

Screening Adolescents, Young Adults for Risk Factors for Chronic Disease

Thesis Project 1.0 – A cross-sectional study on the effects of different levels of exercise intensity on cholesterol levels in healthy individuals

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Preface

The idea to write this bachelor thesis about cholesterol levels came from reading “A sweeter blood”; a book questioning our western lifestyle and health advice regulations. I would like to thank the author Ann Fernholm and my mother, who gave me the book, for inspiring me to learn more about this interesting subject.

Without with my fellow students in this project, Andrea Gustafsson, Ivo van Knippenberg and Niels van Hoof, these five final months of my education would have been very long drawn and difficult. Thanks to you and your support, this time has been both enjoyable and cheerful. I would also like to thank my supervisor Steven Onkelinx, who has with never ending positivity guided me through the process of this thesis. Last but not least I would like to thank Jason Sam Smith for his time, effort and English language expertise.

Abstract

Introduction: Coronary artery disease (CAD) is the leading cause of death worldwide. Dyslipidemia, one of the risk factors for CAD, is a condition in the body where abnormal levels of cholesterol are found in the blood. There are several options to manage cholesterol levels, physical exercise is one of them. Few studies have been done to determine the effect of exercise on blood lipids in asymptomatic subjects. The aim of this study is to investigate whether different exercise intensity levels have differences effects on cholesterol levels in healthy young adults.

Method: All second year physiotherapy students at Fontys University of Applied Science Eindhoven were invited to participate in this observational cross sectional study. Total Cholesterol (TC), high density lipoprotein (HDL) and TC/HDL ratio were measured using a CardioChek® PA. The subjects were divided into different groups depending on their level exercise intensity, high, low or minimal. The intensity was determined using the MET score. The results were compared using the One-Way ANOVA test.

Results: 71 subjects (42 women) aged between 18 and 29 years old participated in the study. There were no statistically significant differences found in cholesterol levels between the high intensity exercise group (n=47) and the low intensity exercise group(n=21).

Conclusion: From the results of this study it is not possible to determine if high intensity and low intensity training have different effects on TC/HDL ratio. No significant differences were found in the cholesterol levels between the training groups. Further research is needed to determine which type of training is most beneficial as prevention for dyslipidemia and for preferable cholesterol levels in young adults.

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Introduction

Coronary artery disease (CAD) is the leading cause of death worldwide. In 2012, CAD caused estimated 7,4 million deaths (1). CAD occurs when there is damage in a coronary artery. An inflammatory response is triggered and from there an atherosclerotic plaque formation starts to grow (2,3). It is a slow process, the time interval is depending on how favorable the environment is for plaque growth in the blood. When the plaque grows bigger it results in narrowed space in the artery leading to higher pressure in the vessel lumen. This condition can lead to myocardial infarction, stable angina, unstable angina or stroke, all of which can be fatal (2).

There are several risk factors identified for CAD: smoking, hypertension, Diabetes mellitus (type II), dyslipidemia, physical inactivity and obesity (which often is associated with hypertension, dyslipidemia, metabolic syndrome and physical inactivity)(4). Dyslipidemia is a condition in the body where abnormal levels of cholesterol are found in the blood. In developed countries this often means high levels of Low density lipoproteins (LDL), the “bad” cholesterol and low level of High density lipoproteins (HDL), the “good” cholesterol. High levels of LDL can lead to plaque formations and growth in the arteries. HDL has the opposite effect; it collects excessive LDL from the blood, pulls it from the plaque formations and brings it to the liver for breakdown, the so called reversed cholesterol transport (5). New research shows that HDL additionally has protective antioxidant, anti-inflammatory, and anticlotting properties (6). A low LDL and a high HDL is beneficial for CAD (7). The predictive value for CAD is identified by calculating the ratio between total cholesterol level (TC) and HDL. A ratio above 5 is considered a risk factor (8).

There are several options to manage cholesterol levels. Minimizing the intake of saturated and trans fats such as margarine, palm oil and partially hydrogenated vegetable oil will reduce LDL levels. Unsaturated fats, e.g. virgin olive oil, canola oil and omega 3 fats have the ability boost the HDL levels and promote cardiac health. Choosing full grain slow absorbing carbohydrates and having a lower sugar intake can protect from the HDL decreasing process of excessive insulin response in the blood (which occurs when refined fast absorbed carbohydrates and sugars are consumed)(6,9). Low amount of alcohol consumption, 1 glass of wine/beer daily is associated with substantial protection for heart attack, since it can increase HDL levels with 0,1mmol/L (6,9,10). Obesity is linked with low HDL levels (6,10). A BMI below 25 would be optimal, but all weight loss is beneficial (6,9). Quitting smoking can raise HDL levels by 15-20% (6,10). Statins, a very efficient LDL lowering medication, is at the moment the most used drug for CAD. Although it does not raise HDL levels significantly, it has a positive effect on the cholesterol level ratio and has anti-inflammatory side effects that can protect the artery walls from further plaque build-ups (6,9). Physical exercise is an important method, sedentary people who starts exercise can increase their HDL levels with up to 20% (6,10).

Several researches have been done to establish what influences different types of exercise have on lipid levels. Tsai et al found that Tai Chi had positive results on cholesterol levels. The participants of the RCT were training in Tai Chi for 50 minutes a day, three times a week during 12 weeks. The intensity of the training was approximately 64% of maximum heart rate. In the intervention group the TC level decreased 0,39 mmol/L and the HDL increased 0,12mmol/L (11). In a systematic review made of Tambalis et al (12), the effect of different training intensities on blood lipids were studied. In total 84 articles were included, the age of participants ranging from 18-80 years and with a minimum of 3 months physical exercise. Moderate intensity aerobic exercise showed a significant increase of HDL levels in 6 out of 28 trials, with a raise in levels between 3-25%. High intensity aerobic exercise showed a significant increase in HDL levels of 2-20% in 22 of 37 studies. Only 8 studies showed decreases in TC levels. However, many of these trials were testing subjects who were overweight, obesity or had abnormal blood lipids (12). In a cross sectional study including 1837 female runners, Williams found a significant increase in HDL levels in relation to greater weekly distance runs. HDL levels were significantly higher with each 16 km increase and the TC/HDL ratio was reduced with ($\beta = -0.005 \pm 0.001$ Km/week)(13).

Few studies have been done to determine the effect of exercise on blood lipids in asymptomatic subjects. Since CAD is an endemic disease, early monitoring and more knowledge concerning how we can reduce risk factors is needed. Can we influence our blood lipids already at a young age to prevent the disease? It is known that physical exercise has positive effects on cholesterol levels (11,12,13). The question remains though regarding if there is any connection between cholesterol levels and exercise intensity in asymptomatic subjects. And if yes, does the level of intensity have any influence? If so, it would be possible to give more accurate recommendations to the adolescent population concerning which intensity level is most effective to lower the risk factor of hyperlipidemia. This leads to the following research question:

What influence does exercise intensity have on the TC/HDL ratio in young adults?

Method

Study design

This cross-sectional observational study took place at Fontys University of Applied Science in Eindhoven, The Netherlands (FUAS). The study was a part of a larger research, focused on screening for risk factors for chronic diseases. In total four students were conducting tests where multiple data was collected concerning physical conditions such as body measurements, flexibility, strength, stamina and reaction time among second year physiotherapy students at FUAS. All tests were conducted in an exercise lab located at FUAS. The collected data was additionally to making it possible to calculate risk factors based on different physical aspects, creating a base for further research.

This particular study will focus on cholesterol levels and the level of physical exercise intensity of the participants, the results of the other tests will not be discussed.

Subjects and selection process

All second year physiotherapy students at FUAS, Dutch and English stream, totaling 137, were recruited for participation in the study. All subjects received written information which explained the aim of the study and the test procedure. A form of consent (Appendix I) was signed prior to the test, guaranteeing the confidentiality of the data. Participation was voluntary and the subjects could withdraw at any time from the testing without giving a reason.

Inclusion criteria for the study required the participants to be between 18 and 30 years old and second year students of FUAS. Subjects suffering from Diabetes I or II or subjects which had an injury that hindered them in performing their regular sport activity were excluded from the study, since both occurrences can influence the cholesterol levels and thereby affect the results.

Participants not matching inclusion and exclusion criteria were still tested, but not included in the study.

Researchers

The research-team consisted of four physiotherapy students at FUAS. This project was a part of the graduation phase.

Three weeks prior to the first test session regular practice of the test procedure took place in the exercise lab to minimize errors being made by the researchers during the actual testing. A total of 8 hours were spent on training. All researchers in the team were present at all test sessions and were responsible for the same tests throughout the training and later on the test occasions to ensure validity of the data collected.

Measurement Tools

Cholesterol levels were measured using a CardioChek® PA, test strips and MEMo Chip® produced by PTS Diagnostics. It is a handheld professional analyzer that uses a test methodology called reflectance photometry to read lipid levels from blood samples of 15 to 40 µL. The strips used analyzed TC and HDL levels. Additionally the CardioChek® calculated the TC/HDL ratio. Protocol for testing is presented in Appendix III.

The results from the CardioChek® PA system meets the accuracy guidelines for Analytical Systems for Cholesterol established by the National Cholesterol Education Program of the National Institutes of Health(US). The instrument is approved by the Cholesterol Reference Method Laboratory Network (US). The Total Analytical Error, the accuracy and precision of the system, is within limits of commercial methods for determining lipid concentration. It is calculated by the difference between the obtained result and the reference value, which is defined by standard methods for lipids (14).

A questionnaire (Appendix II) was used to collect information concerning physical activity, age and gender. Exercise intensity was determined with the Metabolic Equivalent of Task system (MET). It is a physiological measure which expresses the energy consumption of different physical activities. 1 MET is defined as the energy cost of sitting quiet on a chair, an activity with MET 2 would require twice the energy. The values are calculated by measuring the oxygen consumption (and thereby the metabolic rate) during different activities of an average individual. The MET system ranges from 0.9 (sleeping) to 23 (running at 22.5 km/h) (15).

Height, weight and BMI were collected using an automatic measuring scale and length measure. The same measurement tools were used for every participant.

Data Collection

The subjects were divided into subgroups with a maximum of 12 participants. Each subgroup was tested separately during one occasion in the exercise lab at FUAS. The test procedure took approximately one and a half hours. The test sessions were scheduled in advance and took place between 30th March and 8th April 2015. The participants were required to complete the questionnaire describe earlier virtually prior to the test occasion to ensure truthful answers.

The tests were divided into A, B and C stations. All A stations were conducted first since they had to be tested in idle state. Thereafter the participants continued to the B stations. The C station was completed last, to prevent other tests being biased by exhaustion of the participants from the test. See *Figure 1*.

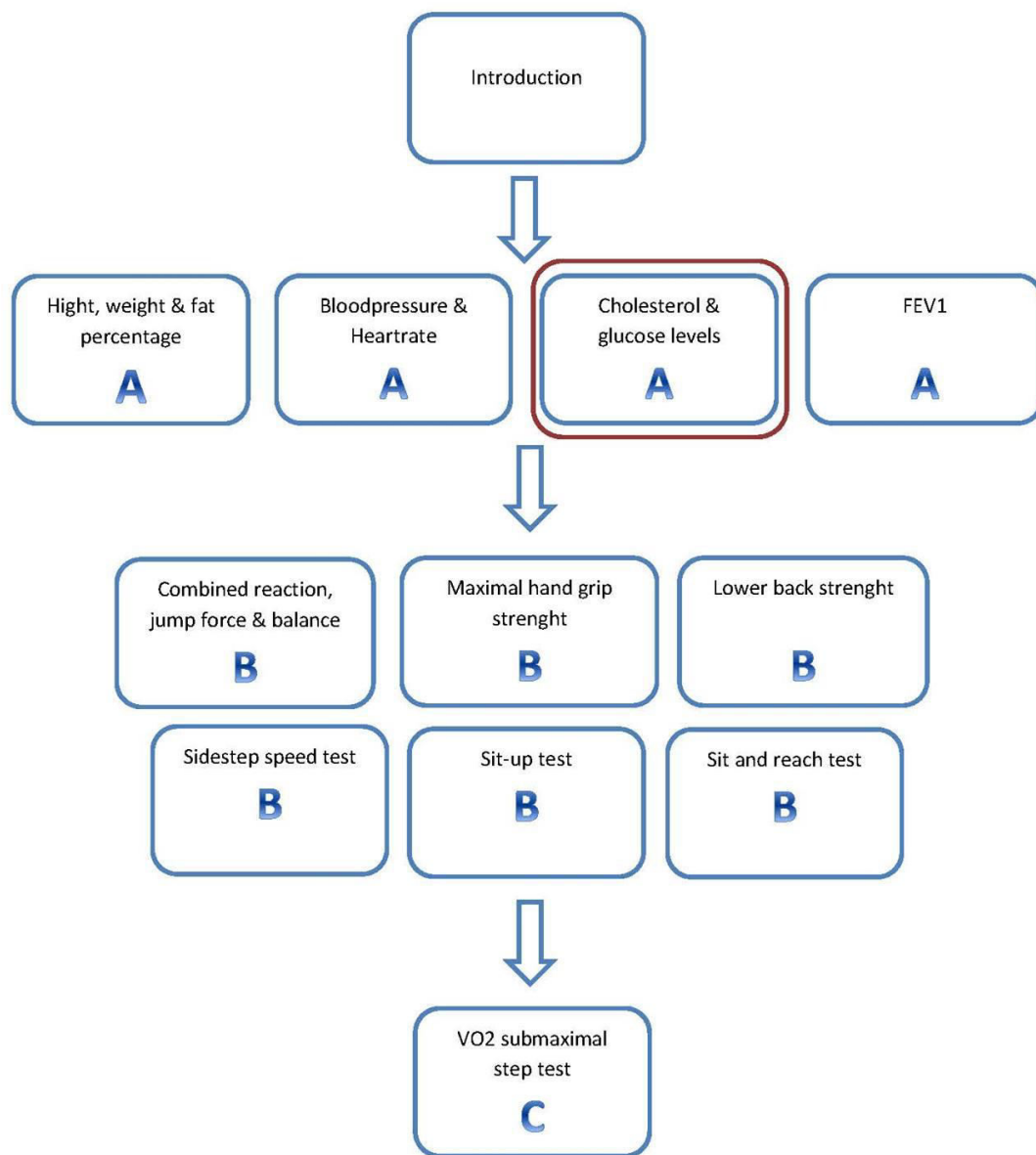


Figure 1. Flowchart of stations.

Data analysis

IBM SPSS version 20 (IBM, Armonk, NY, USA), was used to analyze the data. The main variables were the TC/HDL ratio, TC, HDL and exercise intensity level. The secondary parameters taken into consideration were age, gender, height, weight and BMI. TC/HDL ratio, TC, HDL, age, height, weight and BMI were measures of ratio scale. Gender and level of exercise intensity were measures of a nominal scale. TC and HDL were measured in mmol/l. Age was measured in years. Height was measured in centimeters. Body weight was measured in kilograms. The secondary parameters were used to give a description of the test group. If the data was within normal distribution, mean and standard deviation were used to describe the variables. If not, median and maximum/minimum values were applied.

The participants were divided into three subgroups dependent on the level of exercise intensity.

Group 1: Minimal training (activity with MET >2,5 or activity with MET 2,5-6 less than 180 minutes/week)

Group 2: Low intensity (activity with MET 2,5-6 at least 180 minutes/week or activity with MET >6 less than 180 minutes/week)

Group 3: High intensity (activity with MET >6 at least 180 minutes/week)

Activities MET >6: Soccer, hockey, cross-fit, jogging, running, tennis, squash, racquetball, skiing downhill or cross country, ice or roller skating, fast swimming, boxing and rowing.

Activities MET 2,5-6: Horseback riding, walking, dancing, golf, slow/moderate speed swimming and fitness.(15)

A comparison between the gender groups were additionally to the training groups performed.

The One-Way Analysis of Variance(ANOVA) test was used to analyze the disparities between the training groups and look for statistical significances. The Independent-Samples T test was used to compare the gender groups. TC/HDL ratio, TC and HDL were compared. A p-value of < 0.05 (5%) were considered statistically significant.

Ethical aspects

All participants were second year students studying at Fontys University of Applied Sciences. Taking part in this research project was voluntary and subjects were allowed to retract their participation at any time without giving any reason. All collected data was processed anonymously and stored carefully. All subjects were well informed and signed an informed consent form prior to testing (Appendix I). Data from participants who retracted their participation was catalogued and included up until that point.

Results

Research population

In total 94 second year students participated in the large research. Out of them there were 71 subjects, 29 (40,8%) men and 42 women, which met the inclusion and exclusion criteria and had complete data required for this particular study. The participants were between 18 and 29 years old. Table 1 shows descriptive statistics for the total research population. All data of the variables were normally distributed.

Due to a technical failure the values of the TC and HDL from six participants failed to register and therefore are missing from the results. Nevertheless they were included in the remaining statistics.

Table 1 – Descriptive statistics total group

Total group (n=71)	Mean \pm Standard deviation
Age (years)	21,0 \pm 2,3
Height (cm)	173,19 \pm 8,40
Weight (kg)	67,60 \pm 10,82
BMI (kg/m²)	22,44 \pm 2,49

Training groups

The high intensity training group (Group 3) consisted of 47 subjects, 25 males and 22 females. The low intensity training group (Group 2) consisted of 21 subjects, 4 males and 17 females. The minimal training group (Group 1) consisted of 3 subjects, which is a too small sample size to include in this study. Therefore a comparison between only the low intensity group and the high intensity group was executed using the Independent-Samples T test instead of the One-Way ANOVA test. Descriptive statistics of the groups are shown in Table 2.

Table 2 – Descriptive statistics training intensity groups

	Group 2 (n=21; 4 male, 17 female)	Group 3 (n=47; 25 male, 22 female)	P-value
Age (years)	21,6 \pm 2,5	20,8 \pm 2,9	0,207
Height (cm)	170,15 \pm 7,91	175,28 \pm 7,73	0,017
Weight (kg)	63,09 \pm 8,58	70,53 \pm 10,67	0,004
BMI (kg/m²)	21,79 \pm 2,57	22,87 \pm 2,43	0,111

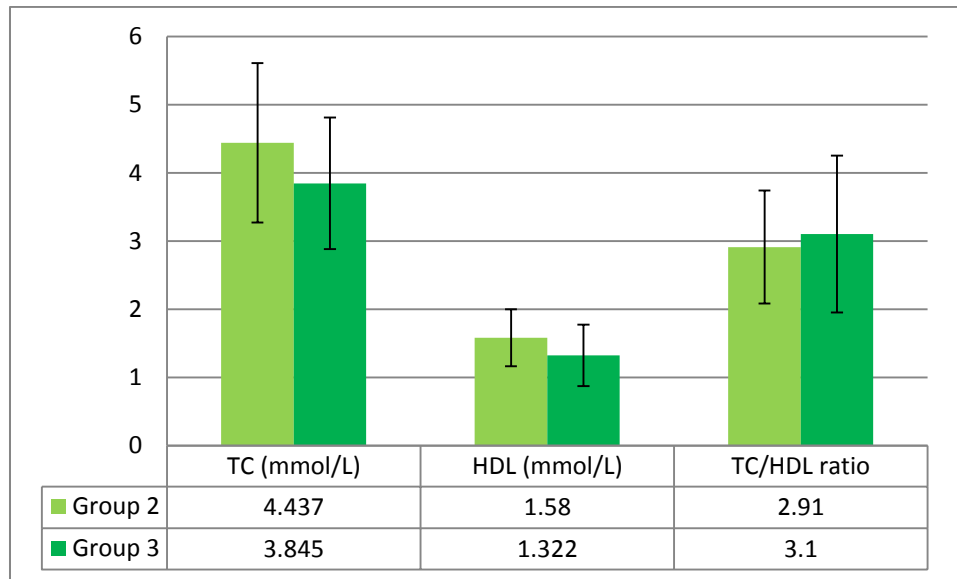
NOTE: values are presented in mean \pm standard deviation.

Group 2: Low intensity training

Group 3: High intensity training

Graph 1 shows mean values of TC, HDL and TC/HDL ratio of the two training intensity groups. The Independent-Samples T test showed that there were no significant differences in TC, HDL or TC/HDL ratio between the groups. Closest to a significant difference was the TC, (Group 2: 4,437 \pm 1,169, Group 3: 3,845 \pm 0,966) where $p = 0,058$.

Graph 1 – Cholesterol levels training intensity groups



Values presented in mean. Standard deviations are to be seen in the bars

Group 2: Low intensity training

Group 3: High intensity training

Gender groups

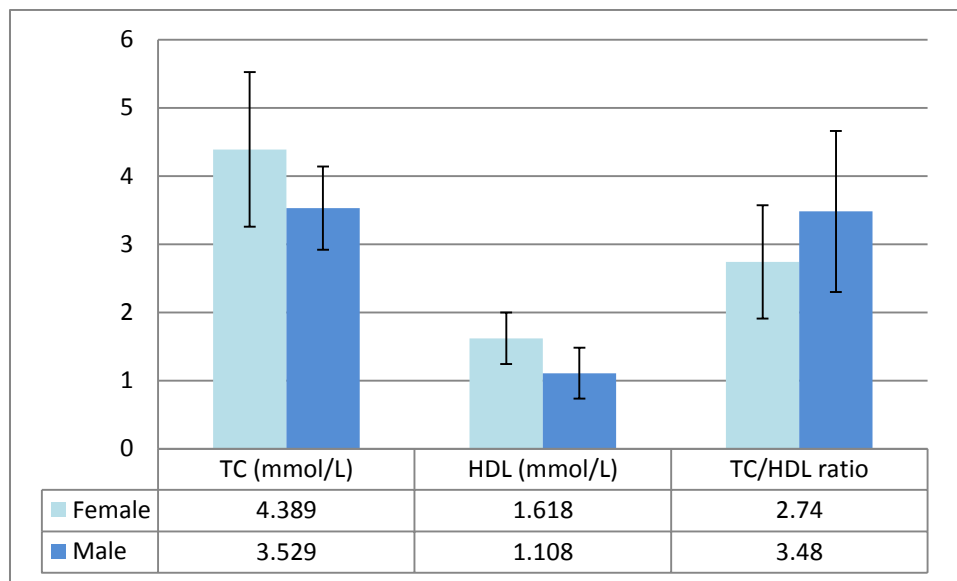
Table 3 shows descriptive statistics of the gender groups. Graph 2 shows the mean TC, HDL and TC/HDL ratio values. Noticeable was that the Independent-Samples T test showed statistically significant differences in all cholesterol values between the gender groups. The female group had a 0,869 mmol/L higher mean TC (Male: $3,520 \pm 0,610$, Female: $4,389 \pm 1,314$), $p < 0,001$. The HDL mean was also higher by the females with 0,510 mmol/L (Male: $1,108 \pm 0,373$, Female: $1,618 \pm 0,373$), $p < 0,001$. The male group had a 0,74 higher mean TC/HDL ratio (Male: $3,48 \pm 1,18$, Female: $2,74 \pm 0,83$), $p = 0,006$.

Table 3 – Descriptive statistics gender

	Female (n=42)	Male (n=29)	P-value
Age (years)	20,9 ± 2,0	21,2 ± 2,8	0,565
Height (cm)	168,46 ± 6,06	180,06 ± 6,32	<0,001
Weight (kg)	62,11 ± 7,22	75,55 ± 10,28	<0,001
BMI	21,87 ± 2,20	23,26 ± 2,69	0,025

NOTE: values are presented in mean ± standard deviation.

Graph 2 – Cholesterol levels gender group



Values presented in mean. Standard deviations are to be seen in the bars.

Discussion

The aim of this study was to determine whether high and low level of exercise intensity have different effects on the TC/HDL ratio in young adults. The results would make it possible to give more accurate recommendations regarding sport activity as prevention for dyslipidemia, a risk factor for Coronary Artery Disease(CAD). The research was executed by examining the total cholesterol (TC), high density lipoprotein (HDL), TC/HDL ratios and level of sport activity of 71 physiotherapy students from the second year at Fontys University of Applied Science. The subjects were divided into three groups, high intensity training (n=47), low intensity training (n=21) and minimal training (n=3). Since the minimal training group only consisted of three subjects, this group was not included when comparing the groups. The groups were comparable according to age and BMI, noticeable was though the higher amount of men compared to women in the high intensity group and vice versa in the low intensity group. There were no statistically significant differences found in the cholesterol levels between the groups. Closest to a significant difference was the TC level, where $p = 0,058$.

Statistically significant differences were though found when comparing the cholesterol levels between the gender groups. The male group had a significant higher BMI mean, (female $21,89 \pm 2,20$, male $23,26 \pm 2,69$, $p = 0,025$). Optimal would have been to compare two gender groups with comparable BMI values, since higher BMI is connected to lower HDL levels and thereby higher TC/HDL ratio.(16) However, comparing these results with the reference intervals of cholesterol levels established in the French population aged 19-64 years (17), similar proportions were present where men had lower HDL values and higher TC/HDL ratios compared to woman. The values were slightly higher than the results of this research (TC – Female: $5,4 \pm 0,5$ Male: $5,4 \pm 0,7$ HDL – Female: $1,7 \pm 0,0$ Male: $1,4 \pm 0,0$ TC/HDL ratio – Female: $3,2$ Male: $3,9$). The difference may be due to the higher age inclusion of the French study, or due to the fact that only physiotherapy students, which we can assume have greater health conscious than the overall population, were included in this study.

A more accurate method would have been to compare the genders separately in the training intensity groups. That would rule out gender differences and give a more comparable result. This was unfortunately not possible due to the small sample size. Out of the 144 initially invited students, only the results of 71 students were eventually included in the study. 50 students did not show up at their

test occasions and 20 students did not, despite multiple reminding emails, fill out the survey with additional information needed to be included in the research. 71 subjects were sufficient to implement the study, even though a bigger research population would have been giving a higher reliability and additionally the possibility to test the gender groups separately. With a larger research population there could be a chance that the minimal training group would be of sufficient size for comparison, that would have given the research additional value.

One of the reasons that there were significant differences in cholesterol levels in many of the trails in the review by Tambalis et al (12) and not in this research could be that the subjects in the trails were of older age, suffered from dyslipidemia or were overweight/obese. Since the subjects of this project were young and healthy, abnormal lipid levels were not expected to be found. That may be a reason why there were no significant differences between the groups. It would be interesting to perform the same test on a group with higher age. Also the fact that the trails (12) were RCT's makes it possible to determine the effects of different exercise intensities better than an observational study. Since the cholesterol values can be of big variance in the subjects, an individual monitoring and comparisons of the changes in cholesterol after an intervention can give more valid results than a one-time measurement where only the cholesterol values are compared.

The MET classification system was used to make an objective assessment of the subjects training/activity habits and divide them into the different training intensity groups. The information was collected using an online survey, where the participants had to fill in their type of sport/activity and active hours in that sport/activity. It was conducted this way to eliminate the risk of the answers being biased as if the subject had to fill in the survey at the test occasion together with the classmates. One flaw of this method was though, as mentioned earlier, that a number of surveys was not filled in. Another aspect to take into consideration is that people perform the same sport differently and there can be big differences in intensity even though the numbers are the same on paper. In future studies a more precise method for measuring sport intensity would be advised to use, such as a training diary and a digital heart rate monitor.

There are several more factors than only sport/activity which are influencing the cholesterol levels(6,9,10). Smoking, diet, alcohol intake and use of cholesterol lowering medication are effecting cholesterol levels but they have not been taken into consideration in this study. There were not many smokers present in the research population, that could be as mentioned earlier – a result of a health conscious student group. Additional information over cholesterol lowering medication was not gathered, since the probability of healthy persons at the age of the participants using that kind of drug is very low. It would have been interesting to include information over diet and alcohol intake, but that would have enlarged the research to an extend which would not have been reasonable to finish within the time frame of this research. However, it would be an interesting aspect to study in combination with cholesterol levels. With a lack of these parameters being included in the research as confounders, there is a possibility that elements additional to the training intensity has influenced the results of the testing. It is almost never possible though to rule out all possible external influences when conducting a research with human test subjects.

Conclusion

From the results of this study it is not possible to determine if high intensity and low intensity training have different effects on TC/HDL ratio. No significant differences were found in the cholesterol levels between the training groups. Further research is needed to determine which type of training is most beneficial as prevention for dyslipidemia and for favourable cholesterol levels in young adults. Hopefully this study will be useful for other researchers and with use of its strengths and limitations conduct further researches in the field of exercise intensity and cholesterol levels.

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Appendix I

Information Letter and Consent Form

Dear student,

You are invited to participate in a study that concerns; screening young adults for risk factors for chronic diseases.

General information:

As a participant in this study, you will be enquired to complete a questionnaire, in which you will be asked to truthfully answer all questions. Thereafter you will be asked to participate in different tests in the exercise lab. Multiple tests will be executed to gather information about body measurements, flexibility, strength, stamina and reactivity. The testing will take approximately one and a half hour and is scheduled as a workshop (FH) in your schedule sometime between 24th march and 8th April 2015. The testing will take place in the exercise lab of Fontys University of Applied Science, Eindhoven. It is important that you as participant follow a couple of clothing prescriptions; clothes and shoes suitable for exercise. Long hair must be tied up and it is essential to remove all kinds of jewelry.

Background / Problem description:

Waist circumference and physical inactivity are risk factors for a.o. pre diabetes mellitus type 2 and cardiovascular disorders. Measuring physical activity, physical fitness and risk factors in a healthy population may increase awareness of health risks. Further, it is interesting to study which factors can be influenced and how, in trying to achieve a lasting healthier lifestyle.

Previous researches have shown that within a student population relatively many risk factors occur. The new Exercise Lab of FPH (Fontys Paramedishe Hogenscholen) offers a range of possibilities to investigate risk factors in young adults. Also, it is possible to follow students throughout their study at Fontys.

Scientific relevancies:

These studies form the beginning of a long term follow-up study. With the results of these studies recommendations can be made for screening and monitoring of young adults with an increased risk on chronic conditions.

Social relevance:

In the light of increasing prevalence of chronic diseases it is important to gain more insight in the presence of risk factors and in how we can modify them in this particular population.

Relevance for the physiotherapy program:

With the new Exercise Lab we can start to integrate clinometric better in the study program. Students will get more insight and get more experience in the use of the different measurement tools.

Personal relevance:

By volunteering for this study, you will learn about psychological research in general and the topic of this study in particular. In addition, you will personally benefit from this research since you can get more insight about your physical status and collect your personal outcomes from the different tests. You may decide to withdraw from this study at any time by counseling one of the researchers and may do so without any penalty.

Participation:

Participation in this research study is voluntary. All information you provide is considered completely confidential; your name will not be included or in any other way associated with the data collected in the study. Data collected during this study will be retained indefinitely, only researchers associated with this study will have access to the given information. There are no known or anticipated risks associated with participation in this study.

Thank you for your assistance in this project, we are looking forward to meet you during your participation of this research.

With kind regards

The research team

Consent form

I agree to participate in this study conducted by Andrea Gustafsson, Miriam Myrsten, Niels van Hoof and Ivo van Knippenberg, students of the Fontys University of Applied Sciences. I have made this decision based on the information I have read in the information letter and have had the opportunity to receive any additional details I wanted about the study. I understand that I may withdraw this consent at any time by telling one of the researchers. I am aware that the data which is being collected is and may be used in current and future researches.

Participant's Name _____

Participant's Signature _____ Date _____

Title of Project: Screening young adults for risk factors for chronic diseases

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Appendix II

“Screening young adults for risk factors for chronic disease”

Questionnaire

Number: _____ (filled in by researcher)

Male/Female

Age: _____

Please circle the most suitable answers describing you and your lifestyle:

I am asthmatic: Yes/No

I was asthmatic during my childhood: Yes/No

I am allergic to grass/pollen: Yes/No

I have diabetes: Yes/No

I have been ill during the past 10 days: Yes/No

I am taking medications interfering with my respiratory function: Yes/No

Hand dominance: Right handed/Left handed

I have been injured the last 3 months and not been able to participate as I normally do in my regular sport/physical activity: Yes/No

Smoking habits:

Not smoking, Yes - 1-15 cigarettes a week, Yes - 16-50 cigarettes/week Yes >50ciggarets/week.

Do you perform any sport or physical exercise? Yes/No

If Yes; which sport/activity? _____

How many hours/week are you performing your sport/activity? _____

For how long have you been performing your sport/activity? 0-3 months / >3 months

Thank you for your cooperation!

Appendix III

Protocol Cholesterol test

CardioChek® PA, test strips measuring TC & HDL and MEMo Chip®.

1. Prepare CardioChek®
2. Inform the test subjects about the procedure
3. Put on gloves
4. Ask the test subject to rub his/her non-dominant hand if the index and/or middle fingers are cold
5. Clean the finger with alcohol and dry it
6. Hold the lancet tight and prick the finger at the side, ask the patient to stand up so the finger will be placed below the heart
7. Remove the first blood drop and fill thereafter the absorption pen. If there is not enough blood to fill the tube, press gently on the hand and finger. **Notice:** Pressing too hard can destroy the blood cells and therefore alter the test result.
8. Apply the blood sampled on the test strip.
9. Give the subject a plaster.
10. Wait for result, +/- 2 minutes. Note the result on the test result sheet.