Summary

Introduction

Cardiovascular diseases (CVD) account for approximately every third death in western as well as developing countries. Despite decades of intensive research many cardiovascular events cannot be predicted by conventional cardiovascular risk factors and can thus not be sufficiently prevented by treating risk factors such as arterial hypertension, hyperlipidemia or diabetes mellitus. Hence, it is important to evaluate novel candidate risk factors for CVD in order to (i) identify high risk patients for CVD which should be subject to intensive treatment and to (ii) identify novel targets for therapeutic interventions.

Accumulating evidence suggests that endocrine disorders are associated with CVD. In this context it was evaluated in this thesis whether the two hormones adiponectin and 25-hydroxyvitamin D (25(OH)D) are associated with cardiovascular risk.

Methods

The general approach was to measure serum levels of adiponectin and 25(OH)D in different cohort studies and to evaluate whether these two hormones are associated with prevalent measures of CVD as well as with future cardiovascular events and mortality.

This issue was addressed by examining a cross-sectional cohort of 140 obese and 100 lean juveniles derived from the Styrian Juvenile Obesity Study (STYJOBS). In addition two prospective study cohorts were investigated: a cohort of 3316 patients routinely referred for coronary angiography (Ludwigshafen RIsk and Cardiovascular Health, LURIC Study) and an older population based cohort comprising 2484 study participants (Hoorn Study).

Prevalent CVD was assessed by ultrasonographic measurement of carotid intima-media thickness, a measure of early CVD and increased cardiovascular risk (STYJOBS and Hoorn Study), by echocardiographic measurement of myocardial structure and function (Hoorn and LURIC Study), and by angiographic determination of coronary artery disease and heart function (LURIC Study). Incident cardiovascular events and mortality were recorded during several years of follow-up in the LURIC and the Hoorn Study.

A main strength of this thesis is that three different study cohorts were investigated, which enabled us to replicate our results in different study populations and to evaluate individuals with low (STYJOBS), intermediate (Hoorn Study) and high cardiovascular risk (LURIC Study).

Statistical analyses were performed to test for associations of adiponectin as well as 25(OH)D with prevalent and incident CVD. These analyses were adjusted for various possible confounders in order to evaluate whether adiponectin and 25(OH)D are independent cardiovascular risk factors. It should, however, be acknowledged that despite careful adjustments residual confounding cannot be ruled out. On the other
hand over adjustments by parameters of the causal pathway might have also occurred. In this context it is also important to note that the results of this thesis are derived from observational data which limits definite conclusions regarding causality.

Adiponectin

Adiponectin is a peptide hormone which is mainly secreted from the adipose tissue and is paradoxically decreased in overweight individuals. Adiponectin receptors are found in various organs with particular high expressions in liver and skeletal muscle. Heart and vessels are also target tissues for adiponectin. Experimental studies suggest that adiponectin may protect against CVD. Accumulating evidence suggests that adiponectin protects against disturbances in glucose and lipid metabolism, exerts anti-inflammatory actions and may prevent direct vascular and myocardial damage. There exists, however, evidence that CVD may also cause a (counterregulatory) increase in adiponectin. Nevertheless, previous clinical studies have almost consistently indicated that low adiponectin serum concentrations are associated with an increased risk of CVD, a hypothesis which has been evaluated in this thesis.

In STYJOBS we found that low adiponectin serum levels are associated with increased carotid intima-media thickness. In 1626 male patients from the LURIC Study it was shown that adiponectin serum levels were significantly lower in patients with compared to patients without significant coronary artery disease. There was, however, no significant relationship between adiponectin serum levels and different stages of coronary artery disease. In prospective analyses of the LURIC study comprising 3146 male and female patients we found an unexpected result: high and not low adiponectin levels were associated with an increased risk of all-cause and cardiovascular mortality. This effect was particularly significant in patients with coronary artery disease as well as in groups with heart failure. Further analyses among 1077 men and 1248 women of the population based Hoorn study confirmed that high adiponectin concentrations were associated with an increased risk of cardiovascular mortality. This association of high adiponectin with increased risk of CVD mortality was particularly evident in patients who were already suffering from CVD at baseline. By contrast, in those study participants free of prevalent CVD there was a reduced risk of mortality in women with higher adiponectin levels and a non-significant U-shaped association in men.

The main conclusion from these adiponectin findings of this thesis is that the association of adiponectin with CVD appears to be modified by the stage of CVD: an inverse association of adiponectin and cardiovascular risk is observed in early stages of CVD (STYJOBS) but is attenuated during the course of CVD (Hoorn Study) and is finally reversed in advanced stages of this disease (LURIC Study). This conclusion draws a new picture of the role of adiponectin in CVD. It is an intriguing hypothesis that the relation or balance of protective effects of adiponectin on CVD and the CVD triggered increase in adiponectin levels may underlie our results. To further evaluate this hypothesis in future studies we suggest to elucidate whether high adiponectin levels in patients with significant CVD represent (i) a counterregulatory increase in order to protect against cardiovascular damage, (ii) are increased as a response to "adiponectin resistance" or (iii) are even harmful and exert deleterious consequences.
These issues need to be clarified before seriously considering the implementation of specific therapies aiming to modify adiponectin.

**Vitamin D**

Vitamin D and its metabolites can be rather regarded as hormones than as vitamins. The main source for vitamin D in humans is sunlight (ultraviolet-B) induced vitamin D production in the skin whereas vitamin D intake by nutrition play usually only a minor role. Our current lifestyle with reduced outdoor activities is generally associated with very limited time of sunlight exposure and this seems to be the main cause for the worldwide pandemic of vitamin D deficiency. Assessment of vitamin D status is performed by measuring 25-hydroxyvitamin D (25(OH)D) levels, which are below the optimal range in almost every second person. Historically, vitamin D supplementation is known to prevent rickets in children and to exert beneficial effects for the maintenance of musculoskeletal health. The discovery that the vitamin D receptor (VDR) is almost ubiquitously expressed throughout the body and reports on the association of vitamin D deficiency with various chronic diseases raised the public health interest in vitamin D.

The cardiovascular system is also an important target tissue for vitamin D effects and it has been shown that vitamin D metabolites regulate the expression of many genes which are important for cardiovascular health. Accumulating evidence suggests that vitamin D may reduce cardiovascular risk in particular by its anti-hypertensive and anti-inflammatory effects, improvements of glucose metabolism, nephro- and cardio-protective actions as well as suppression of parathyroid hormone (PTH) levels. A crucial role of vitamin D for cardiovascular pathophysiology is supported by observations that VDR knock out mice suffer from CVD. In this thesis we aimed to evaluate whether low 25(OH)D concentrations are associated with an increased cardiovascular risk.

In the Hoorn Study it was shown that low 25(OH)D levels were independent of classic cardiovascular risk factors associated with an increased risk of cardiovascular and all-cause mortality. There was, however, no significant association of 25(OH)D concentrations and carotid intima-media thickness. Prospective analyses of the LURIC Study yielded that high 25(OH)D levels are associated with reduced risk of fatal strokes. In the same study it was also shown that patients with prevalent and incident heart failure showed significantly reduced levels of 25(OH)D compared to patients without significant myocardial dysfunction. Risk of sudden cardiac death was also associated with a poor vitamin D status but low 25(OH)D levels were not significantly predictive for fatal myocardial infarctions. In the Hoorn study it was shown that 25(OH)D concentrations were not significantly associated with systolic myocardial function. Vitamin D deficiency was, however, more common in patients with diastolic dysfunction but this association was attenuated towards a non-significant trend after adjustments for age and other cardiovascular risk factors.

In conclusion, these results suggest that individuals with vitamin D deficiency are at increased risk for CVD. These findings significantly add to the current knowledge on observed associations of low 25(OH)D levels with multiple chronic and life-threatening diseases. We can, however, not draw final conclusions on the effect of vitamin
D supplementation on CVD events and we have to wait for results from large-scale randomized controlled trials that specifically address the effects of vitamin D on cardiovascular outcomes.

**General conclusion**

By using data from observational studies, the results of this thesis have improved the understanding of the role of adiponectin and vitamin D in human health and disease. Further studies will address the possible clinical consequences of therapies targeting on adiponectin and vitamin D status.