Chapter 9
General discussion

Evaluating novel risk factors for cardiovascular diseases (CVD) is an important goal to identify individuals at high cardiovascular risk who should be subject to intensive preventive and therapeutic procedures to reduce the morbidity and mortality of CVD [1]. Apart from this, evaluating the relationship of candidate risk factors with CVD is important to improve our understanding of the pathogenesis of CVD and may help to identify novel targets of treatment [1]. In part 1 of this thesis, the association of adiponectin serum levels with CVD was evaluated, and in part 2, the association of vitamin D status with CVD. The main findings and their possible implications for future research as well as for current clinical practice will be discussed in this chapter.

9.1. Adiponectin

Adiponectin is a hormone which is mainly secreted by the adipose tissue. Various experimental studies suggest that adiponectin protects against CVD but the association between adiponectin serum levels and CVD still remains to be clarified in detail [2-5].

Summary of the results on adiponectin

Main findings on adiponectin were that in obese juveniles, low adiponectin levels were significantly associated with increased carotid intima-media thickness (chapter 2). In the Ludwigshafen Risk and Cardiovascular Health (LURIC) Study, it turned out that adiponectin levels were significantly reduced in men with coronary artery disease compared to men without significant stenosis of their coronary arteries (chapter 3). There was, however, no significant relationship between adiponectin serum levels and different stages of coronary artery disease (chapter 3). In prospective analyses of the LURIC Study, high adiponectin levels were independent of other cardiovascular risk factors associated with an increased risk of cardiovascular and all-cause mortality (chapter 3). This association was particularly significant in both patients with coronary artery disease and those with heart failure. Data from the Hoorn study confirmed that risk of CVD mortality was significantly increased in study participants with high adiponectin levels (chapter 4). 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.
Discussion of the methods

The methodological approach used to address the association between adiponectin and CVD in this thesis, was to analyse cross-sectional and prospective cohort studies. Baseline samples from existing cohorts were used for measurement of adiponectin. Three cohorts were included: Styrian Juvenile Obesity Study (STYJOBS), LURIC and Hoorn Study.

STYJOBS is a cross-sectional study which is designed to investigate early stages of atherosclerosis and metabolic disorders among obese juveniles. All study participants were examined at the Medical University of Graz, Austria between 2003 and 2004. The study population consists of 140 obese juveniles and 100 lean age-matched controls.

LURIC is a prospective follow-up study among 3316 patients routinely referred to coronary angiography to a tertiary care centre in Southwest Germany between 1997 and 2000. This study was designed to investigate environmental and genetic risk factors for CVD. Total and cause-specific mortality were recorded during follow-up.

The Hoorn Study is also a prospective cohort study among 2484 individuals of the older general population in a middle-sized town in the Netherlands. That study started in 1989 and was mainly designed to investigate risk factors for type 2 diabetes and its complications. Mortality and fatal as well as non fatal cardiovascular events were recorded during follow-up.

Possible sources for bias, confounding and selection effects

Before drawing final conclusions some possible sources for bias or confounding need to be discussed. In particular selection effects cannot be completely ruled out; e.g. study recruitment procedures might have contributed to the selection of specific cohorts, which meaningfully differ from samples of the individuals who were initially screened for study inclusion.

Possible confounding factors should also be discussed because several established cardiovascular risk factors are associated with adiponectin (predictor variable) but are also causal factors for the development of CVD (outcome variable). True confounding factors are associated with the predictor and the outcome variable but do not mediate the effect of the predictor on the outcome variable. For some cardiovascular risk factors it is, however, difficult to differentiate whether they are true confounding factors or are rather parameters of the causal pathway. These parameters of the causal pathway mediate the effect of the predictor variable on the outcome variable.

In STYJOBS, the willingness to participate in the study might have been guided by obesity related factors such as extent of obesity or previous changes of body weight. This could have contributed to selection of a specific study cohort. It should also be considered that in particular obesity and its related CVD risk factors, which contribute to atherosclerosis, could be confounders for the association of adiponectin and carotid intima-media thickness. It could therefore be conceivable that the link between low adiponectin and early atherosclerosis is merely a reflection of obesity associated increases in carotid intima-media thickness. The association of low adiponec-
tin and increased carotid intima-media thickness remained, however, highly significant even after controlling (adjustment) for body mass index. This suggests a rather low impact of obesity related mechanisms. Apart from this, also other diseases and risk factors for CVD are associated with adiponectin serum levels and might therefore be potential sources for confounding. To account for this, the analyses of STYJOBS were additionally adjusted for a panel of possible confounders. In this context, it should, however, be noted that beyond obesity, the juveniles of STYJOBS cohort did not apparently suffer from various significant cardiovascular relevant diseases or risk factors. Hence, significant confounding is relatively unlikely in that study.

By contrast, in LURIC, the study population of patients with an indication for coronary angiography has a high prevalence of diseases or conditions which are associated with adiponectin levels and contribute to CVD (e.g. reduced adiponectin levels in patients with type 2 diabetes mellitus, dyslipidemia or inflammation). Hence, the possibility of confounding is expected to be much higher than in STYJOBS. To account for this, the statistical analyses of the LURIC Study were also adjusted for a panel of possible confounders. Again, for various of these possible confounders it is unclear whether they are true confounders or whether they lie in the causal pathway. It is therefore conceivable that the statistical analyses were over-adjusted by parameters of the causal pathway. This might have contributed to an underestimation of the true association of adiponectin and CVD. Apart from this, it should also be noted that despite these careful adjustments residual confounding by unmeasured or unconsidered parameters may still exist.

Selection of specific patients might have also occurred in the LURIC Study because those individuals who agreed to participate in that study might have a different attitude and adherence to health-related issues compared to those who refused to participate. It is, however, unlikely that this selection might have contributed to significant bias of our results because there is no convincing evidence that selected groups of patients referred to coronary angiography show differential associations of adiponectin with CVD.

The study cohort of the Hoorn Study resembles the general older population and the risk of selection is relatively low because the study participants were randomly selected from a population register. Considering that the response rate of the eligible subjects was 71.5%, it might be conceivable that some kind of selection might have also occurred in that study. Significant chronic diseases are less prevalent in the Hoorn when compared to the LURIC study. Therefore, the chance that the results of the Hoorn study are confounded by underlying diseases seems to be lower compared to the LURIC Study.

**Assessment of CVD**

Assessment of CVD by ultrasonography (carotid intima-media thickness and myocardial function), coronary angiography (coronary artery disease) and classification of cardiovascular events might have been imprecise. Even under highly standardised conditions as in the above described studies measurement errors and misclassifications can hardly be completely ruled out. It is, however, unlikely that imprecise mea-
Measurements have contributed to bias because the amounts of possible misclassifications are unlikely to be related to the prevailing adiponectin status.

Measurement of carotid intima-media thickness, as it was performed by a single physician in STYJOBS, is observer dependent. Of note is that ultrasonography of obese patients is more difficult than of lean individuals. Imprecision of ultrasonographic measurements might have increased with higher body mass index. This is, however, unlikely to cause a systematic bias by over- or underestimation of carotid intima media thickness. Therefore, the effect on the association of adiponectin and carotid intima-media thickness is most likely towards the null, which strengthens the results of any significant association. Of note, the fact that ultrasonographic measurements were performed by a single physician is a strength of that study because this precludes problems related to inter-observer variability.

In the LURIC study assessment of coronary artery disease was well standardised and every classified coronary angiogram was further evaluated and reviewed by a senior cardiologist in order to minimize the rate of misclassifications. Incident CVD and mortality, as assessed in the LURIC and Hoorn study, are hard and well defined endpoints with only a very low probability of misclassification or bias.

**Laboratory methods**

Laboratory methods should also be critically discussed and it should be noted that in all studies adiponectin was measured from stored samples. In the STYJOBS and the LURIC Study the same immunoassays for adiponectin were used and all measurements were performed at the same laboratory. Mean adiponectin serum concentrations were comparable in both cohorts. This argues against a significant effect on adiponectin measurements by long-term sample storage, as in the LURIC study. In the Hoorn Study, adiponectin was measured by a different immunoassay and in another laboratory. Mean adiponectin levels in the Hoorn Study were higher compared to LURIC or STYJOBS but there was still a remarkable overlap in the range of adiponectin concentrations. Known associations of low adiponectin with e.g. measures of obesity, dyslipidemia (e.g. low HDL-cholesterol), inflammation or type 2 diabetes mellitus were observed in all three cohorts. This strongly argues that the laboratory measurements produced reliable results in all studies.

In all three cohorts only total adiponectin levels were available which is a limitation due to lack of information on the distribution of adiponectin subfractions, which were previously described in the introduction of this thesis. High molecular weight (HMW) forms of adiponectin are of particular interest because they were shown to be biologically highly active [2-5]. It was previously observed that compared to total adiponectin, the concentrations of HMW adiponectin showed more significant correlations with cardiovascular risk factors [6]. There is also evidence that HMW adiponectin or the ratio of HMW to total adiponectin might be better markers for coronary artery disease than total adiponectin alone [7, 8]. In addition, globular adiponectin was shown to exert a variety of effects on the cardiovascular system [9-11]. Interestingly, globular and full-length adiponectin exert partially distinct effects [10, 11]. Hence, missing data on adiponectin subfractions are a limitation of this thesis and future studies on adiponectin and CVD should aim to measure them.
Summary of general methodological limitations and strengths

Inherent to all observational data is that conclusions regarding causality cannot be made uncritically. In detail, it is unclear whether changes in adiponectin levels are the cause or the consequence of CVD. To minimise the chance that the results are significantly driven by confounding factors which are associated with both adiponectin as well as CVD we adjusted our statistical analyses for possible confounders including common cardiovascular risk factors. On the other hand, adiponectin is suggested to exert causal effects on cardiovascular risk factors such as impaired glucose metabolism, dyslipidemia or inflammation. Such "adiponectin modulated" cardiovascular risk factors might lie in the causal pathway and mediate detrimental consequences of hypoadiponectinemia. Given that the effect of adiponectin on various cardiovascular risk factors still needs to be clarified in detail and considering that we wanted to evaluate whether adiponectin status is an independent predictor of CVD we adjusted our analyses for virtually all classic cardiovascular risk factors. Hence, an over-adjustment for effect mediating factors of the causal pathway might have occurred. The inclusion of various cardiovascular risk factors as covariates into our statistical analyses did, however, not substantially alter our results.

The prospective analyses of this thesis are a main strength because prospective cohort studies (LURIC and Hoorn Study) are in general superior compared to cross-sectional or case control studies. Measuring biomarkers at baseline and recording CVD events during a follow-up period (prospective follow-up studies) is a better approach to evaluate whether a biomarker is predictive for a disease than selecting patients already suffering from CVD and comparing them with matched controls (case control studies). In this context, it should be acknowledged that a main limitation of many case control and cross-sectional studies is that the temporal criterion for cause and consequence cannot be discerned.

It is also a main strength of this thesis that three different study cohorts were investigated because this enabled us to address the association of adiponectin and CVD in patients with low (STYJOBS), intermediate (Hoorn Study) and high cardiovascular risk (LURIC Study). It was thus possible to replicate certain results and to evaluate whether the observed associations are modified by the underlying cardiovascular risk pattern.

Mechanistic Discussion and possible clinical relevance

In obese juveniles, the inverse association of adiponectin with carotid intima-media thickness is in line with the hypothesis that low adiponectin levels may contribute to the pathogenesis of atherosclerosis. This notion is supported by experimental studies, which showed that adiponectin exerts various anti-atherosclerotic properties related to pro-atherosclerotic changes of endothelial cells and vascular smooth muscle cells [2-5]. It should also be mentioned that the association of hypoadiponectinemia with increased carotid intima-media thickness has been subsequently confirmed in a similar group of obese children and adolescents as well as in middle-aged men and women [12, 13].
In the LURIC Study reduced adiponectin levels in patients with coronary artery disease were not further influenced by the progression of this disease. Hence, it could be concluded that low adiponectin serum levels are closely related to atherosclerosis, in particular at early stages. Unexpectedly, high adiponectin levels were independent of other cardiovascular risk factors associated with an increased risk of cardiovascular and all-cause mortality in the LURIC Study. On a first sight this finding appeared to contrast the general opinion that high adiponectin levels are protective against CVD when considering e.g. the anti-atherosclerotic and anti-diabetic properties of adiponectin. These prospective results of the LURIC Study do, however, complete the picture on the adiponectin CVD link with an inverse association of adiponectin with atherosclerosis in early stages of CVD that is mitigated in more advanced stages and is finally reversed in end-stages of CVD. In line with this, subgroup analyses of patients with and without coronary artery disease showed that the association of adiponectin and mortality was only significant in those with coronary artery disease and was particularly pronounced in patients with advanced heart failure.

The findings of the Hoorn Study fit well to this above mentioned hypothesis in particular because the association of adiponectin with mortality risk was modified by the presence or absence of prevalent CVD. Towards this it could be speculated that in very advanced stages of CVD the association of high adiponectin levels with increased cardiovascular risk is mainly driven by the fact that CVD itself causes an increase of adiponectin levels which might be regarded as a defence mechanism against cardiovascular damage. This hypothesis is in line with the concept of a wasting process that usually accompanies end-stage CVD patients and that is associated with weight loss, which is known to increase adiponectin levels [14]. Furthermore, it has been shown that some mediators of cardiovascular damage such as inflammation or oxidative stress may increase adiponectin, which in turn exerts anti-inflammatory and anti-oxidative properties [2-4, 15, 16]. In this context, it has been shown that adiponectin is elevated in heart failure patients by increased secretion from cardiomyocytes [17, 18]. Hence, the findings of this thesis on adiponectin are well supported by pathophysiological and clinical data from other groups and have been confirmed by subsequent studies [19-22]. Whether the increase of adiponectin in advanced stages of CVD is just a marker of a poor clinical outcome or whether high adiponectin levels may even be deleterious in this setting deserves further studies. This thesis provides, however, a reasonable hypothetic model for the observed associations of adiponectin with CVD (see figure 3).

Another point to be discussed is the complex regulation of adiponectin receptor expression, which has been partially described in the introduction of this thesis (chapter 1). Numerous previous studies have shown that cardiovascular risk factors or related diseases are associated with differential expression of adiponectin receptors. Hence, even at the same adiponectin level, the effect of adiponectin, as mediated by the adiponectin receptors, might be different. It is therefore conceivable, that there are conditions of either high adiponectin sensitivity (=high expression of adiponectin receptors) or adiponectin resistance (=downregulation of adiponectin receptors). The concept of adiponectin resistance in CVD is supported by a study among patients with chronic heart failure [23]. In these patients adiponectin receptors were significantly downregulated suggesting the development of adiponectin resistance in
chronic heart failure [23]. The results of the thesis are limited because there were no data available on adiponectin receptor expression. Data on adiponectin receptor expressions should, however, be considered in future studies to evaluate whether associations of adiponectin with CVD or its risk factors are modified by the quantitative expression of the different adiponectin receptors.

Figure 3: Hypothetic model for the associations of adiponectin with CVD

Conclusions

In conclusion, the studies on adiponectin and CVD have shown that the association of adiponectin with cardiovascular risk is significantly modified by the course of CVD with an inverse association in early stages and a positive association of adiponectin and cardiovascular risk in advanced stages. This knowledge on the role of adiponectin has significantly contributed to our current picture on the role of adiponectin serum levels in CVD which deviates from the initial view that high adiponectin serum levels are only indicative of low cardiovascular risk. Moreover, the results on this complex association of adiponectin and CVD is an important rationale for further studies to evaluate whether the increasing adiponectin levels in advanced stages of CVD can still be considered as a beneficial defence mechanism against cardiovascular damage or should be rather regarded as an overwhelming upregulation of adiponectin which might even be harmful. Addressing this question will also be important for future conclusions on the proposed therapeutic interventions aimed to increase the levels or effects of adiponectin.

9.2. Vitamin D

Accumulating evidence suggests that vitamin D deficiency might contribute to CVD. The high prevalence of vitamin D deficiency and the apparently relatively easy way by which vitamin D deficiency can be treated or prevented underline the importance to evaluate whether low 25-hydroxyvitamin D (25(OH)D) levels are associated with increased cardiovascular risk [24-26].
Summary of the results on vitamin D

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 (chapter 5). Carotid intima-media thickness was, however, not significantly associated with 25(OH)D levels in this Dutch study (chapter 6). In the LURIC Study, it turned out that high 25(OH)D levels are associated with reduced risk of fatal stroke (chapter 7). In the same study it was also shown that prevalent and incident heart failure patients showed significantly reduced levels of 25(OH)D (chapter 8). In prospective analyses of the LURIC study, patients with low 25(OH)D levels were also at significantly increased risk of sudden cardiac death but there was no significant association of vitamin D status and fatal myocardial infarction (chapter 8). Echocardiographic data (ultrasound of the heart) from the Hoorn Study showed that systolic function, which indicates the function of the heart during the pumping phase, was not significantly associated with vitamin D status (chapter 8). Diastolic function, which indicates the relaxation phase to ensure a sufficient blood inflow into the heart chambers, was reduced in patients with low 25(OH)D levels. This association was, however, attenuated towards a non-significant trend after adjustments for age and other cardiovascular risk factors.

Discussion of the methods

Since observational data from the Hoorn and the LURIC Study were also used to study associations of vitamin D status and CVD, the methods and their limitations are in general comparable to the above mentioned issues discussed in the section on adiponectin. There are, however, some differences and specific aspects of the vitamin D part of this thesis that need to be discussed.

Assessment of CVD

In addition to the assessment of CVD described in the adiponectin part there was also an ultrasonographic assessment of carotid-intima media thickness as well as myocardial structure and function in the Hoorn Study. These measurements were performed according to standardised protocols.

Determination of carotid intima-media thickness was performed by a single observer. Reproducibility of carotid intima-media thickness assessment was performed in 10 individuals and showed a coefficient of variation of only 10.9% (chapter 6).

Echocardiographic measurements of myocardial structure and function were performed by a single experienced research technician (chapter 8). All echocardiographic recordings and readings were further evaluated by a senior cardiologist to ensure the quality of this examination [27], and expected associations were observed.

Assessment of vitamin D Status

Measurement of 25(OH)D levels is an accepted approach to assess vitamin D status. Laboratory methods were a radioimmunoassay in the LURIC Study and a competi-
tive binding protein assay in the Hoorn Study, both provided by the same company (Diasorin). These assays are widely used and in LURIC the 25(OH)D levels measured by radioimmunoassay correlated well with a liquid chromatography tandem mass spectrometry method, the gold standard method for measurements of 25(OH)D concentrations. Validity of the 25(OH)D laboratory methods is also supported by the fact that established associations of 25(OH)D with e.g. age or parathyroid hormone (PTH) could be well replicated in the LURIC and the Hoorn Study.

Beyond laboratory methods, seasonal variation of 25(OH)D levels is also an important issue to consider. In Central Europe the seasonal differences in UV-B exposure lead to significant variations in 25(OH)D levels. In the LURIC Study 25(OH)D levels collected at the end of summer were almost twice as high as those samples from the end of winter (see chapter 8). To account for this, vitamin D status classification was based on the percentile distribution (or deviation from the median concentration) of 25(OH)D values within the respective month or season in which the blood sample was drawn. This 25(OH)D classification system according to month or season is similar to the height and weight percentile classification system according to age in children. A limitation of this 25(OH)D classification is the assumption that (i) the study participant groups within the different seasons or months of blood sampling are comparable and that (ii) the seasonal variation in 25(OH)D levels is similar for each individual.

**Summary of general methodological limitations and strengths**

In general, testing for associations between 25(OH)D levels and CVD is a difficult task due to frailty related limited sunlight exposure. This is in particular a problem for the LURIC cohort because those patients with limited sunlight exposure and subsequently reduced 25(OH)D levels were likewise those with a high mortality risk due to diseases which limit mobility as well as sunlight access. Hence, it is difficult to differentiate whether the association between 25(OH)D levels and CVD reflects a "true" pathophysiologic relationship or whether it is confounded by the fact that patients suffering from CVD or risk conditions for CVD have reduced sunlight exposure due to their physical impairments or their risk factor related social behaviour. In general, lack of physical activity may likewise contribute to a poor vitamin D status. Furthermore, vitamin D deficiency may be associated with other nutritional characteristics as well and frailty in the elderly is often associated with a poor overall nutritional status that may, beyond vitamin D deficiency, involve also other (micro-) nutrients. Given that 25(OH)D levels are a surrogate for sunlight exposure (and lack of physical activity), it is also conceivable that the association of vitamin D status with CVD might partly be driven by cardiovascular relevant mechanisms associated with sunlight exposure, beyond the induction of vitamin D synthesis in the skin. Furthermore, a problem with interpreting vitamin D status is that the same levels of 25(OH)D can be achieved by different ways (sunlight induced vitamin D synthesis in the skin, nutrition and/or supplements) and it is difficult to adequately consider the effects of these different vitamin D sources on the association of vitamin D deficiency and CVD. Even with careful adjustments of the analyses of this thesis by parameters related to physical performance or sunlight exposure, it is difficult to adequately ad-
address these above mentioned issues. The situation is even more complex when considering the impact of nutrition on vitamin D status (e.g. vitamin D intake by nutrition is frequently associated with other nutrients such as vitamin A in cod liver oil) and the increasing knowledge that vitamin D deficiency itself may contribute to musculoskeletal diseases resulting in reduced mobility.

Cardiovascular risk factors such as e.g. renal failure may also have a reciprocal effect on vitamin D status. On the one hand vitamin D deficiency may contribute to renal damage but on the other hand chronic kidney disease is associated with impaired vitamin D synthesis in the skin and urinary loss of vitamin D metabolites [28, 29]. A similar relationship may exist for vitamin D and heart failure: low 25(OH)D concentrations may hypothetically contribute to myocardial dysfunction but heart failure associated limitations in mobility may reduce outdoor activities and sunlight exposure. Therefore, we can conclude that the results of this thesis support the notion that low 25(OH)D levels indicate an increased cardiovascular risk but even after multivariable adjustments we cannot be sure on the direction of causality (see figure 4).

Figure 4: Reciprocal influences of vitamin D deficiency, CVD and cardiovascular risk factors

Mechanistic Discussion and possible clinical relevance

This thesis provides evidence that vitamin D deficiency is associated with increased risk of CVD and suggests the presence of associations with myocardial diseases and strokes (chapters 5 to 8). These results have been largely confirmed by others and are in line with previous observations that UV-B radiation, a surrogate for vitamin D status, is inversely associated with CVD and stroke risk [30-32]. In line with this, a study among 41,504 patients derived from a general healthcare population confirmed a particularly strong association of vitamin D deficiency with cerebrovascular diseases and heart failure [33]. Evidence for low 25(OH)D levels in patients with cerebrovascular diseases is of particular concern because frequent musculoskeletal complications of poststroke patients can be partially prevented by vitamin D supplementation which significantly reduces falls and fractures and might also exert
beneficial effects on cognitive function [34-37]. On the other hand, limited mobility with reduced sunlight exposure and malnutrition are frequently observed in post-stroke patients and may contribute to vitamin D deficiency.

Underlying mechanisms for the relationship of vitamin D deficiency and CVD remain partially speculative. It should be pointed out that there was no significant association of vitamin D status with carotid intima media thickness or fatal myocardial infarction (chapters 6 and 8). This may suggest that the association of vitamin D deficiency with cardiovascular risk might not be mainly mediated by atherosclerotic vessel diseases. In this context, there exists evidence that vitamin D metabolites itself might play a direct role for myocardial function (chapter 8). This may also be relevant for stroke risk because thrombotic emboli of cardiac origin due to heart arrhythmias are a frequent cause of strokes. Concerning vitamin D effects on the heart it should be noted that heart failure in children with rickets could be cured with vitamin D and calcium supplementation [38, 39]. Among adults there exist some studies suggesting that vitamin D treatment may protect the heart, but currently available data are still insufficient and results are partially conflicting [40]. It should also be acknowledged that most studies reporting effects of vitamin D treatment on myocardial structure or function were performed among patients with chronic kidney disease, which are particularly prone to disturbances in vitamin D metabolism [40]. There exists, however, a good rationale to study vitamin D effects on the heart because it is a target tissue for vitamin D with an active vitamin D metabolism. Beyond heart failure, knockout mice for the vitamin D receptor (VDR) and for 1-alpha-hydroxylase suffer from arterial hypertension, which is a significant risk factor to develop myocardial diseases [41, 42]. Meta-analyses of randomized controlled trials have been published, which indicate that vitamin D therapy may lower systolic blood pressure by 2-6 mm Hg [43]. Even reductions of 2 mm Hg in systolic blood pressure may be of clinical relevance because epidemiologic data suggest that lowering systolic blood pressure by 2 mm Hg translates into a reduction of total mortality of 3% [44]. Potential anti-hypertensive as well as other suggested cardioprotective effects of vitamin D have been extensively reviewed and discussed in chapter 7 and 8. In brief, these include parathyroid hormone (PTH) suppression and effects on calcium homeostasis, vasculoprotective and anti-diabetic properties, anti-inflammatory effects as well as prevention of infectious diseases. In addition, vitamin D may also exert renoprotective effects and there exists evidence that VDR activation may reduce albuminuria [45]. These above mentioned data fit well to the observations of this thesis that low 25(OH)D levels are associated with increased risk of heart failure and sudden cardiac death.

The findings, reported in the present thesis, are an additional rationale for further studies to evaluate the precise underlying mechanisms. Importantly, it should be clarified whether vitamin D treatment is useful for the prevention and treatment of CVD or whether vitamin D deficiency is merely a marker of a poor health status with e.g. reduced sunlight exposure. This latter research question is of great importance for public health when considering the high prevalence of vitamin D deficiency and the relatively easy, safe and cheap way by which it can be treated or prevented. It also remains a question which dose of vitamin D should be administered for primary
prevention or treatment of vitamin D deficiency. It should be noted that the human skin can produce vitamin D amounts equivalent to an oral intake of up to 10,000-20,000 international units (IU) of vitamin D per day [46]. This is a large amount of vitamin D when considering that according to a rule of thumb a daily intake of 1,000 IU vitamin D increases 25(OH)D levels by ~ 10 ng/ml [47]. Given that there is no known case of sunlight induced vitamin D intoxication, which is characterised by hypercalcemia, it seems to be relatively easy and safe to correct vitamin D deficiency [48]. However, we should also consider that sunlight induced synthesis of vitamin D in the skin may be regulated by partially unknown mechanisms, which may be bypassed by oral vitamin D supplementation. So far, however, long-term outcome data on i.e. CVD events or CVD mortality for vitamin D doses of over 400-800 IU per day are missing. Furthermore, although acute vitamin D toxicity with hypercalcemia does usually not occur at 25(OH)D levels below 150 ng/mL, some data suggest that even much lower 25(OH)D levels (i.e. >50 ng/mL) might be harmful for long-term outcomes [49-52]. Some RCTs showed increased risk of hip fractures and falls with high-dose vitamin D supplementation and some observational studies showed a U-shaped association of 25(OH)D and mortality with increasing mortality risk at both low and high 25(OH)D levels [49-52]. It should therefore be underlined that we still need more data on long-term outcomes of individuals with high 25(OH)D levels.

9.3 General conclusion

In conclusion, the results of this thesis have contributed to improve our knowledge on the role of adiponectin and vitamin D status as risk factors for CVD. This better understanding on the role of these two hormone systems in CVD is an important rationale for future studies to investigate the underlying mechanisms and to address the causality of the observed associations.

In detail, the data of this thesis suggest that the association of adiponectin with cardiovascular risk is modified by the stage of CVD. An inverse association of adiponectin and cardiovascular risk in early stages is attenuated during the course of CVD and is finally reversed in advanced stages of this disease. This relationship is crucial for our current view of the role of adiponectin serum levels in cardiovascular risk assessment. Since adiponectin is generally considered to protect against CVD it will be important to elucidate whether high adiponectin levels in patients with significant CVD (i) represent 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 effects on the cardiovascular system.

It was also shown in this thesis that vitamin D deficiency is an independent risk factor for CVD, in particular for heart diseases and strokes. Considering the relatively safe and cheap way by which vitamin D deficiency can be prevented or treated there exists now an urgent need for large-scale randomized controlled trials to evaluate whether these associations are of causal nature. Whether vitamin D treatment should be best done by food fortification, general recommendations to supplement vitamin D or increased sunlight (or UV-B) exposure remains a challenge for the
future [53-55]. Nevertheless, it is premature to raise general recommendations for vitamin D therapy for the prevention and treatment of CVD unless large-scale randomized controlled trials have been conducted [56-58]. Therefore, although currently available data on vitamin D and CVD are largely in favour of the notion that a sufficient vitamin D status may protect against CVD, we should be cautious with recommendations for vitamin D therapy for the prevention and treatment of CVD until we have sufficient data on the possible beneficial and/or deleterious long-term effects of vitamin D on CVD.

In conclusion, by using data from observational studies, we have contributed to the understanding of the role of adiponectin and vitamin D in human health and cardiovascular disease. Further studies will address the possible consequences for use in clinical practice.

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