Chapter 1

General introduction

Cardiovascular diseases (CVD) are one of the most challenging global health problems and account for approximately every third death in western as well as developing countries [1-3]. Identifying novel risk factors for CVD is important to improve the risk prediction for CVD which guides the intensity of patient treatment [3]. Discovery of new risk factors for CVD is also crucial to identify and assess potential targets for new therapeutic interventions [3]. In this context, it has been increasingly recognized that endocrine disorders are critically involved into the pathogenesis of CVD [4-6]. This emerging new field of research has been termed cardiovascular endocrinology [4, 5]. Accumulating evidence suggests that the two hormones adiponectin and vitamin D may exert various effects with relevance for the cardiovascular system. The objective of this thesis is to evaluate in part 1 the role of serum levels of adiponectin and in part 2 the role of vitamin D metabolites as risk factors for the prevalence and incidence of CVD. In this general introduction the basic metabolism and pathophysiologic functions of adiponectin and vitamin D metabolites with a main focus on their role in the context of CVD will be described. In addition, the rationale for the specific research questions of this thesis will also be outlined.

1.1. Adiponectin

Research over the last few decades has shown that the adipose tissue is a highly active site of hormone production including the synthesis of adiponectin [7, 8]. This is of particular interest when considering that obese persons are at increased risk of CVD [7, 8]. The function of adipose tissue as an endocrine organ is based on the fact that it secretes hormones into the blood stream which are then transported to their target tissue or target organs where they exert their effects by binding to specific receptors which mediate the hormonal effects. Beyond this endocrine function there is growing evidence that perivascular and epicardial adipose tissue might also exert direct (paracrine) effects on the vessel walls and heart [9-11]. Hence, adipose tissue derived hormones (so called adipokines) might play a role in cardiovascular pathophysiology. Whether disorders of adipokines underlie the well-established associations of obesity as well as adipose tissue distribution with CVD remains to be explored [12]. This is an important research question when considering the current efforts of public health authorities to reduce the burden of obesity in order to improve public health. In this context, adiponectin is a promising adipokine to further clarify the relationship between adipose tissue and CVD [13].

Adiponectin structure and adiponectin receptors

Adiponectin has been simultaneously discovered by different groups in the mid 90s as the most abundant gene transcript in adipose tissue and was initially termed apM1,
Acrp30, AdipoQ, or GBP28 [14-17]. Adiponectin consists of 244 amino acids and can be subdivided into a signal-peptide, a collagen-like domain and a globular domain [18]. Adiponectin peptides undergo intensive pros-translational modifications and form low molecular weight (LMW) trimers through associations between their globular domains [13]. These trimers are stabilized by a triple helix formation of the collagen-like domains [13]. Two adiponectin trimers can form middle molecular weight (MMW) hexamers via disulphide bonds [13]. Further high molecular weight (HMW) forms are the result of interactions between adiponectin hexamers and are considered the biologically most relevant forms of adiponectin [13]. In addition to this LMW, MMW and HMW forms, adiponectin circulates in plasma also to a small amount as a globular form which is produced by proteolytic cleavage of the globular domain from the full-length adiponectin peptide [18] (see figure 1 for adiponectin structure and isoforms). In humans, the overall adiponectin concentration in plasma usually ranges between 3-30 mg/L, accounting for 0.01% of all plasma proteins [18]. This means that adiponectin is much higher concentrated than most other known hormones. Although adiponectin is mainly released by the adipose tissue it has been shown that obese subjects have significantly lower plasma adiponectin concentrations compared to lean controls [19]. Suppression of adiponectin secretion by high fat mass and by enlarged adipocytes, which are the cells of the adipose tissue, has been subsequently confirmed by several studies [20, 21]. Interestingly, it has been shown that apart from adipocytes various other cells such as cells of the heart (cardiomyocytes), skeletal muscle cells or hepatic endothelial cells can also synthesize and release adiponectin [22-24]. There exist three different membrane receptors for adiponectin: adiponectin receptor 1 (AdipoR1), adiponectin receptor 2 (AdipoR2) and T-cadherin. These receptors have been identified in many different tissues including the heart and the vessel walls [25-27]. Highest expressions are found for AdipoR1 in skeletal muscle, for AdipoQ2 in the liver and for T-cadherin in injured vascular endothelial and smooth muscle cells [25-27].

**Adiponectin and cardiovascular risk factors**

Experimental and clinical data suggest that adiponectin reduces various cardiovascular risk factors, in particular the components of the metabolic syndrome which consists of impaired glucose metabolism, dyslipidemia, raised blood pressure and central obesity [13, 20, 21, 28]. Metabolic effects of adiponectin, which are mediated by AdipoR1 induced AMPK activation, include stimulation of fatty acid oxidation, glucose uptake in skeletal muscle and adipose tissue as well as suppression of hepatic glucose production [13, 20, 21, 25, 27]. This in turn reduces insulin resistance and improves insulin sensitivity. Towards this, a meta-analysis of prospective observational studies has already shown that high adiponectin levels are associated with a significantly reduced risk of developing type 2 diabetes mellitus [29]. Among lipids adiponectin is particularly associated with a favourable lipid status composed of high levels of high-density lipoproteins (HDL) and low triglycerides [13, 20, 21, 30, 31].
Adiponectin has also been shown to directly modulate key processes in the pathogenesis of vascular and myocardial diseases [13, 32]. Adiponectin may protect against CVD by anti-inflammatory effects, anti-oxidative properties, improvement of endothelial function by increasing the production of the endothelial derived relaxation factor nitric oxide (NO) or by anti-atherosclerotic actions such as suppression of endothelial adhesion molecules, inhibition of macrophage to foam cell formation and beneficial effects on vascular remodelling [32]. Adiponectin has also been shown to protect the heart against detrimental influences and to improve myocardial function [33-37]. Apart from this, genetic variants of adiponectin (single nucleotide polymorphisms (SNP) of the adiponectin gene on chromosome 3q27) have been linked to obesity, type 2 diabetes mellitus, the metabolic syndrome and CVD [38].

Regulation of adiponectin secretion

Concerning the role of adiponectin in CVD it is also important to consider that the regulation of adiponectin secretion is modulated by various cardiovascular relevant parameters [39, 40]. Obesity itself decreases and weight loss increases adiponectin secretion [18-21]. Insulin has been shown to suppress adiponectin secretion whereas there exist inconsistent data on the influence of oxidative stress or inflammation [41-46]. The regulation of adiponectin secretion is, however, even more complex since drugs for the treatment of type 2 diabetes mellitus or dyslipidemia and certain parameters indicating cardiovascular risk or cardiovascular damage such as endothelin-1 or lipid infiltration enhanced adiponectin expression [46-49].
Outline of the thesis regarding adiponectin

Taken together we can conclude that adiponectin exerts various effects which may protect against CVD. Whether this translates into an association of high adiponectin plasma levels with reduced risk of CVD in humans remains to be elucidated and is a main objective of this thesis. Hence, a main aim of this thesis was to elucidate whether adiponectin is associated with the prevalence and incidence of CVD. This issue was addressed by examining a cross-sectional cohort of obese juveniles derived from the Styrian Juvenile Obesity Study (STYJOBS) as well as two prospective cohorts: a cohort of patients routinely referred for coronary angiography (Ludwigshafen RIsk and Cardiovascular Health LURIC Study) and an older population based cohort (Hoorn Study).

Specific aims were:

1. To elucidate whether adiponectin serum levels are associated with carotid-intima media thickness, a measure of atherosclerosis and an indicator of high cardiovascular risk (STYJOBS)
2. To elucidate associations of adiponectin serum levels with different stages of coronary artery disease (LURIC Study)
3. To elucidate whether adiponectin serum levels are associated with risk of all-cause and cardiovascular mortality (LURIC Study)
4. To elucidate whether adiponectin serum levels predict mortality and cardiovascular events (Hoorn Study)

1.2. Vitamin D

Recent advances in vitamin D research have produced a growing public health interest. This can mainly be attributed to the fact that only the minority of the general population has a sufficient vitamin D status and that vitamin D deficiency has been linked to increased morbidity of several diseases as well as to mortality [50-52].

Structure, sources and historical perspective of vitamin D

Vitamin D is a secosteroid and although initially named as a vitamin its physiologic role justifies the classification of vitamin D metabolites as hormones [53, 54]. The main source for vitamin D (~80 to 90%) is sunlight induced vitamin D synthesis in the skin. In detail, ultraviolet-B (UV-B) induces the conversion of the precursor 7-dehydrocholesterol to previtamin D$_3$ followed by thermal isomerization to vitamin D$_3$ (cholecalciferol). Vitamin D$_3$ or vitamin D$_2$ (ergocalciferol) is also contained in some natural foods (e.g. eggs, fish, cod liver oil or mushrooms) but only in relatively small amounts. In addition, various countries such as the US or Finland have already introduced food fortification with vitamin D. Historically vitamin D (alphabetically named "D" as the fourth known vitamin) was discovered by McCollum et al in 1922 as the substance which could treat rickets, a bone disease in children characterized by deformities of the skeleton [55-57]. Even before the identification of vitamin D by
McCollum et al it has already been known that sunlight was effective in preventing rickets [55]. Subsequent research in this field has shown that the UV-B induced vitamin D production in the skin is significantly decreased in the elderly as well as by use of sunscreen and high skin pigmentation in blacks [55]. Interestingly, it has been observed that UV-B radiation is inversely associated with chronic diseases such as cancer, hypertension or CVD suggesting a role of vitamin D in non-skeletal diseases [58-60]. Given the negative effect of skin pigmentation on vitamin D synthesis it should be mentioned that during evolution our dark skinned ancestors developed a fair skin when they migrated from Africa to northern countries with less sunlight exposure [61]. Hence, it has been hypothesized that lighter skin colour evolved to ensure sufficient vitamin D production under conditions of less UV-B radiation in order to avoid various chronic diseases and premature deaths [62,63]. Our current lifestyle and environment (e.g. air pollution) is, however, frequently associated with very little sunlight exposure of the skin resulting in a worldwide high prevalence of vitamin D deficiency [50-52].

Vitamin D metabolism and vitamin D receptors

Vitamin D, synthesized in the skin or ingested by food, is considered biologically relatively inactive and is therefore hydroxylated to more active metabolites. As a first step vitamin D is hydroxylated to 25-hydroxyvitamin D (25[OH]D), a process that takes mainly place in the liver but can also occur in other tissues such as the skin, kidney or the male reproductive tract [50, 53, 54, 64-66]. 25(OH)D is the major circulating vitamin D metabolite and is used to classify the vitamin D status [67]. According to a common classification, vitamin D sufficiency is defined as 25(OH)D levels ≥ 30 ng/ml, vitamin D insufficiency as 25(OH)D levels between 21 and 29 ng/ml and vitamin D deficiency as 25(OH)D levels ≤ 20 ng/ml (to convert ng/ml to nmol/l multiply by 2.496). The definition of vitamin D sufficiency was initially based on the observation that 25(OH)D levels below 30 ng/ml are associated with disturbed calcium homeostasis characterized by reduced intestinal calcium absorption and increases of parathyroid hormone (PTH), which is released in response to hypocalcemia and aims to increase serum calcium levels. There is, however, an ongoing debate on the classification of vitamin D status. In this context, it should be underlined that after extensive review of the literature, the Institute of Medicine (IOM) in the US and the Health Council of the Netherlands concluded that a 25(OH)D level of 20 ng/mL is sufficient for the general and older population [68, 69].

Although 25(OH)D is used to assess the vitamin D status, the affinity of 25(OH)D to the almost ubiquitously expressed vitamin D receptor (VDR) is relatively low [53, 54]. Further hydroxylation of 25(OH)D results in the formation of 1,25-dihydroxyvitamin D (1,25[OH]2D), which is considered the most active vitamin D metabolite (for basic vitamin D metabolism see figure 2). Circulating 1,25(OH)D levels seem to be mainly dependent on renal conversion of 25(OH)D to 1,25(OH)2D. This renal 1-alpha-hydroxylation of 25(OH)D is tightly regulated by various factors such as PTH or fibroblast-growth factor (FGF-23) to maintain a normal calcium and phosphorus homeostasis. It has, however, also been shown that many extra-renal tissues are capable to produce 1,25(OH)2D on a local level [53, 53, 70, 71]. This latter process is
significantly dependent on circulating 25(OH)D levels and seems to determine local (tissue) levels of 1,25(OH)2D. Finally, 1,25(OH)2D exerts its biological effects by binding to the intracellular located VDR. VDR belongs to the superfamily of nuclear receptors for steroid hormones and acts as a ligand-activated transcription factor. After ligand binding the VDR forms a heterodimer with the retinoid X receptor (RXR) and translocates to the nucleus. There, it binds to so called vitamin D responsive elements (VDRE) on the DNA. This DNA binding process involves the recruitment of further proteins (coregulators). The final result is the transcriptional suppression or upregulation of genes. Overall, the vitamin D endocrine system regulates the gene expression of approximately three percent of the human genome [54]. Apart from this, vitamin D metabolites have also been shown to exert rapid non-genomic effects which need to be further characterized in detail [53, 54].

**Figure 2: Basic vitamin D metabolism**

**Classical skeletal vitamin D effects**

Regulation of calcium homeostasis is a main function of vitamin D metabolites and is important for the mineralization of the skeleton as well as for neuromuscular functions [72]. Vitamin D metabolites play a crucial role in intestinal and renal calcium absorption and can mobilize calcium from the bones. In states of vitamin D deficiency the mineralization of the skeleton can be impaired leading to rickets in children and osteomalacia in adults. In particular older individuals are prone to vitamin D deficiency and its adverse effects on musculo-skeletal health [73]. In this context, it has been shown in the Longitudinal Aging Study Amsterdam (LASA), a prospective cohort study in individuals aged 65 years and older, that low 25(OH)D levels are associated with frailty, fractures and poor bone health [74-76]. Meta-analyses of randomized controlled trials have shown that vitamin D supplementation reduces fractures and falls [77, 78]. Therefore, vitamin D supplementation is an integral part of the treatment of osteoporosis which is characterized by reduced bone mineral density with increased risk of fractures [79].
Non-classical and cardiovascular relevant vitamin D effects

Beyond musculoskeletal diseases vitamin D metabolites have been shown to play a role in the pathogenesis of various diseases including cancer, infections, autoimmune or neurological disorders [80-87]. Data from clinical as well as interventional studies suggest that vitamin D deficiency might be critically involved into the pathogenesis of these latter diseases [80-87].

Concerning the cardiovascular system it has been shown that the VDR as well as key enzymes for vitamin D metabolism have been identified in the heart and in the cells of the vessel walls [54, 88-92]. Knock-out mice for the VDR or for 1-alpha-hydroxylase have been shown to suffer from CVD including heart failure and hypertension [54, 93-96]. This is in line with observations in humans showing that children with rickets suffer from heart failure but can be successfully treated with vitamin D plus calcium supplementation [97-99]. Apart from this, vitamin D metabolites exert various effects on the vessel wall which might protect against atherosclerosis. Beside these proposed direct effects on the cardiovascular system vitamin D metabolites may also modulate cardiovascular risk factors. In this context, vitamin D deficiency has been associated with arterial hypertension, diabetes mellitus, obesity, dyslipidemia, chronic kidney disease and inflammation [100-106]. Whether these latter associations are of causal nature with vitamin D deficiency as a contributing factor for a high cardiovascular risk profile is currently the subject of intensive research [100-106].

Outline of the thesis regarding vitamin D

The cardiovascular system is a target tissue for vitamin D effects and a poor vitamin D status has been associated with cardiovascular risk factors and heart failure. Whether low 25(OH)D levels are associated with myocardial as well as vascular diseases and cardiovascular mortality in large epidemiological studies remains to be explored in detail. Investigating associations of 25(OH)D levels and CVD is of great interest when considering the high prevalence of vitamin D deficiency and the easy, safe and inexpensive way by which 25(OH)D levels can be raised with vitamin D supplementation. To elucidate the associations of 25(OH)D levels and CVD, a cohort of patients routinely referred for coronary angiography (Ludwigshafen RIsk and Cardiovascular Health LURIC Study) and an older population based cohort (Hoorn Study) was investigated.

Specific aims were:

1. To elucidate whether low 25(OH)D levels are associated with all-cause and cardiovascular mortality (Hoorn Study)
2. To elucidate whether 25(OH)D levels are associated with carotid intima-media thickness (Hoorn Study)
3. To elucidate whether 25(OH)D levels are associated with risk of fatal stroke (LURIC Study)
4. To elucidate whether 25(OH)D levels are associated with prevalent and incident heart failure and sudden cardiac death (LURIC Study) and with specific measures of myocardial function (Hoorn Study)

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