Chapter 1

Introduction and aims of the thesis
Long-term challenges in kidney and liver transplantation

End-stage organ failure is a condition in which solid organs such as the kidney and liver are no longer able to maintain their physiological functions. In this stage, affected patients require organ replacement therapy in order to survive. In case of end-stage renal failure, kidney transplantation offers better quality of life and improves survival compared to patients treated with dialysis (1). With regard to end-stage liver disease, liver transplantation is currently the only feasible treatment option. Fortunately, short-term outcomes of transplanted organs and its recipient have improved considerably over the past decades, which is largely due to improvements in immunosuppressive regimens, surgical techniques, treatment and prevention of infectious diseases, and living donation in case of kidney transplantation. Nevertheless, long-term outcomes of solid organ transplant recipients require further improvement, as still 15-25% of all kidney transplant recipients (KTR) and 40% of all liver transplant recipients (LTR) die within the first decade after transplantation (2,3). This sets the stage for shifting the focus of research from improvement of short-term outcomes to the prevention of premature mortality. This novel aim does however come with a new set of challenges, with at its core the prevention of cardiovascular disease as this is the predominant cause of mortality in KTR and among the top three causes of death in LTR (4,5).

Risk factors which predispose patients to develop cardiovascular disease such as hypertension, obesity, and diabetes are often already present pre-transplantation and are even the leading cause of end-stage kidney failure. Furthermore, there are an increasing amount of patients with end-stage liver disease who were transplanted because of non-alcoholic steatohepatitis, which is driven by, amongst others, diabetes and obesity (3,6). Unfortunately, transplantation is generally unable to ameliorate these comorbidities and these conditions will subsequently remain a substantial risk for early allograft dysfunction and mortality in KTR and LTR (4,5,7). This considerable risk is reflected by an approximately three times higher incidence rate of cardiovascular disease in solid organ transplant recipients compared to the general population (8). Additionally, various transplantation related factors including allograft dysfunction and immunosuppressive medication also predispose transplant recipients to the development of novel cardiovascular disease or promote the progression already existing conditions. To date, most effort to prevent cardiovascular disease in transplant populations, has been directed at modification of classical and transplantation related risk factors for cardiovascular disease. Nevertheless, as trends in long-term survival have remained largely unchanged over the past years, a broader research scope investigating the role of non-traditional cardiovascular disease risk factors is necessary (2,3).
The main hypothesis put forward in this thesis is that low muscle mass, low androgens and glucocorticoid treatment are closely interrelated and play an important role in the development of cardiovascular disease and poor long-term outcomes of kidney and liver transplant recipients (see figure 1).

Figure 1.

Potential pathways linking muscle mass and anabolic components such as testosterone and catabolic components including glucocorticoids to outcomes in transplant recipients. The illustrations which were used for this figure are sourced from Servier Medical Art by Servier and are licensed under a Creative Commons Attribution 3.0 Unported License: https://smart.servier.com/. No changes were made to the original artwork.

**Muscle mass**

Muscle mass is an essential part of the human body as it comprises almost 40% of total body weight in men and approximately 30% in women (9). Skeletal muscles are metabolically active and are an important source of amino acids (10). Furthermore, skeletal muscles play a crucial role in the glucose metabolism, as this type of muscle facilitates approximately 85% of all glucose uptake (11,12). Taken together, the quantity and quality of muscles can be an import hallmark of health. This is in line with previous studies which have demonstrated that low muscle mass is
present in a substantial number of patients suffering from chronic diseases and low muscle mass has been linked to major adverse cardiovascular events and adverse outcomes in these chronically affected populations (13,14).

**Muscle mass in transplant populations**

Transplantation often restores solid organ function to a functional level, yet this does not automatically reverse all associated comorbidities. Indeed, low muscle mass, as measured by 24h urinary creatinine excretion rate (CER), was associated with mortality and graft failure in KTR (15). However, whether this phenomenon is restricted to KTR or also present in other transplant population is currently unknown. The 24h urinary CER is a non-invasive measure of muscle mass, as creatinine is produced at a constant rate due to the continuous non-enzymatic conversion of creatine and creatine phosphate in muscle (16). As the amount of creatinine which is formed depends on the quantity of muscles, it is regarded as an excellent proxy for muscle mass both in physiological and in wasting conditions (17,18). Furthermore, in patients with advanced chronic kidney disease it has been observed that CER is not only a marker for muscle mass, but also of frailty, indicating that CER may also reflect muscle performance and therefore strength (19).

**Hypothalamic-pituitary-gonadal axis**

The hypothalamic-pituitary gonadal axis (HPG-axis) is the key regulator of sex hormone production in the human body. In men, the HPG-axis is predominantly responsible for the production of androgens, whereas in women the HPG-axis determines the production of estrogen. The synthesis and subsequent release of sex hormones into the circulation is tightly regulated via the release of gonadotropin-releasing hormone from the preoptic area in the hypothalamus. In turn, this stimulates the pituitary gland to release luteinizing hormone and follicle stimulating hormone, which in men primarily results in synthesis of testosterone and initiation of spermatogenesis respectively.

Androgens are widely known for their anabolic properties and as such stimulate muscle protein synthesis, resulting in a dose-dependent increase in the cross-sectional area of type I and type II muscles (20–22). This indicates that adequate levels of testosterone may be of importance to maintain muscle mass and as a consequence may also play a role in poor outcome in hypogonadal men. Hypogonadism has, through epidemiological studies, been linked to cardiovascular mortality (23). Furthermore, in an intervention trial testosterone supplementation has been shown to have favorable effects on glucose metabolism, even translating in a reduction of the development of newly diagnosed type 2 diabetes in hypogonadal men with pre-diabetes (24).
It has firmly been established that the status of the HPG-axis is altered in patients in chronic kidney disease, as up to 60% of all male dialysis patients have been found to be hypogonadal (25). The cause of low testosterone concentration in this population is likely multifactorial with low kidney function and subsequent or concomitant biochemical changes, comorbidities, and medication as important contributors (26).

**Hypothalamic-pituitary-gonadal axis in transplant populations**

The remarkable improvements in short-term survival in transplant populations have not only shifted the focus of research towards long-term outcomes, but also moved attention towards the question whether damage acquired in the pre-transplantation phase, including damage to the HPG-axis, remains present after transplantation. Studies which have investigated the status of the HPG-axis short-term after transplantation, have concluded that testosterone levels return to normal within one year after transplantation (27,28). Yet, low levels of testosterone at the time of transplantation have been linked to poor patient survival and graft failure in male KTR (29). Furthermore, it is important to note that the studies which assessed the status of the HPG-axis beyond one year posttransplantation demonstrate an aberrant function of the HPG-axis, indicating that return to normal within one year after transplantation may not per sé translate into persistence of normal function on the longer-term (30,31). Further conclusions on the status of the HPG-axis in male KTR should however only be drawn after standardization of study methods, as interpretation of current literature is severely hampered by inconsistent use of measurement methods, inconsistent androgen selection, and variation in applied cut-off values. The status of the HPG-axis in transplant patients may also be of interest from the perspective of development of post-transplantation diabetes mellitus, since an animal study has uncovered a mechanism by which androgens can influence insulin secretion (32). As the prevalence of post-transplantation diabetes mellitus remains high in KTR, maintenance of adequate levels of testosterone may be of importance for lowering the risk of development of post-transplantation diabetes mellitus (33).

**Glucocorticoids**

Exogenous glucocorticoids are often synthetic variants of the physiological hormone cortisol. This class of medication is most commonly known for its anti-inflammatory and immunosuppressive properties and it is a fundamental part of the therapy regimen of many diseases, including autoimmune diseases, respiratory disorders, and chronic kidney diseases. Exogenous glucocorticoids such as prednisolone and dexamethasone are, like cortisol, able to exert their effects on the glucocorticoid receptor due to their 11β-hydroxyl group. Glucocorticoids are notorious for their
catabolic properties, resulting in increased muscle breakdown (34). Clinically, this phenomenon is presented in patients suffering from Cushing’s disease, which typically presents with profound muscle weakness. In addition, these patients suffer from impaired glucose metabolism, as glucocorticoid excess stimulates gluconeogenesis in the liver and lowers insulin sensitivity in skeletal muscles and the liver (35). Subsequently, glucocorticoid excess may lead to a phenotype which is a breeding ground for cardiovascular disease.

Glucocorticoids in the transplant population

The development and use of exogenous glucocorticoids provided a breakthrough in transplantation medicine and remains a cornerstone in the immunosuppressive regimens for transplant patients to date (36). KTR and LTR rely on the glucocorticoid properties of prednisone or prednisolone to prevent allograft rejection. Yet, next to the immunosuppressive glucocorticoid effects, prednisolone leads to activation of the mineralocorticoid receptor, which likely contributes to the development of hypertension in transplant recipients. This latter effect related to the stimulation of the mineralocorticoid receptor comes on top of unwanted side effects of stimulation of the glucocorticoid receptor, which not only also include a propensity for development of hypertension, but also negative effects on glucose and lipid metabolism and on bone quality. As a result, there has been a growing call to switch towards steroid free immunosuppressive protocols or withdrawal of steroids for KTR (37). Yet, the benefit-risk ratio of steroid free protocols still is in favor of steroid maintenance and steroid free protocols are even be frankly unfavorable in patients with a high immunological risk (38).

Nevertheless, within the physiology of glucocorticoid metabolism lies a hidden non-utilized opportunity, which may theoretically resolve part of the cardiovascular risk associated with long-term treatment by glucocorticoids. Prednisone and prednisolone are currently the standard choice of exogenous glucocorticoids for transplant recipients, despite a lack of proper foundation for this choice among numerous available glucocorticoids (39,40). Furthermore, based on the opposing metabolization of different types of glucocorticoids by 11β-dehydroxysteroid dehydrogenase type 1 and 11β-dehydroxysteroid dehydrogenase type 2, it may be hypothesized that the use of glucocorticoids such as dexamethasone may result in a less pronounced development of hypertension or other side effects (41). Nevertheless, before deciding to switch to dexamethasone as main glucocorticoid in immunosuppressive regimens, it should be realized that current bio-equivalence data of prednisolone and dexamethasone are based on an animal study and a non-randomized study with subjective endpoint in patients with rheumatoid arthritis (39,40). It is therefore desirable to re-examine the currently presumed bio-equivalence of these drugs in healthy subjects to gain more insight into the metabolization and effects of different types of
glucocorticoids. This may provide a starting point for improvement of glucocorticoid related outcomes in solid organ transplant recipients and beyond.

**Aims and outline**

In this thesis we aimed to investigate the influence of low muscle mass, low androgens, and glucocorticoid treatment as novel and non-traditional risk factors for cardiovascular disease and poor long-term outcomes in kidney and liver transplant recipients. Due to the low grade inflammation and other transplant related factors which may alter physiological processes, we assessed the association of some of these potential risk factor with outcomes both in the general population and in transplant populations.

First we sought to determine in chapter 2 whether muscle mass, measured by CER, is associated with diabetes in the general population. In chapter 3, we explored the association of muscle mass with long-term outcomes after LTR. After chapter 2 and chapter 3, we deemed the influence of muscle mass of importance for recipients of a solid organ transplantation and aimed to investigate whether markers of muscle mass are also related to muscle strength. Therefore, in chapter 4, we investigated whether CER may also reflect muscle strength in KTR.

The second objective of this thesis was to investigate whether androgens can also contribute to cardiovascular disease in transplant recipients. However, as there is an ongoing debate with regard to the status of the HPG-axis, measurement methods, and a lack of reference intervals, we first aimed to determine in chapter 5 whether a dysfunction of the HPG-axis is present in male KTR long-term after transplantation and how hypogonadism should be assessed in this population. Hereafter, in chapter 6, we studied the association of total testosterone and dihydrotestosterone with the development of post-transplantation diabetes mellitus, a major contributor to cardiovascular disease in male KTR.

Exogenous glucocorticoids can influence both muscle mass and cardiovascular disease and are frequently used by transplant recipients. In chapter 7, we hypothesized that it could be beneficial to switch patients with kidney disease including KTR to a different glucocorticoid with different pharmacodynamics. To solidify our hypothesis it is necessary to perform a clinical trial comparing the effects of glucocorticoids in transplant recipients. However, as the current bio-equivalence data which would be necessary for such as study lack a proper foundation, we first constructed the CORE study, a randomized, double-blind, cross-over clinical trial which investigates the presumed bio-equivalence of prednisolone and dexamethasone in healthy individuals. A detailed rationale and design of this study can be found in chapter 8. Chapter 9 provides the general discussion of the main finding in this thesis and addresses the future perspectives. A summary is given in chapter 10.
Introduction and aims of the thesis


PART I

Studies on muscle mass in the general and transplant population