactose molecules that can interact with receptors, which leads to the removal of the proteins from the circulation (Stryer 1988). LDL has sialic acid -containg carbohydrate chains both in its protein and lipid parts (Swaminathan and Aladjem 1976). Because of the role sialic acid has in the metabolism of glycoproteins its content in LDL is an interesting factor with regard to the atherogenicity of this lipoprotein. Desialylation of LDL has been shown to increase its uptake into cultured cells (Filipovic et al 1979, Orekhov et al 1992) and its interaction with extracellular matrix components (Camejo et al 1985) proportionately to the degree of desialylation. In addition, a low sialic acid content has been found in LDL of CAD patients as compared with healthy control subjects (Orekhov et al 1991b, Ruelland et al 1993), although all studies do not agree on this (Chappey et al 1998). Consequently, desialylation of LDL seems to take place in vivo, and thus it could be an atherogenic modification of the lipoprotein.

The level of LDL cholesterol in blood is regulated by the metabolism of cholesterol and LDL. As the sialic acid content of LDL affects its catabolism at the cellular level, it could be assumed that the content is also associated with LDL metabolism in vivo. This work was conducted to investigate the relations of LDL sialic acid content with various factors connected with atherosclerosis, including the concentration, composition, and metabolism of lipids and lipoproteins, and with states where the risk of CAD is elevated, namely type 2 diabetes mellitus (DM) and type II hyperlipidemias. In addition, the effect of statin treatment on LDL sialic acid content was studied.

2 REVIEW OF THE LITERATURE

2.1 Overview of lipoprotein metabolism

In human plasma, lipids are transported as lipoproteins which consist of cholesterol esters and triglycerides in the hydrophobic core, and of free cholesterol, phospholipids and apolipoproteins on the hydrophilic surface. Plasma lipoproteins are separated into five major classes on the basis of their density: chylomicrons (d < 0.94 g/ml), very low density lipoproteins (VLDL, d = 0.94-1.006 g/ml), intermediate density lipoproteins (IDL, d = 1.006-1.019 g/ml), low density lipoproteins (LDL, d = 1.019-1.063 g/ml), and high density lipoproteins (HDL, d = 1.063-1.210 g/ml) (Havel and Kane 1995). The main features of lipoprotein metabolism are presented in Figure 1.

Chylomicrons are very large lipoproteins that consist mainly of triglycerides and of minor amounts of cholesterol and phospholipids. Their major protein is apolipoprotein (apo) B-48, and they also contain apolipoproteins A, C and E. Chylomicrons are formed postprandially from dietary fats in the intestinal epithelial cells, and they enter the circulation via lymph. In the blood, triglycerides are rapidly hydrolyzed into free fatty acids by lipoprotein lipase. Some cholesterol and phospholipids, and the A and C apolipoproteins are transferred to HDL. The resulting particle, called a chylomicron remnant, is taken up by hepatic receptors.

VLDL is produced in hepatocytes, and consists of a large amount of triglycerides and smaller amounts of cholesterol and phospholipids. Its major protein is apo B-100, and it contains also some E and C apolipoproteins. VLDL carries endogenously synthesized lipids from the liver. In the blood, lipoprotein lipase hydrolyzes triglycerides, and the size of the particles diminishes. Some of these particles, called VLDL remnants, are directly removed from the blood by LDL receptors on hepatocytes. VLDL surface remnants, especially phospholipids, are transported to HDL via the function of phospholipid transfer protein (PLTP) (Jiang et al 1999). The remaining particles are transformed into IDL, and, through loss of apolipoproteins E and C and further hydrolysis of triglycerides by hepatic lipase, further into LDL.

LDL consists of one single copy of apo B-100, and of large amounts of cholesterol esters and smaller amounts of free cholesterol, triglycerides and phospholipids. LDL is the main carrier of cholesterol in blood, and it is responsible for transporting cholesterol to peripheral cells. Apo B interacts

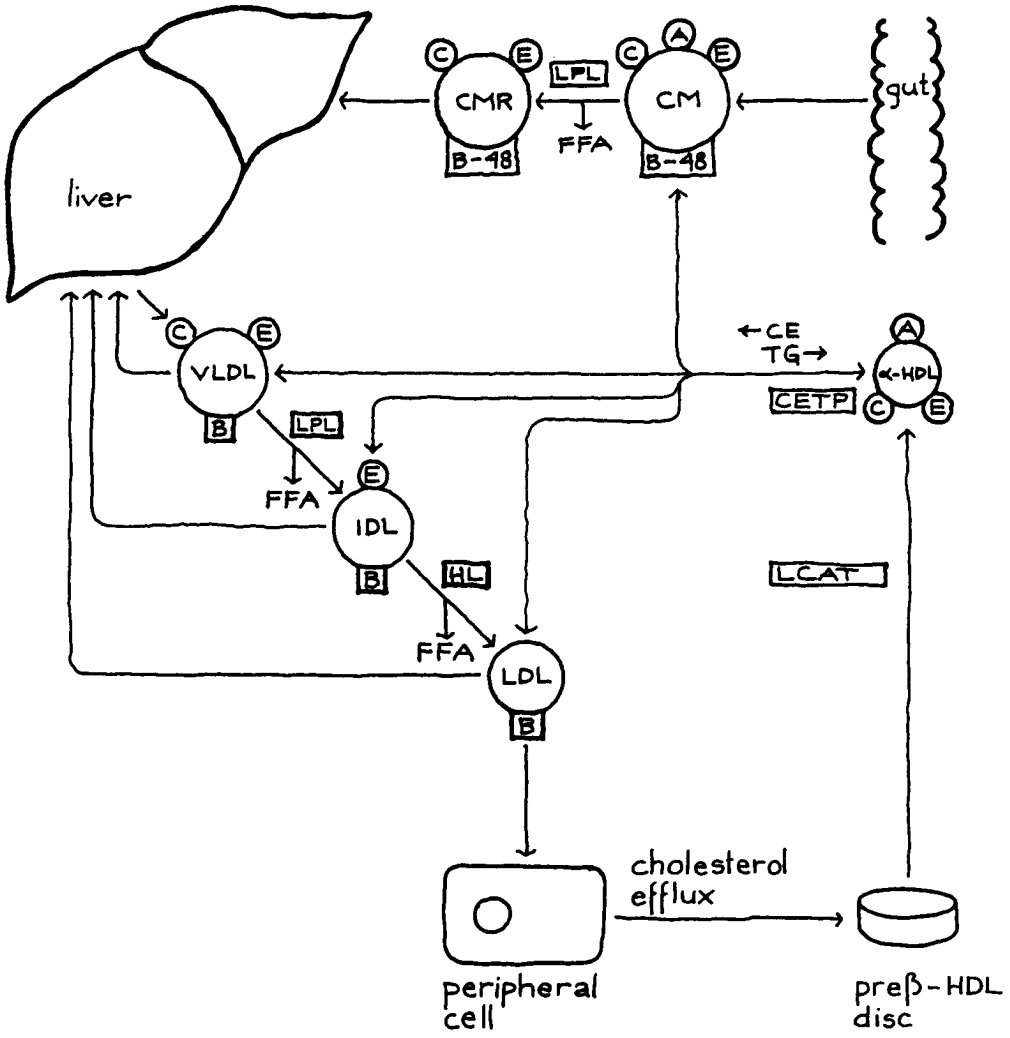

Figure 1. Overview of lipoprotein metabolism. Forward cholesterol transport, cholesterol transport from the liver to peripheral cells. Reverse cholesterol transport, cholesterol transport from peripheral cells back to the liver for excretion. See text for explanation.
A-I, apo A-I; B, apo B-100; B-48, apo B-48; C, apo Cs; CE, cholesterol ester; CETP, cholesterol ester transfer protein; CM, chylomicron; CMR, chylomicron remnant; E, apo E; HL, hepatic lipase; LPL, lipoprotein lipase; TG, triglycerides. α-HDL and preβ-HDL, HDL particles displaying α- and preβ-mobilities.

with specific LDL receptors located on cell surfaces in many tissues, including the liver, and thus LDL particles are mainly removed from the circulation.

HDL is formed as a precursor in cells of the liver and intestine. Nascent HDL contains mainly protein and phospholipids, but in the circulation it gains material, including phospholipids and free cholesterol via lipolysis of triglyceride-rich particles and from cell membranes. The primary apolipoproteins in mature HDL are apo A-I, A-II and A-IV. Free cholesterol in HDL is esterified by lecithin:cholesterol acyltransferase (LCAT), and cholesterol esters are transferred from HDL to apo B -containing lipoproteins by cholesterol ester transfer protein (CETP) in exchange for triglycerides (Tall 1986). Thus, HDL has an important role in reverse cholesterol transport: first specific HDL subclasses (preβ-HDL) function as primary cholesterol acceptors and are able to remove cholesterol from peripheral cells, and after cholesterol esterification cholesterol esters are delivered from HDL to apo B -containing lipoproteins, which can be removed from the circulation by hepatic receptors.

A sixth lipoprotein class, lipoprotein(a) (Lp(a)), consists of one LDL particle associated with one molecule of a glycoprotein called apo(a) (Utermann 1989, Utermann 1995). Its density range is 1.04-1.125 g/ml, overlapping those of LDL and HDL. Apo(a) is secreted by the liver but the site of assembly and that of catabolism of Lp(a) are unclear. Its plasma concentration is genetically determined and there are large interindividual differences. A physiological role for Lp(a) has not been found, but apo(a) has a close structural resemblance with plasminogen, and it has been shown to be able to interfere with fibrinolysis (Utermann 1995).

2.2 Low density lipoproteins

2.2.1 LDL particle structure

Like all mature lipoproteins, LDL is a spherical particle. It has a density of 1.019-1.063 g/ml and a diameter of 22-28 nm. It contains 35-45% of cholesterol esters and 6-12% of triglycerides in the core, and 20-25% of phospholipids, 6-10% of free cholesterol, and 20-25% of protein on the surface (Deckelbaum 1987). The sole protein of LDL is apo B, and one LDL particle always contains one molecule of apo B.

2.2.2 LDL metabolism

LDL is the end product of VLDL metabolism (Sigurdsson et al 1975, Reardon et al 1978, Fisher et al 1980, Thompson et al 1987, Demant et al 1996). It is produced from small VLDL particles, larger VLDL particles being rapidly removed from the circulation (Packard et al 1984). The production rate depends both on the rate at which VLDL is produced and the rate at which VLDL remnants and IDL are removed from the circulation via LDL receptors, and also on the lipolytic activity in the transformation of VLDL and IDL into LDL. In addition, some LDL can be directly secreted by hepatocytes, especially in hyperlipidemic states (Janus et al 1980, Kissebah et al 1984, Fisher et al 1994, Gaw et al 1995), but also in normolipidemia (Cohn et al 1990).

LDL is cleared from the circulation mainly by the specific LDL receptor (Brown and Goldstein 1976, Shireman et al 1977, Brown and Goldstein 1986, Goldstein et al 1995), the rest being removed by unspecific pathways, for example by the scavenger receptor pathway. The LDL receptor is located on the surface of hepatocytes and peripheral cells, and it interacts with LDL via apo B. After the binding of LDL to the receptor, the receptor-lipoprotein complex is endocytosed and LDL dissociates from the receptor. The synthesis of LDL receptors in the cell is suppressed by cholesterol derived from LDL, which phenomenon regulates the amount of cholesterol entering the cell (Brown et al 1981, Goldstein et al 1995).

Serum level of LDL is dependent both on the LDL production rate and LDL's clearance from the circulation (Grundy et al 1985, Kesäniemi et al 1987). LDL cholesterol level has been reported to have both a positive correlation with the production of LDL apo B and a negative correlation with the clearance of LDL from the circulation in some studies (International Collaborative Study Group 1986, Miettinen et al 1992, Gylling et al 1994), while others have reported the level to be associated only with LDL production (Kesäniemi and Grundy 1982, Vega et al 1985). The activity of LDL receptors affects the rate of clearance of LDL from the circulation but also its production rate, because VLDL remnants are taken up from the circulation by the same receptors, and when their activity is low, more VLDL is transformed into LDL.

Serum levels of LDL cholesterol usually increase with age, and both the catabolism (Kesäniemi et al 1987) and the production (Gylling et al 1994) of LDL is lower in elderly men. Women seem to have a slightly faster catabolism of LDL apo B than men, and estrogen therapy raises LDL catabolic rate (Kesäniemi et al 1987). In type 2 DM, LDL cholesterol levels are regulated by the catabolism but not the production of LDL apo B (Gylling and Miettinen

1996a, Gylling and Miettinen 1997), and compared to non-diabetics the production of LDL is normal but its catabolism is low (Howard et al 1987), possibly resulting from glucosylation of LDL (Howard 1987, Kesäniemi et al 1987).

High serum levels of LDL cholesterol, i.e. hypercholesterolemia or type IIa hyperlipidemia, can be a result of elevated LDL production, low LDL clearance, or both. Familial hypercholesterolemia (FH) is a condition caused by a defect in the gene encoding the LDL receptor (Goldstein et al 1995). Heterozygotes have about half the normal receptor activity, and homozygotes have practically no functional LDL receptors. Defective clearance during the whole length of the lipolytic pathway (VLDL $\rightarrow$ IDL $\rightarrow$ LDL) leads to high levels of LDL cholesterol in the blood (Shepherd and Packard 1989); heterozygotes usually have two- to threefold higher LDL cholesterol levels than normal population. A far more common form of hypercholesterolemia is polygenic in origin and in this case the serum levels of LDL cholesterol are mildly to moderately high.

High levels of LDL cholesterol can be associated with high serum triglyceride levels; this condition is called combined hyperlipidemia, or type IIb hyperlipidemia. Familial combined hyperlipidemia (FCHL) is a genetically heterogenic entity, and affected individuals in a family may have either hypercholesterolemia (type IIa), hypertriglyceridemia (type IV), or both (type IIb) (Grundy et al 1987, Kane and Havel 1995). The basic defect in FCHL appears to be overproduction of apo B (Kissebah et al 1984, Teng et al 1986, Brunzell et al 1987, Ericsson et al 1992), and serum apo B levels are characteristically elevated. Accordingly, it has been suggested that the disorder should be called hyperapobetalipoproteinemia instead of FCHL (Sniderman et al 1992). However, with the present diagnostic criteria there is no simple method to discriminate between affected and unaffected individuals in a family (Porkka et al 1997), but recent findings implicate that the presence of FCHL can be genetically determined in the near future (Pajukanta et al 1998, Pajukanta et al 1999). The catabolism of LDL in FCHL is low to normal (Teng et al 1986, Ericsson et al 1992, Aguilar-Salinas et al 1997), but overall the kinetics of apo B are heterogenic (de Graaf and Stalenhoef 1998).

2.2.3 LDL subclasses

Plasma LDL is not a homogeneous lipoprotein population, but consists of multiple subclasses with differing size and density (Lindgren et al 1969, Shen et al 1981, Krauss and Burke 1982). The density and molecular weight of

LDL particles are negatively correlated so that as the density of the particles increases, their weight and size decrease (Crouse et al 1985). LDL derived from the VLDL-IDL lipolytic cascade is initially large and buoyant, and as a result of exchange of lipids between lipoproteins it becomes smaller and denser (Fisher et al 1980, Thompson et al 1987). Small dense LDL particles consist of relatively more protein and less cholesterol than bigger and more buoyant LDL particles (Teng et al 1983). Serum triglyceride levels are positively related with LDL density so that hypertriglyceridemia is often accompanied by small dense LDL particles (Nelson and Morris 1983, Crouse et al 1985, Swinkels et al 1989b, Campos et al 1992a, Campos et al 1995, Nikkilä et al 1996). Predominance of a small dense LDL subtype, LDL subclass pattern B, is partially genetically determined; about 15% of American population have an allele that leads to LDL subclass pattern B (Austin and Krauss 1986). The subclass pattern is also affected by such factors as age and gender (de Graaf et al 1992, Austin 1993). LDL subclass pattern B is associated with high serum triglyceride and low HDL cholesterol levels (McNamara et al 1987, Austin et al 1988).

2.2.4 LDL and atherosclerosis

High serum total cholesterol concentration has been strongly connected with atherosclerosis in numerous studies (e.g. Keys 1970, Martin et al 1986, Brunner et al 1987). Being the main carrier of cholesterol in blood, LDL is also the principal lipoprotein causing atherosclerosis (Grundy 1995b). LDL has been found in atherosclerotic plaques (Hoff et al 1979), and it has been shown in vitro to be able to transform smooth muscle cells and macrophages into foam-cells (Goldstein et al 1979, Brown and Goldstein 1983), the major cells of atheromatous lesions. Vast epidemiological studies have attested a strong independent association between LDL cholesterol levels and the risk of atherosclerotic disease (e.g. the Framingham Study, Kannel et al 1979). Furthermore, clinical trials of cholesterol-lowering treatments have led to a marked fall in the incidence of cardiac events due to CAD and in mortality from CAD (Lipid Research Clinics Program 1984, Frick et al 1987, Huttunen et al 1988, Scandinavian Simvastatin Survival Study Group 1994, West of Scotland Coronary Prevention Study Group 1995, Cholesterol and Recurrent Events Trial Investigators 1996, AFCAPS/TexCAPS Research Group 1998, The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group 1998).

2.2.4.1 Arterial proteoglycans

Camejo et al first documented that LDL forms complexes with a protein present in aortic and coronary intima-media (Camejo et al 1975, Camejo et al 1980). Furthermore, the serum of subjects with acute myocardial infarction (AMI) has been reported to form more of these insoluble complexes than the serum of control subjects (Camejo et al 1976, Linden et al 1989). The concentration of LDL and its structural properties affect its binding to arterial proteoglycans (Camejo et al 1989). Small dense LDL has a higher affinity for the proteoglycans (Bondjers et al 1990, Camejo et al 1991), and a predominance of small dense LDL is associated with a higher rate of LDL-proteoglycan complex formation (Anber et al 1996, Anber et al 1997). Reversible association with arterial proteoglycans seems to cause changes in LDL that render it atherogenic (Hurt and Camejo 1987, Ismail et al 1994), and at least one of these changes is an increase in the susceptibility to oxidation (Hurt-Camejo et al 1992). Arterial proteoglycans have also been reported to increase the rate of proteolytic fusion of LDL particles taking place in the arterial intima (Pentikäinen et al 1997). Part of the cellular uptake of LDL-proteoglycan complexes has been shown to be mediated by an unspecific scavenger pathway possibly not subject to regulation like the LDL receptor pathway (Hurt et al 1990, Vijayagopal et al 1993). It has been suggested that the retention of lipoproteins in the arterial wall by their binding to proteoglycans causes lipoprotein deposition in arterial tissues (Bondjers et al 1990, Camejo et al 1991, Hurt-Camejo et al 1997, Camejo et al 1998). Complex formation between LDL and arterial proteoglycans has been shown to be strongly correlated with serum cholesterol, LDL cholesterol and apo B concentrations and to diminish significantly during lipid-lowering medication (Wiklund et al 1996).

2.2.4.2 Small dense LDL

An association between small dense LDL and CAD has been established in many studies. CAD patients have been shown to have smaller and denser LDL particles than controls (Crouse et al 1985, Swinkels et al 1989a, Tornvall et al 1991, Campos et al 1992b, Coresh et al 1993, Tornvall et al 1993, Griffin et al 1994, Gardner et al 1996), and an increased risk of AMI has been connected with LDL subclass phenotype B (Austin et al 1988) and with small LDL particle diameter (Stampfer et al 1996). However, in some studies the association has not been so clear (Lahdenperä et al 1996, Sherrard et al 1996), but in all these studies a strong inverse relation between LDL particle size and serum triglyceride level has been evident. Thus, the association between small dense LDL and CAD may not be independent, but linked to

other CAD risk factors. Small dense LDL is associated, besides with high serum triglyceride levels, also with high LDL and low HDL cholesterol levels (Austin et al 1990b, Campos et al 1992a, Krauss 1998).

Men, who are more prone to CAD than premenopausal women, have a smaller LDL particle diameter than women of same age (McNamara et al 1987, Swinkels et al 1989b, Campos et al 1992a, Nikkilä et al 1996, Sherrard et al 1996). After menopause the size of LDL particles shrinks in women as the risk of CAD grows, indicating that female hormones have an effect on LDL particle size (Campos et al 1988, Campos et al 1992a). Smoking and the use of beta-blocking agents are associated with a relative rise in dense LDL (Campos et al 1992b, Griffin et al 1994). Subjects with combined hyperlipidemia (Austin et al 1990a, Dejager et al 1993, Hokanson et al 1995) or hyperapobetalipoproteinemia (Kwiterovich 1988) have an increased prevalence of small dense LDL phenotype. Type 2 DM patients, who have an elevated risk of CAD (e.g. Kannel and McGee 1979, Uusitupa et al 1990, Koskinen et al 1992), have smaller and denser LDL particles than non-diabetics (Feingold et al 1992, Stewart et al 1993), and LDL subclass phenotype B has been shown to be associated with an increased risk of type 2 DM (Austin et al 1995). Furthermore, also non-diabetic subjects with glucose intolerance have a predominance of small dense LDL particles (Suehiro et al 1995).

Oxidation of LDL renders it more atherogenic, and small dense LDL seems to be more liable to oxidative modifications than larger LDL (de Graaf et al 1991, Tribble et al 1992, Chait et al 1993, de Graaf et al 1993). Jaakkola et al (1993) documented that dense LDL subfractions increased intracellular cholesterol concentration in cell culture, and this effect was significantly stronger for the dense LDL of CAD patients than for that of controls. Shrinking of LDL particle size seems to cause progressive changes in the conformation of LDL apo B, thus possibly affecting its receptor affinity (Teng et al 1985). However, Knight et al (1986) found no differences in the binding and degradation of light and dense LDL in fibroblasts and macrophages. They also reported that both subfractions used the LDL receptor pathway and not the scavenger pathway (Knight et al 1986), while Thompson et al (1987) documented similar binding and degradation of light and dense LDL through both the LDL receptor and the scavenger receptor. On the contrary, in two other studies small dense LDL had a weaker affinity for the LDL receptor than larger LDL (Galeano et al 1994, Galeano et al 1998), and higher affinity for LDL receptor independent binding sites (Galeano et al 1998). Therefore it could preferably be taken up via scavenger receptors on macrophages of extrahepatic tissues, like artery walls, thus promoting the formation of foam cells.

2.2.4.3 Other risk factors of atherosclerosis

Low serum level of HDL cholesterol is a well documented CAD risk factor (Nikkilä 1953, Miller and Miller 1975, Miller et al 1977, Castelli et al 1986, Frick et al 1990, Lehto et al 1993). Serum triglyceride levels have also been positively associated with CAD risk, especially in women (Nieminen et al 1992, Bengtsson et al 1993, Stensvold et al 1993), and high levels are often linked with low HDL cholesterol concentration (Robinson et al 1987, Austin 1989) and LDL subclass pattern B (Krauss 1998).

Serum levels of Lp(a) have been widely reported to be higher in CAD patients than in healthy controls (Genest et al 1991, Sandholzer et al 1992, Kario et al 1994, Bostom et al 1996), and thus Lp(a) has been suggested to be a CAD risk factor (Utermann 1989, Loscalzo 1990, Stein and Rosenson 1997). However, this association between CAD and Lp(a) has not been found in the Finnish population (Jauhiainen et al 1991, Alfthan et al 1994). Another trial failing to establish any value of serum Lp(a) for predicting the risk of AMI was the Physicians' Health Study (Ridker et al 1993). At present the interaction of CAD and Lp(a) remains unconfirmed (Maher and Brown 1995, Jialal 1998). In addition, Lp(a) seems to be partially dependent on other cardiovascular risk factors (Labeur et al 1992). The mechanism of action of Lp(a) in atherosclerosis is still not clear, but Lp(a) has been shown to be able to interfere with various factors involved in thrombogenesis and thrombolysis (Scanu 1992, Stein and Rosenson 1997).

2.3 Sialic acids

2.3.1 General

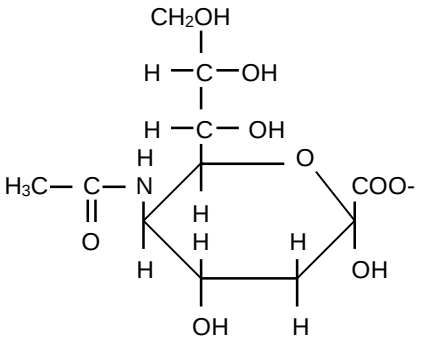

Figure 2. N-acetyl neuraminic acid.

Sialic acids are small aminosaccharides. They consist of a neuraminic acid backbone with one or multiple O- or N-linked side chains, the most abundant derivative in humans being N-acetylneuraminic acid (Figure 2). Sialic acids are numerous in many human tissues and fluids (Warren 1959b, Huttunen 1966, Sillanaukee et al 1999a). In serum, sialic acids are mostly bound to the carbohydrate chains of

glycoproteins and glycolipids. Sialic acid residues are located in the terminal ends of carbohydrate chains of many glycoproteins, for instance immunoglobulins and peptide hormones, and when the terminal sialic acid is removed by neuraminidase of the vascular endothelium, a galactose molecule is revealed. Specific receptors on hepatocytes recognize these asialoglyco-proteins and remove them from the circulation (Stryer 1988).

2.3.2 Serum sialic acid

A high serum total sialic acid (TSA) concentration has been associated with a number of conditions (Sillanaukee et al 1999a). Many studies have demonstrated a positive association between high TSA levels and the incidence of CAD (Lindberg et al 1991, Watts et al 1995a, Råstam et al 1996). TSA seems to be linked with other CAD risk factors, namely high serum cholesterol and triglyceride concentrations and low HDL cholesterol concentration (Wakabayashi et al 1992, Wu et al 1999), high serum concentration of apo B and smoking and physical inactivity (Lindberg et al 1993), and high serum Lp(a) levels (Kario et al 1994). TSA concentration has been also shown to be higher in American than Japanese subjects even after adjustment for other CAD risk factors, this being in accordance with the higher prevalence of CAD in American population (Lindberg et al 1997). However, other studies have not found any difference in TSA level between angiographically separated CAD and non-CAD subjects (Salomone et al 1998, Wu et al 1999).

Serum TSA level has been shown to be elevated in DM (Crook et al 1993, Crook et al 1996), and associated with CAD risk in diabetic subjects (Pickup et al 1995). TSA is also an indicator of microalbuminuria in DM both in cross-sectional (Crook et al 1994, Chen et al 1996) and follow-up studies (Yokoyama et al 1996). Besides, TSA concentration is higher in women than in men with DM, which could explain why DM increases the relative risk of CAD more in women than in men (Pickup et al 1997). TSA level is also elevated in many malignant diseases (Sillanaukee et al 1999a), during pregnancy (Crook et al 1997a), and in alcohol abuse (Sillanaukee et al 1999b). It is also high in inflammation (Sillanaukee et al 1999a) and positively correlated with erythrocyte sedimentation rate (Miettinen and Nikkilä 1960, Crook et al 1997b) and serum concentration of C-reactive protein (Wu et al 1999). Accordingly, serum TSA level seems to be an unspecific but sensitive marker of acute phase reactions, probably because of the pronounced content of sialic acid in many acute phase proteins.

2.3.3 Sialic acid in vascular tissue

Freshly infarcted myocardium has been shown to have a greater sialic acid content than normal myocardium (Huttunen et al 1972). Both vascular endothelium and red blood cells are rich in sialic acid and thus have a high negative charge creating a repulsion between endothelium and blood cells, which can inhibit blood clotting (Born and Palinski 1985). Treatment of cells with neuraminidase that removes a major part of their surface sialic acids has been shown to promote the uptake of LDL into the cells (Görög and Born 1982, Görög and Pearson 1984).

2.3.4 Sialic acid in lipoproteins

2.3.4.1 Chylomicrons, VLDL and IDL

The sialic acid content of chylomicrons has not been studied, while a few researchers have investigated the sialic acid content of other triglyceride-rich lipoproteins. An early study found the highest sialic acid content in VLDL, whereas levels in IDL, LDL and HDL did not differ from each other (Fontaine and Malmendier 1975). Similarly, two recent studies reported that the sialic acid content decreased with increasing lipoprotein density from light VLDL to dense IDL, then being similar until dense LDL (Anber et al 1997, Millar et al 1999). Another study described decreasing sialic acid content from VLDL to LDL and further to HDL (Harada et al 1998). In these studies, the sialic acid contents of the protein and lipid parts were not separated.

2.3.4.2 LDL

Glycoprotein. LDL has sialic acid residues both on its lipid and protein parts. The largest part of the sialic acid has been reported to be in the protein part of LDL, that is, in apo B (Tertov et al 1993). Apo B is a glycoprotein with 20 potential N-glycosylation sites, out of which up to 16 asparagine (Asn) residues are glycosylated (Yang et al 1986, Taniguchi et al 1989). About 5-9 mass-% of apo B is carbohydrate, and there are two major types of carbohydrate chains, one of which is neutral and the other acidic (Swaminathan and Aladjem 1976, Taniguchi et al 1989). They consist of the monosaccharides mannose, galactose, N-acetylglucosamine and sialic acid. The structures of the two chains are shown in Figure 3. The acidic chain has one or two terminal sialic acid residues followed by galactose molecules. Sialic acid constitutes 10% of the total carbohydrate in apo B, but unlike the

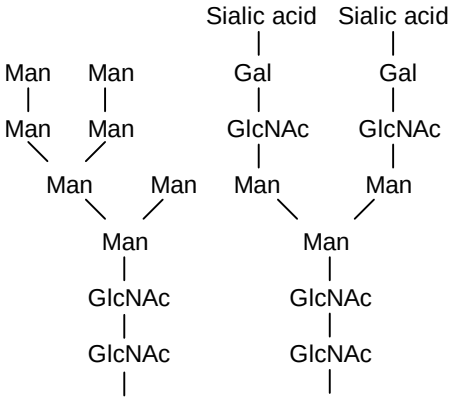

Figure 3. The two major carbohydrate chains of LDL apo B. Both are N-linked sugar chains and bound to 16 Asn residues distributed along the apo B-100 polylpeptide chain. Gal = galactose; GlcNAc = N-acetylglucosamine; Man = mannose.

other monosaccharides, the contents of which are fairly constant, the amount of sialic acid varies markedly between individuals (Swaminathan and Aladjem 1976). The apo B molecule in LDL is calculated to contain usually 12-14 sialic acid residues per particle (Taniguchi et al 1989). LDL apo B differs from most serum glycoproteins by having more galactose than sialic acid residues (Taniguchi et al 1989), and the amount of monosialylated oligosaccharides, reflecting the number of free galactose residues, can be calculated to be on the average four per LDL particle. A recent study confirmed that all LDL particles in plasma have free galactose residues at the ends of the carbohydrate chains, indicating that LDL apo B is always partially desialylated (Bartlett and Stanley 1998). The sialic acid content of LDL has a very strong positive correlation with LDL apo B concentration (Fontaine and Malmendier 1978), attesting that a large part of the sialic acid in LDL is bound to apo B.

Glycolipids. Gangliosides are sialic acid -rich glycosphingolipids transported in serum by lipoproteins, 66% of total serum gangliosides being transported by LDL and smaller amounts by other lipoproteins, and thus they also contribute to LDL sialic acid content (Dawson et al 1976, Senn et al 1989). Gangliosides probably contain all the sialic acid associated with the lipid part of LDL. In addition to the monosaccharides of LDL apo B, the lipid part of LDL contains galactosamine and glucose but no mannose (Tertov et al 1993). Sialic acid constitutes 6-7% of the total carbohydrate in LDL lipid. The content of galactose is markedly higher than that of sialic acid also in the lipid part of LDL (Tertov et al 1993), thus contributing to the number of free galactose residues in the lipoprotein particle.

2.3.4.3 Cell culture studies

Many forms of modifications have been observed to increase the atherogenicity of LDL (Steinberg et al 1989). Regarding the role of sialic acid in the metabolism of glycoproteins, its content in LDL could affect the atherogenicity of LDL. This has been studied in cell cultures. Incubation with neuraminidase leading to desialylation of either aortic cells, LDL, or both, caused a rise in aortic uptake of LDL (Day 1976). Furthermore, desialylation of LDL was shown to double its binding and uptake into human fibroblasts (Filipovic and Buddecke 1979), and there was a positive correlation between the degree of desialylation and the enhancement of uptake (Filipovic et al 1979). In addition, a recent study described accelerated uptake of cholesterol from desialylated LDL to macrophages (Harada et al 1998). Harada et al (1998) also reported that desialylation of LDL increased the transport of cholesterol esters from HDL to LDL by CETP, and desialylation of HDL decreased its capacity to remove cholesterol from cells, both effects promoting atherogenesis. However, two other studies found no difference in the cellular binding and degradation of native and desialylated LDL (Attie et al 1979, Shireman and Fisher 1979).

LDL with low sialic acid content formed insoluble complexes with arterial proteoglycans avidly, and there was a significant negative correlation between the sialic acid content of LDL and the relative avidity to form these complexes (Camejo et al 1985). This finding was confirmed in a recent study, where a fall in the sialic acid content of LDL raised its ability to interact with arterial proteoglycans (Millar et al 1999). Since the arterial proteoglycans have a strong electronegative charge (Camejo et al 1991) and desialylation of LDL diminishes its negative charge, the increased affinity could partially be due to reduced electronegative repulsion.

Enzymatic treatment of LDL with the combination of trypsin, cholesterol esterase and neuraminidase turns LDL into non-homogeneous lipid particles similar to those found in atherosclerotic lesions (Bhakdi et al 1995). This modified LDL does not contain oxidized lipids but has a high negative charge. It accumulates at a high rate into macrophages, this being mediated at least partially by the scavenger receptor pathway, and induces immunological reactions that promote early development of atherosclerotic lesions, such as activation of the complement system, and chemotaxis, adhesion and transendothelial migration of blood monocytes (Bhakdi et al 1995, Klouche et al 1998, Klouche et al 1999). All these effects were stronger for the enzymatically modified LDL than for acetylated or oxidized LDL, but were not detectable in LDL treated with only one or two of the above-mentioned enzymes.

Apart from desialylation, another modification of LDL, acetylation, augments its cellular uptake and degradation, and this is mediated not by LDL receptors but by scavenger receptors (Goldstein et al 1979, Wiklund et al 1991). Such receptors also seem to be involved in the catabolism of desialylated glycoproteins, including LDL. Desialylated glycopeptides bind to a hepatic lectin, the avidity of this binding being highly dependent on the number of free terminal galactose residues, that is, on the degree of desialylation (Lee et al 1983). Furthermore, a high density of galactose residues increases the binding of asialo-orosomucoid both to a lectin receptor on hepatocytes, and to an asialoglycoprotein receptor on macrophages (Ozaki et al 1995). Assumably, desialylated LDL can also be taken up by these receptors. Indeed, LDL and chylomicron remnants have been reported to be able to bind to an asialoglycoprotein receptor on hepatocytes (Windler et al 1991), and desialylation of chylomicrons increases their hepatic uptake and degradation, probably due to interaction with this asialoglycoprotein receptor (Guldur et al 1997). Moreover, LDL is documented to enter cells besides through the LDL receptor pathway, also through a galactose-specific lectin receptor pathway (Grewal et al 1996).

2.3.4.4 Coronary artery disease

Since the sialic acid content in LDL seems to be associated with atherogenesis, it might have a role in CAD. A Russian group has carried out extensive studies on the relations of LDL sialic acid content with atherosclerosis and CAD. They first discovered that serum or LDL of CAD patients caused a significant rise in cholesterol content of normal aortic cells during incubation, while serum or LDL from healthy subjects did not change it (Orekhov et al 1988, Tertov et al 1989a). Lipid and protein compositions of these LDLs were similar, but the sialic acid content was markedly lower in LDL of CAD patients (Orekhov et al 1989, Orekhov et al 1991b, Tertov et al 1992a, Tertov et al 1992b). Neuraminidase treatment of LDL from healthy subjects made it atherogenic, and there was a strong negative correlation between LDL sialic acid content and the amount of cholesterol accumulated intracellularly (Orekhov et al 1989, Orekhov et al 1992, Tertov et al 1992a, Tertov et al 1992b). In addition, LDL separated from normal aortic intima and from fatty streaks had a lower sialic acid content than plasma LDL (Tertov et al 1996a). These findings together support the hypothesis that desialylation is an atherogenic LDL modification taking place in vivo (Sobenin et al 1991, Orekhov et al 1992). During incubation, a decrease in the sialic acid content was the first modification observed in LDL, followed by gain of atherogenicity and decrease in the lipid content and size of the particles (Tertov et al 1998).

Moreover, it has been proved that sialic acid is implicated in the antigenic sites of LDL (Goldstein and Chapman 1981), and Orekhov et al (1991a) found, compared with healthy controls, in sera of CAD patients a thirty-fold greater amount of autoantibodies against LDL that had a strong affinity for desialylated LDL. Circulating immune complexes from blood of CAD patients were discovered to be atherogenic (Tertov et al 1990, Tertov et al 1996b), and LDL from circulating immune complexes was sialic acid -poor (Tertov et al 1996b). LDL was separated from both CAD patients and healthy subjects to sialic acid -rich and sialic acid -poor fractions by affinity chromatography, and it turned out that the proportion of desialylated LDL was considerably higher in CAD patients than in controls (Tertov et al 1992b). Desialylated LDL from both groups raised the intracellular lipid content, but this effect was much stronger for desialylated LDL from CAD patients. Sialic acid -poor LDL and LDL from CAD patients also formed aggregates during incubation while sialylated LDL or LDL from healthy subjects did not (Tertov et al 1992b, Tertov et al 1992c), and a strong positive correlation between LDL aggregation and intracellular cholesterol accumulation was demonstrated (Tertov et al 1989b, Tertov et al 1992c).

Other groups have come up with controversial results about differences in the sialic acid content of LDL between CAD patients and controls. Ruelland et al (1993) found lower a LDL sialic acid content in CAD patients than in angiographically verified non-CAD subjects, but LDL sialic acid content was not correlated with the severity of CAD. Furthermore, other studies with asymptomatic hypercholesterolemic subjects revealed no differences in LDL sialic acid content between subjects who in angiography were seen to have either no atherosclerotic plaques or one or more plaques (Chappey et al 1995, Chappey et al 1998). These results imply that the sialic acid content of LDL is not associated with the extent of CAD. In addition, LDL sialic acid content was even slightly higher in subjects with both CAD and peripheral atherosclerosis and in patients suffering from AMI than in those with CAD only and in controls (Chappey et al 1998), while another research group reported decreased LDL sialic acid content immediately after coronary artery by-pass surgery (Jahangiri et al 1999). However, a recent study found lower sialic acid content in both light and dense LDL of CAD patients compared with healthy controls (Millar et al 1999).

LDL from DM patients also turned out to be poor in sialic acid and able to cause intracellular cholesterol deposition, and as in CAD patients and controls there was a strong negative correlation between the sialic acid content of LDL and the degree of atherogenicity (Tertov et al 1992a, Sobenin et al 1993, Sobenin et al 1994). A more recent study again found no

difference in LDL sialic acid content between DM patients and control subjects (Ruelland et al 1997).

Little is known about the importance of LDL sialic acid content in the metabolism of LDL, but two studies have reported that a reduction of LDL sialic acid content by neuraminidase treatment leads to faster LDL catabolism (Hendrix et al 1975, Malmendier et al 1980).

Only a couple of studies have investigated LDL sialic acid content in hyperlipidemia. Barbosa et al (1995) stated the sialic acid content of both light and dense LDL to be high in combined hyperlipidemia but low in hypercholesterolemia compared with normolipidemia. Another study showed low LDL sialic acid content associated with hypercholesterolemia in DM patients (Maruhama et al 1983).

Two studies have shown an inverse connection between LDL sialic acid content and serum triglyceride levels (La Belle and Krauss 1990, Chappey et al 1995), and a negative correlation with LDL cholesterol and positive with HDL cholesterol level have also been documented (La Belle and Krauss 1990). However, Millar et al (1999) found no correlation between serum cholesterol or triglyceride concentrations and LDL sialic acid content.

The sialic acid -containing gangliosides, transported in serum mainly by LDL, have also been shown to be associated with atherosclerosis (Prokazova and Bergelson 1994). Gangliosides stimulate LDL aggregation (Mikhailenko et al 1991), and LDL incubated with gangliosides is avidly taken up by macrophages (Prokazova et al 1991) and fibroblasts (Filipovic et al 1981) but poorly by hepatocytes (Prokazova et al 1986) thus probably interfering with the clearance of LDL from the circulation to the liver. Furthermore, serum ganglioside concentration is high in hypercholesterolemia proportionately to the increase in cholesterol concentration (Dawson et al 1976, Senn et al 1992), and antibodies against gangliosides have been detected in serum of CAD patients (Golovanova et al 1998). In addition, high amounts of gangliosides have been found in atherosclerotic lesions compared to unaffected intima (Breckenridge et al 1975, Mukhin et al 1989).

2.3.4.5 LDL density classes

Within the LDL density range, the sialic acid content falls with growing particle density and diminishing size (La Belle and Krauss 1990, Millar et al 1999). Furthermore, Tertov et al demonstrated that desialylated LDL separated from total LDL was smaller and denser than sialic acid -rich LDL,

and poor in cholesterol and triglycerides (Tertov et al 1992b, Tertov et al 1996b). In addition, as the density of the particles rose, their sialic acid content decreased and their ability to accumulate cholesterol into cells increased (Tertov et al 1992b). In accordance with these results, La Belle and Krauss (1990) also reported that subjects with LDL subclass pattern B had a lower sialic acid content in LDL than those with subclass pattern A, and the difference appeared to be in the glycolipid and not in the protein part of the particle.

2.3.4.6 Oxidation and electronegativity

Tertov et al (1992b) found no differences in the content of oxidation products in sialic acid -poor and sialic acid -rich LDL particles, and another study found that the desialylation of LDL had no effect on its susceptibility to peroxidation (Sattler et al 1991). However, in two other studies neuraminidase treatment decreased the susceptibility of LDL to oxidation (Dousset et al 1994, Myara et al 1995). A recent study reported that desialylation of LDL with neuraminidase caused physical and chemical modifications in the structure of apo B, which could account for its lowered susceptibility to oxidation (Dousset et al 1998). Barbosa et al (1995) showed that the effect of desialylation on LDL oxidizability was dependent on the density of LDL and on the type of hyperlipidemia; light LDL was more resistant to oxidation in both type IIa and IIb hyperlipidemia than in controls, but dense LDL of IIb hyperlipidemic subjects was more susceptible to oxidation than that of normolipidemic subjects, and its desialylation raised its resistance to oxidation, while it decreased the resistance to oxidation of dense LDL in type IIa hyperlipidemia. On the other hand, a fall in LDL sialic acid content during peroxidation has also been reported (Tanaka et al 1997).

An electronegative subfraction of LDL was found to be sialic acid -poor and atherogenic, and it was suggested that this electronegative subfraction could be identical with the desialylated subfraction (Tertov et al 1995, Tertov et al 1996a). However, another group separated an electronegative LDL subfraction with a high sialic acid content, and its proportion of total LDL was positively correlated with LDL cholesterol concentration (Demuth et al 1996).

2.3.4.7 Lp(a)

Lp(a), which consists of LDL and apo(a), contains six times as much sialic acid as LDL (Ehnholm et al 1972, Utermann 1989). Since its density range overlaps that of LDL, it is possible that LDL separated by ultracentrifugation

contains some Lp(a), this possibly affecting its sialic acid content in analysis. However, the sialic acid content of LDL has been proved not to be correlated with serum Lp(a) levels (Chappey et al 1995).

Lp(a) is more resistant to peroxidation than LDL (Sattler et al 1991, Beaudeux et al 1996), but neuraminidase treatment was shown to either not affect (Beaudeux et al 1996) or reduce (Sattler et al 1991) the resistance, the latter result leading to the suggestion that the higher resistance of native Lp(a) to peroxidation could be due to its higher sialic acid content as compared with LDL. Like LDL, Lp(a) from CAD patients was reported to be sialic acid -poor and atherogenic in cell culture compared with Lp(a) from healthy controls; unlike native Lp(a) desialylated Lp(a) formed aggregates, and their formation was strongly correlated with the extent of intracellular cholesterol deposition (Tertov and Orekhov 1994).

2.4 Cholesterol metabolism

2.4.1 Cholesterol absorption

Cholesterol is derived to the body from two sources, diet and cellular synthesis. Cholesterol elimination from the body is mediated by the liver, which can transform cholesterol into bile acids. Both free cholesterol and bile acids are secreted into bile. Dietary and biliary cholesterol is absorbed from the small intestine into enterocytes, where it is incorporated into chylomicrons and secreted into the circulation. Unabsorbed cholesterol and bile acids are excreted from the body in feces as neutral steroids and bile acids. The absorption of cholesterol from the intestine depends on the amount of cholesterol in diet and in bile, and on the absorption efficiency of cholesterol, which varies considerably interindividually, the usual range being 30 to 80 % (Grundy 1983). Cholesterol absorption efficiency has been reported to regulate serum cholesterol levels in Finnish men (Kesäniemi and Miettinen 1987), so that cholesterol absorption efficiency was correlated positively with serum and LDL cholesterol levels (Kesäniemi and Miettinen 1987, Miettinen and Kesäniemi 1989, Vanhanen and Miettinen 1995), and negatively with cholesterol synthesis (Kesäniemi and Miettinen 1987, Gylling and Miettinen 1989, Miettinen and Kesäniemi 1989). However, another study reported no correlation of cholesterol absorption with serum and LDL cholesterol levels (Gylling and Miettinen 1988). Lowered cholesterol absorption efficiency has been observed in many conditions, including obesity (Miettinen and Kesäniemi 1989) and DM (Gylling and Miettinen 1997).

Small amounts of plant sterols are present in human serum, and the most common of these are campesterol and sitosterol. They are totally of dietary origin, and are absorbed in the small intestine similarly to cholesterol, but less effectively. In serum, they are transported by lipoproteins and thus their concentration is highly dependent on serum cholesterol concentration. The ratios of campesterol and sitosterol to cholesterol are positively correlated with cholesterol absorption efficiency (Tilvis and Miettinen 1986, Vuoristo et al 1988, Miettinen et al 1990), and the measurement of their concentrations from serum represents a simple method for the estimation of cholesterol absorption efficiency.

Cholestanol is a metabolite of cholesterol found in small amounts in blood, and transported by lipoproteins. Its ratio to cholesterol is strongly correlated with the ratios of campesterol and sitosterol (Miettinen et al 1998), and, like these plant sterols, it is a reliable indicator of cholesterol absorption efficiency (Miettinen et al 1989).

2.4.2 Cholesterol synthesis

Cholesterol synthesis takes place mainly in the liver, but nearly all human cells are capable of synthesizing cholesterol. The initial step in cholesterol synthesis is the conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) into mevalonic acid by HMG-CoA reductase, which is the rate limiting enzyme in cholesterol synthesis. Mevalonic acid is then transformed into squalene, and after many stages finally into cholesterol. Some of the cholesterol precursor sterols that are part of the chain leading from squalene to cholesterol are present in detectable amounts in serum, particularly Δ^8-lathosterol, desmosterol and lathosterol (Miettinen 1968, Miettinen 1969). They are transported in serum by lipoproteins similarly to cholesterol. The ratios of these precursor sterols to cholesterol are correlated positively with cholesterol synthesis rate (Björkhem et al 1987, Miettinen et al 1990), and negatively with cholesterol absorption efficiency. Thus, the precursor sterol ratios can be used as indicators of cholesterol synthesis rate.

Cholesterol synthesis in the body is strictly regulated (Brown and Goldstein 1986). Circulating lipoproteins, especially LDL, deliver cholesterol to peripheral and liver cells, and enter the cells principally via the LDL receptor. In cells, cholesterol esters are hydrolyzed to free cholesterol, which can be used as a constituent of cell membranes, in the biosynthesis of steroid hormones, or re-esterified for storage. Cholesterol entering the cell from LDL suppresses the cell's own cholesterol synthesis thus keeping up a homeostasis in the cholesterol content of the cell. In hepatocytes, cholesterol

can also be secreted back to the blood in lipoprotein particles or it can be converted into bile acids or secreted as free cholesterol with bile acids to the bile.

Cholesterol synthesis averages 9-13 mg/kg of body weight in humans per day (Nestel et al 1969). It is high in obesity (Miettinen 1970), hypertriglyceridemia (Briones et al 1986), and DM (Bennion and Grundy 1977, Briones et al 1986, Gylling and Miettinen 1997), and low in hypercholesterolemia (Miettinen 1970, Miettinen 1971). In FH, low cholesterol synthesis rate is associated with high CAD mortality (Miettinen and Gylling 1988). Both positive (Gylling and Miettinen 1989) and negative (Miettinen et al 1992, Gylling and Miettinen 1997) correlations have been reported between cholesterol synthesis rate and serum and LDL cholesterol concentrations, but most studies (Gylling and Miettinen 1988, Miettinen and Kesäniemi 1989, Miettinen et al 1989, Gylling et al 1994) found no association between these. Thus it seems that serum cholesterol levels are more dependent on cholesterol absorption than synthesis. However, in type 2 DM cholesterol synthesis seems to have an inverse association with LDL cholesterol level (Gylling and Miettinen 1996a, Gylling and Miettinen 1997).

2.4.3 Cholesterol and lipoprotein metabolism

Few studies have investigated the associations between cholesterol synthesis and absorption on the one hand, and production and catabolism of lipoproteins on the other hand. Miettinen et al (1987) reported no clear correlations between the synthesis and elimination of cholesterol and, on another side, concentration and catabolism of LDL apo B, which suggests that the changes in cholesterol metabolism are compensated in the liver without affecting the metabolism of LDL apo B. However, bile acid malabsorption enhanced both cholesterol synthesis and the catabolism of LDL apo B, while cholesterol malabsorption caused by different mechanisms only increased cholesterol synthesis but affected LDL apo B kinetics inconsistently (Miettinen et al 1987). Later, other studies found positive correlations between cholesterol synthesis rate and the catabolism of LDL apo B, and between fractional cholesterol absorption and production of LDL apo B (Miettinen et al 1992, Gylling et al 1994). Positive correlations found recently between the hepatic secretion of VLDL apo B and indicators of cholesterol synthesis suggest that the production of apo B from the liver is regulated by cholesterol synthesis rate (Watts et al 1995c, Riches et al 1997).

2.5 Statin treatment

The first statin, initially called mevinolin, was discovered over twenty years ago (Endo et al 1976, Alberts et al 1980). Statins are specific inhibitors of HMG-CoA reductase, the rate limiting enzyme in cholesterol synthesis. Inhibition of cholesterol synthesis accelerates the activity of LDL receptors (Kovanen et al 1981, Traber and Kayden 1984, Berglund et al 1989, Raveh et al 1990, Reihner et al 1990, Angelin 1991). However, the clearance of LDL particles from the circulation has been reported to either increase (Kovanen et al 1981, Bilheimer et al 1983, Malmendier et al 1989, Vega and Grundy 1991, Gylling and Miettinen 1996b), decrease (Vega et al 1988), or remain unchanged (Grundy and Vega 1985, Arad et al 1990, Vega et al 1990, Cuchel et al 1997) during statin treatment. Lowered production of LDL apo B during statin treatment has been more consistently reported (Kovanen et al 1981, Grundy and Vega 1985, Vega et al 1988, Arad et al 1990, Vega et al 1990, Gaw et al 1993, Gylling and Miettinen 1996b, Cuchel et al 1997), and it is considered to result from increased clearance of VLDL and IDL particles by LDL receptors before conversion into LDL. However, in combined hyperlipidemia a rise in the catabolic rate of LDL apo B without a significant change in its production during statin treatment has been demonstrated (Vega and Grundy 1991, Parhofer et al 1993, Schonfeld et al 1998). Some studies have also shown decreased production of VLDL apo B by the liver during statin treatment (Cortner et al 1993, Watts et al 1995b, Burnett et al 1997), possibly due to the reduction in cholesterol synthesis regulating the production of apo B in the liver to some extent (Thompson et al 1996, Riches et al 1997, Watts et al 1997). Furthermore, the basal rate of lipoprotein metabolism and the dose of the statin probably affect the changes caused by statins (Aguilar-Salinas et al 1998). In addition to increased LDL receptor activity and decreased production of LDL apo B, statin treatment was recently shown to decrease the affinity of LDL particles for the LDL receptor, which could explain why the catabolism of LDL is only modestly increased by statins (Berglund et al 1998). Thus, the mechanism by which statins lower levels of LDL cholesterol seems to be more complicated than just up-regulation of LDL receptors. The effect of statins on the sialic acid content of lipoproteins has not been studied, whereas fibrates seem to diminish it (Anber et al 1997, Millar et al 1999).

Nowadays, statins are widely used in the treatment of hypercholesterolemia. Their efficiency in lowering serum and LDL cholesterol levels and thus the morbidity and mortality related to CAD has been confirmed in many large studies (Scandinavian Simvastatin Survival Study Group 1994, West of Scotland Coronary Prevention Study Group 1995, Cholesterol and Recurrent Events Trial Investigators 1996, AFCAPS/TexCAPS Research Group 1998,

The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group 1998). They have also been shown to inhibit progression of atherosclerosis in coronary arteries (Watanabe et al 1988, Khachadurian et al 1991, Kroon et al 1993). Lately, other mechanisms apart from the lowering of cholesterol levels have been suggested to account partially for the good and relatively rapid efficiency of statins in diminishing the incidence of CAD events and CAD mortality, e.g. inhibition of the action of macrophages, promotion of endothelial relaxation, and decrease in hypercoagulability by affecting multiple factors associated with blood clotting (Vaughan et al 1996).

Out of LDL subfractions, statin treatment reduced only the concentration of the most dense fraction in one study (Nozaki et al 1990), but evenly the concentrations of all subfractions in another study (Yuan et al 1991). The ratio of cholesterol to apo B in LDL has been reported to either decrease (Nozaki et al 1990) or remain unchanged (Vega et al 1990, Yuan et al 1991, Gylling and Miettinen 1996b) during statin therapy, whereas the mean size of LDL particles enlarges (Yuan et al 1991, Zhao et al 1991) or keeps unchanged (Vega et al 1990, Cheung et al 1993, Superko et al 1997). Overall, LDL subclass pattern seems to persist during statin treatment (Vega et al 1990, Franceschini et al 1994, Homma et al 1995, Superko et al 1997). Statins have been shown to diminish the ratios of cholesterol precursor sterols to cholesterol and elevate the ratios of plant sterols, reflecting decreased synthesis and increased intestinal absorption of cholesterol (Vanhanen and Miettinen 1995, Gylling and Miettinen 1996b). A high ratio of cholestanol to cholesterol at baseline, indicating low cholesterol synthesis rate, predicts poor response to statin treatment (Miettinen et al 1998).

While statins efficaciously reduce the concentrations of serum and LDL cholesterol, their effect on serum Lp(a) levels is controversial. Many studies have found no change in Lp(a) levels during statin treatment (Berg and Leren 1989, Fieseler et al 1991, Cheung et al 1993, Hunninghake et al 1993, Haffner et al 1995, Raal et al 1997), some have reported even elevated levels (Jürgens et al 1989, Kostner et al 1989, Broijersen et al 1994, Branchi et al 1995), while also lowered levels have been described (Leren et al 1992). The importance of the apparent uneffectiveness of statins on high Lp(a) levels is unclear, but possibly subjects with very high levels would benefit from reduction of Lp(a).

3 AIMS OF THE STUDY

The deposition of cholesterol from lipoproteins to artery walls leads to atherosclerosis, and this is undoubtedly mediated by multiple mechanisms. According to data from previous studies referred to above, sialic acid as structural part of LDL particles seems to have a role in the evaluation of the atherogenic potential of LDL. A low sialic acid content in LDL of CAD patients has been reported in many but not all studies, leaving the subject controversial. Serum total and LDL cholesterol levels are regulated in general by the metabolism of cholesterol and LDL apo B, but there is no information on whether the sialic acid content of LDL is related with this. There is some evidence that LDL sialic acid content has associations with serum and lipoprotein lipid concentrations, but former data are not consistent, and there are no data on the connections of sialic acid with lipid and apo B contents in LDL subfractions. Furthermore, the effect of statins, widely used as efficacious hypocholesterolemic agents, on LDL sialic acid content has not been studied.

Thus, the aims of this study were:

- To evaluate the relations of LDL sialic acid content with the concentrations of lipids and lipoproteins, and with the composition of LDL particles
- To investigate the sialic acid content of LDL in CAD and in conditions associated with an increased risk of CAD, namely type 2 DM and type IIa and IIb hyperlipidemia
- To find out possible associations of LDL sialic acid content with the metabolism of LDL, and with synthesis and absorption of cholesterol
- To study the effect of statin treatment on LDL sialic acid content

4 PATIENTS AND METHODS

4.1 Patients and study designs

Subjects for the studies were collected from the outpatient clinics of Helsinki University Central Hospital. All subjects volunteered for the study and gave an informed consent. The study protocols were accepted by the Ethics Committee of this hospital. Clinical characteristics of the study subjects are given in Table 1.

Table 1. Clinical characteristics of the study patients. BMI = body mass index

Variables	Study I		Study II		Study III	Study IV
	DM+	DM-	IIa	IIb		
Number of subjects	20	10	22	21	98	26
Males/females	20/0	10/0	18/4	12/9	45/53	21/5
Age, years	56.0±1.2	54.2±2.7	53.5±2.2	58.2±1.1	54.0±0.7	55.8±1.6
BMI, kg/m^2	28.9±0.9	28.2±1.1	27.8±1.0	28.6±0.9	27.1±0.4	28.8±0.9

mean±SE

Study I, type 2 diabetes mellitus. The study group consisted of 20 men with type 2 DM (DM+) and 10 non-diabetic men of the same age (DM-). The diagnosis of type 2 DM was based on repeated fasting blood glucose level ≥7.0 mmol/l (WHO Consultation 1998). The duration of DM was 4.5±1.0 (mean±SE) years, and diabetes control was moderate with a mean glycated hemoglobin value of 7.8±0.5%. None of the diabetics had insulin treatment, 8 were on diet only, 9 on glibenclamide, 1 on metformin, and 2 on the combination of glibenclamide and metformin. The medications were unchanged during the study. The presence of clinically evident CAD was found in 13 diabetic subjects verified by coronary angiography (n=11), exercise bicycle test (n=1) (ST-segment or T-wave changes with typical chest pain), or with a history of myocardial infarction (n=1). The absence of CAD was observed in 7 patients verified by angiography (n=2) or exercise bicycle test (n=5). Out of the 10 non-diabetic men, 4 had CAD, of which 2 were verified angiographically and 2 with a history of myocardial infarction. In the

remaining 6 subjects, CAD was excluded by exercise bicycle test. Three DM patients and 1 control subject were smokers.

Study II, type IIa and IIb hyperlipidemia. Forty-three patients, 30 men and 13 women, were divided into two groups according to their lipid phenotype obtained in two fasting serum samples a week apart. Twenty-two subjects (18 men and 4 women) were categorized into the primary moderate hypercholesterolemia group (type IIa; LDL cholesterol 3.5-5.5 mmol/l and serum triglycerides <2.5 mmol/l), and 21 subjects (12 men and 9 women) into the combined hyperlipidemia group (type IIb; LDL cholesterol 3.5-5.5 mmol/l and serum triglycerides >2.5 mmol/l). Thirty-three subjects had CAD verified either by exercise bicycle test (n=4), or by coronary angiography and/or a history of myocardial infarction at least six months prior to the study (n=29), while the remaining 10 subjects had no evidence of CAD in exercise bicycle test. One patient in the IIa group had oral medication for type 2 DM, and 3 in IIa and 4 in IIb group had DM treated only with diet. Eleven volunteer subjects from IIa group and 16 from IIb group participated in the kinetic studies.

Study III, coronary artery disease. Ninety-eight subjects, 53 women and 45 men, were recruited for the study. Fifty-six subjects (CAD+), 30 men and 26 women, had CAD diagnosed either by a history of myocardial infarction (n=37), by coronary angiography (n=14) or by exercise bicycle test (n=5), while the remaining 42 subjects (CAD-) had no symptoms or manifestations of CAD in clinical interview and examination, or in ECG. None of the subjects had DM. They had been councelled a low-fat low-cholesterol diet at least six months earlier, and they kept the diet unchanged. Forty-four subjects were on beta-blocking agents, 6 subjects were taking thiazide diuretics, and 15 out of the 53 women had hormone replacement therapy. Twenty-four subjects were current smokers. In order to examine differences in CAD+ and CAD- subjects at different sialic acid levels, both CAD+ and CAD- subjects were divided into quartiles according to the sialic acid to apo B ratios of total and dense LDL, with equal numbers of subjects in each quartile. The quartiles are designated as Q_1, Q_2, Q_3 and Q_4 from low to high sialic acid ratios. Fifty-eight volunteers out of the 98 subjects participated in the kinetic study.

Study IV, statin treatment. The study group consisted of 26 moderately hypercholesterolemic patients (LDL cholesterol >3.3 mmol/l), 21 males and 5 females. All except 4 patients had CAD verified either by angiography and/or a history of acute myocardial infarction at least six months before the study (n=20), or by exercise bicycle test (n=2). Five patients had DM treated with diet only. Sixteen patients were on beta-blocker treatment at the time of the study, and 2 used thiazide diuretics for hypertension. The medications were unchanged during the study. None of the women used hormone replacement

therapy. Four patients were current smokers. After recruitment to the study, possible lipid lowering medication was discontinued, and the patients started a low-fat low-cholesterol diet, containing a scheduled amount of 30 energy-% of fat and less than 300 mg/day of cholesterol. After six weeks on the diet, baseline lipid analyses and metabolic studies were performed. Subsequently, the patients continued the diet and started statin treatment, with 10 patients receiving on the average 0.2 mg/day (0.1-0.3 mg) of cerivastatin, 9 patients on the average 30 mg/day (20-40 mg) of fluvastatin, and 7 patients 20 mg/day of simvastatin. When the patients had been on statins for two to three months, the lipid analyses and metabolic studies were repeated. Thirteen subjects volunteered to participate in both kinetic studies.

None of the subjects in the studies had gastrointestinal, liver, renal, or thyroid disease, except 3 subjects in Study III who had thyroxin treatment for hypothyroidism, and had been euthyroid for at least two years before the study. No one had tendon or cutaneous xanthomas or xanthelasmas. The subjects were not on any lipid lowering medication at the baseline of the studies.

4.2 Methods

4.2.1 Separation of lipoproteins

Serum samples were drawn after a 12-hour fast. Serum lipoproteins were separated with serial ultracentrifugations into VLDL, IDL, LDL and HDL (Havel et al 1955, Lipid Research Clinics Program 1982). After the separation of total LDL (d = 1.019-1.063 g/ml), it was fractionated into three subfractions: light (d = 1.019-1.036 g/ml), dense (d = 1.037-1.055 g/ml) and very dense (d = 1.056-1.063 g/ml), by density gradient ultracentrifugation. The density gradient was prepared in a 40-ml polyallomer Quick-Seal tube as follows: 11.3 ml of NaBr-NaCl salt solution (d = 1.065 g/ml) was carefully overlayered by 11.3 ml of LDL (d = 1.04 g/ml), which in turn was overlayered by NaBr-NaCl salt solution of d = 1.025 g/ml. The fractionation was completed by ultracentrifugation (44 h at 58,000 rpm at +10°C) in a Ti 60 fixed-angle rotor (Beckman Instruments).

4.2.2 Analyses of lipids, apolipoproteins and non-cholesterol sterols

The concentrations of total and free cholesterol, triglycerides, and phospholipids were analyzed from serum and from all lipoproteins with

commercial kits (Boehringer Diagnostica, Mannheim, Germany; and Wako Chemicals GMBH, Neuss, Germany). Concentrations of apo B, apo A-I and Lp(a) were analyzed with commercial kits (Orion Diagnostica, Espoo, Finland; and Mercodia AB, Uppsala, Sweden) from serum, and that of apo B also from total LDL and its subfractions. The coefficients of variation of the analyses were <2% for lipids and <5% for apolipoproteins. Mean values of three analyses from samples taken at one-week intervals are given for serum and lipoprotein lipids and apolipoproteins. Recoveries for serum lipoprotein lipid concentrations were always >90% and mostly >95%. In LDL density gradient the recovery percents were occasionally more variable, and therefore the lipid concentrations of LDL subfractions are given recovery corrected except in Study I. Apo E phenotypes were analyzed by electrophoresis and isoelectric focusing from serum (Havekes et al 1987). Total protein contents of the lipoproteins were analyzed in Study II with the Lowry method (Lowry et al 1951).

Serum non-cholesterol sterols, including the cholesterol precursor sterols Δ^8-lathosterol, desmosterol and lathosterol, the plant sterols campesterol and sitosterol, and the cholesterol metabolite cholestanol, were determined from non-saponifiable serum extract by gas-liquid chromatography on a 50-m long SE-30 capillary column (Hewlett Packard, Ultra I column) (Miettinen 1988). Being transported in serum by lipoproteins similarly to cholesterol, the concentrations of the non-cholesterol sterols are highly dependent on serum cholesterol levels. Thus the values are expressed as 10^2 x mmol/mol of serum cholesterol, i.e. as ratios to cholesterol, to eliminate the effect of variation in cholesterol levels.

4.2.3 Analysis of sialic acids

The concentration of sialic acids in total LDL and its subfractions, and in Study II, also in VLDL, IDL and HDL, was analyzed according to the resorcinol method by Svennerholm (1957) modified by Miettinen and Takki-Luukkainen (1959), using N-acetylneuraminic acid (Sigma Chemical Company, St Louis, MO, USA) as the standard. Shortly, sialic acids were first dissociated from LDL by acid hydrolysis in 0.1-N H_2SO_4 at +80°C for one hour. Subsequently, resorcinol-hydrochloric acid was used to cause a color reaction with sialic acid. Finally, the pigment was extracted with butyl acetate and its intensity measured with a spectrophotometer. In Study I, the sialic acid concentration was determined with the thiobarbituric acid assay (Warren 1959a) in addition to the resorcinol method, and the results were consistent with each other with a correlation coefficient of r=0.960 (n=16). Because of the high dependence of LDL sialic acid concentration on LDL apo B

concentration (Fontaine and Malmendier 1978), observed also in the present study, the sialic acid content of total LDL and its subfractions is given as the ratio of sialic acid to apo B and not as sialic acid concentration as in all earlier studies, and it is designated as the sialic acid ratio. In Study II, the sialic acid content of lipoproteins in the comparison of VLDL, IDL, LDL and HDL is given as the ratio of sialic acid to total protein.

4.2.4 Lipoprotein kinetic studies

For the kinetic studies, 50 ml of fasting EDTA plasma was drawn, and autologous total LDL and HDL were separated by serial density ultracentrifugations. Apo A-I was isolated from HDL as described in detail by Gylling et al (1992). Briefly, HDL was incubated with guanidine hydrochloride, dialyzed, density-adjusted with NaBr, and ultracentrifugated, after which pure apo A-I was collected from the bottom and extensively dialyzed. LDL was fractionated by serial density ultracentrifugations into three subclasses as previously described, and dialyzed extensively. Dense LDL apo B and apo A-I were iodinated with ^{125}I, and total LDL apo B with ^{131}I by a modification of the iodine-monochloride method (McFarlane 1958, Bilheimer et al 1972). Three days before injection the subjects started to take peroral potassium iodide to protect the thyroid gland. Approximately 1 mg of a mixture of the labeled total and dense LDL apo B and apo A-I were mixed with 5% human serum albumin, filtered, and injected. The total amount of radioactivity did not exceed 60 μCi.

After the injection, serial blood samples of 10 ml were collected for 14 days and counted. The die-away curves were constructed from plasma for ^{131}I-LDL and after ultracentrifugal separation for ^{125}I-dense LDL and ^{125}I-HDL. Fractional catabolic rates (FCRs) for total and dense LDL apo B and HDL apo A-I were determined using a two-pool model (Matthews 1957). Transport rate (TR) was calculated by multiplying FCR by the pool size. Pool size was the apoprotein plasma concentration multiplied by plasma volume, which was calculated to be 4.5% of body weight. FCR is expressed in pools/day and reflects the rate at which the lipoprotein is removed from the circulation, while TR, expressed in mg/kg/day, reflects the rate of production of the lipoprotein.

4.2.5 Statistics

Analyses were performed with Microsoft Excel, Primer of Biostatistics, and Biomedical Data Program (BMDP). Statistical significancies of differences between groups were tested with analysis of variance (ANOVA) and, when

appropriate, with Student's two-tailed t-test. X^2-test or Fisher exact test was used for nominal scale variables. In Study IV, paired t-test was used to determine the change caused by the statin treatment. Correlations were analyzed by calculating Pearson's product-moment correlation coefficient for normally distributed variables. In case of skewed distributions, either logarithmic transformations or Spearman's rank-order correlation coefficient was applied. Values are given as mean±SE. A P-value of less than 0.05 was considered statistically significant.

In Study I, the variability in the FCR for LDL apo B was examined with a multiple linear regression analysis with the presence of DM, LDL and HDL cholesterol and serum triglyceride concentrations, and LDL sialic acid ratio as the independent variables. Similarly, another linear regression model was made with FCR for dense LDL apo B as the dependent variable, and the presence of DM and cholesterol and triglyceride concentrations and the sialic acid ratio of dense LDL as the independent variables. In Study III, a stepwise logistic regression analysis was carried out with the presence of CAD as the dependent variable, and LDL cholesterol and triglyceride concentrations, LDL sialic acid ratio, and FCR and TR for LDL apo B as the independent variables. To explain the variability in LDL sialic acid ratio, a stepwise regression analysis was performed with the presence of CAD, the concentrations of cholesterol and triglycerides in LDL, and FCR and TR for LDL apo B as the independent variables.

5 RESULTS

Table 2. Serum and lipoprotein lipid and apo B levels.

Variables	Study I		Study II		Study III	Study IV
	DM+ n=20	DM- n=10	IIa n=22	IIb n=21	n=98	n=26
Serum cholesterol, mmol/l	6.10±0.16	5.93±0.23	6.84±0.17*	7.39±0.17	6.31±0.11	7.06±0.16
Serum triglycerides, mmol/l	2.90±0.40*	1.69±0.18	1.78±0.09	3.43±0.18	1.89±0.10	2.50±0.22
LDL cholesterol, mmol/l	3.44±0.14	3.49±0.19	4.38±0.11	4.16±0.13	3.75±0.08	4.33±0.09
LDL apo B, mg/dl	72.7±2.5	72.5±3.3	99.7±2.8	99.8±2.5	86.8±2.1	100.9±2.3
HDL cholesterol, mmol/l	0.98±0.04	1.21±0.13	1.18±0.06	1.06±0.04	1.23±0.03	1.12±0.04

mean±SE. * P<0.05 for difference between groups within study sections.

5.1 Type 2 diabetes mellitus (Study I)

Serum and lipoprotein lipids. The presence or type of hypoglycemic therapy had no effect on serum lipid and sialic acid levels or metabolic parameters. The diabetic subjects had higher triglyceride levels in serum (Table 2) and VLDL than the non-diabetics, and a larger proportion of triglycerides in all LDL subfractions. Cholesterol and apo B concentrations were similar between diabetics and non-diabetics in total LDL and all its subfractions.

LDL sialic acid ratio. The sialic acid per apo B ratio (mass/mass ratio, μg/mg) was higher in the diabetics than in the non-diabetics in total LDL and all its subfractions (Figure 4). There were no correlations between sialic acid ratios and serum triglyceride or LDL cholesterol levels. In the diabetic subjects, the sialic acid ratios were similar between CAD- (n=7) and CAD+ subjects (n=13) in total LDL and in the subfractions, being e.g. 65.9±16.1 and 69.2±7.9 μg/mg, respectively, in dense LDL. In the non-diabetics, there was a

tendency towards lower ratios in CAD+ subjects; for instance, in dense LDL the ratio was 46.5±9.8 μg/mg in CAD- (n=6) and 31.9±5.2 μg/mg in CAD+ (n=4), but the difference between the ratios was not significant.

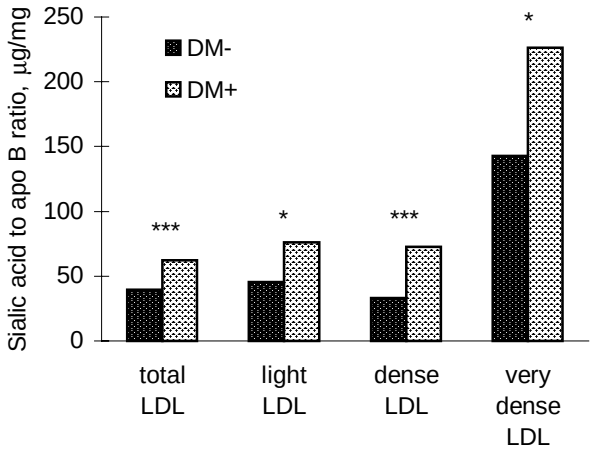

Figure 4. The sialic acid to apo B ratios of total LDL and its light, dense and very dense subfractions in subjects without (DM-, n=10) and with DM (DM+, n=20). * P<0.05; *** P<0.001 for difference between DM- and DM+.

LDL metabolism. FCRs for both total and dense LDL apo B were more elevated in the diabetic subjects and so was the TR for dense LDL apo B, indicating a higher turnover rate for dense LDL in the diabetics (Table 3).

Table 3. Kinetics of total and dense LDL apo B in non-diabetic (DM-) and type 2 diabetes mellitus (DM+) patients. FCR = fractional catabolic rate, TR = transport rate.

Variables	DM- n=10	DM+ n=20	
Total LDL			
FCR, pools/d	0.283±0.010	0.329±0.010	**
TR, mg/kg/d	7.8±0.4	8.6±0.5	
Dense LDL			
FCR, pools/d	0.298±0.031	0.399±0.028	*
TR, mg/kg/d	5.0±0.6	6.8±0.6	*

mean±SE. * P<0.05, ** P<0.01

FCR for total LDL apo B had a positive correlation with serum triglyceride level (r=0.631, P<0.001) and a negative correlation with LDL cholesterol level (r=-0.654, P<0.001) in the whole study group. FCR for dense LDL apo B was positively correlated with the sialic acid ratio of dense LDL (Figure 5). In the multiple linear regression analysis, the variability in FCR for total LDL apo B was explained by LDL cholesterol and serum triglyceride concentrations, and the variability in FCR for dense LDL apo B was explained only by dense LDL cholesterol concentration.

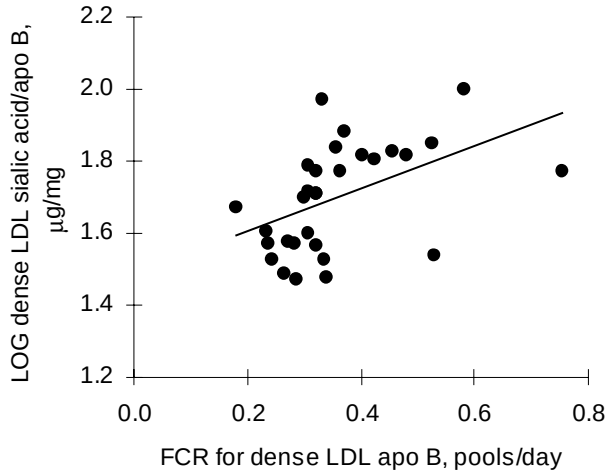

Figure 5. Correlation between fractional catabolic rate for dense LDL apo B and dense LDL sialic acid to apo B ratio (log scale) in DM+ and DM- subjects. n=30, y = 0.603x + 1.49, r=0.485, P<0.05.

5.2 Type IIa and IIb hyperlipidemia (Study II)

Serum and lipoprotein lipids. In addition to higher serum triglyceride levels, the subjects with IIb hyperlipidemia also had higher cholesterol (Table 2) and phospholipid concentrations in serum, VLDL and IDL, and higher triglyceride levels in all lipoproteins than the IIa hyperlipidemia subjects. In the LDL subfractions, the concentrations of cholesterol, phospholipids and apo B were lower in light LDL and higher in dense and very dense LDL of IIb than IIa subjects, and the concentration of triglycerides similar in light but higher in dense and very dense LDL of IIb subjects (Table 4). The light LDL particles of IIb subjects had less cholesterol and phospholipids per apo B, and very dense particles less triglycerides and phospholipids per apo B than those of

IIa subjects, while the composition of the dense LDL particles were alike in the two groups (Table 4).

Table 4. Lipid and apo B concentrations and lipid composition per apo B in light, dense and very dense LDL subfractions in type IIa and IIb hyperlipidemia patients.

Variables	IIa n=22	IIb n=21	
Light LDL			
cholesterol, mmol/l	2.10±0.16	1.36±0.12	***
triglycerides, mmol/l	0.17±0.01	0.17±0.01	
apo B, mg/dl	44.5±3.6	31.8±2.5	**
Dense LDL			
cholesterol, mmol/l	2.07±0.14	2.43±0.12	
triglycerides, mmol/l	0.12±0.01	0.18±0.01	***
apo B, mg/dl	50.6±3.3	60.6±1.9	*
Very dense LDL			
cholesterol, mmol/l	0.21±0.02	0.36±0.06	*
triglycerides, mmol/l	0.025±0.002	0.039±0.003	**
apo B, mg/dl	4.6±0.4	7.5±0.6***	
Light LDL			
cholesterol/apo B	1.96±0.02	1.75±0.04	***
triglycerides/apo B	0.42±0.03	0.48±0.03	
phospholipids/apo B	1.26±0.02	1.13±0.02	***
Dense LDL			
cholesterol/apo B	1.68±0.03	1.68±0.10	
triglycerides/apo B	0.26±0.01	0.25±0.01	
phospholipids/apo B	1.05±0.02	0.98±0.04	
Very dense LDL			
cholesterol/apo B	1.98±0.09	1.88±0.14	
triglycerides/apo B	0.60±0.05	0.44±0.03	**
phospholipids/apo B	1.76±0.17	1.31±0.09	*

mean±SE. * P<0.05, ** P<0.01, *** P<0.001

The percentage of esterified cholesterol out of total cholesterol was higher in total, dense and very dense LDL of IIb than IIa subjects. Apo E phenotype distributions were similar in IIa and IIb groups. The kinetics of total LDL apo B were comparable in IIa and IIb subjects, but the TR for dense LDL apo B was higher in IIb as compared with IIa (5.55±0.37 vs 4.20±0.29 mg/kg/day, P<0.01).

Lipoprotein sialic acid ratios. The ratio of sialic acid to total protein lowered gradually with increasing lipoprotein density, being highest in VLDL and IDL, intermediate in LDL and lowest in HDL (Figure 6). In VLDL and IDL, the ratio was lower for IIb than IIa subjects, but similar in LDL and HDL between the groups. In LDL subfractions, the ratio of sialic acid to apo B was lower in the dense and very dense LDL of IIb than IIa subjects (Figure 7).

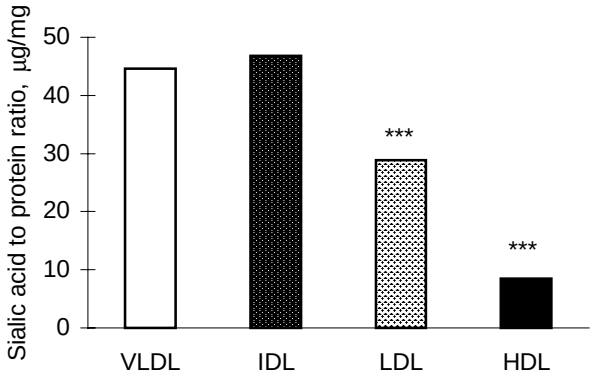

Figure 6. The sialic acid to total protein ratios of VLDL, IDL, LDL and HDL in the combined IIa and IIb groups (n=43). *** P<0.001 from the preceding lipoprotein density class.

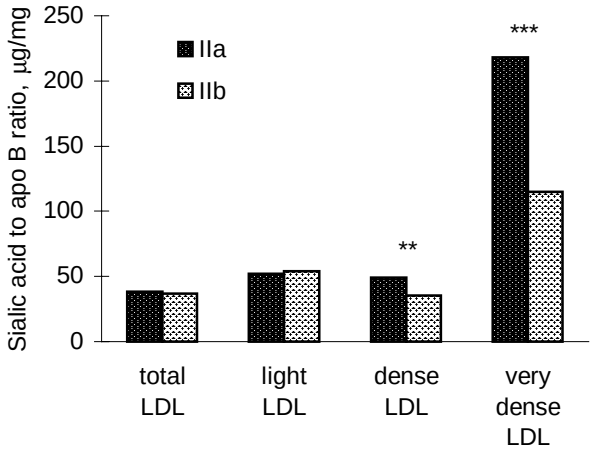

Figure 7. The sialic acid to apo B ratios of total LDL and its light, dense and very dense subfractions in subjects with type IIa (n=22) and IIb (n=21) hyperlipidemia. ** P<0.01; *** P<0.001 for difference between IIa and IIb.

CAD+ and -CAD subjects had similar sialic acid ratios in IIa and IIb groups separately, and when the groups were combined, the ratio did not differ between CAD+ and CAD- subjects in total, light or dense LDL, but was higher in the very dense LDL of CAD (n=33) than non-CAD (n=10) subjects (178.9±20.1 and 129.7±12.6 μg/mg, respectively, P=0.04). This is presumably associated with the presence of Lp(a) in this fraction, and serum Lp(a) levels were unsignificantly higher in CAD+ than CAD- subjects (345±59 vs 199±55 U/l, respectively, P=0.08). The 8 subjects with DM had similar LDL sialic acid ratios than the rest of the subjects.

Correlations. In IIa and IIb hyperlipidemia groups combined, the sialic acid ratios of dense and very dense LDL were negatively related with their cholesterol (r=-0.526 and r=-0.597, respectively, P<0.001 for both) and triglyceride concentrations (r=-0.486 and r=-0.463, respectively, P<0.01 for both), and with the TR for dense LDL apo B (r=-0.575 and r=-0.624, P<0.01 for both). TR for dense LDL apo B was positively correlated with the cholesterol concentration of dense LDL, serum triglyceride level, and the esterification percentage of total LDL cholesterol (r=0.675, P<0.001).

5.3 Coronary artery disease (Study III)

Clinical characteristics and LDL sialic acid ratios. Sialic acid ratios of total LDL and its subfractions did not differ between males and females, or smokers and non-smokers. The use of thiazide diuretics or, in females, hormone replacement therapy, had no effect on LDL sialic acid ratios. In the whole study group and in the CAD+ group, LDL sialic acid ratios were not affected by beta-blocker treatment, but in hypertensive CAD- subjects on beta-blockers (n=6) the sialic acid ratio was lower in total LDL than in CAD- subjects not on beta-blockers (n=34) (33.5±3.1 vs 44.9±2.3 μg/mg, respectively, P<0.01). CAD+ and CAD- subjects were of similar age, body mass index (BMI), and sex and apo E phenotype distributions.

LDL sialic acid ratio and CAD. LDL cholesterol concentration was higher in CAD+ than CAD- subjects, while levels of HDL cholesterol, serum triglycerides and Lp(a) were similar between the groups. The mean sialic acid ratios of total LDL and its dense and very dense subfractions were lower in CAD+ than CAD- subjects (Figure 8). When the CAD+ and CAD- subjects were divided into quartiles by LDL sialic acid ratio, the ratios of both total and dense LDL were markedly lower in CAD+ than CAD- subjects of the three highest quartiles but not in those of the lowest quartile. However, in the stepwise regression analysis with the presence of CAD as the dependent

variable, only LDL cholesterol concentration and not its sialic acid ratio explained its variability.

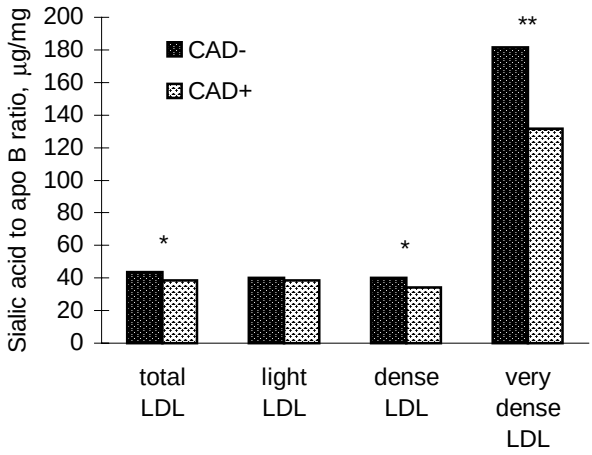

Figure 8. The sialic acid to apo B ratios of total LDL and its light, dense and very dense subfractions in subjects without (CAD-, n=42) and with CAD (CAD+, n=56). * P<0.05, ** P<0.01.

Serum and lipoprotein lipids. In general, in serum and in total, light and dense LDL all the lipid concentrations had negative correlations with LDL sialic acid ratio (Table 5), while the concentrations of HDL lipids and Lp(a) were not associated with it. The ratios of cholesterol and phospholipids per apo B were positively associated with the sialic acid ratio in total LDL, and in the subfraction analysis this was seen in the dense and very dense subfractions (Table 5).

Table 5. Correlation coefficients of total LDL sialic acid to apo B ratio with lipid and apo B concentrations and lipid to apo B ratios of total LDL and its light, dense and very dense subfractions. n=98.

	Sialic acid to apo B ratio			
Variables	Total LDL	Light LDL	Dense LDL	Very dense LDL
cholesterol	-0.377 ***	-0.126	-0.305 **	0.066
triglycerides	-0.296 **	-0.274 **	-0.225 *	-0.003
apo B	-0.526 ***	-0.246 *	-0.372 ***	-0.144
cholesterol/apo B	0.268 **	0.188	0.227 *	0.351 ***
triglycerides/apo B	0.226 *	0.062	0.125	0.132
phospholipids/apo B	0.385 ***	0.192	0.302 **	0.395 ***

* P<0.05, ** P<0.01, *** P<0.001

Non-cholesterol sterols and LDL sialic acid ratio. The ratios of non-cholesterol sterols to cholesterol did not differ between CAD- and CAD+ subjects, and were not related to the sialic acid ratio of total LDL. However, the sialic acid ratio of dense LDL was correlated negatively with Δ^8-lathosterol, desmosterol and lathosterol ratios (r=-0.389, P<0.001; r=-0.244, P<0.05; and r=-0.311, P<0.01, respectively), and positively with campesterol, sitosterol and cholestanol ratios (r=0.219, P<0.05; r=0.239, P<0.05; and r=0.407, P<0.001, respectively). At high dense LDL sialic acid ratios (>40 μg/mg), CAD+ subjects had lower lathosterol ratios than CAD- subjects (135.9±14.4 vs 191.0±15.4, P<0.05).

LDL kinetics and sialic acid ratio. The 58 subjects participating in the kinetic studies had higher BMI and serum triglyceride concentration, and higher mean sialic acid ratio in total LDL than the non-participants (43.8±1.7 vs 36.0±0.8 μg/mg, P<0.001). Similarly to the total study group, in the participants of the kinetic study cholesterol and apo B concentrations of both total and dense LDL had inverse associations with the sialic acid ratios of total and dense LDL. The sialic acid ratios of total and dense LDL were unrelated to the FCRs for total and dense LDL apo B, but they correlated inversely with the TRs for both total and dense LDL apo B (Table 6, Figure 9). In addition, the TR for dense LDL apo B had significant positive correlations with Δ^8-lathosterol and lathosterol ratios (r=0.322 and r=0.307, respectively, P<0.05 for both), and a negative correlation with cholestanol ratio (r=-0.410, P<0.01). The CAD+ subjects had higher TRs for total and

Table 6. The correlation coefficients of kinetic parameters of total and dense LDL apo B with sialic acid to apo B ratios of total and dense LDL. FCR = fractional catabolic rate, TR = transport rate. n=58.

	Sialic acid to apo B ratio	
Variables	total LDL	dense LDL
Total LDL		
apo B	-0.583 ***	-0.468 ***
FCR	0.241	0.158
TR	-0.384 **	-0.305 *
Dense LDL		
apo B	-0.476 ***	-0.582 ***
FCR	-0.003	-0.174
TR	-0.351 **	-0.456 ***

mean±SE. * P<0.05, ** P<0.01, *** P<0.001

dense LDL apo B - this was seen only in the two highest quartiles - while the FCRs did not differ between CAD+ and CAD- subjects. The stepwise regression analysis showed that both the TR and the FCR for total LDL apo B accounted for the variability in LDL sialic acid ratio, but the presence of CAD and the concentrations of cholesterol and triglycerides in LDL did not affect it.

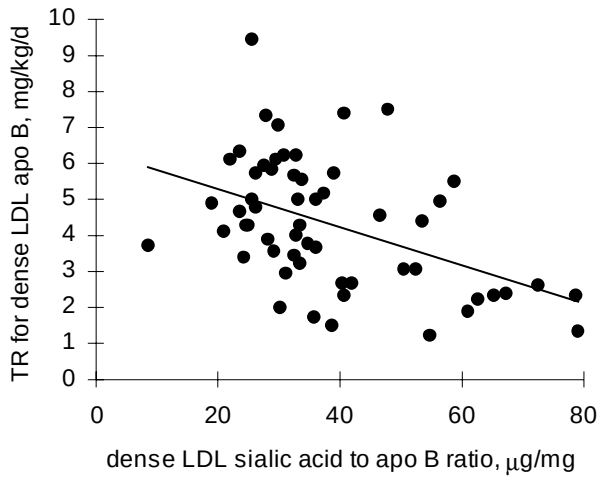

Figure 9. Correlation between dense LDL sialic acid to apo B ratio and transport rate (TR) for dense LDL apo B. n=58, y = -0.053 x + 6.36, r=-0.456, P<0.001.

5.4 Statin treatment (Study IV)

Serum and lipoprotein lipids. No differences were seen at baseline and in the changes caused by the various statins on lipids or sialic acids, and thus the groups with the three different statins (fluvastatin, simvastatin, cerivastatin) were examined together. The concentrations of cholesterol, triglycerides and phospholipids were significantly lowered in serum, VLDL, IDL and LDL during statin treatment, and the relative proportion of cholesterol diminished in all apo B -containing lipoproteins. The concentrations of lipids and apo B decreased remarkably also in all the LDL subfractions during statin treatment, and the higher the baseline lipid levels were, the more markedly did they decrease. The lipid/apo B ratios of LDL subfractions did not change, indicating that statins decreased only the number and did not change the composition of LDL particles.

LDL sialic acid ratio. The sialic acid ratio increased significantly in total LDL and its three subfractions during statin treatment (Figure 10). The post-treatment ratios did not vary much between the groups treated with different statins, and the change was significant in total LDL for all the statin groups separately (Figure 11).

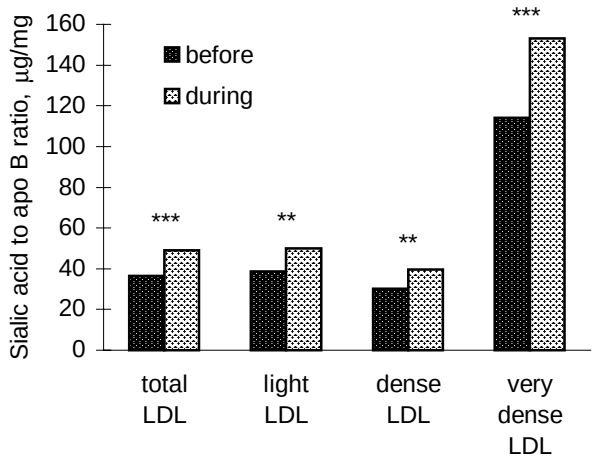

Figure 10. The sialic acid to apo B ratios of total LDL and its light, dense and very dense subfractions before and during statin treatment. n=26, ** P<0.01; *** P<0.001 for change during statin treatment.

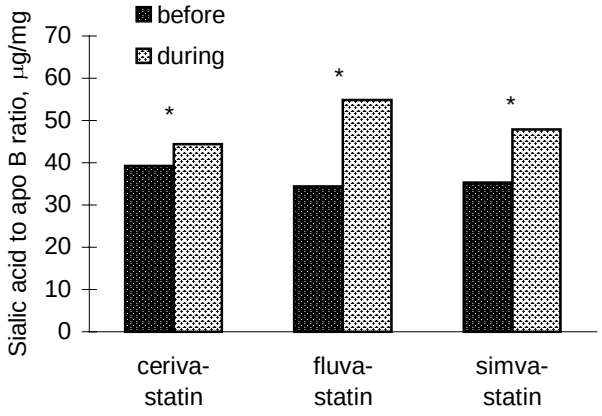

Figure 11. The sialic acid to apo B ratio of total LDL before and during treatment with cerivastatin (n=10), fluvastatin (n=9), and simvastatin (n=7). * P<0.05.

The sialic acid ratio was negatively correlated with cholesterol, phospholipid and apo B concentrations in light and dense LDL subfractions both before and during statin treatment, and with triglyceride concentration only during statin treatment in all the subfractions. The changes in cholesterol, phospholipid and apo B concentrations of dense LDL were negatively correlated with the change in dense LDL sialic acid ratio (r=-0.501 for cholesterol, r=-0.464 for phospholipids, and r=-0.667 for apo B, Figure 12). In light and dense LDL, also the baseline sialic acid ratios were correlated with the changes in cholesterol, phospholipid and apo B concentrations, and in dense LDL additionally with the change in triglyceride concentration, so that the lower the baseline sialic acid ratio was, the greater were the reductions in the lipid and apo B concentrations (Figure 13).

Metabolism of LDL and cholesterol. FCRs for both total and dense LDL apo B were increased, and TR for dense LDL apo B was decreased during the statin treatment (Table 7). None of these variables were significantly related to the sialic acid ratios or to the changes in them. The ratios of Δ^8-lathosterol, desmosterol and lathosterol to cholesterol diminished remarkably during the statin treatment indicating decreased cholesterol synthesis, while those of the plant sterols campesterol and sitosterol, and that of cholestanol increased. The baseline ratios of the non-cholesterol sterols or the changes in them were not connected with baseline sialic acid ratios nor with the changes in sialic acid ratios during statin treatment.

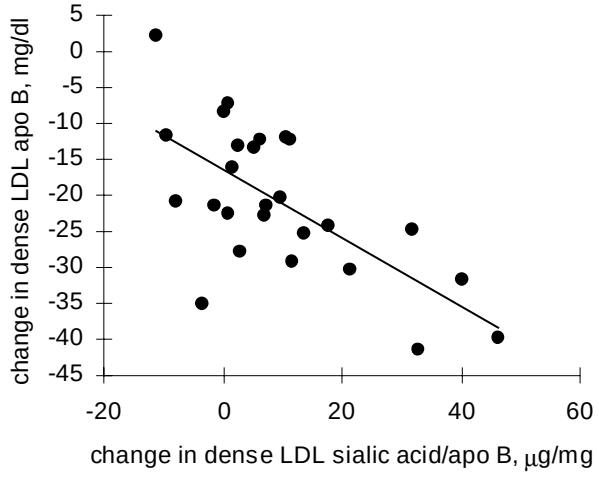

Figure 12. Correlation between the change in dense LDL sialic acid to apo B ratio and the change in dense LDL apo B concentration. n=26, y = -0.47x - 16.4, r=-0.667, P<0.001.

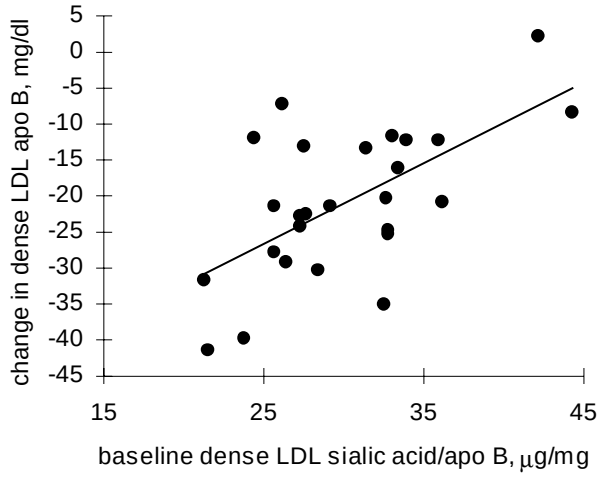

Figure 13. Correlation between the baseline dense LDL sialic acid to apo B ratio and the change in dense LDL apo B concentration. n=26, y = 1.13x - 54.8, r=0.614, P<0.001.

Table 7. Apo B concentrations and kinetics of total and dense LDL apo B before and during statin treatment. FCR = fractional catabolic rate, TR = transport rate. n=13.

Variables	Before	During	
Total LDL			
apo B, mg/dl	102.9±2.9	69.4±3.6	***
FCR, pools/d	0.277±0.011	0.380±0.010	**
TR, mg/kg/d	12.7±0.48	11.6±0.77	
Dense LDL			
apo B, mg/dl	63.6±4.4	37.9±3.3	***
FCR, pools/d	0.269±0.030	0.325±0.016	**
TR, mg/kg/d	5.29±0.45	3.70±0.30	**

mean±SE. ** P<0.01, *** P<0.001

6 DISCUSSION

6.1 General

The function of sialic acids in the metabolism of some glycoproteins (Stryer 1988) and their presence in lipoproteins, especially LDL (Swaminathan and Aladjem 1976), make their role in atherosclerosis an intriguing subject. In the late 1970's a few studies reported that desialylation of LDL affects it catabolism both in vitro (Filipovic and Buddecke 1979, Filipovic et al 1979) and in vivo (Hendrix et al 1975, Malmendier et al 1980). Ten years later the sialic acid content of LDL and its association with atherosclerosis came to focus again, first in the numerous studies by Orekhov et al (1989, 1991b, 1992) and Tertov et al (1992a, 1992b), and later in the mid- to late 1990's also in studies by other groups (Ruelland et al 1993, Chappey et al 1995, Grewal et al 1996, Anber et al 1997, Bartlett et al 1998, Harada et al 1998, Millar et al 1999).

6.2 Clinical characteristics

In this study, the sialic acid ratios of total LDL and its subfractions did not differ consistently between males and females. The mean age of the subjects was around 55 years, and the females were practically all postmenopausal. In Study III, 15 females out of 53 were on hormone replacement therapy, and their LDL sialic acid ratios were similar to those of the women not taking hormones and to those of the men. The age of the subjects was not connected with LDL sialic acid ratio, nor were there consistent associations between BMI and LDL sialic acid ratio. Current smokers had LDL sialic acid ratios approximately corresponding to those of non-smokers. Apo E phenotypes had no relation with LDL sialic acid content. Consequently, LDL sialic acid ratio seems to be independent of demographic factors associated with an increased risk of CAD, that is, male sex, old age, overweight, and smoking, and also of apo E phenotypes 4/3 and 4/4 known to associate with high CAD risk (Davignon et al 1988).

Beta-blocking agents are known to change plasma lipid profiles to a more atherogenic direction (Lehtonen and Viikari 1979, Rohlfing and Brunzell 1986, Cruickshank 1990, Superko et al 1993), but they reduce CAD mortality (Cruickshank 1990, Wikstrand et al 1991, Goldstein 1996). Overall, no significant lipid variations were found between beta-blocker users and non-

users in any of the present studies. However, in Study III in the CAD- subgroup, beta-blocker users had lower total LDL sialic acid ratio than non-users, while in the CAD+ subjects, who included most of the beta-blocker users, there was no difference whatsoever in the sialic acid ratios between users and non-users (39.0±1.6 vs 37.1±2.1 μg/mg for total LDL, respectively). The small number of beta-blocker users in the CAD- subgroup (6 out of 42 subjects) does not allow to draw firm conclusions from the difference in this group and, according to all available data, it is inferred that beta-blockers seem not to have a significant effect on LDL sialic acid levels. Furthermore, the use of thiazide diuretics, or the type of hypoglycemic therapy in DM subjects did not affect the LDL sialic acid ratio.

6.3 Measurement of sialic acid ratio

The sialic acid content of lipoproteins was measured mainly with the resorcinol method (Svennerholm 1957, Miettinen and Takki-Luukkainen 1959), and in Study I, also with the thiobarbituric acid assay (Warren 1959a). Both methods are very practicable and reliable. Results from repeated analyses with the resorcinol method were constant, with an intra-assay and interassay variability of less than 5% in the present study. The resorcinol method was preferably used because no time-consuming dialysis of the samples is necessary, and the reagents are more stable than in the thiobarbituric acid assay. Most other investigators have employed the thiobarbituric acid assay, while the resorcinol method has been used by a minority of groups (Fontaine and Malmendier 1975, Fontaine and Malmendier 1978, La Belle and Krauss 1990, Sobenin et al 1993, Tertov et al 1996b, Millar et al 1999). The two methods, utilized in parallel in Study I, gave similar results (r=0.960) in conformity with other reports (Orekhov et al 1991b, Orekhov et al 1992).

The overall sialic acid to apo B ratios of LDL were on similar level in all the subject groups, the mean values of the groups ranging from 36.7 to 43.7 μg/mg apo B for total LDL, excluding the diabetic subjects in Study I who had a clearly higher ratio (mean of 62.3 μg/mg). These levels were close to those reported in some studies (Orekhov et al 1989, La Belle and Krauss 1990, Orekhov et al 1991b, Grewal et al 1996), but markedly higher than in most other papers (Maruhama et al 1983, Orekhov et al 1992, Tertov et al 1992a, Ruelland et al 1993, Sobenin et al 1993, Tertov et al 1993, Chappey et al 1995, Myara et al 1995, Ruelland et al 1997, Chappey et al 1998, Harada et al 1998), in which the sialic acid levels were between 6 and 13 μg/mg protein. Thus, there are large variations in the sialic acid ratios in studies by different groups, and the results of this study are not the only ones that deviate from

the majority of reports. The variability is not a question of methodology, because the two methods employed in the earlier studies and the present one give similar and reliable results as already discussed. Therefore, the large differences in the sialic acid levels can not be explained, but with a similar range in all the sections of the present study the measurements used in them can be considered reliable.

The sialic acid content of LDL is expressed as ratio to the concentration of apo B in LDL because of the high dependence of its concentration on that of apo B, as already pointed out in the Methods chapter, and this having been the praxis in all previous studies. In the present study, the concentration of apo B was measured with an immunoturbidometric method (Riepponen et al 1987), while in most other studies the total protein of LDL has been measured with the Lowry method (Lowry et al 1951). Apo B is the only protein in LDL, and its concentration is very close to the total protein concentration in LDL. The reproducibility of results with the apo B analysis method is good, and the levels are closely similar to those obtained with the Lowry method (apo B 35.1±1.6 mg/dl and total protein 38.7±1.7 mg/dl in dense LDL, n=90, r=0.967). Thus, variability in protein measurements cannot explain the differences in the sialic acid to apo B ratios observed in different studies.

6.4 Lipoproteins and sialic acids

Negative associations between serum and lipoprotein lipid levels and LDL sialic acid ratio have been described also earlier (La Belle and Krauss 1990, Chappey et al 1995). In the present study, serum total and LDL cholesterol levels had negative correlations with the sialic acid ratio of total LDL in the large population of Study III. This indicates that high LDL cholesterol level is associated with low LDL sialic acid ratio, both presumably accelerating atherogenesis. However, in the smaller patient groups of Studies I, II and IV, the negative associations between serum or LDL cholesterol concentrations and LDL sialic acid ratios were not significant, similarly to two other studies (Chappey et al 1995, Millar et al 1999), while La Belle and Krauss (1990) also found a weak but significant negative association between the cholesterol concentration and the sialic acid ratio of LDL. This can simply be due to the connection not being strong enough to be seen in all populations.

Serum triglyceride levels have been inversely associated with LDL sialic acid ratio (La Belle and Krauss 1990, Chappey et al 1995). In the present study, the sialic acid ratio of total LDL had no association with serum triglyceride levels, but in Study III it was negatively related with the triglyceride

concentration of LDL. Furthermore, a clear negative association was seen for dense and very dense LDL sialic acid ratios and serum triglyceride levels in Studies II and III, and only for very dense LDL sialic acid ratio in Study IV. In Study II, the subjects were divided into groups according to their serum triglyceride level, and in the IIb hyperlipidemics high triglyceride levels were associated with low sialic acid ratios in dense and very dense LDL, but not in total LDL. Consequently, an inverse association seems to exist between serum triglyceride levels and the sialic acid ratios of the denser LDL subfractions.

High concentrations of apo B in serum and LDL are considered a remarkable risk factor for CAD (Sniderman et al 1980). The sialic acid ratios of total and dense LDL had strong negative associations with serum and LDL apo B levels in Study III. However, in the smaller populations of Studies II and IV, the relation with serum apo B level was significant only for very dense, and in Study II, also for dense LDL sialic acid ratio. Again, the significant findings in the large population suggest that there is a correlation that is not seen in smaller populations. Overall, these associations of LDL sialic acid ratios with serum and LDL lipid and apo B levels indicate that atherogenic lipid profiles, specifically high cholesterol, triglyceride and apo B concentrations in LDL, are connected with low LDL sialic acid ratios, especially in the dense and very dense subfractions. On the contrary, no association between LDL sialic acid ratios and HDL cholesterol level was seen in any of the studies.

The sialic acid ratios of the light, dense and very dense LDL subfractions were in general negatively associated with the respective cholesterol, triglyceride, phospholipid and apo B concentrations, the inverse association being strongest for apo B concentrations. The correlations were significant in dense LDL for all the lipid and apo B levels in every study section, and for light and very dense LDL in almost all of them. As each LDL particle contains one apo B molecule, the concentration of apo B reflects the number of LDL particles. Thus, the inverse correlation between apo B concentration and sialic acid ratio suggests that when the number of LDL particles is high, their sialic acid content is low. In addition, the sialic acid ratios were positively associated with the lipid to apo B ratios of total LDL and its subfractions, which reflect the size of LDL particles. These observations together suggest that low LDL sialic acid content is associated with a large number of relatively small LDL particles, generally known to be atherogenic, and are also in concordance with an earlier finding that subjects with small dense LDL have a lower LDL sialic acid content (La Belle and Krauss 1990).

The ratio of sialic acid to protein was negatively associated with the density of lipoproteins so that the ratio lowered from VLDL and IDL to LDL. Similar decreases were described in three recent studies (Anber et al 1997, Harada

et al 1998, Millar et al 1999). There is a number of possible explanations for this loss of sialic acid from lipoproteins when density increases. First, apolipoproteins other than apo B are lost during the conversion of VLDL into IDL and further into LDL; second, the removal of sialic acid -rich apo B -containg lipoproteins could be enhanced, and third, apo B could be desialylated by a neuraminidase during this conversion into denser particles owing to longer residence time in the circulation. The recently reported finding that in plasma, LDL apo B is always partially desialylated (Bartlett and Stanley 1998) suggests that desialylation of apo B does take place in plasma and can contribute to the observed decrease in lipoprotein sialic acid ratio with increasing density. On the other hand, LDL is richer in gangliosides than VLDL (Senn et al 1989), and as the amount of sialic acid in the glycolipid part of lipoproteins is thus higher in LDL than VLDL, the decrease in the sialic acid content of the lipoproteins with delipidation indeed seems to ensue from a loss of sialic acid from the glycoprotein, that is, apo B.

HDL was found to have a lower sialic acid ratio than any of the apo B -containg lipoproteins, in concordance with a previous study (Harada et al 1998). This is undoubtedly due to the major apolipoproteins in HDL, apo A-I and A-II, not being glycoproteins, the small amount of sialic acid thus residing in the glycolipids of HDL and in some tightly bound glycoproteins like LCAT, PLTP and CETP.

The sialic acid ratios have been reported to diminish also by increasing density of LDL particles (La Belle and Krauss 1990, Millar et al 1999), in continuation of the diminution in sialic acid ratio from triglyceride-rich apo B -containing lipoproteins to LDL. Results from the present study partly support previous findings, but are not totally concordant. In Study II, the sialic acid ratio fell significantly from light to dense LDL only in the IIb but not in the IIa group; however, the decrease was significant when both groups were combined (53.2±2.2 to 42.1±2.2 μg/mg, $P=0.002$). In Study IV, the ratio decreased clearly from light to dense LDL both at baseline and during the statin treatment ($P<0.01$ for both). On the contrary, in Study I the sialic acid ratios of the diabetic patients were similar in light and dense LDL, and in the non-diabetic group, there was a non-significant decrease from light to dense subfraction ($P=0.09$). In Study III, no significant decrease was seen either for all the patients together or for CAD+ and CAD- subjects separately. However, the positive associations of LDL sialic acid ratios with lipid to apo B ratios suggest that when the sialic acid ratios are low, the particles are lipid-depleted and thus denser and smaller in size. In summary, at least a tendency towards decreasing sialic acid ratios with increasing particle density was seen in most of the present study populations, and desialylation of LDL apo B possibly contributes to this loss of sialic acid, supported by the multiple findings of desialylated LDL in human plasma (Orekhov et al 1992, Tertov et

al 1992a, Tertov et al 1992b, Sobenin et al 1993, Tertov et al 1993, Bartlett and Stanley 1998).

In all the study sections and patient groups, the sialic acid ratio was clearly highest in very dense LDL. This is most presumably due to the presence of Lp(a), which is highly sialylated (Ehnholm et al 1972) and overlaps with the density range of LDL. Separation of dense and very dense LDL was performed to minimize the amount of Lp(a) in the dense subfraction and apparently this succeeded, because the dense LDL subfraction, having a remarkably lower sialic acid ratio, probably did not contain significant amounts of Lp(a). Furthermore, separate measurements showed that less than 30% of the subjects, all of whom had relatively high Lp(a) concentrations in serum, had detectable amounts of Lp(a) in dense LDL, and in these subjects its mean concentration in dense LDL was 16.6±2.9 U/l or 4.7% of serum total Lp(a). Similarly, very dense LDL contained 8.3% of serum Lp(a).

6.5 LDL sialic acid content in different conditions

6.5.1 Type 2 diabetes mellitus

The sialic acid ratio of total LDL and its three subfractions was higher in DM subjects than in the controls in Study I. The few DM patients in Studies II and IV (8 and 5, respectively) did not differ significantly from the non-DM subjects, but in both studies there was a non-significant tendency towards higher sialic acid ratios in the DM subjects. These results are contradictory to earlier observations by Tertov et al (1992a) and Sobenin et al (1993), who found lower content of sialic acid in the LDL of DM patients compared with normoglycemic controls. In addition, other studies have reported no differences in LDL sialic acid ratio in normolipidemic subjects with or without DM (Maruhama et al 1983, Ruelland et al 1997). The reason for the very high sialic acid levels in the diabetic subjects of the present study might be in the catabolism of LDL, which also differed from that of the non-DM subjects. Both in Studies I and II the FCR for LDL apo B, and especially the FCR for dense LDL apo B, was significantly higher in the DM subjects compared with normoglycemic controls. This is opposite to what was expected, because the FCR for LDL apo B has been reported to be decreased in DM (Howard et al 1987). Thus, the higher sialic acid ratio in LDL was associated with an accelerated removal of LDL from the circulation in DM. On the other hand, hypertriglyceridemia is often related with enhanced clearance of LDL (Vega and Grundy 1989). The diabetic subjects in Study I had higher serum triglyceride levels than the non-DM controls, which could explain their faster LDL catabolism. The FCR for LDL apo B also had a positive correlation with

serum triglyceride levels, so that both high LDL sialic acid ratio and high serum triglyceride levels were connected with increased catabolism of LDL apo B in DM.

6.5.2 Type IIa and IIb hyperlipidemia

Even though the study population was selected according to their lipid profile and matched for age, sex and BMI, the composition of lipoproteins, especially of LDL, was similar in type IIb to that presented for familial combined hyperlipidemia (Kane and Havel 1995). As compared with IIa, in IIb hyperlipidemia light LDL particles were lipid-depleted and fewer. The composition of dense particles was similar in the two groups while in IIb the numbers of both dense and very dense LDL particles were higher, and very dense particles were poor in triglycerides and phospholipids. Thus, in IIb subjects both the number and the production rate of dense LDL particles were elevated.

The sialic acid ratios of dense and very dense LDL subfractions were lower in IIb than IIa hyperlipidemia, in accordance with the negative correlation of the sialic acid ratios of dense and very dense LDL with serum triglyceride levels. In addition, LDL sialic acid ratio was low both in IIa and IIb hyperlipidemia compared with a healthy female population. Both type IIa and IIb hyperlipidemic subjects have an increased risk of CAD. In IIb hyperlipidemia, elevated triglyceride levels are often associated with high concentrations of dense LDL and low HDL cholesterol levels, this lipid profile being especially atherogenic (Grundy 1995a). This was the case also in the IIb hyperlipidemic subjects of the present study with high concentrations of dense LDL and non-significantly lower HDL cholesterol levels compared with IIa subjects. It can be assumed that the risk of developing CAD is even higher for IIb than IIa subjects, and the lower sialic acid content in the atherogenic dense LDL subfraction of IIb subjects could contribute to their increased risk.

6.5.3 Coronary artery disease

Low sialic acid content has been found in LDL of CAD patients in many (Orekhov et al 1989, Orekhov et al 1991b, Tertov et al 1992a, Tertov et al 1992b, Ruelland et al 1993, Millar et al 1999) but not in all studies (Chappey et al 1998). It has been suggested that desialylation is an atherogenic modification of LDL taking place in the circulation, since sialic acid -poor LDL has been found in blood, especially in that of CAD patients (Tertov et al 1992b). LDL with a low sialic acid content causes lipid deposition into cells

(Filipovic et al 1979, Orekhov et al 1992) and binds to arterial proteoglycans (Camejo et al 1985), which both phenomena are characteristic to atheromatous lesions. Desialylized LDL can be considered as a minimally-modified LDL that avidly internalizes in macrophage-foam cells. Thus, sialic acid -poor LDL could be one relevant factor leading to the development of CAD.

In the large patient groups of Study III, CAD+ patients had significantly lower sialic acid ratios in total, dense and very dense LDL than CAD- subjects. This gives further support to the theory that the sialic acid content of LDL might affect its atherogenicity and thus predispose to the development of CAD. However, at low levels of total and dense LDL sialic acid ratio (in Q_1), CAD+ and CAD- subjects had similar mean sialic acid ratios, while at higher values (Q_2-Q_4) CAD+ patients had lower mean sialic acid ratios than CAD- subjects. This implies that the generally lower LDL sialic acid ratios in CAD+ than CAD- subjects may not be seen in populations with low LDL sialic acid ratios. This could partially explain the inconsistencies of previous studies in finding differences in LDL sialic acid ratio between CAD+ and CAD- subjects. Furthermore, LDL cholesterol level was inversely associated with LDL sialic acid ratio and was higher in CAD+ than CAD- subjects. Thus, in stepwise logistic regression analysis only the concentration of cholesterol in LDL, and not its sialic acid ratio, was associated with the presence of CAD, which indicates that LDL cholesterol concentration is more strongly associated with CAD than is its sialic acid ratio.

However, in the other sections of the present study no clear association between low sialic acid ratio and CAD was observed, but this can be explained for instance by the following factors. The diabetic subjects, known to have a very high CAD risk (Kannel and McGee 1979), had high LDL sialic acid levels in Study I, and since the difference in LDL sialic acid ratio seems to be strongest at high levels of sialic acid ratios, the diabetics should exhibit marked differences between CAD+ and CAD- subjects. However, diabetic CAD+ and CAD- subjects had equally high sialic acid ratios, and thus they seem not to be comparable with non-diabetics. In the 10 normoglycemic subjects in Study I, the sialic acid ratio did not differ significantly between CAD+ and CAD- subjects in total LDL or its subfractions, but there was a tendency towards lower sialic acid ratios in dense LDL of CAD+ subjects. In Study II, the sialic acid ratios of total and dense LDL were very similar between CAD+ and CAD- subjects, but this could be due to 8 out of the 10 CAD- subjects belonging to the IIb hyperlipidemia group and thus having a high serum triglyceride level which was associated with a low LDL sialic acid ratio. In Study IV, only 4 subjects did not have CAD, and their small number does not allow to draw conclusions about the lack of differences in LDL sialic acid ratio between CAD- and CAD+ subjects.

Considering these confounding factors in the other sections of the present study on this aspect, and the association being evident in Study III with the largest number of patients, it can be deduced that non-diabetic CAD patients have in general lower LDL sialic acid ratios than comparable subjects without CAD. On the other hand, since to this day there are no follow-up studies on the matter, it can not be excluded that subjects with a high LDL sialic acid ratio could have an elevated rate of fatal AMIs, those with low values having non-fatal AMIs; this would lead to surviving CAD patients having low LDL sialic acid content compared with the general population. In the future, follow-up studies will be crucial in the evaluation of this aspect.

In the combined IIa and IIb group in Study II, the sialic acid ratio was higher in very dense LDL of CAD+ than CAD- subjects, while no differences were detected in total LDL or in the other subfractions. This is probably related to a higher level of Lp(a), known to be associated with CAD in many populations (Genest et al 1991, Sandholzer et al 1992, Kario et al 1994, Bostom et al 1996). If total LDL or its light and dense subfractions were contaminated with significant amounts of Lp(a), it probably would raise their sialic acid content. Accordingly, it could be assumed that CAD+ patients would have a higher LDL sialic acid ratio. However, even though CAD+ patients tended to have a higher mean Lp(a) level in Studies II and III, they had generally lower LDL sialic acid ratios than CAD- subjects. In addition, there was no correlation between LDL sialic acid ratio and serum concentration of Lp(a) in the present study, consistently with previous results (Chappey et al 1995). Moreover, subjects with serum Lp(a) levels in the low or high end of the range had sialic acid ratios in LDL and all its subfractions similar to those of all the subjects together. These findings strongly suggest that the possible contamination of LDL with Lp(a), except in the case of the very dense subfraction, was so small that it can be considered insignificant. Furthermore, it can be presumed that the fractions were equally contaminated with Lp(a) in CAD+ and CAD- subjects, and in the case of a real contamination, the difference in the sialic acid ratio between CAD+ and CAD- subjects would be even more marked in pure LDL.

6.6 LDL sialic acid content and lipoprotein metabolism

6.6.1 LDL catabolism

Multiple types of scavenger receptors with the ability to mediate uptake and degradation of modified lipoproteins have been found in atherosclerotic lesions (Ylä-Herttuala 1996, Greaves et al 1998), while cells of these lesions seem not to express LDL receptors (Ylä-Herttuala et al 1991, Luoma et al

1994). The expression of scavenger receptors is induced by modified lipoproteins, and cholesterol uptake by these receptors is not subject to down-regulation thus allowing extensive intracellular cholesterol accumulation (Greaves et al 1998). Sialic acid -poor LDL is capable of binding, besides to the LDL receptor, to both an asialoglycoprotein receptor (Windler et al 1991) and a galactose-specific lectin receptor (Grewal et al 1996). Both of these receptors could act as scavenger receptors, and so a large part of desialylized LDL could be cleared from the circulation through this unregulated scavenger pathway, leading to the formation of foam cells. Accordingly, the catabolism of sialic acid -poor LDL by the LDL receptor would be slower than that of sialic acid -rich LDL. This hypothetical mechanism is depicted in Figure 14. Earlier studies have reported increased removal from the circulation for desialylated LDL, but the receptor pathway used by desialylated LDL was not specified (Hendrix et al 1975, Malmendier et al 1980).

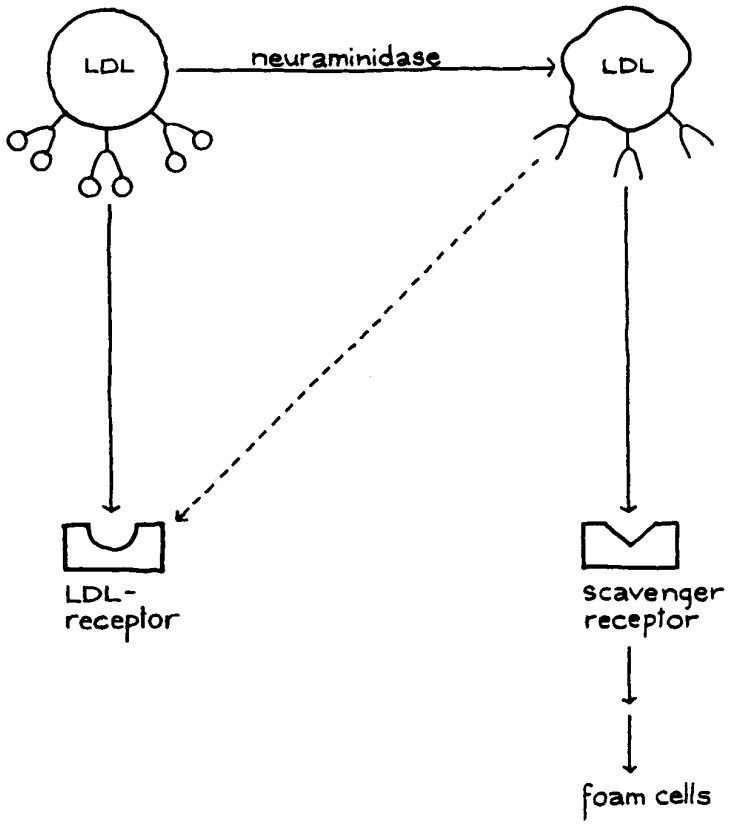

Figure 14. Hypothetical scheme of the receptor interaction of sialylated LDL on the left and desialylated LDL on the right.

In Study I, a positive correlation between the sialic acid ratio of dense LDL and the FCR for dense LDL apo B was seen, while the correlation between these parameters in total LDL did not quite reach significance. The subjects in this study were mainly diabetics with high LDL sialic acid ratios and high FCRs for total and dense LDL. However, in the mostly non-diabetic subjects of the other study sections no clear association came out. In Study II with 8 out of 43 subjects being diabetic, a significant positive correlation was established between the FCR for total LDL apo B and the sialic acid ratio of light but not that of total or dense LDL. Due to the small number of subjects in the kinetic analyses of Study IV no conclusion can be drawn from the lack of correlations between LDL sialic acid and metabolic parameters. In the normoglycemic population in Study III, the FCRs for total and dense LDL apo B were not significantly associated with their sialic acid ratios, although there was a tendency towards a positive correlation between total LDL sialic acid ratio and the FCR for LDL apo B ($r=0.241$, $P=0.08$). Interestingly, however, in stepwise regression analysis the FCR for LDL apo B entered the model explaining the variability of LDL sialic acid ratio along with the TR for LDL apo B when the effect of the latter was removed. This suggests that both the production and the catabolism of LDL are connected to some extent with its sialic acid content. Globally, these data indicate that the sialic acid content of LDL may have a weak positive correlation with its catabolism, the association being stronger in diabetic subjects.

6.6.2 LDL production

The TR for dense LDL apo B was higher in IIb than IIa hyperlipidemia, and had a strong positive correlation with serum triglyceride levels, implying that in the presence of high triglyceride levels, the production of dense LDL particles increases. This is supported by the fact that in FCHL (Kissebah et al 1984, Teng et al 1986, Venkatesan et al 1993) and in hypertriglyceridemia (Vega et al 1985, Vega and Grundy 1989, Vega and Grundy 1998), there is an enhanced production of apo B from the liver, and production rates for VLDL and LDL are high. Thus, also more dense LDL particles are likely to be formed. The TR for dense LDL apo B was also strongly correlated with the esterification percentage of total LDL cholesterol in the IIa and IIb hyperlipidemia subjects, which indicates that the more LDL and specifically dense LDL is produced, the greater part of its cholesterol content is esterified. Cholesterol esterification and ester transfer rates have been reported to be higher in subjects with hypertriglyceridemia or combined hyperlipidemia than in hypercholesterolemic or normolipidemic subjects (Kudchodkar and Sodhi 1976, Murakami et al 1995), and to be positively correlated with serum triglyceride level (Murakami et al 1995). This is in

concordance with the finding of the present study that in hypercholesterolemic subjects a high serum triglyceride level is associated with a high esterification percentage in LDL.

The sialic acid ratios of both dense and very dense LDL manifested significant negative correlations with the TR for dense LDL apo B in Studies II and III. As in the case of LDL catabolism, the small number of participants in the kinetic part of Study IV probably explains the fact that the association did not reach significance in these subjects. In the large patient group in Study III, also the sialic acid ratio of total LDL was inversely related with the TR for dense LDL apo B, and the sialic acid ratios of total, dense and very dense LDL were similarly connected with the TR for total LDL apo B; the negative association between the production and the sialic acid ratio of LDL was even stronger for dense LDL. This association of elevated production of LDL with a low sialic acid ratio could be due to more sialic acid -poor than sialic acid -rich VLDL, IDL and light LDL being converted into LDL and dense LDL, and/or to sialic acid -rich lipoproteins being cleared more avidly from the circulation before conversion into dense LDL. High production rate of LDL apo B could also be linked with high neuraminidase activity causing accelerated desialylation of LDL in the circulation. All of these mechanisms would lead to a lower sialic acid ratio in total and especially in dense LDL. Thus, it would seem that when the sialic acid ratio of lipoproteins is low, their catabolism is slow leading to high production of dense LDL with a low sialic acid ratio. The association of high production, slow catabolism, and large numbers of small LDL particles with low LDL sialic acid ratio is presented in Figure 15. Slow lipoprotein catabolism also leads to a long residence time, allowing desialylation of LDL to occur in the circulation, and could lead to a bigger proportion of LDL being cleared through the scavenger pathway and eventually to an increase in the cholesterol content of macrophages in the intimal layer of endothelium.

As the CAD patients in Study III had low LDL sialic acid ratios, and these ratios were negatively associated with the TRs for total and dense LDL apo B, they also had accelerated production of total and dense LDL apo B, in concordance with findings in one previous study (Kesäniemi and Grundy 1983). It can thus be suggested that low sialic acid ratios in LDL and especially in its dense subfraction associated with high TRs for total and dense LDL apo B are atherogenic. Specifically, the higher production and similar clearance of LDL apo B in CAD+ than CAD- subjects indicate longer residence time in the circulation for LDL in CAD, and could allow more time for desialylation of LDL. The mechanism of this desialylation is not known, but neuraminidase is an enzyme having the ability to remove sialic acid residues from carbohydrate chains, and its concentration in serum has been found to be higher in CAD+ than CAD- subjects (Sonmez et al 1998).

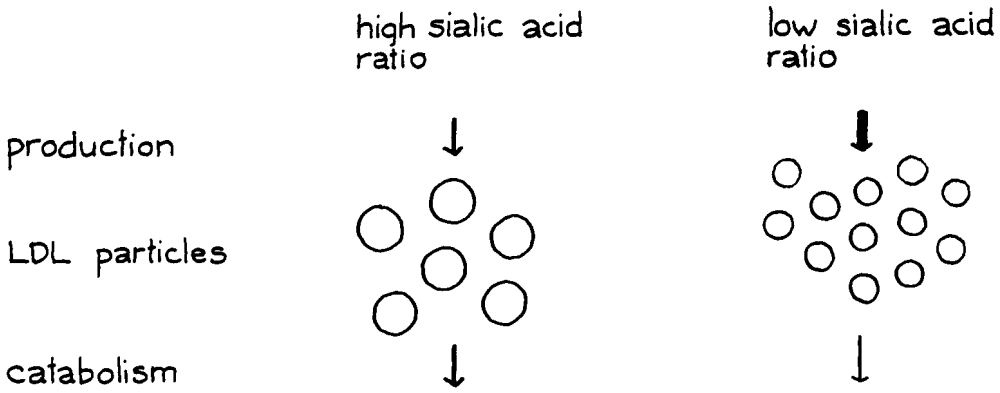

Figure 15. Schematic presentation of the metabolism of LDL and the size and concentration of LDL particles in the case of high and low sialic acid to apo B ratio.

6.7 LDL sialic acid content and cholesterol metabolism

Serum ratios of the cholesterol precursor sterols Δ^8-lathosterol, desmosterol and lathosterol to cholesterol reflect cholesterol synthesis, while the ratios of the plant sterols campesterol and sitosterol and the ratio of cholestanol reflect cholesterol absorption (Miettinen et al 1990). The correlations of dense LDL sialic acid ratio with serum non-cholesterol sterol ratios in the present study indicate that a low sialic acid ratio in dense LDL was associated with high synthesis and low absorption of cholesterol, and these were all connected with a high TR for dense LDL apo B. A positive correlation between cholesterol synthesis and TR for LDL apo B has also been observed earlier in elderly men (Gylling et al 1994), but in middle-aged men no such correlation was found, and contrary to the results now obtained, a positive correlation between cholesterol absorption and TR for LDL apo B has been reported (Miettinen et al 1992, Gylling et al 1994). However, recent studies with stable isotopes have demonstrated a positive correlation between cholesterol synthesis and hepatic production of VLDL apo B (Watts et al 1995c, Riches et al 1997), and since in hypercholesterolemia both TRs for VLDL apo B and LDL apo B are elevated (Teng et al 1986), also more dense LDL apo B is presumably formed. This is in accordance with the present results which suggest that the more cholesterol is synthesized, the more dense LDL is produced, and the lower is its sialic acid ratio.

No consistent differences in cholesterol metabolism have been reported between CAD+ and CAD- subjects previously, but low cholesterol synthesis rate was found to predispose to CAD in a study with FH patients (Miettinen and Gylling 1988). This is in concordance with the finding of this study that at high dense LDL sialic acid ratios, CAD+ subjects had lower lathosterol ratios than CAD- subjects. The reason for this not being seen at lower sialic acid ratios may be due to all the subjects with low sialic acid ratios having high TR for dense LDL apo B, which in turn was associated with high lathosterol ratios, and thus subjects with low LDL sialic acid ratios had high lathosterol ratios independently of CAD status.

6.8 The effect of statin treatment on LDL sialic acid content

The change in the sialic acid ratios of total LDL and all its subfractions was approximately similar in the groups treated with different statins, i.e. cerivastatin (n=10), fluvastatin (n=9) and simvastatin (n=7). The cholesterol concentrations of light and dense LDL subfractions at baseline were negatively correlated with the respective baseline sialic acid ratios so that when the cholesterol contents were decreased by statins, the sialic acid ratios increased. Further, the more marked the decrease in cholesterol or apo B concentration of dense LDL was, the greater was the increase in its sialic acid ratio. Thus, since the strongest lipid lowering of LDL subfractions was found in the most hypercholesterolemic subjects, it could be assumed that these patients also had the biggest rise in sialic acid ratios. However, basal lipid levels were not correlated with the change observed in the sialic acid ratios.

Considering that desialylated LDL is supposed to be atherogenic, the new finding that LDL sialic acid ratio rises during statin treatment could contribute to the well-known observation that statin treatment is associated with a regression of atherosclerosis. The decrease in dense LDL lipid and apo B concentrations was related to the basal sialic acid ratio of dense LDL so that a low sialic acid ratio at baseline predicted effective lowering of lipid and apo B concentrations.

In a recent study by Orekhov et al (1997), the sialic acid content of LDL increased in patients treated with amlodipine alone or with the combination of amlodipine and lovastatin. The augmentation was seen more rapidly with the combination treatment than with amlodipine alone, but after a treatment of four weeks, the final level of sialic acid was the same whether or not lovastatin was used. In contrast to these findings, results of the present study imply that statin treatment alone causes a rise in LDL sialic acid ratio. Millar et

al (1999) reported that ciprofibrate treatment reduced the sialic acid ratio of lipoproteins, and although the sialic acid content was inversely associated with the formation of atherogenic lipoprotein-proteoglycan complexes, the formation of these complexes diminished during ciprofibrate treatment.

LDL sialic acid ratio rose in most but not in all subjects during the statin treatment. The 5 subjects with a decreasing sialic acid ratio had a higher LDL sialic acid ratio at baseline than the other subjects, but otherwise they had identical clinical characteristics, baseline lipid and apo B concentrations and LDL kinetic parameters, and the changes in these parameters during statin treatment were comparable to those in patients with increasing LDL sialic acid content. Because of the good reproducibility of sialic acid measurements discussed earlier, the decreasing sialic acid ratios in some subjects can not either be explained by error in the measurement. Consequently, it is difficult to determine why their LDL sialic acid content responded differently.

As the lipid contents of lipoproteins fell during the statin treatment, their metabolism was also affected. The FCRs for total and dense LDL apo B increased, implying an accelerated catabolism. Furthermore, there was a decrease in the TR for dense LDL apo B, reflecting a diminished production of this subfraction, while the TR for total LDL apo B was not affected. This suggests that statin treatment reduced the conversion of light LDL into the more atherogenic dense subfraction, which has also been associated with a lower sialic acid content (La Belle and Krauss 1990). However, there was no connection between baseline sialic acid ratios and the changes in LDL metabolic variables, but it should be noted that the number of subjects in the kinetic study was limited.

The observed increases in LDL sialic acid ratio and LDL catabolic rate are in concordance with the finding in Study I that high LDL sialic acid ratio is associated with high FCR for LDL apo B, especially in diabetics. It is not known which of these changes causes the other. Statin treatment augments LDL catabolism by upregulating the activity of LDL receptors (Kovanen et al 1981, Reihner et al 1990), and TR for dense LDL apo B is apparently decreased because of effective removal of lipoproteins of lower density. So, it could be assumed that when the sialic acid content of LDL is high, its catabolism by the LDL receptor accelerates, which also leads to lower production of dense LDL. On the other hand, high LDL catabolic rate could lead to a higher sialic acid content by leaving less time for desialylation of LDL in the circulation. Both of these mechanisms can link high LDL sialic acid ratio to enhanced catabolism of LDL and to low production of dense LDL. Further confirmation to this would be given by investigating FH subjects, who have a slow LDL catabolism and therefore presumably a low LDL sialic acid ratio.

7 SUMMARY AND CONCLUSIONS

The sialic acid content of lipoproteins has been suggested to affect lipoprotein metabolism and the development of atherosclerosis. Therefore, the sialic acid content of lipoproteins, especially that of LDL and its three subfractions, and its relations to different lipid variables and lipoprotein and cholesterol metabolism, as well as to DM, type IIa and IIb hyperlipidemias, and CAD were investigated. Furthermore, the effect of statin treatment on LDL sialic acid content was studied.

To study these aspects, subjects were examined as follows: 20 type 2 DM subjects and 10 non-diabetic controls (Study I), 22 type IIa and 21 type IIb hyperlipidemic subjects (Study II), 56 CAD and 42 non-CAD subjects (Study III), and 26 hypercholesterolemic subjects before and during statin treatment (Study IV). Lipoproteins were separated by ultracentrifugation, and sialic acid contents were measured with the resorcinol method. The metabolism of lipoproteins was investigated with radioactive iodine labeling, and that of cholesterol by measuring non-cholesterol sterols from serum.

The sialic acid content of apo B -containing lipoproteins decreased with increasing particle density from VLDL to LDL, and less consistently from light to dense LDL. The sialic acid content of LDL was negatively correlated with the concentrations of cholesterol, triglycerides, phospholipids, and apo B especially in the dense LDL subfraction, and low sialic acid content was associated with large amounts of relatively small LDL particles.

LDL sialic acid content had a weak positive correlation with the catabolism of LDL apo B, the association being stronger in type 2 diabetes mellitus. The production rate of LDL apo B was inversely related with LDL sialic acid content, especially in dense LDL. Low sialic acid content in dense LDL was also associated with high synthesis and low absorption of cholesterol, which in turn were also related with high production rate of dense LDL apo B.

CAD patients had a lower sialic acid content in LDL, especially at high sialic acid ratios, than non-CAD subjects. Type IIb hyperlipidemia was associated with a low sialic acid content in dense and very dense LDL compared with type IIa hyperlipidemia. Type 2 diabetic patients had higher sialic acid contents in total LDL and its subfractions than non-diabetics, connected with higher catabolism of LDL apo B. Age, sex, BMI, smoking and apo E

phenotype had no effect on LDL sialic acid ratio, neither had the use of beta-blockers, thiazide diuretics or hormone replacement therapy.

Statin treatment increased the sialic acid ratio of LDL and its subfractions proportionately to the decrease in cholesterol levels, and in dense LDL low sialic acid content at baseline predicted effective lowering of cholesterol level in the subfraction. Production of dense but not that of total LDL apo B decreased during statin treatment, while catabolism of both total and dense LDL apo B increased. The number of subjects in the kinetic study before and during statin treatment was, however, so small that no associations were found between the changes in kinetics and the sialic acid ratios of LDL.

In summary, a low sialic acid content in LDL and especially in its dense subfraction was associated with CAD and with multiple variables related with an increased risk of CAD, namely high serum and lipoprotein lipid levels, high numbers of small LDL particles, and high production rate of dense LDL apo B. In addition, statins increased the sialic acid content of LDL. Thus, low LDL sialic acid content seems to be a risk factor of atherosclerosis and connects with high lipoprotein lipid levels, and LDL sialic acid level is associated with the metabolism of LDL apo B and cholesterol and is increased by statin treatment proportionately to the decrease in lipid levels.

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