Physicial activity, sedentary behaviour and markers of cardiovascular and brain diseases

Chapter seven

Summary and general discussion

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Cardiovascular and brain diseases are in the top 10 of leading causes of death globally.[1] The pathophysiology of these diseases is complex, but lifestyle factors (like diet and physical activity) are important modifiable risk factors.[2,3] International guidelines recommend at least 150 minutes of moderate-to-vigorous intensity physical activity per day and limiting sedentary time.[4] However, the knowledge about the effect of different intensities and patterns of physical activity on health related outcomes is limited.[4] Also, in individuals with type 2 diabetes the effect of physical activity can be more beneficial, possibly due to the more severe endothelial dysfunction in type 2 diabetes.[5]

In this final chapter of this thesis, the main findings of the manuscripts are summarised and discussed in the context of current scientific literature and methodological considerations. Additionally, future directions of research will be discussed in the view of this thesis.

Main findings

In the first part, all analyses were done in a data set of The Maastricht study. This is a population-based observational cohort with an oversampling of individuals with type 2 diabetes.[6]

In **chapter two**, we investigated the association between physical activity and sedentary time on the one hand and cardiac biomarkers (cardiac troponins and NT-proBNP) on the other. We did not find an association between physical activity and sedentary time and cardiac troponins. In contrast, regular vigorous intensity physical activity and possibly moderate-to-vigorous intensity physical activity was associated with lower levels of NT-proBNP.

In **chapter three**, we investigated the association between physical activity and sedentary behaviour and arterial stiffness (which was assessed by carotid-to-femoral pulse wave velocity and carotid distensibility coefficient). We found an association between total higher intensity physical activity and carotid-to-femoral pulse wave velocity but not with carotid distensitbility coefficient, which was stronger in individuals with type 2 diabetes. The insufficiently actives, weekend warriors and regularly actives had statistically significantly lower carotid-to-femoral pulse wave velocity, compared to the inactives (<75minutes of higher intensity physical activity), but there were no statistical differences between these three groups.

In **chapter four**, we investigated the association between sedentary behaviour and physical activity and biomarkers of endothelial dysfunction and low-grade inflammation.

Higher levels of different intensities of physical activity were associated with lower levels of biomarkers of endothelial dysfunction and low-grade inflammation. Also, more regularity of moderate-to-vigorous intensity physical activity was advantageous for the biomarkers of endothelial dysfunction and low-grade inflammation. Higher sedentary time was associated with higher levels of biomarkers of endothelial dysfunction and low-grade inflammation. All the associations between physical activity and biomarkers of endothelial dysfunction were stronger in individuals with prediabetes and type 2 diabetes than in people with normal glucose metabolism.
In **chapter five**, we investigated the association between physical activity and sedentary time and structural brain abnormalities. In general, more and more regular physical activity was associated with less lacunar infarcts and higher grey matter volume. There were no significant associations between physical activity and white matter volume and hyperintensities; nor between sedentary behaviour and structural brain abnormalities. However, we did find a harmful but potentially important association between moderate-to-vigorous intensity physical activity with cerebral microbleeds, which requires conformation.

In the second part, for **chapter six**, we used data of a randomized controlled trial with individuals with type 2 diabetes (LiPAT: Light intensity Physical Activity Trial). During this trial, we investigated whether a 6-month program to increase light intensity physical activity and decrease sedentary time (with 6 months follow-up) had effects on macrovascular and microvascular function, blood pressure, lipids, glucose, HbA1c, body composition, physical function, depressive symptoms and quality of life. In general, there was no difference between the control and intervention group in physical activity and sedentary time after 6 and 12 months. Also, there were no differences between the control and intervention group for the primary and secondary outcomes.

Interpretation of the main Findings

Physical activity and sedentary behaviour and cardiac biomarkers (chapter 2)

Cardiac biomarkers (defined as cardiac troponins and NT-proBNP) are markers for cardiac injury.[7,8] As shown by previous research, cardiac troponins can be released by acute bouts of physical activity[9-12], possibly caused by reversible injury, increased cardiomyocyte turnover, apoptosis and myocardial necrosis but the underlying mechanism is not completely understood.[13-15] However, physical activity is considered essential for a healthy lifestyle.[4] We studied “usual” or habitual levels or patterns of

physical activity and sedentary time, which is another way of exercise than acute bouts. So we did not find an association between physical activity (intensity and pattern) and sedentary time and cardiac troponins in our study. This indicates that usual levels of physical activity do not give cardiac damage (indicated by higher levels of cardiac troponins) and physical activity can be seen as a healthy habit.

Our results for NT-proBNP showed that vigorous (>11 minutes) and possibly moderate-to-vigorous intensity physical activity was associated lower levels of NT-proBNP, which was in line with earlier research.[16,17] This result suggest that the intensity of physical activity (in this case at least moderate-to-vigorous intensity) is important for the level of NT-proBNP, which is also supported by the associations between the pattern of moderate-to-vigorous intensity physical activity and NT-proBNP. This suggests that performing regular physical activity reduces cardiac stress (as measured by NT-proBNP).

There was no difference in individuals with or without type 2 diabetes for all these results. So the beneficial effect was of similar strength in both groups. Individuals with type 2 diabetes could have (in general) higher levels of cardiac biomarkers.[18-23] A possible explanation can be the relative low levels of cardiac biomarkers in The Maastricht study, which indicates a population with low levels of cardiac damage.

Physical activity and sedentary behaviour and arterial stiffness (chapter 3)

Our results show an inverse association between higher intensity physical activity and carotid-to-femoral pulse wave velocity, which was stronger in individuals with type 2 diabetes. In general, these results are consistent with earlier research.[24-27] However, the observation that the association is stronger in individuals with type 2 diabetes is novel. Also the results with regard to different patterns is an extension on previous research.[26]
There are different mechanisms that are thought to be the involved in the relationship between physical activity and arterial stiffness.[28-32] Physical activity reduces vascular oxidative stress, and shear stress increases the production of endothelial nitric oxide (NO) and increases blood flow which also increases NO and improves vessel wall homeostasis.[28-30] Another mechanism is the decrease of sympathetic hyperactivity and an improved sympatho-vagal balance which may decrease arterial stiffness.[31,32] It is also thought that regional arterial stiffening is affected differently by risk factors (as physical activity), which can explain that there was no association between physical activity and carotid distensibility coefficient.[33] However, the exact mechanism is poorly understood.

The pattern (regularity) of moderate-to-vigorous intensity physical activity was not important in relation to arterial stiffness, which was also shown in earlier research for

different health outcomes (CVD, metabolic syndrome and mortality).[25,34-36] Our results show that some moderate-to-vigorous intensity physical activity is better than none for arterial stiffness.

Physical activity and sedentary behaviour and endothelial dysfunction and low-grade inflammation (chapter 4)

As consistent but more extensive (characterisation of physical activity and biomarkers of endothelial dysfunction and low-grade inflammation) with earlier research, all intensities of physical activity were associated with biomarkers of endothelial dysfunction and low-grade inflammation.[37-44] Also the regularity of moderate-to-vigorous intensity physical activity is important in relation to endothelial dysfunction and low-grade inflammation. The mechanism for endothelial dysfunction is the same as explained in the part on arterial stiffness above. For low-grade inflammation, the underlying mechanism is not completely understood, but thought to relate to reduced adipose tissue, changes in number of (immune) cells and a reduction of toll-like receptors.[45,46] Earlier research showed an association between sedentary time and biomarkers of endothelial dysfunction and low-grade inflammation[47-51], as is consistent with our results. Shear stress is reduced by sedentary behaviour, which decreases endothelial oxide nitric synthase and decreases bioavailability of NO.[47]

For individuals with prediabetes and type 2 diabetes, the association between physical activity and biomarkers of endothelial dysfunction were consistently stronger compared to the individuals with normal glucose metabolism. The mechanism behind the stronger association is not completely understood, but physical activity can possibly interrupt the vicious cycle of hyperglycaemia and impaired microvascular endothelial function, by improving endothelial function. However, a short-term study did not find a difference in biomarkers of endothelial dysfunction between individuals with and without type 2 diabetes.[44] In our study, physical activity is a reflection of long-term habits, which makes it possible that long-term physical activity is needed to improve endothelial dysfunction. But further research is required.

Physical activity and sedentary behaviour and structural brain abnormalities (chapter 5)

In general, the finding that more moderate-to-vigorous intensity physical activity is associated with less lacunar infarcts is consistent with literature[52-54], although our data suggest that the intensity is important (minimal moderate-to-vigorous intensity physical activity). Our results showed a protective effect of physical activity on grey matter volume. Earlier research was not completely consistent and some studies showed a

similar result[55-57], whereas others did not.[58,59] The pathophysiological mechanism is not completely understood, but it is thought that physical activity increases cerebral blood flow and angiogenesis and promotes upregulation of neurotrophic factors which is involved in the neurogenesis.[57,60,61] Also, our results suggest that regularity of moderate-to-vigorous intensity physical activity is important for less lacunar infarcts and more grey matter volume, which had never been studied before (as far as we are aware).

Earlier research was inconsistent about the associations between physical activity and white matter volume and hyperintensities.[54,58,59,62-64] Our data showed no association between physical activity and white matter volume/hyperintensities.

Our study suggest that moderate-to-vigorous intensity physical activity can have a potential harmful effect on the development of cerebral microbleeds, which was not found in earlier research.[54,65] However, the peak blood pressure during physical activity is a potential mechanism to provoke cerebral microbleeds.[66] Also peak blood pressure is a predictor for cardiovascular complications and incident hypertension.[66] However, in our study we did not find an interaction with systolic blood pressure (office and 24h) hypertension. So further investigation is needed to confirm these results.

LiPAT (chapter six)

Our study showed no differences between the control and intervention group after 6 and 12 months in any intensity of physical activity and sedentary time. Earlier research is limited and mostly short-term for increasing light intensity physical activity and reducing sedentary time in individuals with type 2 diabetes. One 3 year study in individuals with type 2 diabetes showed a positive effect with intensive coaching on increasing light intensity physical activity, reducing sedentary time and some cardiometabolic outcome.[67,68] Also other studies in individuals with type 2 diabetes have shown a reduction in sedentary time by a lifestyle intervention[69] or coaching with a wearable intervention.[70] As we compare these results with our study, the intensity and way of coaching is an essential point in making a long-term behaviour change in individuals with type 2 diabetes, which is also seen in the DIRECT trial.[71] Therefore, in further research it is important to take the intensity and way of coaching into account. Further, in retrospect, our intervention might have been too difficult for the target population (2 applications and a watch).

Methodological considerations

In the first part of this thesis, the studies used data of The Maastricht study, a population based cohort study in the Netherlands. In the second part, we used data of a clinical

trial. With all the results, methodological strength and limitations have to be taken into account. In the section below, this will be discussed.

Internal validity

Information bias

Assessment of physical activity and sedentary behaviour

Physical activity and sedentary behaviour are in all chapters measured by activPAL. As mentioned before, activPAL is an accelerometer that was worn for 8 consecutive days and is an appropriate measurement for research. It was attached waterproof to the right leg.

In chapter 2,3,4 and 5, the measurement represents the 'usual/habitual' physical activity and sedentary behaviour. However, we only measure 8 days, which may be limited to represent the 'usual' physical activity and sedentary behaviour. Earlier research showed, however, that accelerometer measurements from five days are already reliable to represent the 'usual' physical activity[72], which makes it reasonable that eight days of measurement is enough.

In chapter 6, we measured physical activity and sedentary behaviour only at baseline, 3 months, 6 months and 12 months and saw no differences over that time. Although this was done to make the study as minimally invasive as possible, maybe the time between the measurements was too long. It is also known that behavioural changes are the greatest in the beginning when the participant is the most motivated. So there is a possibility that we missed the change in physical activity and sedentary behaviour during the first three months. However, this change, if any, was not sustainable. Also, the COVID-19 lockdowns were during the trial. This limited the options for physical activity by the participants and our participants had type 2 diabetes, which was a risk factor in the development of severe COVID-19. So, this also may reduced physical activity of the participants.

Assessment of markers for cardiovascular disease

In chapters 2 and 4, we measured biomarkers. All these biomarkers are well known for their relationship with cardiovascular diseases.[8,13,73-80]

In chapters 3 and 6 we used carotid-to-femoral pulse wave velocity to represent aortic stiffness. These measurements were performed by a well-trained nurse/researcher and are the most non-invasive accurate way to get information on the aortic stiffness. We also performed measurements on the carotid artery. In chapter 6, due to technical problems, we changed the equipment, which could have led to differences. However, a validation study was performed by K. Reesink et al ('unpublished observations') showed no differences in the measurement outcomes.

Assessment of markers for brain disease

In chapter 5, we measured structural brain abnormalities by MRI. MRI is known as the best non-invasive way to measure structural brain abnormalities.[81]
In chapter 6, we only used questionnaires for quality of life and depression. All questionnaires were validated.[82-84] The participant was asked to fill in this questionnaires at the end of every study visit, while having a breakfast.

Confounding/overadjustment bias

Confounding bias is a one of the major concerns for the internal validity of a study.[85] In the observational Maastricht study, many potential confounders were measured and in chapters 2,3,4 and 5 we adjusted the associations for potential confounders. However, it is impossible to fully exclude residual confounding.[85] In our studies, we performed multiple sensitivity analyses to reduce the possible residual confounding showing the robustness of our findings.
By adjusting for multiple covariates, it is possible that we have also adjusted for mediators, which is overadjustment. Therefore, we used different models to adjust the associations. In chapters 2,3,4,5, model 2 involved potential confounders, while model 3 involved potential confounders/mediators; in this way, potential overadjustment can be assessed.

Selection bias

In The Maastricht study, participants were recruited by mass media campaigns and via mailing from the municipal registries and diabetes patient registry. This way of recruitment was done to receive a good representation of the general population. However, the means of inclusion may have led to overselection of healthy individuals - who tend to be more likely to participate in clinical trials (86). The Maastricht study was promoted as a 'health check', so it may be that individuals who are more interested in their own health would participate more likely than individuals who are not interested in their own health, which could result in a healthier cohort than the general population.

Some measurements had specific in- and exclusion criteria in The Maastricht study. For example, the MRI measurements (chapter 5) had several exclusion criteria such as too large body size or metal inside the body. This excludes some unhealthier individuals, which can result in selection bias. The measurement of aortic stiffness (chapter 3), was only performed in individuals without a carotid plaque for safety reasons, which can also give selection bias. In most studies (except for chapter 5), we only used complete-cases analyses. However, in most chapters (2,3,4) we compared the in- and excluded individuals. In general, the characteristics were similar, which limits the effect of selection bias from the participants in the study.

For the recruitment of LiPAT (chapter 6), we recruited participants by online and paper advertisements and by alerting general practitioners. The goal was to include a reflection of the population of individuals with type 2 diabetes. When we look at the LiPAT participants, participants had different treatments (diet, oral medication, insulin or combination), which limits the selection bias. However, due to the inclusion criteria (BMI<35 kg/m^2, being able to walk 10 minutes and not more than 150minutes of physical activity/week), healthier and unhealthier individuals were excluded, which limits the generalizability of this study.

External validity

In the first part (chapter 2,3,4,5), we used data of The Maastricht study. This is an observational population-based cohort study in the southern part of the Netherlands which included individuals between the age of 40 to 75. The inclusion criteria of The Maastricht Study imply that the results of the studies conducted using The Maastricht Study cannot necessarily be extrapolated to other individuals. Especially the age of the participants makes it difficult to project the results on younger individuals. In The Maastricht study most of the participants were Caucasian, which limits the generalizability. Also, in The Netherlands, lifestyle factors can be different than in other cohorts. For example, the infrastructure for cycling or walking is good, which allow people to go somewhere by bike/foot, which contributes to physical activity.[87] This is not the case in every country, which can make a difference in physical activity in another population cohort. Also, it is known that the individuals in Maastricht and the surrounding area have a more burgundian lifestyle, which includes different types and amount of food that may differ from other cultures. Also this can give a difference, as diet is one of the other major modifiable risk factors in the development of cardiovascular and brain diseases.

In two of the four studies (from part one), we found an interaction with type 2 diabetes. This indicates that individuals with type 2 diabetes potentially have a more beneficial effect of physical activity than individuals without type 2 diabetes. However, we cannot confirm this with our trial (chapter 6).

Statistical considerations

Most of the chapters are based on observational data. Observational studies have limitations. Conclusions about causation should be made with caution and it may be difficult to exclude reverse causality.[85] Therefore, it remains important to study the biological mechanism of the association. Also correcting for possible confounding makes

it more reasonable that the associations that we found possibly can be causal in the chapters.
For chapter 6, we used data of the intervention trial (LiPAT). In principle, causal inference in an intervention trial is more robust than in observational studies. However, the intervention did not result into more physical activity and less sedentary time, so in this case, it is not possible to draw any conclusion on the causation.
A sufficiently large sample size is important in order to obtain sufficient statistical power to detect significant associations. The absence of statistically significant associations in a large number of our analyses in chapters two and five may be the result of a limited sample size. However, given the large sample size of The Maastricht study makes this less likely. In chapter six (LiPAT), the results of our study may be limited by a small sample size. We tried to reduce the risk of insufficient power by conducting an a priori power calculation.

Conclusion and future directions

In conclusion, in this thesis we studied the relationship between physical activity and sedentary behaviour on the one hand and markers for cardiovascular and brain diseases on the other hand. In general, we can say that physical activity has a positive effect on markers for cardiovascular and brain diseases, especially in individuals with type 2 diabetes, although the effect of more intense physical activity can be potential harmful in that it may contribute to cerebral microbleeds. Changing behaviour of individuals with type 2 diabetes is very difficult and complex. The way and amount of coaching is important to implement more physical activity and less sedentary time.
For the future, physical activity has to be promoted to everyone, because it has a lot of advantageous. It is important to perform more research in longitudinal and clinical trial settings. Also, more research about the pathophysiological pathway that the effects of physical activity are larger in individuals with type 2 diabetes would be interesting. A large randomised controlled trial in individuals with prediabetes and type 2 diabetes to assess different types and intensities of coaching in combination with electronically devices (e.g. watches, applications) in different age categories, including the health outcomes, sustainability and costs of the coaching. This would be the ideal trial, but will be expensive.

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