Effect of muscle mass, androgens, and glucocorticoids on health outcomes

Stam, Suzanne

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Chapter 9

Discussion and future perspectives

Solid organ transplantation is the treatment of choice for patients with end-stage organ disease (1,2). Nevertheless, after transplantation, solid organ transplant recipients remain at risk for premature mortality, graft failure, and complications. Over the past decades substantial progress has been made in improvement of short-term outcomes. Unfortunately, the advancement of long-term outcomes after for instance kidney and liver transplantation is only a fraction of the improvement in short-term outcomes (3,4). To date, the main focus for improvement of long-term outcomes in kidney transplant recipients (KTR) and liver transplant recipients (LTR) has been directed at the prevention of cardiovascular disease, as this is the most prevalent cause of mortality in KTR and is one of the top three causes of death in LTR (5,6). To ameliorate the burden of cardiovascular disease, the focus of research has mainly been on the impact of classical risk factors for cardiovascular disease such as hypertension and diabetes. However, as the current insights and treatment options of classical risk factors for cardiovascular disease have not fully eradicated classical risk factors and their detrimental downstream consequences, a novel approach is warranted to ameliorate the impact of hypertension and diabetes in KTR and LTR. In this thesis, we aimed to investigate whether muscle mass, androgens, and exogenous glucocorticoid treatment also play a role in the outcomes of KTR and LTR. Androgens and glucocorticoids are, respectively, considered to have anabolic and catabolic effects on muscle mass and as such are part of an interplay, in which alterations in one of these factors may lead to detrimental effects on the other components. Taken together, this may lead to a downward spiral impairing cardiovascular health. A more in-depth understanding may provide novel insight and potentially lead to novel intervention opportunities.

PART I

Studies on muscle mass in the general and transplant population

Muscle mass, as measured by 24h urinary creatinine excretion rate (CER), has previously been associated with mortality in the general population, in patients with heart failure, coronary artery disease, and type 2 diabetes (7–10). Yet, next to its association with mortality, we demonstrated in **chapter 2** that low muscle mass measured by CER is also associated with the prevalence and incidence of diabetes in the general population. Interestingly, we observed that this association is both present in subjects with elevated and with normal body mass index (BMI). Thus far, classical risk factors such as a western diet, low physical activity, and obesity have been the focus in prevention of diabetes, with obesity being one of the strongest risk factors for diabetes (11). However, if interventions to prevent and treat diabetes would only focus on patients with obesity, people with a normal weight as a consequence of low muscle mass rather than low fat mass might receive suboptimal care. In addition, from a physiological point of view, muscle

mass is of significant importance as skeletal muscles are the primary site of peripheral glucose uptake (12,13). To reduce the incidence of diabetes, screening might be considered in vulnerable people with a normal BMI. With regard to the transplant populations, muscle mass as measured by CER, has previously been shown to be of importance after kidney transplantation (14). Accordingly, in **chapter 3** we observed that muscle mass, as measured by CER is also associated with mortality in LTR long-term after transplantation and that recipients in the lowest tertile of CER had a 2.5-fold increased risk of all-cause mortality compared to patients in the highest tertile of CER. Although pre-transplantation mechanisms leading to a diminished muscle mass are reasonably well defined, the mechanisms responsible for post-transplantation muscle loss in LTR remain to be fully identified (15). One possibility is that muscle anabolism is impaired as a consequence of immunosuppressive medication. Although not all LTR use equal regimens, drugs such as calcineurin inhibitors and exogenous glucocorticoids are often utilized by LTR (16). The latter drug class has been demonstrated to upregulate myostatin, which is a known negative regulator of muscle mass (17). Myostatin is generally suppressed by insulin-like growth factor 1 and testosterone (18,19). Although insulin-like growth factor 1 appears to increase to the normal range quite quickly after liver transplantation, testosterone values do not fully reach normal standards (20,21). Additionally, testosterone supplementation therapy has been shown to improve muscle strength after liver transplantation (22). There are also indications that muscle mass is adversely affected by an increase in muscle catabolism. Circulating cytokines and inflammation concomitant to chronic diseases result in an inappropriate increase in muscle autophagy (23). It has previously been demonstrated that this process of muscle autophagy is regulated through the ubiquitin-proteasome pathway, which is upregulated by increased levels of inflammatory cytokines such as interleukin-6 (23). Transplant recipient are often exposed to increased levels of circulating inflammatory cytokines and as such are prone to develop a state of chronic low grade inflammation (24). This pro-inflammatory state also contributes to an increased whole-body protein turnover (25), which may be exacerbated by physical inactivity, which has been observed to be prevalent in LTR (26). This is of particular importance because inactivity has also been shown to activate the ubiquitin-proteasome pathway, thereby contributing to increased muscle breakdown (18). Importantly, there are some ongoing intervention studies which investigate the effect of exercise programs in waitlist candidates and LTR of which the results have not yet been made public (27,28). To further improve the survival of solid organ transplant recipients with a low muscle mass, and to increase the potential effect of lifestyle interventions, there is a need to identify other potentially modifiable risk factors. In **chapter 4**, we observed that CER is not only a marker for muscle mass, but also for muscle strength, which increases the relevance for improvement of CER as potential focus for intervention in KTR. The importance of such interventions is also suggested by the results demonstrated in **chapter 4**, as we additionally

observed that CER and handgrip strength were lower in stable KTR than in healthy controls. In general this part of the thesis emphasizes that the role of low muscle mass as an emerging risk factor deserves more attention, not only in KTR but also in the general population. The reasons for loss of muscle mass need to be clarified and two important aspects herein in KTR are further studied in part II and III, namely hypogonadism and use of glucocorticoids.

PART II

Long-term complications in kidney transplant recipients: focus on androgens

It has been suggested that hypothalamic-pituitary-gonadal axis (HPG-axis) dysfunction is reversed after kidney transplantation and that this warrants no further attention (29–31). However, this conclusion is based on a few studies with large heterogeneity between their respective study designs, with the focus each time limited to the status of the HPG-axis at short-term after kidney transplantation. In the second part of this thesis, we wanted to assess whether the suggestion that HPG-axis dysfunction is reversed is justified, by investigating the status of the HPG-axis long-term after transplantation in male KTR. Furthermore, we aimed to assess whether, besides from the perspective of reproduction, the HPG-axis is clinically relevant for male KTR by investigating its association with diabetes as a cardiovascular risk factor.

In **chapter 5** we demonstrated that long-term after kidney transplantation, testosterone values are lower compared to healthy controls and that when cut-off values of the general population were applied almost half of all stable male KTR were classified as having insufficient androgen values. This demonstrates that HPG-axis dysfunction has a high prevalence long-term after kidney transplantation. To date, the primary trigger leading to low testosterone values in male KTR remains unknown. In other populations such as hypogonadal patients with type 2 diabetes and in the general population, obesity has been deemed as one of greatest risk factors for secondary hypogonadism (32,33). Interestingly, compared to these populations, we observed in **chapter 5** that the difference in BMI between the hypogonadal male KTR and normogonadal KTR was not very striking. Instead, there was an evident difference in waist circumference, indicating that central obesity is an important problem in male KTR with hypogonadism. In **chapter 5** we furthermore demonstrate that in male KTR there is important bias between the cheaper and faster immunoassay method for assessment of testosterone and the current gold standard method for assessment of testosterone, which is by liquid chromatography tandem mass spectrometry (LC-MS/MS). The bias was present in such a way that the immunoassay overestimates testosterone values in male KTR compared to the LC-MS/MS method. Although the superiority of the LC-MS/MS method has long ago been established, the call for sole use of the LC-MS/MS method

with regard to steroid analyses has not yet been appraised in the field of kidney transplantation (34,35). Thus far, only one other study did investigate sex hormone levels with this analytical method, yet it did not make a comparison between different analytical techniques (36). In this latter study, Grossmann and colleagues did observe that total testosterone values were higher in male KTR long-term after transplantation compared to patients with chronic kidney disease stages III and IV and dialysis patients, but still very low compared to the general population.

In **chapter 5** we also described our finding that male KTR with hypogonadism have a higher prevalence of cardiovascular risk factors such as hypertension and post-transplantation diabetes mellitus. To investigate whether testosterone is also prospectively associated with the incidence of the latter important risk factor for poor long-term outcomes, we performed additional analyses which are described in **chapter 6**. In these analyses, we observed that both total testosterone and dihydrotestosterone (DHT) are associated with the development of post-transplantation diabetes mellitus in male KTR. It has previously been demonstrated in pre-clinical and animal studies that by activating the androgen receptor, DHT upregulates insulin secretion through the glucacon-like peptide 1 pathway (37). Along this line, we hypothesized that male KTR with low androgen values are unable to adequately upregulate their insulin secretion in response to insulin resistance. In **chapter 6** we observed that male KTR in the lowest tertiles of DHT and total testosterone have an approximately 4.7-fold and 4.2-fold increased risk to develop PTDM, respectively. Although our study is observational in nature and as such is unable to definitively determine the pathway and influence of testosterone on β-cells, it may provide a starting point for future studies. Despite that this study is the first and to date the only study to investigate the association between androgens and post-transplantation diabetes mellitus in male KTR, the role of testosterone in cardiovascular disease and its risk factors has been investigated in other populations (38,39). When reviewing this literature, it appears that most of these studies have focused on a link of low testosterone values with increased insulin resistance. Interestingly, our observed associations of total testosterone and DHT with the development of post-transplantation diabetes mellitus were independent of BMI, waist circumference, and triglyceride levels, which are strongly related to insulin resistance, with analyses therefore pointing in another direction. It is good to realize that in circumstances of insulin resistance normally functioning β-cells will be able to compensate for the increased demand in insulin production provoked by insulin resistance, making the development of hyperglycemia unlikely. However, if β-cells are unable to adequately upregulate insulin section in response to increased insulin resistance, hyperglycemia may ensue. This indicates that for the development of diabetes to develop, some degree of β-cell failure is required (40–42), which likely is the other direction to which our analyses were pointing.

PART III

Improving glucocorticoid treatment in kidney transplant recipients: a first reconnaissance

Since the discovery of compound E (currently better known as cortisone) around 70 years ago, glucocorticoids have been used to treat many diseases, particularly inflammatory and immunological disorders (43). In solid organ transplantation, the synthetic glucocorticoids prednisone or prednisolone are used to prevent and treat allograft rejection (16,44). This makes KTR and LTR, at least partially, dependent on the use of exogenous glucocorticoids. The use of exogenous glucocorticoids is, however, not without consequences, as it comes with numerous side-effects (45). Fortunately, advancement of medical and biological knowledge have resulted into novel insights and pathways through which different glucocorticoids vary in their influence on cardiovascular health, opening up opportunities to lower glucocorticoid related cardiovascular health risks. First, as hypothesized in **chapter 7**, the incidence or severity of glucocorticoid-induced hypertension may be lowered if treatment of patients with chronic kidney disease, including KTR, would be switched to another exogenous glucocorticoid than currently used prednisone and prednisolone, namely dexamethasone. Dexamethasone is a synthetic glucocorticoid which in clinical settings usually solely activates the glucocorticoid receptor, whereas prednisone and prednisolone also activate the mineralocorticoid receptor (46). The binding of this latter receptor results in enhanced sodium absorption and consequently an increase in extracellular volume. Subsequently, it may be hypothesized that less activation of the mineralocorticoid receptor leads to an attenuated effect on blood pressure of the applied glucocorticoid. The second potential advantage is the opposite handling of dexamethasone compared to prednisone and prednisolone by the isoenzymes 11β-hydroxysteroid dehydrogenase type 1 and 11β-hydroxysteroid dehydrogenase type 2 (47). The latter enzyme is regarded as protector of the mineralocorticoid receptor and has a large presence in kidney. As 11β-hydroxysteroid dehydrogenase type 2 converts active prednisolone to its in inactive counterpart prednisone and converts inactive 11-dehydrodexamethasone to its active counterpart dexamethasone, this results in local inactivation of prednisolone and local activation of dexamethasone in the kidney. As a result this mechanism has the potential for a more localized immunosuppressive effect in addition to existing systemic effects and thereby offering superior immunosuppression in the case of kidney transplantation. Taken together, the influence of exogenous glucocorticoids on cardiovascular risk factors such as hypertension, warrants re-evaluation of current standard glucocorticoid therapy in KTR and such evaluation may provide a basis to change the current standard glucocorticoid therapy in KTR.

Nevertheless, before such a major change in therapy regimen can be accomplished, the proposed hypothesis should be tested with a randomized controlled trial. This is necessary as it remains widely acknowledged that prednisone and prednisolone remain a vital part of the immunosuppressive regimen to prevent graft rejection (48,49). Furthermore, it is good to note, that the hypothesis to switch KTR from prednisone and prednisolone to dexamethasone as standard glucocorticoid has been proposed once before in the 1970s (50). In a small pilot, four KTR were switched from prednisolone to dexamethasone, resulting in a substantial increase in serum creatinine in three of the four KTR. Although this study demonstrates the need for caution, developments in the field of transplantation medicine, including improved regimens of immunosuppression, make it possible to re-evaluate this topic. Interestingly, the feasibility of this hypothesis was recently demonstrated during the COVID-19 pandemic. During this pandemic a number of KTR were switched from prednisolone to dexamethasone as the latter was provided to severely ill patients with COVID-19 (51). Although small in sample size, an observational study showed no difference in the incidence of graft failure between transplant recipients, irrespective whether they were using prednisolone or dexamethasone (52). Furthermore, a recent phase 2a study in LTR demonstrated that use of budesonide, a glucocorticoid with similar properties as dexamethasone, was save and resulted in similar rates of biopsy proven acute cellular rejection compared to prednisone after twenty-four weeks of treatment (53). Yet, before a randomized controlled trial investigating the potential benefits and harms of prednisolone compared to dexamethasone in KTR can take place, there first is a need for an updated understanding of the bio-equivalence of prednisolone and dexamethasone. Fascinatingly, the current bio-equivalence data of glucocorticoids are based on an animal study and a non-randomized study with subjective endpoints in patients with rheumatoid arthritis, performed in the 50s of the last century (54,55). When comparing the designs, endpoints, subjects, and utilized laboratory techniques of these studies with modern-day standards, both studies are at best suboptimal from a methodological and a physiological point of view. Therefore, in **chapter 8** we present the design and rationale of the CORE study, a randomized, cross-over, investigator-initiated, clinical trial investigating the presumed bio-equivalence of 7.5 mg prednisolone/1.125 mg dexamethasone and 30 mg prednisolone/4.5 mg dexamethasone in healthy subjects.

Future perspectives

In **chapter 2** and **chapter 3** we demonstrated the importance of muscle mass for maintenance of health and in **chapter 4** we show that CER is low in KTR compared to healthy controls, indicating the need for intervention. The results of these studies may provide a base for the design and rationale of future post-transplantation intervention studies to improve muscle mass and muscle strength and ideally muscle related outcomes. Yet, before such interventional studies are performed, it is also warranted to investigate the role of CER compared to other markers of muscle mass on outcomes in solid organ transplant recipients. For example, the use of imaging techniques such as magnetic resonance imaging and computed tomography have a lower availability and are far more expensive that CER, while DEXA and muscle ultrasonography share the major disadvantage of not allowing for the differentiation between muscle mass and intramuscular adipose tissue (56,57). Moreover, while bioelectric impedance analysis is often viewed as inexpensive and save, its results are easily influenced by fluid retention and general health status(57,58). If future studies were to include CER as outcome measure next to current imaging based assessments of muscle mass, the additive value or even superiority of CER as marker for muscle mass and strength in solid organ transplant recipients could be assessed.

Chapter 5 and **chapter 6** of this thesis demonstrate the overwhelming numbers of male patients after KTR with hypogonadism and the associated comorbidities. This may give an opportunity for intervention with testosterone or DHT to replace the HPG-axis dysfunctionality of male KTR. However, as testosterone supplementation may come with side-effects such as erythrocytosis, it may be worthwhile to investigate the potential added benefit of testosterone supplementation combined with or compared to other interventions. For example, future studies may want to examine the effect of lifestyle interventions with and without testosterone supplementation in obese male KTR. In addition, in male KTR who are affected by post-transplantation diabetes, it may be of interest to investigate whether long-term injection of glucagon-like peptide 1 solely or combined with testosterone is save and able to improve glycemic control. Interestingly, there is an ongoing trial investigating the effect of testosterone replacement therapy on the incidence of major adverse cardiovascular events in hypogonadal men (59). If this study demonstrates that testosterone replacement therapy prevents novel major cardiovascular events, it may be worthwhile to investigate whether similar results can be achieved in hypogonadal male KTR. With regard to androgen measurement related research in KTR, future studies should aim to measure androgen values with LC-MS/MS. Not just because it is the gold standard, but also as it allows for simultaneous and accurate measurement of precursor and product hormones. Measuring these concomitant androgens has a two-fold advantage. First, it gives a more in-depth view of the status of androgen synthesis. Second, it allows for accurate measurement of the

product hormone DHT. As DHT is the most potent androgen acting on the androgen receptor, it is responsible for the majority of effects downstream of its receptor, suggesting that future research could move towards DHT as prominent marker to assess the effects of the HPG-axis. An argument for this is also that DHT is not subjected to conversion to other product hormones, whilst testosterone may still be converted to estradiol. This latter hormone has a plethora of effects on its own which could cloud results (23,60).

Exogenous glucocorticoids can exert effects through both genomic and non-genomic pathways. The former is mediated by binding of exogenous glucocorticoids to the glucocorticoid receptor. This receptor is a member of the nuclear receptor family that controls a myriad of functions, including the stress response, inflammation, metabolism, and other biological processes in the cardiovascular system (61). The efficacy of the glucocorticoid receptor within these processes is at least partially determinant by the repression and activation of transcriptional regulation and is subsequently influenced by several polymorphisms in the glucocorticoid receptor gene (62–64). As a result, genetic variations may impact both disease pathophysiology and efficiency of glucocorticoid therapy as has been shown in patients treated asthma, graft-versus-host disease, and inflammatory bowel disease (65–67). It may therefore be worthwhile to investigate the role of the glucocorticoid receptor in the cardiovascular health of KTR and LTR and whether differences in glucocorticoid receptor polymorphisms between donors and recipients influence outcomes in KTR and LTR. With the CORE study as presented in **chapter 8**, we aim to provide an updated understanding of glucocorticoid bio-equivalence and a first step towards improved health for patients who are subjected to chronic glucocorticoid use. After completion of the CORE study, the results of the study first will have to be validated in patient populations. Future studies investigating the bio-equivalence of prednisolone and dexamethasone or the substitution of prednisolone with dexamethasone, may want to focus on specific patient groups, such as KTR with hypertension, KTR with post-transplantation diabetes mellitus, and KTR with hypogonadism as these patients have the most to gain from potential interventions within immunosuppressive regimens.

Although most of the interventions and future perspectives focus on one component, it is of clinical importance to also investigate potential therapeutic options which target a cluster of modifiable risk factors. For example, patients with a low standard of physical activity and high glucocorticoid levels predispose themselves to a lower quantity of muscle mass and a higher risk to develop central obesity. Likewise, patients who suffer from hypogonadism may have a predisposition to develop diminished muscle mass and central obesity, of which the latter in turn also increases the risk of an even more pronounced HPG-axis dysfunction. Furthermore, patients with supraphysiological levels of glucocorticoids may be at risk to develop hypogonadism, also

initiating this downward slope leading to poor health. Interventions such as a potential switch in glucocorticoids, which have the potential to influence both the HPG-axis and muscle breakdown, may therefore also be a topic of interest for future studies.

Conclusion

Muscle mass as well as hypogonadism and treatment with synthetic glucocorticoids are all factors that have the potential to be modified and optimized and as such may improve long-term outcomes in kidney and liver transplant recipients. Future studies in these patients should focus on interventions and, when successful, implement programs to optimize muscle mass, treat hypogonadism and optimize glucocorticoid treatment.

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