BACHELOR THESIS

HANZEHOGESCHOOL GRONINGEN – UNIVERSITY MEDICAL CENTER GRONINGEN

Is there a significant association between iron deficiency and physical activity in renal transplant recipients, and is this association independent of anemia?

"Is er een significante associatie tussen ijzerdeficiëntie en fysieke activiteit in niertransplantatiepatiënten, en is deze associatie onafhankelijk van bloedarmoede?"

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Preface

This bachelor thesis was written as part of the study 'physical therapy' of the Hanzehogeschool Groningen. I wrote my thesis on assignment of the Transplant Center of the University Medical Center Groningen.
The reason I chose to apply for this assignment, is because I am very interested in medicine. I wanted to learn more about medicine, transplantation and physical activity in transplant recipients. With this assignment, I have challenged myself into linking medicine with physical therapy. This assignment has been very educational for me, and therefore I would like to thank both my supervisors:

Drs. M.F. Eisenga, on behalf of the University Medical Center Groningen, and
Dr. E.J. van Adrichem, on behalf of the Hanzehogeschool Groningen.

-Lisa Bühler

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Abstract

Background: Physical activity is a contributing factor to long-term survival after transplantation. Studies have shown that iron deficiency (ID) is an important determinant of lower levels of physical activity in healthy persons. Since ID is a modifiable factor, improvement in physical activity and subsequently survival could be obtained. To this date, it is unknown what the role of ID is on physical activity in renal transplant recipients (RTR). Within this cross-sectional study, we aimed to investigate whether there is a significant association between ID and physical activity in RTR, to be able to improve long-term survival after renal transplantation, and therefore healthy ageing, in RTR.

Methods: We analyzed data from the 'TransplantLines'-study of the University Medical Center Groningen, The Netherlands. Blood pressure was measured according a standard protocol, and blood analyses were drawn using a standard laboratory protocol. Participants were examined using a composed Standard Operating Protocol (SOP), which included four physical tests, including the hand grip strength test, the 2-Minute Walk Test (2MWT), the Five Time Sit To Stand test (FTSTS) and the Timed Up and Go test (TUG). Univariate analyses, followed by multivariate stepwise backward linear regression, were used to analyze the predictive variables of physical activity in RTR.

Results: We included 128 RTR (age 55.6 ± 12.8; 56.3 % males). ID occurred in 47 RTR (37 %), anemia in 26 RTR (20 %). Mean hand grip strength was 33.9 ± 13.3 kg, 2MWT was 155.5 ± 53.1 meter, FTSTS was 12.4 ± 3.9 seconds and TUG was 6.9 ± 2.2 seconds. In linear regression analyses, ID was not significantly associated with all four physical tests.

Conclusions: We did not identify an association between iron status and physical activity in RTR. Remarkably, we did find a strong relationship between CRP and physical activity, suggesting that inflammation is detrimental for physical performance in this patient setting.

Introduction

A deviation in an organ's function, is referred to as organ dysfunction. When this dysfunction reaches the point where the normal homeostasis can only be maintained with help from an external intervention, we speak of organ failure [1]. In patients with end stage organ failure, transplantation is a life-saving intervention [2]. In The Netherlands, a total of 1275 organ transplantations took place in 2015. Of all these transplantations, renal transplantation comprises the largest group, with a total of 720 transplantations. Of these 720 renal transplantations, 207 (29%) were cadaveric donors, 198 (28%) were living related donors, and 315 (44%) were living unrelated donors [3].

The last decade, the survival rate has markedly improved in the acute phase after transplantation, due to improved techniques for organ preservation, improved surgical techniques, and better immunosuppressive medication [4]. In average, 70% of all kidneys from post-mortal donors still function 5 years after transplantation. The success-rate of kidneys from living-donors is even higher, with an average of 84% renal transplant recipients still having a functioning graft 5 years after transplantation [5]. Due to the marked improvement in short-term outcomes, research nowadays focuses more on long-term survival after transplantation. Many complications still arise on the long-term after transplantation, such as post-transplant obesity, post-transplant diabetes mellitus, malignancy, and increased cardiovascular risk [4,6-9].

Previous studies have revealed that post-transplant outcomes are improved when the renal transplant recipient (RTR) has a greater physical activity [10]. In general population, studies have shown that regular physical activity is associated with an increased life expectancy [11]. Lower levels of physical activity is one of the main risk factors of mortality. Zelle et al. investigated the association between physical activity and mortality in RTR, and identified a strong association between lower levels of physical activity and an increased risk for cardiovascular and all-cause mortality [12].

Lower levels of physical activity can be linked to iron parameters in blood. Hemoglobin (Hb), ferritin, transferrin and transferrin-saturation (TSAT) are important indicators for iron status in blood [13]. Iron deficiency (ID) can lead to anemia when the Hb levels drop below reference levels [14]. The World Health Organization (WHO) defined anemia as following: Hb level <8.1 mmol/l in men, and <7.5 mmol/l in women [15]. The most common symptoms of anemia are fatigue and faintness, which influence physical activity in healthy population [14].

Recent studies in chronic heart failure patients have shown an association between ID and lower levels of physical activity [16,17]. Therefore, it is hypothesized that an association is to be found in RTR as well. This hypothesis lead to the following research question: “Is there a significant association between ID and physical activity in RTR, and is this association independent of anemia?”. Since ID is a modifiable factor that could be corrected [18], it is important to investigate whether or not there is a significant association between ID and lower levels of physical activity in RTR, which can possibly improve long-term survival. Subsequently, the long-term survival rates will positively contribute to healthy ageing in RTR.

Methods

Study design and participants

This cross-sectional study was carried out within the Groningen transplant center of the University Medical Center Groningen (UMCG), Groningen, The Netherlands. This department of the UMCG founded the research program 'TransplantLines' in 2014. TransplantLines comprises a large biobank, used to improve long-term survival and quality of life after transplantation [19]. The study protocol was approved by the Institutional Review Board (METc 2014/077).

Data used for this study were obtained from June 1st, 2015 until March 27th, 2017. Inclusion criteria were that RTR were transplanted at the UMCG, were currently at least 1 year post transplantation, and signed a written informed consent before participating in this study. Initially, 309 RTR were included. RTR were excluded when data on ferritin was missing (n=89), and when RTR completed less than three out of the four tests to objectify physical activity (N=92). This resulted in 128 RTR (72 male, 56.3%, age 56.9 ± 12.5) suitable for analysis.

Measurement instruments

Blood pressure (mmHg) was measured according to a standard protocol, which is used in the regular patient care program of the UMCG. To perform bio-impedance analysis, the multifrequency Bio-Impedance Analyses (BIA) (Quadscan 4000, Bodystat Ltd, Douglas, British Isles) was used. Blood analyses were drawn using standard laboratory protocol. TSAT (%) was calculated as 100 x serum iron (µmol/L) / 25 x transferrin (g/L). To determine renal function, the estimating glomerular filtration rate (eGFR) was assessed applying the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) [20]. ID was defined as TSAT<20% and ferritin <300µg/L [18].

To objectify the physical activity levels of the participants, the composed Standard Operating Protocol (SOP) included four physical tests. Participants started with a hand grip strength test using the The Jamar® Hydraulic Hand Dynamometer. Mathiowetz et al. (1984) described the validity of the hand grip strength test with an accuracy of ±3%, with an inter-rater reliability of at least .97, and a test-retest reliability of .80 on the mean of three tests [21]. RTR were tested in a seated position with their shoulder in 0° abduction, elbow in 90° flexion and wrist in neutral position. The test was executed for each hand, and repeated three times, with dominant hand being stated.

Participants followed with the 2-Minute Walk Test (2MWT). Bohannon et al. (2015) investigated normative values and reliability, and showed a test-retest reliability of .82 [22]. To calculate distance covered by RTR on the 2MWT, two pylons were set apart 15 meters. The RTR were instructed to walk as fast as possible without running, until examiners said stop. Participants were updated on the time left after 1.00 and 1.45 minutes, and the final five seconds were indicated by a countdown. The distance RTR covered after 2 minutes was measured and documented.

After the 2MWT they were asked to perform the Five Time Sit To Stand test (FTSTS). The FTSTS has an test-retest reliability of .81 [23]. The test was executed three times, with one additional trial round. RTR were instructed to stand up five times as fast as possible, from sitting position, with their arms across their chest. Time was measured in seconds.

The last physical test performed was the Timed Up and Go test (TUG). The TUG has a test-retest reliability of .56 in elderly adults [24]. For the test, a pylon and chair were put apart 3 meters. The test was repeated four times, the first round being a trial. RTR were instructed to stand up from the chair without support of the arms, walk with their normal gait speed around the pylon, and go back to the chair to sit down again. In case RTR used a walking aid in normal day life, the test was performed with said aid. The TUG was measured in seconds, from the moment the participant was instructed to get up until the moment the participant sat down again.

Procedure
For 'TransplantLines', RTR transplanted at the UMCG received an information package and were asked to participate in the study. After signing written consent, a consultation at 'TransplantLines' was planned either before or after the regular hospital visitation. Blood was drawn in the morning, urine was collected from the 24-hour urine RTR brought to their regular hospital visitation. Trained examiners tested the RTR using the SOP for each participant, starting with blood pressure measurement and the bio-impedance analysis, before performing the physical tests.

Statistics
Data were analyzed using IBM SPSS software, version 23.0 (SPSS Inc., Chicago, IL, USA). Normally distributed data were presented as mean standard deviation (±SD), and skewed data as median with interquartile range. Baseline characteristics are shown in Table 1, dichotomized for ID or anemia. Results shown for blood pressure and physical activity tests were calculated as an average out of the performed trials. Skewed data on results for ferritin, RDW-cv and CRP were log transformed before applying statistical analysis. Univariate linear regression analyses were performed to assess the determinants of the physical activity tests, followed by multivariate stepwise backward linear regression. For inclusion and exclusion in multivariate linear analysis, *P*-values were set at 0.2 and 0.1, respectively. In all tests, a two-sided *P*-value <0.05 was considered significant.

Results

Baseline characteristics
We included 128 RTR (age 55.6 ± 12.8; 56.3 % males) with a mean duration of 5.0 (1.5-10.1) years after transplantation. Mean eGFR was 48.0 ± 15.9 ml/min/1.73m². ID occurred in 47 RTR (37 %), anemia in 26 RTR (20 %). Mean serum iron concentration was 13.5 ± 5.6 µmol/l, ferritin concentration was 96 (41.3-191.3)µg/l, transferrin concentration was 2.4 ± .46 µg/l, and TSAT was 23.5 ± 11.0 %. Mean hand grip strength was 33.9 ± 13.3 kg, 2MWT was 155.5 ± 53.1 meter, FTSTS was 12.4 ± 3.9 seconds and TUG was 6.9 ± 2.2 seconds. Further baseline characteristics are described in Table 1, and are provided for the total population, for RTR with or without ID, and for RTR with anemia.

Anemic RTR had highest systolic and diastolic blood pressure, and lowest BMI (kg/m²). Iron deficient RTR had the shortest period of time after transplantation, but scored higher on hand grip strength, the FTSTS and the TUG in comparison to non-iron deficient and anemic RTR, while anemic RTR scored highest in the 2MWT in comparison to non-iron deficient and iron deficient RTR.

Table 1. Baseline characteristics

Variables*	All RTR (N=128)	Non-Iron Deficient (N=81)	Iron Deficient (N=47)	Anemic (N=26)
Demographics				
Sex (male, %)	72 (56.3%)	47 (58%)	25 (53.2%)	8 (30.8%)
Age (years)	55.6 ± 12.8	55.3 ± 13.4	56.3 ± 11.7	48.8 ± 12.8
BMI (kg/m²)	26.6 ± 4.7	26.5 ± 4.0	26.9 ± 5.8	26.1 ± 7.1
Systolic blood pressure (mmHg)	137.9 ± 17.0	136.5 ± 15.5	140.2 ± 19.4	143.0 ± 21.1
Diastolic blood pressure (mmHg)	80.8 ± 11.8	80.6 ± 11.7	81.2 ± 12.2	83.0 ± 14.2
eGFR (ml/min//1.73m²)	48.0 ± 15.9	46.7 ± 16.0	50.2 ± 15.7	44.0 ± 16.5
Time since transplantation (years)	5.0 [1.5-10.1]	6.3 [1.9-11.1]	2.7 [1.3-6.6]	5.7 [1.8-10.4]
Laboratory measurements				
Iron (µmol/l)	13.5 ± 5.6	16.5 ± 4.5	8.4 ± 2.8	11.5 ± 5.2
Ferritin (µg/l)	96 [41.3-191.3]	133 [77-309.5]	37 [17-76]	78 [17.8-194.8]
Transferrin (µg/l)	2.4 ± .46	2.2 ± .35	2.7 ± .48	2.5 ± .64
Transferrin-saturation (%)	23.5 ± 11.0	29.7 ± 8.6	12.7 ± 4.5	20.1 ± 9.8
Hemoglobin (Hb) (mmol/L)	8.4 ± 1.2	8.4 ± .98	8.2 ± 1.4	7.0 ± .73
MCV (fl)	89.5 ± 6.5	91.8 ± 5.5	85.6 ± 6.4	87.0 ± 8.0
RDW-cv (%)	14.3 ± 1.8	13.7 ± 1.2	15.2 ± 2.2	14.8 ± 1.7
24h-urinary creatinin clearance (ml)	65.8 ± 25.7	64.8 ± 27.3	67.3 ± 23.0	56.0 ± 17.8
CRP (mg/l)	1.7 [.70-5.0]	1.7 [.70-3.9]	2.4 [.80-8.0]	2.9 [1.1-5.4]
Folic acid (nMol/L)	17.1 ± 7.1	16.4 ± 7.4	18.5 ± 6.5	15.3 ± 3.9
Vitamin B12 (pMol/L)	322.3 ± 183.0	322.6 ± 161.1	321.8 ± 223.0	383.3 ± 242.9
LDL Cholesterol (mMol/L)	3.0 ± 1.0	3.1 ± 1.1	3.0 ± .90	2.7 ± .74
HDL Cholesterol (mMol/L)	1.5 ± .56	1.5 ± .56	1.5 ± .57	1.5 ± .59
Physical activity tests				
Grip strength test (kg)	33.9 ± 13.3	34.2 ± 14.4	33.5 ± 11.1	33.0 ± 15.9
2-Minute Walk Test (meters)	155.5 ± 53.1	157.4 ± 55.4	152.2 ± 49.3	174.5 ± 51.7
Five Time Sit To Stand (seconds)	12.4 ± 3.9	12.3 ± 3.2	12.7 ± 4.9	11.8 ± 3.5
Timed Up and Go (seconds)	6.9 ± 2.2	6.9 ± 2.1	7.1 ± 2.3	6.6 ± 2.0

BMI, body mass index; eGFR, estimated glomerular filtration rate; MCV, mean corpuscular volume; RDW-cv, red blood cell distribution width; CRP, C-reactive protein.

**Normally distributed data presented as mean ±SD, skewed data presented as median with interquartile range.*

Determinants of Physical Activity Tests

We assessed the associations of different variables with the primary performed physical activity tests, i.e. hand grip strength test, the 2MWT, the FTSTS and the TUG. In the statistical analyses of all physical activity tests, age and CRP showed a significant association using univariate analyses, but after multivariate stepwise backward linear regression only age remained to be statistically significant.

As main determinants of hand grip strength, we identified in univariate analyses significant associations of sex, age, eGFR, time since transplantation, 24h-urinary creatinin clearance and CRP with hand grip strength. In multivariate stepwise backward linear regression, sex, age, eGFR and 24h-urinary creatinin clearance remained the most important determinants of hand grip strength (Table 2).

Table 2. Determinants of hand grip strength test in renal transplant recipients

Hand Grip Strength Test (kg)	**Univariate analyses**		**Multivariate analyses**	
Parameter	**Std. β**	***P*-value**	**Std. β**	***P*-value**
Sex (male)	- 1.741	<0.001*	-2.071	<0.001
Age (years)	-.310	<0.001*	-.324	<0.001
BMI (kg/m²)	.034	.645		
eGFR (ml/min/1.73m²)	-1.189	.008*	-1.485	.010
Time since transplantation (years)	-.103	.160*		
Iron (µmol/l)	.054	.553		
Ferritin (µg/l)	-.066	.466		
Transferrin-saturation (%)	-.006	.945		
Hemoglobin (Hb)	.086	.401		
MCV (fl)	-.075	.409		
RDW-cv (%)	.028	.764		
24h-urinary creatinin clearance (ml)	.195	.046*	.157	.077
CRP (mg/l)	-.123	.181*		
Iron deficient (yes/no)	-.025	.781		
Anemia (yes/no)	.002	.985		

BMI, body mass index; eGFR, estimated glomerular filtration rate; MCV, mean corpuscular volume; RDW-cv, red blood cell distribution width; CRP, C-reactive protein.
**P-value <0.20*

As main determinants of the 2MWT, we identified in univariate analyses significant associations of age, BMI, iron, MCV, 24h-urinary creatinin clearance, CRP, and anemia with the 2MWT. In multivariate stepwise backward linear regression, age and CRP remained the most important determinants of the 2MWT (Table 3.)

Table 3. Determinants of 2-Minute Walk Test in renal transplant recipients

2-Minute Walk Test (meters)	**Univariate analyses**		**Multivariate analyses**	
Parameter	**Std. β**	***P*-value**	**Std. β**	***P*-value**
Sex (male)	.408	.443		
Age (years)	-.334	<0.001*	-.250	.030
BMI (kg/m²)	-.250	.005*		
eGFR (ml/min/1.73m²)	.541	.310		
Time since transplantation (years)	.008	.923		
Iron (µmol/l)	.130	.153*		
Ferritin (µg/l)	-.083	.362		
Transferrin-saturation (%)	.088	.335		
Hemoglobin (Hb)	-.057	.583		
MCV (fl)	-.215	.017*		
RDW-cv (%)	-.082	.379		
24h-urinary creatinin clearance (ml)	.140	.162*		
CRP (mg/l)	-.130	.162*	-.250	.030
Iron deficient (yes/no)	-.046	.611		
Anemia (yes/no)	.146	.155*		

BMI, body mass index; eGFR, estimated glomerular filtration rate; MCV, mean corpuscular volume; RDW-cv, red blood cell distribution width; CRP, C-reactive protein.
**P-value <0.20*

As main determinants of the FTSTS, we identified in univariate analyses significant associations of age, BMI, iron, MCV, 24h-urinary creatinin clearance, CRP, and anemia with FTSTS. In multivariate stepwise backward linear regression, age and CRP remained the most important determinants of the FTSTS (Table 4).

Table 4. Determinants of Five Time Sit To Stand test in renal transplant recipients

Five Time Sit To Stand (seconds)	**Univariate analyses**		**Multivariate analyses**	
Parameter	**Std. β**	***P*-value**	**Std. β**	***P*-value**
Sex (male)	.020	.970		
Age (years)	.320	.001*	.295	.002
BMI (kg/m²)	.236	.009*		
eGFR (ml/min/1.73m²)	-.105	.845		
Time since transplantation (years)	.075	.401		
Iron (µmol/l)	-.127	.163*		
Ferritin (µg/l)	.052	.569		
Transferrin-saturation (%)	-.102	.266		
Hemoglobin (Hb)	-.070	.505		
MCV (fl)	-.079	.389		
RDW-cv (%)	.244	.009*		
24h-urinary creatinin clearance (ml)	-.113	.266		
CRP (mg/l)	.301	.001*	.226	.016
Iron deficient (yes/no)	.041	.654		
Anemia (yes/no)	-.052	.617		

BMI, body mass index; eGFR, estimated glomerular filtration rate; MCV, mean corpuscular volume; RDW-cv, red blood cell distribution width; CRP, C-reactive protein.
**P-value <0.20*

At last, as main determinants of the TUG, we identified in univariate analyses significant associations of sex, age, BMI, eGFR, time since transplantation, CRP, and anemia with TUG. In multivariate stepwise backward linear regression, age and time since transplantation remained the most important determinants of the TUG (Table 5).

Table 5. Determinants of Timed Up and Go test in renal transplant recipients

Timed Up and Go (seconds)	**Univariate analyses**		**Multivariate analyses**	
Parameter	**Std. β**	***P*-value**	**Std. β**	***P*-value**
Sex (male)	.813	.121*		
Age (years)	.361	<0.001*	.308	.003
BMI (kg/m²)	.152	.080*		
eGFR (ml/min/1.73m²)	.715	.172*		
Time since transplantation (years)	.251	.004*	.322	.002
Iron (μmol/l)	-.091	.316		
Ferritin (μg/l)	.028	.762		
Transferrin-saturation (%)	-.069	.445		
Hemoglobin (Hb)	.130	.207		
MCV (fl)	-.006	.948		
RDW-cv (%)	.075	.422		
24h-urinary creatinin clearance (ml)	-.070	.485		
CRP (mg/l)	.130	.162*		
Iron deficient (yes/no)	.054	.549		
Anemia (yes/no)	-.182	.076*		

BMI, body mass index; eGFR, estimated glomerular filtration rate; MCV, mean corpuscular volume; RDW-cv, red blood cell distribution width; CRP, C-reactive protein.
**P-value <0.20*

Discussion

In this study, we investigated if there was a significant association between ID and physical activity in RTR, and if is this association was independent of anemia. We identified no significant association between iron status and physical activity in RTR. However, we found interesting univariable associations of RDW with performance of the FTSTS test, and MCV with the performance of 2MWT.

For the formation of Hb, iron is an important factor. Hb is responsible for transportation of oxygen throughout the body [14]. Iron storage in Hb exists for about two-thirds of ferritin [14], and about 40% of ferritin levels is storaged in muscle tissues and cells of the reticuloendothelial system, which is part of the body's immune system [25]. If iron storage for the production of Hb is affected, it has great influence on physical capacity since transport of oxygen to muscle tissues is compromised [25].

Bellizzi et al. showed that great physical function is an important factor for long-term survival in RTR [10]. If there were underlying factors present prohibiting great physical activity levels, it would be important to target these complications in order to achieve advanced survival rates after transplantation. Eisenga et al. investigated the association of ID with all-cause mortality in RTR, since ID, iron deficiency anemia (IDA) and anemia are highly prevalent among RTR, with 30%, 13% and 34%, respectively. They concluded that there is an association between ID and an increased risk of mortality, independent of anemia [18]. Since ID is a modifiable factor, it was important to investigate whether or not there is a significant association between ID and lower levels of physical activity in RTR, to improve long-term survival rates [18].However, we did not identify a significant association between ID and lower levels of physical activity in RTR.

Surprisingly, with our investigations we identified a strong relationship between CRP and outcomes on the physical activity tests in this patient setting. Previous studies have revealed that physical activity levels are associated with CRP concentrations in the general population [26,27]. Ford (2002) describes the association between physical activity and CRP concentrations, and suggests that physical activity may alleviate inflammation [26]. Other studies identified that post-transplant CRP levels can predict among others eGFR after transplantation, and therefore making it a useful marker to anticipate graft survival and cardiovascular morbidity in RTR [28].

There are some limitations that should be acknowledged. First of all, the study population ended up being smaller than previously anticipated. Since we used a raw datasheet, we lost a big amount of subjects due to missing data on ferritin and results on physical activity tests. We would need a bigger sample size to maximize power for statistical analysis. We checked data on normal distribution, and for skewed data on ferritin, RDW-cv and CRP we applied log transformations before performing statistical analyses. We did not exclude any outliers found in skewed data. Because there were very little outliers found, we questioned if this would have had significantly influenced our results. Nevertheless, we were the first to investigate an association between iron status and physical activity in RTR, to determine if lower levels of physical activity in RTR need to be targeted from a multidimensional approach.

In conclusion, we did not identify an association between iron status and physical activity in RTR. Since we did not find an association between iron status and physical activity in RTR, we conclude there are no special limitations that physical therapists need to take into account in a rehabilitation program for this patient group. Remarkably, we did find a strong relationship between CRP and physical activity, suggesting that inflammation is detrimental for physical performance in this patient setting. Further investigations should identify what other factors contribute to healthy ageing in RTR.

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