CHAPTER 7

GENERAL SUMMARY, DISCUSSION
AND FUTURE PERSPECTIVES
General summary

The aim of this thesis was to detail our understanding of the multi-factorial etiology of human diabetic cardiomyopathy. The introduction in Chapter 1 reviews the current knowledge on the structural, functional and metabolic changes in the diabetic heart. In addition, the effects of dietary intervention, as well as several blood glucose lowering agents, and bariatric surgery, on cardiac function in diabetic cardiomyopathy are discussed. Recent studies have proposed that hormones released from adipose tissue, the so-called (adipo)cytokines impact on cardiometabolic function, and alterations thereof in patients with type 2 diabetes mellitus (T2DM). The role of circulating levels of several of these cytokines in cardiometabolic function in male patients with T2DM without cardiovascular disease was evaluated in Chapters 2, 3, and 4. Furthermore, we investigated the role of the fat depot located most closely to the heart, the epicardial adipose tissue (EAT) in more detail. Chapter 5 describes the outcome of the analysis to the relation between EAT, coronary vasomotor function and coronary artery calcium score which was investigated in 46 T2DM patients and 153 non-T2DM patients who were being evaluated for coronary artery disease. Finally, Chapter 6 describes the effects of glucagon-like peptide (GLP)-1 receptor agonist therapy, exenatide, on cardiac function, perfusion, and energetics examined in 26 T2DM patients with left ventricular (LV) systolic dysfunction.

Summary of the major findings

The review in Chapter 1 focused on the aspects of myocardial structure, cardiac function, and metabolism in diabetic cardiomyopathy. LV hypertrophy has been regarded as a common structural change in early diabetic cardiomyopathy. Hypertension, obesity and coronary artery disease contribute to the development of LV hypertrophy in patients with T2DM. These structural changes will lead to diastolic abnormalities in early diabetic cardiomyopathy, with preserved systolic function. Hence, systolic dysfunction in patients with diabetic cardiomyopathy can be regarded as a late event in the pathogenesis. Changes in myocardial metabolism in diabetic cardiomyopathy include a further shift from glucose to predominantly fatty-acid metabolism, with higher myocardial triglyceride content. This could result in reduced cardiac efficiency, as a greater reliance on fatty-acid metabolism is related to increased oxygen consumption. However, no direct relation between these changes in metabolism and cardiac function has been established in human diabetic cardiomyopathy. Although extensive knowledge in diabetes is gained from animal, especially rodent, studies, the direct translation should be done with caution. The major differences between animal models of diabetic cardiomyopathy and human cardiomyopathy are the genetic and environmental heterogeneity among humans as compared to the controlled situation of animal experiments, for example the homogeneity of inbred species or the
extreme metabolic abnormalities the rodent myocardium is exposed to in a relatively short time. Furthermore, another important difference is that rodents hardly develop atherosclerosis, which is probably due to changes in lipid metabolism as compared to humans. Moreover, T2DM patients are frequently treated with drugs other than glucose-lowering agents, including statins or antihypertensive drugs with its effects on dyslipidemia, blood pressure, atherosclerosis, and cardiac remodeling. Therefore, it is expected that the human situation will divert from the animal models over time.

Next, several metabolic interventions were described. Diet studies showed equivocal effects, varying from increased myocardial triglyceride content accompanied by impaired LV diastolic function following 3-days of starvation in healthy lean men to decreased myocardial triglyceride content and improved LV diastolic function after prolonged caloric restriction in obese T2DM patients. LV diastolic function in T2DM patients without complications is improved upon treatment pioglitazone. This thiazolidinedione compound has an insulin-sensitizing action, and has anti-steatotic effects. However, due to increased risk of heart failure as a consequence of thiazolidinedione-induced edema, these agents are less suitable in patients with heart failure. Small-scaled studies with short-term GLP-1 treatment reported beneficial effects on systolic function and performance in heart failure patients. Although positive cardiac effects of dipeptidyl peptidase (DPP)-4 inhibitors have been reported, treatment with a number of DPP-4 inhibitors has been associated with an increased risk of hospitalization for heart failure. Finally, bariatric surgery has been shown to be a promising treatment in T2DM patients with diabetic cardiomyopathy with beneficial effects on LV structure and function associated with a lower risk of cardiovascular morbidity.

A large part of this thesis focused on the role of (adipo)cytokines. In Chapter 2, the associations of osteoprotegerin and adiponectin with arterial function, cardiac function and myocardial glucose metabolism were determined in 78 T2DM patients and 14 healthy controls. Osteoprotegerin has been proposed to be involved in atherosclerosis, whereas adiponectin has been associated with insulin sensitivity. In this study, T2DM patients had lower aortic distensibility, LV volumes, and impaired LV diastolic function and myocardial metabolic rate of glucose uptake (MMRglu; all P < 0.05). Circulating osteoprotegerin levels were increased (1.4 (1.1-1.8) μg/L versus 0.9 (0.9-1.1) μg/L, P = 0.02) and adiponectin levels were decreased (5.1 (3.7-6.7) mg/L versus 5.9 (5.1-6.5) mg/L, P = 0.04) in T2DM patients as compared to controls. In T2DM patients, plasma osteoprotegerin levels correlated inversely with aortic distensibility (r = -0.327), LV endsystolic volume (r = -0.248), E/A peak ratio (r = -0.272) and MMRglu (r = -0.307), and positively with age (r = 0.343), HbA1c (r = 0.249) and LV mass/volume ratio (r = 0.311, all P < 0.05). In pooled analysis, plasma osteoprotegerin correlated negatively with LV enddiastolic volume (r = -0.213, P < 0.05).
osteoprotegerin, adjusted for systolic blood pressure and age, was negatively associated with aortic distensibility ($\beta = -0.268, P < 0.01$), and positively with LV mass/volume ratio ($\beta = 0.216, P = 0.05$). Age and blood pressure significantly affected the association between osteoprotegerin and LV enddiastolic volume in the T2DM patients ($\beta = -0.104, P = 0.32$). In addition, age and HbA1c were contributors of the association between osteoprotegerin and E/A peak ratio ($\beta = -0.071, P = 0.47$). Plasma adiponectin levels were inversely associated with body mass index (BMI, $r = -0.299$) and waist ($r = -0.314$), and positively with HDL-cholesterol ($r = 0.397$) and M-value ($r = 0.485$, all $P < 0.05$) in T2DM patients. Correlations of adiponectin and HbA1c ($r = -0.253$), rate pressure product ($r = -0.364$) and MMRglu ($r = 0.288$, all $P < 0.05$) were only significant when both patients with T2DM and controls were included in the analysis. Adiponectin was not significantly correlated with LV functional parameters. M-value, HbA1c and waist were contributors of the association between adiponectin and MMRglu ($\beta = 0.055, P = 0.66$). These findings suggest that osteoprotegerin could be used as a marker in cardiac dysfunction and adiponectin as a cardioprotective marker regarding myocardial metabolism in early diabetic cardiomyopathy.

In Chapter 3, the relationship between circulating activin A levels and LV dimensions, function, and myocardial metabolism was assessed. Activin A was described to be released in higher amount from EAT from patients with T2DM versus controls, and to be involved in the development of cardiac fibrosis. Circulating activin A levels were comparable (293 (264-360) pg/mL versus 315 (268-388) pg/mL, $P = 0.42$) in 78 T2DM patients and 14 controls. In multivariate analysis, activin A levels were independently inversely associated with MMRglu ($\beta = -0.373, P < 0.01$), and positively with LV mass/volume-ratio in T2DM patients ($\beta = 0.276, P = 0.02$). Twenty-four weeks of intervention with metformin decreased activin A levels (from 293 (257-374) pg/mL to 261 (231-322) pg/mL, $P < 0.01$), whereas pioglitazone treatment did not alter activin A levels (from 293 (270-361) pg/mL to 302 (278-385) pg/mL, $P = 0.13$). The changes in plasma activin A levels were not correlated with the changes in MMRglu following either pioglitazone ($r = -0.122, P = 0.55$) or metformin treatment ($r = 0.067, P = 0.74$). A borderline significant correlation of changes in plasma activin A levels and changes in LV mass/volume-ratio was observed after pioglitazone treatment ($r = 0.338, P = 0.05$). Activin A levels were not associated with any determinant of LV diastolic function. Based on these findings, we conclude that activin A could play a potential role in the impaired myocardial glucose metabolism and cardiac remodeling in diabetic cardiomyopathy.

Omentin is a potentially protective adipocytokine, like adiponectin. Accordingly, circulating levels of omentin-1 are lower in patients with T2DM versus controls. In Chapter 4, omentin-1 levels were determined in 78 T2DM patients and 14 healthy controls, as well as in cardiac fat depots from 14 T2DM and 11 non-T2DM patients undergoing open heart surgery. Omentin-1
was highly expressed and secreted by EAT as compared to subcutaneous adipose tissue (P < 0.01), and was reduced in T2DM patients compared to non-T2DM patients (P < 0.01). Circulating omentin-1 levels were lower in T2DM versus healthy controls (313 (196-446) ng/mL versus 426 (337-614) ng/mL, P < 0.01). Furthermore, omentin-1 levels positively correlated with the diastolic parameters early peak filling rate (r = 0.246), early deceleration peak (r = 0.221) and early deceleration mean (r = 0.218, all P < 0.05). The improvement in early peak filling rate, early deceleration peak, and early deceleration mean following 24-week intervention with pioglitazone associated with increases in omentin-1 levels (resp. r = 0.415, r = 430, and r = 0.426, all P < 0.05). These associations were not found after metformin treatment. In vitro, exposure of cardiomyocytes to conditioned media derived from EAT from patients with T2DM induced contractile dysfunction, as depicted in reduced peak sarcomere shortening, and reduction in the departure and return velocity of contraction (all P < 0.01). Insulin-mediated Akt-phosphorylation was inhibited by the exposure of cardiomyocytes to EAT-derived conditioned media as well (P < 0.01). Both contractile dysfunction and insulin resistance were prevented by the addition of recombinant omentin (P < 0.01). Collectively, these findings indicate that omentin acts as cardioprotective adipokine, which is associated with LV diastolic function in patients with uncomplicated T2DM.

Excess EAT has been associated with coronary vasomotor dysfunction. In Chapter 5, the impact of T2DM on the interaction between EAT, coronary vasomotor function and coronary artery calcification was investigated. In 46 T2DM and 153 non-T2DM patients with low to intermediate risk for coronary artery disease, myocardial blood flow (MBF) was assessed at rest and during adenosine-induced hyperemia using $^{15}$O-H$_2$O positron emission tomography (PET) combined with computed tomography (CT) to quantify EAT volumes and coronary artery calcium score. EAT volumes and coronary artery calcium score were comparable between the non-T2DM and T2DM patients (resp. 129 (99 - 168) mL versus 113 (78 -171) mL, P = 0.31, and 9 (0 - 108) versus 55 (0 – 173), P = 0.13). Both patient groups showed comparable resting MBF (non-T2DM, 1.04 ± 0.32 mL/min/g versus T2DM, 1.07 ± 0.40 mL/min/g, P = 0.58) and coronary vascular resistance (non-T2DM, 79.7 ± 20.3 mm Hg/mL/min/g versus T2DM, 82.5 ± 20.8 mm Hg/mL/min/g, P = 0.42), while lower hyperemic MBF (non-T2DM, 3.18 ± 1.21 mL/min/g versus T2DM, 2.76 ± 0.97 mL/min/g, P = 0.03), coronary flow reserve (non-T2DM, 3.22 ± 1.24 versus T2DM, 2.73 ± 0.95, P = 0.02), and higher hyperemic coronary vascular resistance (non-T2DM, 28.4 ± 12.2 mm Hg/mL/min/g versus T2DM, 33.5 ± 14.5 mm Hg/mL/min/g, P = 0.02) were observed in T2DM patients. Pooled analysis showed a positive association of EAT volume with hyperemic coronary vascular resistance (r = 0.17, P = 0.02), but not with resting MBF (r = 0.02, p = 0.75), hyperemic MBF (r = -0.11, P = 0.14), or resting coronary vascular resistance (r = 0.04, P = 0.55). In subgroup analysis, EAT volume was inversely associated with hyperemic MBF (r = -0.16, P
coronary flow reserve \( r = -0.17, P = 0.04 \), and positively with hyperemic coronary vascular resistance \( r = 0.26, P < 0.01 \) only in non-T2DM patients. Multivariate regression analysis, adjusted for age, sex and BMI, showed an independent association of EAT volume with hyperemic MBF \( (\beta = -0.19, p = 0.01) \), coronary flow reserve \( (\beta = -0.16, p = 0.04) \), and hyperemic coronary vascular resistance \( (\beta = 0.25, p < 0.001) \) in the non-T2DM group. In T2DM patients, EAT volume was not associated with any parameter of coronary vasomotor function. These results suggest a role of EAT in myocardial microvascular dysfunction, but, once T2DM develops, the pathophysiology is more complex and multifactorial.

As mentioned earlier in Chapter 1, GLP-1 treatment has been associated with beneficial effects on systolic function in patients with heart failure. However, the underlying mechanisms are incompletely understood. It has been proposed that GLP-1 improves endothelial function and perfusion.

Shifting myocardial metabolism toward augmented utilization of glucose as energy substrate is associated with favourable effects on cardiac efficiency. The effects of GLP-1 receptor agonist t .01, between-groups, P = 0.49). However, neither exenatide nor insulin glargine treatment resulted in alterations in cardiac function, perfusion, and energetics. In conclusion, myocardial oxidative metabolism and efficiency were not impaired in T2DM patients. In addition, no changes in cardiac function, perfusion and energetics were observed in T2DM patients with an impaired LV systolic function after 26-weeks of exenatide or insulin glargine.

Conclusions

To summarize, in diabetic cardiomyopathy structural and metabolic changes could lead to cardiac dysfunction. Although a lot of knowledge is gained from animal studies, the translation should be done with caution, because of the more complex and multifactorial pathophysiology in human diabetic cardiomyopathy. Early metabolic interventions may favorably alter myocardial metabolic and functional remodeling, thereby delaying the progression to heart failure. Nonetheless, it may also interfere with the early adaptive processes that aim to preserve cardiac function or by its pleiotropic effects, causing an increased risk of heart failure. Adipocytokines could be cardioprotective or play a negative role in myocardial metabolism or cardiac function. Furthermore, excess EAT is linked to myocardial microvascular dysfunction, however in T2DM other factors are more dominant contributing to its pathophysiology. In T2DM patients with modest systolic dysfunction, myocardial oxidative metabolism and energetics are not impaired as compared to healthy
BMI-matched controls. Twenty-six weeks of exenatide treatment does not result in alterations in cardiac function, perfusion or energetics in these patients.

Methodological considerations

Several studies described in the present thesis had a cross-sectional design and are therefore limited to describing associations. However, by including age- and BMI-matched controls and using state-of-the-art imaging techniques, these studies did provide novel knowledge in diabetic cardiomyopathy. The randomized controlled trial, in which the effects of exenatide on cardiac function, metabolism, and perfusion were investigated, was limited by its small population size and inclusion of only male patients. Moreover, these patients already had advanced cardiovascular disease, and treatment with GLP-1 receptor agonist could be more beneficial in early diabetic cardiomyopathy.
Future perspectives

As the numbers of patients with obesity and T2DM steadily increases worldwide, the number of patients with associated cardiovascular morbidity will rise as well. Therefore, effective treatments that can safely be used in T2DM patients with cardiovascular disease are equally important as effectively lowering blood glucose levels. The results of this thesis, contributed to knowledge of the pathophysiology of human diabetic cardiomyopathy. Furthermore, 26-weeks of treatment with a GLP-1 receptor agonist in a small population of male T2DM patients with LV systolic dysfunction, did not improve or worsen LV function, and did not affect myocardial perfusion and oxidative metabolism. The inclusion of only male patients with mild LV systolic dysfunction limits its generalizability. Future research on glucose-lowering therapy must include both male as well as female patients with and without cardiovascular complications. Besides, this research should focus on effects on myocardial vasculature and metabolism, as well as the influences of these effects on cardiac function.