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

Duivenvoorden, I.

Chapter 6

Discussion

&

Future Perspectives

The metabolic syndrome is an increasing problem in our Western society. Many of the features of the metabolic syndrome like obesity, hepatic steatosis, insulin resistance and dyslipidemia are established risk factors for cardiovascular diseases. Growing evidence shows that handling of free fatty acids (FA) and/or body distribution of triglycerides and free FA plays a central role in the pathogenesis of the problems associated with the metabolic syndrome. In this thesis we present different studies aimed at unraveling the pathophysiological mechanisms underlying the development of obesity, dyslipidemia, insulin resistance and hepatic steatosis.

Several mouse studies indicate that decreased lipoprotein lipase (LPL) activity in adipose tissue decreases the propensity to develop obesity[1-5]. However, it was unclear whether the opposite was true as well, *i.e.*, whether activation of LPL leads to an enhanced susceptibility to diet-induced obesity and associated insulin resistance. We showed that, during high-fat feeding, the absence of apolipoprotein (apo) C3, a strong inhibitor of LPL, indeed leads to a higher adipose tissue mass, concomitant with insulin resistance and mild hepatic steatosis compared with wild-type littermates (**chapter 2**). It would be of interest, to investigate whether apoC3 overexpression leads to less adipose tissue mass.

Obesity does not necessarily lead to insulin resistance in the liver. As long as the FA flux toward the adipose tissue does not lead to increased FA flux to the liver, hepatic insulin resistance is not expected to occur. For instance, peroxisome proliferator-activated receptor (PPAR) γ agonist treatment leads to increased adipose tissue LPL expression[6], concomitant with more adipocyte differentiation[7] and increased whole body insulin sensitivity. It may be concluded that growing obese *per se* is not detrimental to the development of diabetes type 2 and cardiovascular health. Diminishing FA fluxes from liver and plasma to adipose tissue, as well as increasing adipose tissue lipolysis are two possibilities to prevent obesity, but may be detrimental, rather than favorable to reducing the incidence of the metabolic syndrome. It is obvious, that not the adipose tissue mass *per se* but rather the FA fluxes determining the adipose tissue mass are fundamental to the metabolic relationship between obesity and insulin resistance.

However, it is not only FA fluxes. Recent studies clearly showed that various endocrine factors are secreted by adipose tissue, like leptin, resistin and adiponectin. These hormones are known to affect insulin sensitivity and are correlated with adipose tissue mass[8-11]. In this thesis we described that apoC3-deficient mice indeed had increased plasma leptin levels, in accordance with an increase in adipose tissue mass. Although it is likely that the hyperleptinemia observed in these mice is the consequence rather than the cause of insulin resistance, as has been observed earlier in humans[8,12-15].

Our results show that the liver can modulate plasma lipid levels and at the same time fat mass and insulin sensitivity through production of just one protein, apoC3. It warrants further investigation, whether in man apoC3 is a potential therapeutic target for

treatment of obesity and/or obesity-related insulin resistance and at the same time for prevention of cardiovascular risk.

Next to adipose tissue mass, lipogenesis, chylomicron- and very low density lipoprotein (VLDL)-remnant uptake, VLDL production and secretion, as well as β-oxidation of FA, are fundamental to hepatic steatosis and, eventually, hepatic insulin resistance. We wondered whether inhibition of hepatic β-oxidation increases hepatic steatosis, VLDL production and/or secretion, or both (**chapter 3**). Therefore, we used methyl palmoxirate (MP), an inhibitor of carnitine palmitoyl transferase I (CPTI), to acutely inhibit hepatic FA oxidation. Indeed, within 2 hours after oral dosing of MP, plasma keton bodies dropped and remained less than 10% for up to 8 hours after gavage. Since plasma keton bodies are solely derived from hepatic β-oxidation, we concluded that hepatic β-oxidation of long-chain FA was almost completely inhibited by the applied dose of MP.

As expected, inhibition of hepatic β-oxidation led to significant accumulation of TG in the liver. This increased hepatic TG accumulation was not associated with increased hepatic VLDL-TG production and/or changes in VLDL-composition. However, we did observe an increase in mRNA expression of microsomal triglyceride transfer protein (*mttp*), involved in hepatic VLDL assembly and secretion, in the livers of MP-treated mice. Therefore, we cannot exclude that chronic, long-term inhibition of hepatic β-oxidation does induce hepatic VLDL-TG production.

Several studies have demonstrated that β-oxidation inhibitors (like etomoxir and MP) are effective at lowering both keton body and glucose levels in rodents, dogs and humans[16-20]. In our overnight-fasted mice plasma glucose levels were similar between MP-treated and control mice. Also, we observed that acute inhibition of β-oxidation was associated with strongly decreased plasma insulin levels. This is in line with Boden et al.[21] who show that in humans there is a positive correlation between plasma keton body concentrations and insulin secretion capacity.

Our study clearly showed that, in contrast to adipose tissue-mediated hepatic steatosis, hepatic steatosis as a consequence of inhibition of β-oxidation does not lead to hepatic insulin resistance. In addition, β-oxidation related hepatic steatosis does not result in increased VLDL production. Thus, the metabolic relationship between hepatic steatosis on one hand, and insulin resistance and VLDL production on the other hand, seems to be dependent on the pathway, via which the TG have been accumulated. These findings argue against a common assumption that the production of VLDL in the liver is substrate-driven. In that respect it would be interesting to know whether stimulation of β-oxidation, by for instance tetradecylthioacetic acid administration[22], would result in decreased hepatic steatosis, concomitant with increased hepatic insulin sensitivity and decreased VLDL production.

Whole-body FA metabolism is driven by the FA homeostasis in adipose tissue and liver, and is a strong determinant of plasma lipid levels and cardiovascular risk. In this respect, much attention has been paid to the effect of specific dietary FA: saturated, (poly)unsaturated, *trans*- and *cis*-unsaturated FA and conjugated linoleic acids (CLA). The mechanisms underlying the various effects of these FA on plasma lipid levels and/or cardiovascular risk has to date not become clear, and the results obtained from dietary studies are often inconsistent due to differences in study design and different animal models used. Therefore, we decided to study the effect of various specific FA on plasma and hepatic lipid levels using a single animal model, with a human-like lipoprotein metabolism, that has been proven to be sensitive to relatively mild perturbations in the diet, the APOE*3Leiden mouse (**chapter 4**). Indeed, our results showed that the various FA differ clearly in their effects on plasma and liver lipid levels in this animal model. To obtain more insight in the underlying mechanisms, we focused on the liver as a central organ in lipid/lipoprotein metabolism by applying a proteomics approach. The results showed that the different specific dietary FA have different effects on protein composition of the liver. Although the combination of proteomics with physiology gave us more insight in the mechanisms by which these FA (may) regulate lipid metabolism and related pathways, the current study is an example of the very beginning of the application of the “omics” approach in finding new relevant molecular pathways.

The statistical analyses of our results revealed many associations, some of which are well known, including the associations with aspects of the metabolic syndrome, whereas many others will be the basis of intriguing new leads for further studies. In the future, these studies should be repeated and extended with other “omics” approaches, like metabolomics and transcriptomics. By doing so, many new promising and less promising molecular pathways underlying the metabolic syndrome and cardiovascular diseases are expected to be found. Subsequently, additional (classical) biochemical/physiological studies have to be executed to evaluate the relevance of the respective pathways. Since the number of possible pathways will be quite extensive, it will be a great challenge to choose the most promising pathway right from the start of this inevitable and necessary “post-omics” era.

It is obvious, that the liver plays a pivotal role in both lipid and glucose homeostasis. Glucose and lipid metabolism are tightly interrelated and a steatotic liver is often the culprit for disturbances in both glucose and lipid metabolism. Interventions to improve liver TG content, and as a consequence, insulin sensitivity and plasma lipid levels is highly needed, especially in Western society where the obesity-related metabolic syndrome is highly prevalent and responsible for the high risk of cardiovascular diseases.

At present, several drugs are available to treat one or maximally two aspects of the metabolic syndrome at a time. Treatment of multiple aspects of the metabolic syndrome with a single natural dietary compound would be an attractive alternative.

Although far from clearly conclusive, various animal studies have been published in the past claiming health benefits for dietary sphingolipids regarding lowering plasma lipid levels. Sphingolipids are membrane constituents in plants, yeasts and animals and are present in our daily diet. In **chapter 5** we first questioned whether sphingolipids supplemented to the Western-type diet indeed decrease plasma cholesterol and/or triglycerides in our "humanized" hyperlipidemic APOE*3Leiden mouse model. We found that both simple and complex sphingolipids decrease plasma lipid levels in this mouse model, the primary underlying mechanism being the inhibition of the intestinal absorption of both cholesterol and TG.

More importantly, we clearly observed that the livers of phytosphingosine (PS)-fed mice weighed significantly less than livers of control mice, and contained less cholesteryl esters and TG, and less lipid-filled vacuoles in the parenchymal cells. In addition, plasma levels of ALAT and SAA, markers for liver damage and liver inflammation, respectively, were strongly decreased. These results point to a true hepatoprotective effect of dietary PS under conditions of Western-type diet feeding. Since inflammatory parameters are involved in both atherosclerotic and diabetes/insulin resistance related processes, dietary sphingolipids may therefore be considered as compounds useful in treating or ameliorating not only the lipid component of cardiovascular disease, but also the insulin-resistance components of the metabolic syndrome.

In studies not presented in this thesis we indeed showed that PS added to the diet improves obesity-related insulin resistance in mice. In a pilot study with human volunteers we showed that daily supplementation of one gram of PS also resulted in a reduction of total plasma cholesterol. A more extended clinical study with metabolic syndrome patients is currently being designed. In that study, next to evaluation of the effect of PS on plasma lipid, glucose and insulin levels, strong focus will be on hepatic steatosis as measured by non-invasive magnetic resonance imaging (MRI) analyses. We expect to conclude from such a study, that sphingolipids hold great potential to treat or prevent metabolic syndrome and, eventually cardiovascular disease.

Besides obesity, administration of some drugs and also alcohol consumption often lead to steatotic livers. Although these forms of hepatic steatosis may be metabolically different from obesity-related steatosis, it is tempting to investigate whether the addition of sphingolipids to the diet can also prevent or cure these forms of hepatic triglyceride accumulations. Chronic liver diseases as caused by hepatitis B, hepatitis C and heavy alcohol consumption have previously been shown to be major risk factors for developing liver cancer[23,24]. Until recently, diabetes alone was also seen as a risk factor for liver cancer, most probably via the high prevalence of obesity-related fatty liver. In this respect, it is important to note that in the developed Western world the incidence of liver cancer is rising parallel to the prevalence of obesity. Whether dietary sphingolipids help in preventing liver cancer might be worth studying in the near future.

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