Aspects involved in the (patho)physiology of the metabolic syndrome

Duivenvoorden, I.

Summary

The metabolic syndrome is an increasing problem in our Western society. Many of the features of the metabolic syndrome, like obesity, insulin resistance, dyslipidemia, and hepatic steatosis are established risk factors for cardiovascular disease. Growing evidence supports the important role of body free fatty acid handling and/or body distribution of triglycerides in the pathogenesis of the metabolic syndrome-associated problems. Since many of the features of the metabolic syndrome are major threats to human health, prevention of the development and/or treatment of the metabolic syndrome is a desirable goal. We used several different approaches to study the development of obesity, insulin resistance, dyslipidemia, and liver steatosis.

In **chapter 2** our aim was to study whether the absence of apolipoprotein (apo) C3, a strong inhibitor of lipoprotein lipase (LPL), accelerates the development of obesity, and consequently insulin resistance. After 20 weeks of high-fat feeding *apoc3*$^{-/-}$ mice showed decreased plasma triglyceride levels and increased body weight compared with wild-type littermates. The observed increase in body weight was entirely explained by increased body lipid mass. We showed that the underlying mechanism for the increased fat mass was increased LPL-dependent triglyceride-derived fatty acid (FA) uptake by adipose tissue in *apoc3*$^{-/-}$ mice, while LPL-independent albumin-bound FA uptake did not differ. As expected, the increased body weight and fat mass led to decreased insulin sensitivity, both peripheral and liver-specific, as measured by hyperinsulinemic-euglycemic clamps. We concluded that the absence of apoC3, a natural LPL inhibitor, enhances FA uptake from plasma triglycerides in adipose tissue. This leads to increased susceptibility to diet-induced obesity, followed by more severe development of insulin resistance. Therefore, we have shown that regulation of body distribution of triglycerides, in a LPL-dependent process, plays an important role in obesity development. Down-regulation of adipose tissue LPL activity might contribute to treatment and/or reduction of obesity development. ApoC3 may be a potential target in this strategy. Nevertheless, because of the risk of cardiovascular diseases the effects of reduced LPL activity on plasma lipoprotein levels need to be carefully monitored.

In another set of experiments, we used methyl palmoxirate (MP), an inhibitor of carnitine palmitoyl transferase I (CPTI), to acutely inhibit hepatic FA β-oxidation in hyperlipidemic APOE*3Leiden mice. We investigated whether FA in the liver are rerouted into very low density lipoprotein (VLDL) production and secretion, and if so, whether this rerouting affects hepatic insulin sensitivity regarding glucose production in **chapter 3**. Administration of MP to the mice led to a strong inhibition of the hepatic β-oxidation (as measured by a reduction in plasma β-hydroxybutyrate [= keton body] levels) compared with vehicle-treated mice. Plasma free FA and cholesterol levels were increased, while insulin levels were decreased in MP-treated mice compared with controls. Although MP treatment led to

an increase in liver triglyceride content, no effect on hepatic VLDL-triglyceride production was observed between both groups. In addition, the capacity of insulin to suppress endogenous glucose production was unaffected in MP-treated mice compared with controls. We concluded from these studies, that acute inhibition of the β-oxidation of FA indeed increases hepatic lipid content, but neither stimulates hepatic VLDL secretion nor reduces insulin sensitivity. Apparently, accumulation of FA-metabolites induced by impaired β-oxidation *per se* does not affect VLDL-production or insulin sensitivity in the liver. It seems likely, that the FA-metabolites trigger a chronic (inflammatory) signal that eventually leads to VLDL overproduction and insulin resistance.

FA are known to have different effects on health. In **chapter 4** we studied the effects of a saturated-fat diet supplemented with fish oil, *trans*10,*cis*12 conjugated linoleic acid (CLA), elaidic acid, or fenofibrate on lipid and glucose metabolism and on liver protein levels in hyperlipidemic APOE*3Leiden mice. We found that all treatments significantly lowered serum cholesterol. Fish oil and fenofibrate significantly lowered triglyceride levels, while CLA significantly increased triglyceride levels in plasma. These changes in plasma cholesterol and triglyceride levels were mirrored by the changes in liver lipid composition: fish oil and fenofibrate decreased, and CLA increased liver triglyceride levels. Serum glucose was decreased by fish oil and fenofibrate, and CLA and fish oil significantly increased serum insulin. Proteomics analyses identified significant changes in the levels of several liver cytosolic and membrane proteins. Principal component analysis revealed a major treatment effect of fish oil on cytosolic protein levels and of elaidic acid on membrane protein levels. Proteins that provided the largest contribution to the treatment effects were involved in glycolysis/gluconeogenesis, lipid metabolism and oxidative stress. This study shows that the combination of proteomics with relevant physiological parameters in a sensitive animal model, is a powerful tool, which will aid in identifying workingmechanisms of various dietary FA. Understanding these workingmechanisms is of interest because some FA are known to have beneficial effects on plasma lipids/lipoproteins, and therefore on human health. It seems that, although effects of different FA on plasma lipid/lipoprotein levels measured are equal, the underlying mechanisms are completely different. Further research in defined metabolic and physiological parameters, such as hepatic VLDL-triglyceride production, absorption, LPL-dependent triglyceride clearance, will enable us to specifically define what changes are induced by specific FA.

In **chapter 5** we questioned whether dietary sphingolipids decrease plasma cholesterol and/or triglycerides in hyperlipidemic APOE3*Leiden mice. The six sphingolipids tested dose-dependently decreased both plasma cholesterol and triglyceride levels. The hypolipidemic effect of specifically phytosphingosine (PS) was the net result of: (a) decreased absorption of dietary cholesterol and free FA; (b) increased hepatic VLDL-triglyceride

production; and (c) increased hepatic uptake of VLDL-remnants. These changes also resulted in less pale livers, which weighed less and contained fewer cholesteryl esters and triglycerides compared with livers of control mice. Furthermore, in PS-fed mice markers for liver inflammation (SAA) and liver damage (ALAT) were decreased. We concluded that sphingolipids protect the liver from fat and cholesterol-induced steatosis. Since sphingolipids are nutritional compounds present in several daily foods, such as milk and meat, addition of sphingolipids to the diet may decrease traditional cardiovascular risk factors, such as plasma cholesterol and triglycerides. Furthermore, the ability of sphingolipids to lower liver inflammation markers and liver-specific steatosis may contribute in targeting the metabolic syndrome. It seems that, sphingolipids may prove to be very beneficial for both cardiovascular and diabetes risk factors. Further research on beneficial effects of sphingolipids on hepatic steatosis and insulin sensitivity might reveal an interesting potential.