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„Predictive value of testosterone as marker for cardiovascular disease and overall mortality”

Dissertation

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1 Introduction

1.1 Cardiovascular Disease Epidemiology

With 17.9 million annual cases of death in 2016, cardiovascular diseases (CVD) (coronary heart disease (CHD), cerebrovascular disease, peripheral-arterial-disease (PAD), rheumatic- and congenital-heart-disease, deep-vein-thrombosis, pulmonary-embolism) is attributable for one third of all causes of death around the world (Joseph et al, 2017) and thus have great importance for the World Health Organisation. Globally, death due to CVD “has increased during the past decade by 12.5%” (Joseph et al, 2017), especially in southern countries and Asia due to the growing and aging nations (Joseph et al, 2017).

However, during the last 26 years, the age specific decline in mortality due to CVD is higher in countries with high-income and therefore the highest economic standard of living compared to low and middle-income countries (Joseph et al, 2017).

The decreasing mortality rate and the increasing size of the elderly population is followed by an increasing CVD-related disability status with substantially increasing health expenditures, productivity loss all over the world for individuals and economies (Heidenreich et al, 2011).

The reason for this increase in CVD can be found mostly in lifestyle-changes during the last 70 to 80 years: Unhealthy diet, lack of exercise, non-moderate alcohol- and tobacco-use have contributed to the metabolic syndrome (adiposity, arterial-hypertension, lipid-metabolic-disorder with hyperlipidaemia, insulin-resistance and glucose intolerance) (Mendrick et al, 2018).

The WHO presented the "Global Monitoring Framework" in 2013 as a plan that includes "9 targets and 25 indicators" to achieve a sustainable prevention and control of CVD, cancer, chronic lung disease and T2D to reduce mortality and morbidity of CVD up to 25% by the year 2025 (Joseph et al, 2017).

Since the commencement of counting in 1980, the prevalence of type 2 diabetes mellitus (T2D) has increased fourfold (NCD Risk Factor Collaboration (NCD-RisC), 2016). Being one of the most important global causes for disability, T2D and its complications are “responsible for over two million deaths every year” (NCD Risk Factor Collaboration (NCD-RisC), 2016)

With atherosclerosis as the underlying cause of CVD, it can take decades to develop symptoms of CVD as well as T2D.

Thus CVD can establish, it is necessary to apply customized lifestyle modifications for those individuals at risk. Additionally, every government can offer individual opportunities to improve primary prevention programs (tobacco-control-policies). More precise methods of CVD risk

prediction models and early detection need to be invented to determine the risk for those at high total cardiovascular risk. (Damen, Johanna A A G et al, 2016 May 16).

1.2 Risk factors and risk prediction

Among those key points of the Global Monitoring Framework, "abnormal lipids, smoking, hypertension, T2D/insulin resistance, abdominal obesity (body mass index > 30kg/m²), psychosocial factors, insufficient consumption of fruits and vegetables, alcohol-consumption and irregular physical activity are powerful predictors of atherosclerosis, myocardial infarction, stroke and cardiovascular morbidity and also have great excess in mortality (Damen, Johanna A A G et al, 2016 May 16).

These factors are summarized as biomarkers, defined in 1989 as: “a measurable and quantifiable biological, physiology related or imaging parameter” (Ruwanpathirana et al, 2015)

Biomarkers can facilitate screening, diagnosis and prognosis of probands with and without known CVD. As acute myocardial infarction (AMI) or heart failures are very complex and heterogeneously presenting diseases, an early and rapid evaluation of myocardial-necrosis or heart-failure requires biomarkers with good discrimination and fast analysis. Accordingly, objective assessments using cardiac biomarkers like Troponin I and NT-pro-BNP have become a common attribute to clinical examinations (Jenny et al, 2016).

In addition to the acute setting of a chest pain unit, biomarkers can be useful regarding the risk estimation and prevention of CVD.

The restricted comparability of over 360 risk prediction models for non-fatal CVD leads to an uncertain usefulness, as they are only partially comparable with each other (Damen, Johanna A A G et al, 2016 May 16).

The most included predictive variables from all existing multivariable models among others are sex, age, tobacco use and lipid biomarkers. The more factors are included, the more laborious and time and cost consuming the trial becomes.

Several epidemiological studies like the INTERHEART study the INTERSTROKE study as well as the Framingham Heart Study (FHS) proved their performance as a risk estimation system with clearly defined endpoints and within a representative population. However, even after extern validation, accepted models like the Framingham Risk Score (FRS) or the United Kingdom Prospective T2D Study (UKPDS) model show only limited power for cardiovascular risk estimation (Cooney et al, 2009).

Based on known cardiovascular risk factors, these models classified at least 15 –20% patients as low risk, although they have suffered from MI (Khot et al, 2003).

This demonstrates the need of novel biomarkers to improve risk prediction. The prediction due to a new biomarker might add new information which have not been explained by verified cardiovascular risk factors. Besides a statistically significant association, the studies need to pay attention to requirements like discrimination, calibration and reclassification.

1.3 Difference between risk-factor and risk-marker

Risk factors and risk markers increase the risk for CVD. However, it is important to distinguish them. A feature of an individual is called a risk-marker, if it can be correlated to CVD but has not been proven to be the cause for CVD. Therefore, it only predisposes to CVD. Some risk marker like adiposity deserve a special attention as it is the results of hyperlipidaemia and therefore indirectly participate in the development of CVD.

Attributes which have been demonstrated to be causally related to CVD (e.g. hypertonia or hyperlipidaemia) are called risk factors (Hackett and Kirby, 2018).

Modifiable risk factors are part of the individual lifestyle. It has been demonstrated that changing these risk factors can modify the cardiovascular risk. Taking some precautions and changing unhealthy habits reduce the risk for CV-events. This includes a healthy diet, reducing alcohol consumption, regular physical exercise, avoiding smoking and reducing weight. However, these modifiable risk factors like hyperlipidaemia or hypertension are mostly not noticed by patients and their physicians until irreversible damage has already been caused to organs. Over the last years, a lot of assessment tools for these factors have been developed. Among those, the British interactive JBS3 tool or the FRS as well as other observational studies include very little data from patients younger than 40 years. But those can be already at increased risk and need to be considered particularly.

1.4 Testosterone

1.4.1 Anatomy and Regulation

Testosterone is based on cholesterol which is derived from acetate or directly resorbed from blood. The hormone is delivered from low density-lipoprotein (LDL) and stored in lipid-drops.
In men, the suprarenal glands as well as Leydig-cells produce dehydroepiandostendione which is converted into andostendion by suprarenal-glands and into andostendiol by testicles. These two very similar intermediate states are converted into testosterone (Heinrich et al, 2014) (Steinhausen, 1984) .

The woman’s corpus luteum produces testosterone in a very limited dose during the productive years using it as an intermediate product for the synthesis of estrogen. Circulating

androstenedione and dihydroepiandostendione (DHEA), which are synthesized by the ovaries as well as adrenal glands, can be transformed by aromatase into estrogen. The concentration of testosterone increases in girls with the age of 6 to 8 years, leading to the highest level in the middle of ovulation (Ilondo et al, 1982).

40% of all circulating testosterone is bound to Sex Hormone Binding Globulin (SHBG). 58% are bound to Albumin and the remaining 0.5% –2% circulate in its free form. The albumin plasma concentration is 1000 times more stable compared to the concentration of SHGB, although the affinity of testosterone to Albumin is three to four times weaker compared to SHGB. Therefore SHGB is very important for the dynamics of TT (Hammond, 2016). The free testosterone (fT) is biologically active. It is supplied by albumin-bound testosterone. The biological activities of albumin and SHGB bound testosterone are subject to discussion (Hammond, 2016). FT needs to split from albumin to become biologically active. Changes in SHBG or albumin have an influence on the TT level.

Patients with reduced albumin concentrations as seen in Acquired Immune Deficiency Syndrome (AIDS), cancer, cirrhosis of the liver, nephrotic syndrome and T2D have been associated to lower testosterone levels (Dunn et al, 1981). Usually, albumin buffers testosterone due to transient changes of testosterone as well as SHBG (Hammond, 2016).

Normal values for fertile women are < 2.1 nmol/L and < 2.8nmol/L for postmenopausal women and for men: 12 –30 nmol/L (Siegenthaler and Aeschlimann, 2005).

The total testosterone (TT) synthesis and secretion is regulated by a complex interaction between hypothalamic hormones and pituitary hormones. Incoming stimuli from the central nervous system trigger the secretion of gonadotropin-releasing-hormone (GnRH) which itself triggers the secretion of luteinizing hormone (LH). LH binds to a special receptor on Leydig-cells promoting the production of enzymes. These enzymes are necessary for testosterone synthesis. Both testosterone as well as estradiol lower the secretion of LH with a negative feedback on the Hypophysis where LH is produced (Heinrich et al, 2014).

GnRH, LH and testosterone follow a rhythmic and pulsatile secretion with 17 –18 testosterone pulses within 24 hours (Steinhausen, 1984). Monthly and seasonally changes of TT values have been demonstrated in several cross-sectional studies for both genders which differ in terms of social and ethnical matter as well as geographical location. While all studies confirm TT peak levels in the early morning (6–8 AM: 26 –28 nmol/L) and lower levels in the evening (6 –8 PM: 17 nmol/L), the results regarding seasonal variation of TT show heterogenic results. A seasonal variation of androgens can be assumed, but further investigation is needed (Gupta et al, 2000; Davis and Wahlin-Jacobsen, 2015).

1.4.2 Hypogonadism

Hypogonadism describes the symptoms resulting from testosterone levels beneath the lower limits of young healthy men between the age of 20 and 30 years. The insufficient production of testosterone can affect testicles (primary hypogonadism) or the hypothalamus /pituitary axis (secondary hypogonadism) as well as both organs. Both can appear at any age and hypogonadism can also appear to an organ specific insensitivity or resistance for testosterone.

1.4.2.1 Late Onset Hypogonadism

The late-onset hypogonadism (LOH) affects aging men. It combines testicular (primary) hypogonadism with hypothalamic-pituitary (secondary) hypogonadism. According to the Baltimore Longitudinal Study of Aging, “20% of men over 60 years, 30% over 70 and 50% over 80 years” show hypogonadal TT (Fink et al, 2014). Of those aging men who present a suppressed serum testosterone-level in the morning. only a small part develops the genuine syndrome of hypogonadism with diffuse sexual. physical and psychological symptoms (Davis and Wahlin-Jacobsen, 2015).

As LOH symptoms and testosterone levels rarely occur simultaneously. the European Male Aeging Study (EMAS) determined LOH as the combination of low TT (< 11 nmol/L), fT (< 220 pmol/L) and three sexual symptoms namely -erectile dysfunction, less frequently sexual thoughts and fewer morning erections. Other concurrent reasons for hypogonadism have to be excluded before (Dimopoulou et al, 2016).

Non-sexual symptoms involve fatigue, low energy as well as weakness, decreased bone-density and less muscle mass, physical frailty and depression (Fink et al, 2014). These are symptoms nonspecific for testosterone deficiency but they lower life quality.

Women show decreasing testosterone levels beginning with the age of 20 to 30. Low levels of TT may be associated with psychological as well as physical symptoms. After menopause TT is an important source for estrogen via aromatization (Petering and Brooks, 2017).

1.4.3 Results of Testosterone Treatment

Despite inconsistent results on bone mineral density, cognitive function and muscle strength, the treatment with TT has strongly raised especially in the USA during the last years. The TT industry targets especially aging men suffering from unspecific and varying symptoms (Petering and Brooks, 2017). More than three million prescriptions worth more than $1 billion in 2012 demonstrate the impact of the TT -business in the USA (Petering and Brooks, 2017).

The application of TT in patients with low testosterone levels, induced by primary or secondary hypogonadism such as Klinefelter's syndrome, pituitary injury or loss of testicles or medical conditions has been proven by the Food and Drug Administration (FDA) (Center for Drug Evaluation and Research). However, the FDA refrains to give a statement regarding the safety of testosterone application if no other reason than aging can be found to explain a low level of TT (Petering and Brooks, 2017).

1.4.4 Testosterone Replacement and Cardiovascular Risk

High levels of circulating TT have been associated with an increased risk for CVD as seen in users of anabolic androgenic steroids (Vanberg and Atar, 2010). In 2010 the randomized controlled Testosterone in Older Men with limited mobility trial (TOM-trial) (Basaria et al, 2010) was stopped due to an increased number of cardiovascular events in patients treated with testosterone compared to the placebo-group. The FDA gave serious considerations to a possibly increased cardiovascular risk and higher risk for mortality during testosterone treatment (Petering and Brooks, 2017). These findings launched a strong debate about the advantages and disadvantages of testosterone therapy. While in 2013 and 2014 the retrospective cohort study of Finkle et al. (Finkle et al, 2014) as well as the large trial of Vigen et al. (Vigen et al, 2013) found a higher rate of adverse cardiovascular events during testosterone therapy, two meta-analyses (Haddad et al, 2007 Jan), (Corona et al, 2014) that included observational trials, could not find an increased risk in men treated with testosterone. Finally, another meta-analysis of 2013 claimed that the results of the studies regarding the impact of testosterone therapy depended on the source of financing. Trials which have not been sponsored by the pharmaceutical industry have found an adverse effect of testosterone application on the cardiovascular system (Xu et al, 2013).

The possibly rejuvenating agent testosterone has been recommended hastily without having a full knowledge of both the impact of endogenous testosterone on the cardiovascular system and the imbalance of hormone homeostasis by applicating testosterone. Participating trials have been very heterogenous and differed in patient characteristics, formulation, dose and therapeutic length of testosterone (Petering and Brooks, 2017; Xu et al, 2013).

The current contradictive state of studies does not allow a clear answer whether testosterone application is beneficial or harmful for the cardiovascular system. Large and standardized trials are needed. In the first instance it is necessary to understand the impact of testosterone in healthy individuals on risk prediction.

1.4.5 Predictive Value of Testosterone for CVD and Overall Mortality

Age and male sex are strong risk factors for coronary artery disease (CAD) (Niccoli and Partridge, 2012; Hyun et al, 2017). Testosterone in men declines with increasing age (Burger et al, 2000). Young to middle aged women produce ten times less testosterone (Heinrich et al, 2014) and show a distinctly reduced risk for cardiovascular events compared to men equally aged (Hyun et al, 2017). Due to the rapid decrease of estrogen in menopausal women, the estrogen-testosterone ratio changes to the benefit of testosterone. These women show an increased risk for cardiovascular diseases. Therefore, it is suggested that testosterone plays an important role in the development of CVD.

Inspired by heterogenous results of testosterone therapy on the cardiovascular system, several investigations have been set up to assess the possible association between endogenous testosterone levels and CVD and to understand testosterone induced effects. A growing number of studies suggests that men with lower levels of testosterone and women with higher levels of testosterone are more predisposed to develop CVD (Vikan et al, 2009; Ohlsson et al, 2011; Schaffrath et al, 2015) and coronary heart diseases (CHD) (Zhao and Li, 1998) (Rosano et al, 2007). Some trials have found a positive association between testosterone and the severity of CAD (Dobrzycki et al, 2003). Besides, lower testosterone is assumed to be associated with a higher risk of developing type 2 diabetes mellitus (T2D) in men (Vikan et al, 2010; Grossmann et al, 2015; Hamilton et al, 2016; Schipf et al, 2011). While higher levels of testosterone have a negative impact on T2D2 in women (Davis and Wahlin-Jacobsen, 2015; Muka et al, 2017), men with low testosterone levels show a higher risk for stroke, ischemic stroke (IST) and transient ischemic attack (TIA) (Yeap et al, 2009), atrial fibrillation (AF) (Rosenberg et al, 2018) and a higher risk for overall mortality (Shores et al, 2012; Araujo et al, 2011; Khaw et al, 2007; Meyer and Wittert, 2018; Schaffrath et al, 2015; Sievers et al, 2010). Traditional lipid -risk -factors for CVD have been associated with low testosterone levels (Yarnell et al, 1993). In contrast, several trials have not found an association for CVD and mortality (Araujo et al, 2011; Meyer and Wittert, 2018), but for additional cardiovascular risk factors as T2D, obesity and the metabolic syndrome (Zhao et al, 2014).

The Framingham-Risk-Score (FRS) is a very popular tool for risk prediction in asymptomatic as well as symptomatic patients regarding further cardiovascular events within 10 years. However, men younger than 40 years or ethnical minorities of the western world are not taken into consideration. Moreover, there are no questions about family history, fasting glucose, testosterone level or erectile dysfunction (Araujo et al, 2010). To prevent cardiovascular events in middle aged to older men and women, it is necessary to recognize healthy probands with an increased risk early to start prevention in time. Several risk factors of the FRS are affected

by testosterone or affect testosterone. Higher levels of testosterone have shown to be independently associated with lower values of the FRS (Chock et al, 2012).

In summary, the impact of testosterone on the cardiovascular system and mortality is very controversial. There is lack of sufficiently powered prospective and longitudinal population-based trials to find out whether testosterone has impact on cardiovascular health and if it could even be an independent risk factor for risk -prediction -models.

1.4.6 Aim of the study

The following dissertation describes the predictive value of total testosterone for I. type 2 diabetes mellitus, II. atrial fibrillation and/or ischemic stroke, III. overall mortality, IV. coronary heart disease. Additionally, the correlation of testosterone and age as well as other cardiovascular risk factors is determined. The strengths and limitations of FINRISK are described as well.

2. Material and Methods

2.1 BiomarCare

Coordinated by the UKE Hamburg, The **Biomar**ker for **Ca**rdiovascular **R**isk Assessment across **E**urope (BiomarCaRE) determines the significance of impact of various biomarkers for cardiovascular risk prediction. Biomarker levels are measured in a centralized biomarker laboratory to standardize measurement conditions. Within BiomarCaRE, biomarker levels as well as epidemiological and clinical data are harmonized in a large database.

The FinRisk97 Study is part of the BiomarCaRE project, co-funded by the European Union and focused on biomarker research for cardiovascular risk prediction with the objective to develop a “European biomarker panel” focusing on Risk estimation for CVD due to established and new biomarker (Blankenberg et al, 2010).

2.2 Definition of Endpoints with BiomarCaRE

Endpoints for this thesis were defined as: receiving the diagnosis of type 2 diabetes mellitus (T2D), coronary heart disease (CHD) (Def.4), atrial fibrillation (AF), ischemic stroke (IST), or mortality within the observation period and after the probands had been enrolled in the study.

CHD (Def.4) included: first myocardial infarction, coronary death, hospitalised unstable angina pectoris and any coronary revascularisation (percutaneous transluminal coronary angioplasty or coronary bypass surgery) (Hughes et al, 2014).

All individuals who already had the endpoint as prevalent disease or reported as prior disease were excluded from further calculations for that particular endpoint but not for all other analyses with other endpoints.

2.3 Study population

The FINRISK studies are large, independently sampled, prospectively followed and representative population cohort studies from Finland. They are set up every five years to compare significant risk factor changes for non-communicable diseases, and changes in health behavior (Borodulin et al, 2015). By monitoring risk factors, it is possible to observe risk factor levels and their influence on morbidity and mortality from CHD (Cornoldi et al, 2010).

In 1997, 8444 subjects participated in the FINRISK 97 study for clinical examination. Aged from 25 to 74 years, the individuals were randomly chosen from the six regions of Finland, namely Helsinki and Vantaa (the metropolitan area), Turku and Loimaa, Northern Savo (former Kuopio), North Karelia, Oulu Province and southwestern Finland.

FINRISK 97 was followed up for over 13 years with a follow up rate of 99.5%. 0.5% moved abroad and were not considered for calculations anymore.

Pregnant women as well as persons receiving testosterone supplementation or having cancer were excluded from further analyses. Additionally, probands who have already had the specific endpoint at baseline, were excluded (Cornoldi et al, 2010). The longest observation period was 13.9 years and the median 13.8 years.

The FINRISK-survey was granted by the Ethics Committee of the National Public Health Institute and performed in accordance with the Declaration of Helsinki.

2.4 Laboratory measurements

Blood was taken from each subject after a fasting period of at least four hours. The blood was stored at -80°C in the THL (National Institute for Health and Welfare) biobank. Helsinki, Finland until the analysis of specific parameters was carried out (Vartiainen et al, 2000).

2.4.1 Testosterone within FINRISK 97

Testosterone levels were analyzed in the BiomarCaRE Laboratory (University Heart Center Hamburg, Germany). The serum was used to quantify TT by a chemiluminescent-microparticle-immunoassay using the ARCHITECT i2000sr (ARCHITECT 2nd Generation Testosterone; Abbott Diagnostics, Wiesbaden, Germany)

Chemiluminescent microparticle immunoassay (CMIA) makes it possible to detect substances in the blood, using the competitive antigen-antibody principle.

Using this method, an anti-testosterone antibody reacts with two antigens which are added to the fixed antibody one after another. One of these antigens is part of the testosterone molecule and directly binds to the anti-testosterone antibody, while the other competes with the first antigen for a binding site. Pre-trigger (acid, H2O2) as well as trigger complement the mixture leading to a chemiluminescent reaction. The measured relative light units are proportional to the sample's TT level and have to be compared to a calibration curve. In FINRISK 97, the limit for detection of TT was determined as 4.33 ng/dL and the measuring range reaches from 4.33 to 1500 ng/dL (Marquette and Blum, 2009).

In FINRISK 97 the inter-assay coefficient of variation (CV) was determined between 2.35 to 3.57 % and the intra-assay CV between 8.75 to 10.93%.

Quality controls were performed daily before and after sample measurements to achieve comparable results. Calibrator measurements were performed weekly. These calibrators as well as control samples target specific TT levels within the possible range (0 –15.0 ng/mL) of measurement.

All calibrators were provided by Abbott Diagnostics and had to be tested against internal standards before using them as calibrators.

The ARCHITECT Testosterone Reagent Kits were stored at 2 –8 °C with no reagents used after the expiration date (; M. Moustapha, K. Hoad, BR. Cooke, C.Mandelt).

2.5 Clinical parameters

Additional information about established cardiovascular risk factors were collected by questionnaire. Patients were asked if they smoked, if they knew about an arterial hypertension, or if they took any drugs.

The questionnaire, as well as the invitation to the health examination were released by email to all chosen subjects. Anthropometric measurements and blood sampling were performed by specially trained nurses in local health centres or other survey sites, after written informed consent by all participants were collected.

In each local centre, the data was coded and analysed. After the preparation which was conducted according to standard formats, all information were sent to the Data Centre.

Information about mortality or incident/prevalent endpoints were supplied by the National Hospital-Discharge Register (NHDS), the Cause of Death Registry, Cancer Registry, and the Drug Reimbursement Records from the Social Insurance Institution of Finland (Pajunen et al, 2005) (Sund, 2012).

2.6 Statistical analyses

With multiple imputation techniques and the use of the WinBUGS software, it was possible to create complete datasets and prevent the loss of estimation power (Moons et al, 2006; Blankenberg et al, 2010). Missing values (n =875) were calculated with MACE (Multivariate imputation by chained equations) (Azur et al, 2011).

In Table 1, all variables are presented as counts and percentages separated for each sex.

The asymmetric distribution of total cholesterol made it necessary to logarithmically transform TT as well as high density lipoprotein (HDL). Afterwards it was possible to create age-adjusted Pearson correlation coefficients.

TT was used as a continuous as well as categorized and untransformed variable for men and as a transformed variable for women. To compare high versus low levels, TT was categorized into quartiles. After separation for sex, Kaplan-Meyer survival analysis curves were set up with age on the x-axis.

Cox regression models were calculated for continuous as well as categorized TT, each separated for gender.

To incorporate possible confounding factors, three different evaluations were implemented, described as model 1, model 2 and model 3.

The first model considers only the geographical region of Finland, age and sex. Model 2 takes total cholesterol, HDL, systolic blood pressure, hypertension, current smoking, waist-to-hip ratio, body mass index and time of blood draw into consideration. Model 3 takes all confounding factors of model 2 and additionally time of blood draw into consideration. For this purpose, total-cholesterol and HDL need to be log-transformed (Zeller et al, 2018).

In order to evaluate the validity of the cox regression models, the goodness of fit was represented by C-Index.

R version 3.3 (R Foundation for Statistical Computing, Vienna, Austria) was used for all analyses.

A p value <0.05 was considered statistically significant and all analyses were two-tailed.

3. Results

3.1 Study characteristics

Table 1 displays relevant multiple imputed clinical characteristics of FINRISK 97. A total of 8444 samples were assayed at baseline, distributed equally for men and women. 49.7% of all individuals were men. The mean age of men was 49.8 years, the mean age for women was 47.62 years. TT levels were much higher in men compared to women 17.10 nmol/L (12.87 – 22.04) versus 1.15 nmol/L (0.87 – 1.56). Body-Mass-Index (BMI), Waist-To-Hip-Ratio (WHR), use of hypertensive medication and systolic blood pressure were higher in men compared to women (see Table 1). Mean total cholesterol levels were almost equally distributed among both genders. Although mean HDL-C levels were lower in men, smoking was much more popular in the male group (see Table 1).

During a median follow-up of 13.8 years, a total of 637 incident T2D cases (7.8 %) (389 men and 248 women) as well as a total of 554 incident CHD cases (Def. 4) (6.8 %) (397 male and 157 women) were registered. Moreover, 991 deaths (12.1%) (673 men and 318 women) occurred. 326 (4%) strokes (213 male and 113 female) as well as 454 cases of AF (5.6%), (287 men and 167 women) appeared within 14 years. During 14 years of follow up, twice as many men as women developed a CHD, a stroke or died (Table 1).

Table 1 displays the multiple imputed baseline-characteristics for each endpoint before statistical analyses.

Table 1: Characteristics of subjects in FINRISK 97

	ALL	MEN	WOMEN
n	**8444**	**4064**	**4380**
Age (years)	48.67 (22.08)	49.8 (23.26)	47.62 (21.25)
Male gender (%)	4064 (49.7%)	4064 (100%)	0 (0%)
Cardiovascular risk factors			
Current smoker (%)	1772 (21.7%)	1063 (26.2%)	709 (17.2%)
Hypertension medication (%)	1381.05 (16.89%)	766.1 (18.85%)	614.95 (14.96%)
4064 Body mass index (kg/m²)	26.15 (5.64)	26.62 (4.87)	25.58 (6.41)
Systolic BP (mmhg)	134 (27)	137 (26)	130 (27)
Biomarker			
Testosterone (nmol/L)	8.04 (16.34)	17.01 (9.17)	1.15 (0.69)
Total cholesterol (mmol/L)	5.4 (1.4)	5.5 (1.4)	5.4 (1.5)
HDL (mmol/L)	1.35 (0.49)	1.22 (0.4)	1.51 (0.47)
Outcome			
Prevalent T2D (%)	469 (5.7%)	254 (6.2%)	215 (5.2%)
Prevalent MI (%)	326 (4%)	246 (6.1%)	80 (1.9%)
Prevalent stroke (%)	212 (2.6%)	131 (3.2%)	81 (2%)
Prevalent CVD (%)	504 (6.2%)	354 (8.7%)	150 (3.6%)
Prevalent AF (%)	78 (1%)	61 (1.5%)	17 (0.4%)
DEATH (%)	991 (12.1%)	673 (16.6%)	318 (7.7%)
Incident CHD (Def. 4)	554 (6.8%)	397 (9.8%)	157 (3.8%)
Incident stroke (%)	326 (4%)	213 (5.2%)	113 (2.7%)
Incident AF (%)	454 (5.6%)	287 (7.1%)	167 (4.1%)
Incident T2D (%)	637 (7.8%)	389 (9.6%)	248 (6%)

For continuous variables, median (25th percentile; 75th percentile) are shown. For binary variables percentage is given, BMI = body mass index, HDL-C = high-density lipoprotein-cholesterol, T2D = type 2 diabetes mellitus; AF= Atrial Fibrillation, Systolic BP= Systolic Blood Pressure, MI= Myocardial Infarction, CVD= Cardiovascular DiseaseCHD (Def.4)= First myocardial infarction, coronary death, hospitalized unstable angina pectoris and any coronary, revascularization (percutaneous transluminal coronary angioplasty or coronary, bypass surgery)

Table 2 describes the classification of TT values into quarters separated for sex.

Table 2 Classification of TT (normalized median values)

	Quarter 1 (nmol/L)	Quarter 2 (nmol/L)	Quarter 3 (nmol/L)	Quarter 4 (nmol/L)
Men	0 –12.82	12.82–17.01	17.01–21.99	21.99–35.0
Women	0 –0.87	0.87 –1.15	1.15 –1.56	1.56 –35.0

Figure 1 and **Figure 2** describe the distribution of TT values in FINRISK 97 separated for sex

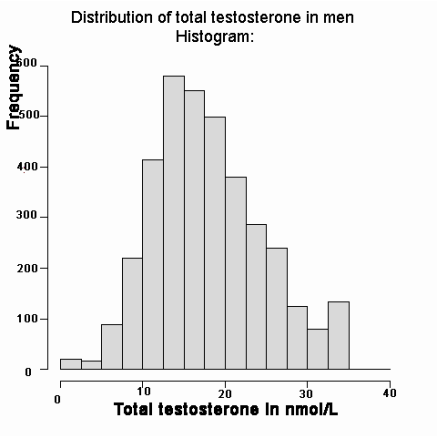

Figure 2: Distribution of total testosterone in men

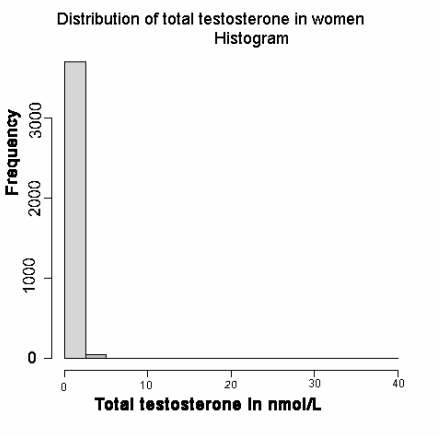

Figure 1: Distribution of total testosterone in women

3.2 Pearson Correlation Coefficient for TT and clinical variables

Table 3 explores the correlation between age-adjusted total testosterone and clinical characteristics by Pearson correlation coefficients.

Smoking men had higher levels of TT (R = 0.09; p < 0.001). Besides, increased levels of TT were associated with higher levels of HDL (R= 0.21. p < 0.001). Men with lower TT had a higher BMI (R= -0.23; p<0.001) and a higher WHR (R= -0.21; p<0.001). Women with higher TT levels showed an increased level of systolic blood pressure (R= 0.04; p = 0.046). Despite late-onset hypogonadism, age itself did not show a correlation to TT levels in men, but a slight correlation with baseline TT in women (R = 0.04; p = 0.012). Age was positively associated with TT level. Based on the results, body configuration, smoking and HDL-C did not show an association with TT levels in women. Interestingly, higher TT levels in men were associated with lower systolic blood pressure (R= -0.04; p = 0.025), whereas higher TT levels in women were related to higher blood pressure (R = 0.04; p = 0.046). Total cholesterol was not statistically associated with TT in both genders. Blood which was collected before 2 PM showed higher TT levels in both genders (**Table 3**).

Table 3: Age-adjusted Pearson correlation coefficients of testosterone levels with clinical variables.

Clinical variable	Men	Women
Time of day of the blood draw	−0.11	−0.03
p-value	<0.001	<0.001
Age (crude analysis)	0.02	0.04
p-value	0.19	0.012
Smoking	0.09	–0.01
p-value	<0.001	0.69
Total cholesterol	0.009	−0.01
p-value	0.98	0.43
HDL-C	0.21	−0.03
p-value	<0.001	0.052
Systolic Blood Pressure	−0.04	0.04
p-value	0.025	0.046
BMI	−0.23	0.03
p-value	<0.001	0.13
WHR	−0.21	0.03
p-value	<0.001	0.098

BMI = body mass index. HDL-C = high-density lipoprotein-cholesterol, T2D = type 2 diabetes mellitus, WHR = waist-to-hip-ratio.

3.3 Cox-Regression for T2D

Observation of 14 years offered an association of TT and T2D in Model 1. Based on the results, men with higher TT at baseline and adjusted for age as well as adjusted for region of Finland have a reduced risk for upcoming T2D. Men with TT in the lowest quartile were more likely to develop T2D compared to the highest quartile of TT (HR: 2.65; 95% CI: 1.89 – 3.69; $p < 0.001$). Therefore, adipose men with lower TT levels, low HDL levels and higher systolic blood pressure showed the highest risk for T2D. (**Table 4, Figure 3, Figure 4**).

While the region of Finland did not seem to have an influence on the development of T2D (HR: 0.98; 95% CI: 0.79–1.21; $p = 0.85$), higher total cholesterol (HR: 3.63; 95% CI: 2.02–6.53; $p < 0.001$), higher Waist-Hip-Ratio (HR: 8317.68; 95%CI: 1384.26 –49979.04; $p < 0.001$) and higher systolic blood pressure (HR: 3.82; 95% CI:1.71 –8.53; $p < 0.0011$) were associated with a higher risk for incident T2D, while increased HDL was associated with a reduced risk for T2D. Smoking (HR: 0.97; 95% CI: 0.75 –1.25; $p= 0.81$) as well as blood draw before 2 PM (HR: 0.76; 95% CI: 0.62–0.94; $p = 0.012$) did not change the risk for T2D **(Table 5)**.

Women however showed opposite results. Increasing testosterone levels showed an increased risk for TD2 (HR: 1.18; 95% CI: 1.03 – 1.35; $p = 0.021$). TT in the lowest quartile presented a lower hazard for future T2D compared to the highest quartile (HR: 0.53; 95% CI: 0.37 – 0.77; $p = 0.003$) (Table 4). After additional adjustment for cardiovascular risk factors (model 2) and time of blood draw (model 3), the statistical significance risk for lower TT levels in 1 quartile vs. quartile 4 (HR: 0.72; 95% CI: 0.49 – 1.05; $p = 0.091$) as well as the statistical significance of continuous increasing testosterone levels (HR: 1.03; 95% CI: 0.89 – 1.20; $p = 0.65$) got lost (**Table 4, Figure 3, Figure 4**).

Increasing cholesterol levels, increasing systolic blood pressure levels, increasing intake of antihypertension medication as well as increasing Waist -Hip ratio showed an increased risk for incident T2D in women regarding the continuous calculation of testosterone. The earlier the blood has been taken, the lower the risk for T2D has been (for continuous testosterone). In categorized testosterone, only HDL, the intake of antihypertensive medication, smoking and Waist- Hip Ratio showed an increased risk for incident T2D **(Table 6)**.

The results showed that the association of TT and T2D remained statistical significance in men and lost significance in females after adjustment for cardiovascular risk factors (**Table 4,5)**. Higher levels of HDL, higher cholesterol levels as well as higher Waist-Hip ratio and increasing intake of antihypertension of medication increased the risk for both gender to develop T2D. Smoking did not chance the risk for incident T2D in men, but increased the risk for incident T2D in women.

Table 4: Cox Regressions: TT and T2D for men and women

Men (n=3810). T2D events (n=363)								
Continous TT		**Categorized TT**						
		Quarter 1		**Quarter 2**		**Quarter 3**		
HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**Model**
0.95 (0.93. 0.97)	<0.001	2.65 (1.89. 3.69)	<0.001	1.93 (1.37. 2.73)	<0.001	1.19 (0.80. 1.76)	0.40	**Model 1**
0.98 (0.96. 0.99)	0.010	1.50 (1.06. 2.12)	0.023	1.29 (0.91. 1.83)	0.15	0.99 (0.66. 1.47)	0.95	**Model 2**
0.97 (0.96. 0.99)	0.0059	1.56 (1.10. 2.21)	0.014	1.33 (0.94. 1.89)	0.11	0.99 (0.67. 1.48)	0.97	**Model 3**
Women (n=3896). events (n=236)								
Continous TT:		**Categorized TT:**						
		Quarter 1		**Quarter 2**		**Quarter 3**		
HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**Model**
1.18 (1.03. 1.35)	0.021	0.53 (0.37. 0.77)	<0.001	0.77 (0.55. 1.08)	0.13	0.66 (0.46. 0.95)	0.026	**Model 1**
1.04 (0.90. 1.20)	0.62	0.71 (0.48. 1.04)	0.078	0.97 (0.68. 1.37)	0.84	0.79 (0.55. 1.15)	0.22	**Model 2**
1.03 (0.89. 1.20)	0.65	0.72 (0.49. 1.05)	0.091	0.98 (0.69. 1.38)	0.89	0.80 (0.55. 1.16)	0.23	**Model 3**

Model 1: *Region of Finland*
Model 2: *Region of Finland, log (Total cholesterol, log(HDL), log(Systolic BP), Hypertension medication, Current smoker, Waist-hip ratio, Prevalent T2D.*
Model 3*: Region of Finland, log (Total cholesterol), log(HDL), log(Systolic BP), Hypertension medication, Current smoker, Waist-hip ratio, Prevalent T2D, Time period of blood draw < 14:00.*

Table 5: Cox Regressions: clinical variables and T2D for men

Men (n= 3810); T2D events (n= 363)									
Clinical Variables:									**Model**
Region of Finland HR (95% CI); p-value	**Log (Total Cholesterol nmol/L)** HR (95% CI); p-value	**Log (HDL nmol/L)** HR (95% CI); p-value	**Log (systolic BP. mmHg)** HR (95% CI); p-value	**Hypertension medication** HR (95% CI); p-value	**Current Smoking** HR (95% CI); p-value	**Waist -Hip Ratio** HR (95% CI); p-value	**Time of blood draw** HR (95% CI); p-value		
1.09 (0.88. 1.34); 0.45								**Cont. TT**	**Model 1**
1.09 (0.88. 1.34); 0.44								**Categ. TT**	
0.98 (0.79. 1.21); 0.83	3.65 (2.02– 6.58); < 0.001	0.27 (0.18– 0.42); <0.001	3.51 (1.58– 7.81); 0.0021	1.60 (1.29– 1.98); <0.001	0.97 (0.75– 1.25); 0.81	8083.37 (1340.37– 48748.46); <0.001		**Cont. TT**	**Model 2**
0.97 (0.79. 1.21); 0.82	3.61 (2.00– 6.51); < 0.001	0.27 (0.18– 0.42); <0.001	3.45 (1.55– 7.68); 0.0024	1.60 (1.29– 1.98); <0.001	0.98 (0.76– 1.26); 0.87	7620.97 (1261.97– 46022.52); <0.001		**Categ. TT**	
0.98 (0.79. 1.21); 0.85	3.63 (2.02– 6.53); < 0.001	0.27 (0.18– 0.42); <0.001	3.82 (1.71– 8.53); 0.0011	1.58 (1.27– 1.95); <0.001	0.97 (0.75– 1.25); 0.80	8317.68 (1384.26– 49979.04); <0.001	0.76 (0.62– 0.94); 0.012	**Cont. TT**	**Model 3**
0.98 (0.79. 1.21); 0.83	3.59 (2.00– 6.46); < 0.001	0.27 (0.18– 0.42); <0.001	3.74 (1.67– 8.36); 0.0013	1.58 (1.28– 1.96); <0.001	0.98 (0.76– 1.26); 0.87	7784.93 (1294.26– 46826.02); <0.001	0.76 (0.61– 0.93); 0.0091	**Categ. TT**	

Model 1: *Region of Finland*
Model 2: *Region of Finland, log (Total cholesterol, log(HDL), log(Systolic BP), Hypertension medication, Current smoker, Waist-hip ratio, Prevalent T2D.*
Model 3*: Region of Finland, log (Total cholesterol), log(HDL), log(Systolic BP), Hypertension medication, Current smoker, Waist-hip ratio, Prevalent T2D, Time period of blood draw < 14:00.*

Table 6: Cox Regressions: clinical variables and T2D for women

Women (n= 3896); T2D events (n= 236)									
Clinical variables: HR (95% CI); p-value									**Model**
Region of Finland HR (95% CI); p-value	**Log (Total Cholesterol nmol/L)** HR (95% CI); p-value	**Log (HDL nmol/L)** HR (95% CI); p-value	**Log (systolic BP. mmHg)** HR (95% CI); p-value	**Hypertension medication** HR (95% CI); p-value	**Current Smoking** HR (95% CI); p-value	**Waist -Hip Ratio** HR (95% CI); p-value	**Time of blood draw** HR (95% CI); p-value		
1.17 (0.90. 1.53); 0.23								**Cont. TT**	**Model 1**
1.18 (0.91. 1.53); 0.22								**Categ. TT**	
1.07 (0.82. 1.39); 0.62	1.94 (0.90. 4.17); 0.090	0.18 (0.11. 0.31); <0.001	2.28 (0.85. 6.09); 0.10	1.48 (1.12. 1.97); 0.0064	1.82 (1.31. 2.52); <0.001	843.36 (220.33. 3228.09); <0.001		**Cont. TT**	**Model 2**
1.08 (0.83. 1.41); 0.58	1.92 (0.89. 4.13); 0.096	0.18 (0.11. 0.31); <0.001	2.11 (0.79. 5.67); 0.14	1.47 (1.10. 1.94); 0.0081	1.81 (1.31. 2.52); <0.001	788.31 (205.22. 3028.19); <0.001		**Categ. TT**	
0.98 (0.79. 1.21); 0.85	3.63 (2.02. 6.53); <0.001	0.27 (0.18. 0.42); <0.001	3.82 (1.71. 8.53); 0.0011	1.58 (1.27. 1.95); <0.001	0.97 (0.75. 1.25); 0.80	8317.68 (1384.26. 49979.04); <0.001	0.76 (0.62. 0.94); 0.012	**Cont. TT**	**Model 3**
1.08 (0.83. 1.41); 0.55	1.89 (0.88. 4.07); 0.10	0.18 (0.11. 0.31); <0.001	2.21 (0.82. 5.97); 0.12	1.45 (1.10. 1.93); 0.0095	1.80 (1.30. 2.50); <0.001	778.10 (202.37. 2991.76); <0.001	0.90 (0.69. 1.17); 0.43	**Categ. TT**	

Model 1: *Region of Finland*
Model 2: *Region of Finland, log (Total cholesterol, log(HDL), log(Systolic BP), Hypertension medication, Current smoker, Waist-hip ratio, Prevalent T2D.*
Model 3*: Region of Finland, log (Total cholesterol), log(HDL), log(Systolic BP), Hypertension medication, Current smoker, Waist-hip ratio, Prevalent T2D, Time period of blood draw < 14:00.*

Figure 3 and **Figure 4** present the survival curves for the endpoint T2D distributed in quartiles of TT and separated for sex. The x-axis is used as observational time since baseline. Therefore, cox regression models of T2D have been adjusted only for age. For a better view, truncation of the y-axis has been used in **Figure 4**.

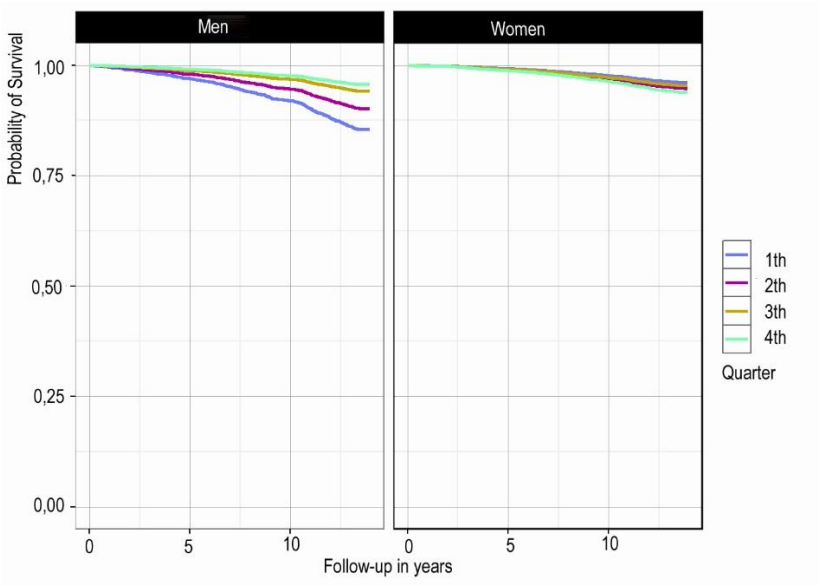

Figure 3: age-adjusted Kaplan-Meier-Survival-Curves for absence of T2D during observational time. Categorized testosterone is shown. First quartile represents the lowest, the fourth quartile represents the highest quartile.

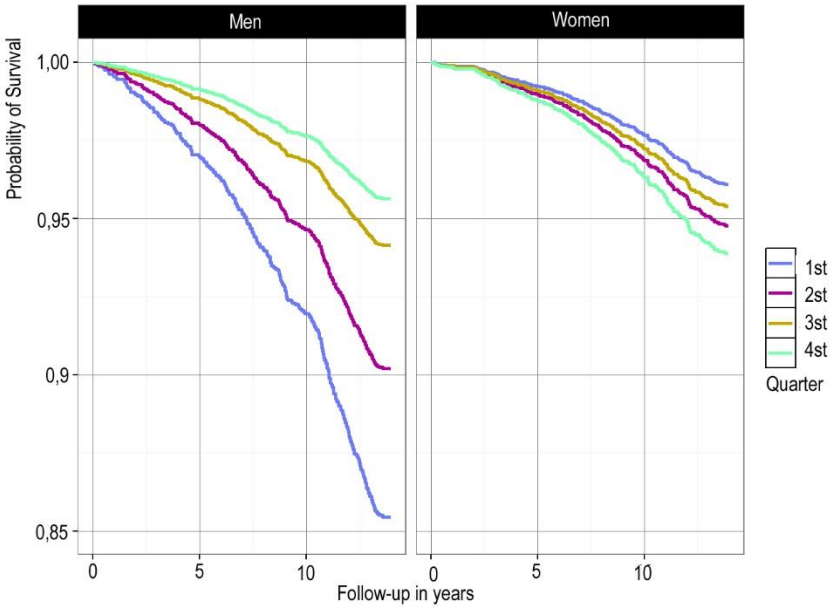

Figure 4: age-adjusted Kaplan-Meier-Survival-Curves for absence of T2D during observational time (with truncated y-axis)

Categorized testosterone is shown. First quartile represents the lowest, the fourth quartile represents the highest quartile.

3.4 Cox Regression for AF and IST

Within 13.8 years of observational time, altogether 629 probands developed an IST (n=276) and/or AF (n=426). Three different cox regression models were set up for linear TT. In Model 1 (adjuste d for age and region of Finland) men with lower TT were at higher risk for AF and/or IST (HR: 0.97; 95% CI: 0.96 – 0.99; p < 0.001). After the adjustment for cardiovascular risk factors in Model 2 and Model 3, the risk decreased by 1% (HR: 0.98; 95% CI: 0.97 – 1.00; p= 0.049 (**Table 7**).

These results could not be confirmed in categorized TT. While men within quartile 1 (HR: 1.52; 95% CI: 1.13 –2.03; p= 0.0049) and men within quartile 2 vs quartile 4 showed a significantly increased risk for AF/IST when adjusted for Model 1 (HR: 1.36; 95% CI: 1.02 –1.81; p = 0.036), this association got lost for all quartiles in Model 2 and Model 3. Apparently, the region of Finland still had a significant impact on the development of AF/IST in men after the adjustment for cardiovascular risk factors (HR: 1.29; 95% CI: 1.05 –1.58; p = 0.016), while smoking (HR: 1.63; 95% CI: 1.29 –2.05; p < 0.001) and the intake of hypertension medication (HR: 1.44; 95%CI: 1.18–1.76; p <0.001) as well as WHR (HR: 12.35; 95% CI: 2.23 – 68.51; p= 0.0040) showed an increased risk for the endpoint, total cholesterol (HR: 0.53; 95% CI: 0.30 – 0.92;p = 0.025) were inversely associated to incident T2D. HDL (HR: 0.80; 95% CI: 0.53 –1.22. p = 0.30), prevalent T2D (HR: 1.00; 95% CI: 0.71 –1.40. p = 0.98) and time of blood-draw (HR: 0.98; 95% CI: 0.81 –1.20; p = 0.88) failed to show an impact on risk prediction **(Table 8)**.

Higher linear TT levels in women were associated with an increased risk for AF/IST (HR: 1.28; 95% CI: 1.12 –1.46; p < 0.001) in Model 1. After additional adjustment for cardiovascular risk factors (Model 2+ 3), the risk prediction remained significant (HR: 1.17; 95% CI:1.01 –1.36; p = 0.031) **(Table 7)**.

The categorization of total testosterone in women confirmed these results after the adjustment for region of Finland (Model 1). TT in quartile 1 (HR: 0.68; 95% CI: 0.47 – 0.97; p= 0.036) and quartile 2 (HR: 0.63; 95% CI: 0.43 – 0.93; p = 0.019) showed a significant lower risk for AF/IST compared to quartile 4. However, this significance vanished in quartile 3 (HR: 0.85; 95% CI: 0.59 –1.22; p = 0.38) as well as for all quartiles after the adjustment for additional factors (model 2 and model 3) **(Table 7)**.

Therefore, the region of Finland seemed to have an impact on the risk of AF/IST (HR: 1.37; 95% CI: 1.04 –1.80; p = 0.026), but this significance was not detected after the adjustment for Model 2 (HR: 1.24; 95% CI: 0.93 –1.63; p = 0.14). Neither total cholesterol (HR: 0.81; 95% CI: 0.36 –1.79; p = 0.60), nor HDL (HR: 0.60; 95% CI: 0.33 –1.10; p = 0.097) changed the risk for AF/IST. Based on this results, higher blood pressure as well as intake of hypertension medication increased the risk for AF/IST. Interestingly, higher blood pressure increased the risk for AF/IST after adjusting for time of blood draw (HR: 2.93; 95% CI: 1.01 –8.52; p = 0.048),

whereas time of blood draw did not have an impact on the development of AF/stroke (HR: 0.77; 95%CI: 0.58 –1.01; p= 0.056). Smoking as well as WHR did not influence the risk for AF/IST **(Table 9)**.

Table 7: Cox Regressions: TT and AF and/or IST

Men (n = 3876). AF/ IST events (n=408)								
Continous TT:		**Categorized TT:**						
		Quarter 1		**Quarter 2**		**Quarter 3**		
HR (95% CI):	**p-value:**	**HR (95% CI)**	**p-value:**	**HR (95% CI):**	**p-value:**	**HR (95% CI):**	**p-value:**	**Model:**
0.97 (0.96. 0.99)	<0.001	1.52 (1.13. 2.03)	0.0049	1.36 (1.02. 1.81)	0.036	1.19 (0.88. 1.60)	0.26	**Model 1**
0.98 (0.97. 1.00)	0.049	1.25 (0.92. 1.69)	0.16	1.18 (0.88. 1.58)	0.27	1.11 (0.82. 1.50)	0.51	**Model 2**
0.98 (0.97. 1.00)	0.049	1.25 (0.92. 1.70)	0.16	1.18 (0.88. 1.58)	0.27	1.11 (0.82. 1.50)	0.51	**Model 3**
Women (n = 4016). AF/IST events (n=221)								
Continous TT:		**Categorized TT:**						
		Quarter 1		**Quarter 2**		**Quarter 3**		
HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**Model:**
1.28 (1.12. 1.46)	<0.001	0.68 (0.47. 0.97)	0.036	0.63 (0.43. 0.93)	0.019	0.85 (0.59. 1.22)	0.38	**Model 1**
1.19 (1.03. 1.38)	0.016	0.81 (0.56. 1.18)	0.28	0.73 (0.49. 1.07)	0.11	0.93 (0.65. 1.35)	0.72	**Model 2**
1.17 (1.01. 1.36)	0.031	0.86 (0.59. 1.26)	0.45	0.76 (0.52. 1.13)	0.18	0.97 (0.67. 1.40)	0.86	**Model 3**

Model 1: *Region of Finland*
Model 2: *Region of Finland, log (Total cholesterol, log(HDL), log(Systolic BP), Hypertension medication, Current smoker, Waist-hip ratio, Prevalent T2D.*
Model 3*: Region of Finland, log (Total cholesterol), log(HDL), log(Systolic BP), Hypertension medication, Current smoker, Waist-hip ratio, Prevalent T2D, Time period of blood draw < 14:00.*

Table 8: Cox Regressions: clinical variables and AF and/or IST in men

Men (n= 3876); AF/IST events (n= 408)										
Clinical Variables: HR (95% CI); p-value									**Model**	
Region of Finland	**Log (Total Cholesterol nmol/L)**	**Log (HDL nmol/L)**	**Log (systolic BP. mmHg)**	**Hypertension medication**	**Current Smoking**	**Waist -Hip Ratio**	**Prevalent T2D**	**Time of blood draw**		
1.39 (1.13. 1.71; 0.0016									**Cont. TT**	**Model 1**
1.39 (1.14. 1.71); 0.0015									**Categ. TT**	
1.29 (1.05. 1.58); 0.016	0.53 (0.30. 0.93); 0.026	0.80 (0.53. 1.22); 0.30	1.49 (0.70. 3.14); 0.30	1.44 (1.18. 1.76); <0.001	1.63 (1.29. 2.05); <0.001	12.30 (2.22. 68.21);0.0041	1.00 (0.71. 1.40); 0.99		**Cont. TT**	**Model 2**
1.29 (1.05. 1.58); 0.016	0.53 (0.30. 0.93); 0.026	0.79 (0.52. 1.20); 0.26	1.47 (0.70. 3.10); 0.31	1.45 (1.19. 1.77); <0.001	1.62 (1.29. 2.04); <0.001	13.53 (2.44. 74.94); 0.0029	1.00 (0.71. 1.40); 0.99		**Categ. TT**	
1.29 (1.05. 1.58); 0.016	0.53 (0.30. 0.92); 0.025	0.80 (0.53. 1.22); 0.30	1.49 (0.71. 3.15); 0.29	1.44 (1.18. 1.76); <0.001	1.63 (1.29. 2.05); <0.001	12.35 (2.23. 68.51); 0.0040	1.00 (0.71. 1.40); 0.98	0.98 (0.81. 1.20); 0.88	**Cont. TT**	**Model 3**
1.29 (1.05. 1.58); 0.016	0.53 (0.30. 0.92); 0.025	0.80 (0.53. 1.22); 0.30	1.49 (0.71. 3.15); 0.29	1.44 (1.18. 1.76); <0.001	1.63 (1.29. 2.05) <0.001	12.35 (2.23. 68.51) 0.0040	1.00 (0.71. 1.40); 0.98	0.98 (0.81. 1.20); 0.88	**Categ. TT**	

Model 1: *Region of Finland*
Model 2: *Region of Finland, log (Total cholesterol, log(HDL), log(Systolic BP), Hypertension medication, Current smoker, Waist-hip ratio, Prevalent T2D.*
Model 3*: Region of Finland, log (Total cholesterol), log(HDL), log(Systolic BP), Hypertension medication, Current smoker, Waist-hip ratio, Prevalent T2D, Time period of blood draw < 14:00.*

Table 9: Cox Regressions: TT and AF and/or/IST in women

Women (n= 4016); AF/IST events (n= 221)										
Clinical Variables: HR (95% CI); p-value										**Model**
Region of Finland	**Log (Total Cholesterol nmol/L)**	**Log (HDL nmol/L)**	**Log (systolic BP. mmHg)**	**Hypertension medication**	**Current Smoking**	**Waist -Hip Ratio**	**Prevalent T2D**	**Time of blood draw**		
1.37 (1.04. 1.80); 0.026									**Cont. TT**	**Model 1**
1.34 (1.02. 1.76); 0.037									**Categ. TT**	
1.22 (0.92. 1.61); 0.16	0.83 (0.37. 1.84); 0.64	0.62 (0.34. 1.12); 0.12	2.65 (0.92. 7.68); 0.072	1.46 (1.11. 1.92); 0.0063	1.19 (0.78. 1.83); 0.42	6.58 (0.89. 48.42); 0.064	1.44 (0.98. 2.13); 0.063		**Cont. TT**	**Model 2**
1.20 (0.91. 1.59); 0.19	0.83 (0.37. 1.85); 0.65	0.60 (0.33. 1.09); 0.093	2.55 (0.88. 7.38); 0.085	1.54 (1.17. 2.01); 0.0019	1.19 (0.77. 1.82); 0.43	6.53 (0.89. 47.66); 0.064	1.38 (0.94. 2.04); 0.10		**Categ. TT**	
1.24 (0.93. 1.63); 0.14	0.81 (0.36. 1.79); 0.60	0.60 (0.33. 1.10); 0.097	2.93 (1.01. 8.52); 0.048	1.44 (1.10. 1.90); 0.0088	1.19 (0.77. 1.82); 0.44	6.08 (0.82. 44.88); 0.077	1.43 (0.97. 2.11); 0.069	0.77 (0.58. 1.01); 0.056	**Cont. TT**	**Model 3**
1.22 (0.92. 1.61); 0.17	0.81 (0.36. 1.80); 0.60	0.58 (0.32. 1.06); 0.078	2.84 (0.98. 8.27); 0.055	1.52 (1.16. 1.99); 0.0026	1.19 (0.77. 1.83); 0.43	6.01 (0.82. 44.16); 0.078	1.37 (0.93. 2.02); 0.11	0.76 (0.58. 1.00); 0.049	**Categ. TT**	

Model 1: *Region of Finland*
Model 2: *Region of Finland, log (Total cholesterol, log(HDL), log(Systolic BP), Hypertension medication, Current smoker, Waist-hip ratio, Prevalent T2D.*
Model 3*: Region of Finland, log (Total cholesterol), log(HDL), log(Systolic BP), Hypertension medication, Current smoker, Waist-hip ratio, Prevalent T2D, Time period of blood draw < 14:00.*

Figure 5 and **Figure 6** present the survival curves for the endpoint AF and/or IST distributed in quartiles of TT and separated for sex. The x-axis is used as observational time since baseline. Therefore, cox regression models of AF and/or/IST have been adjusted only for age. For a better view, the y-axis in Figure 6 was truncated.

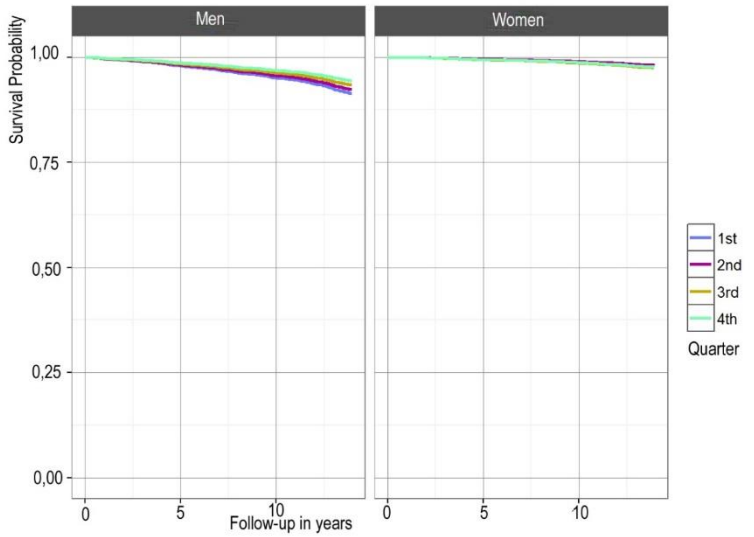

Figure 5: age-adjusted Kaplan-Meier-Survival-Curves for absence of AF and/or IST during observational time. Categorized testosterone is shown. First quartile represents the lowest, the fourth quartile represents the highest quartile.

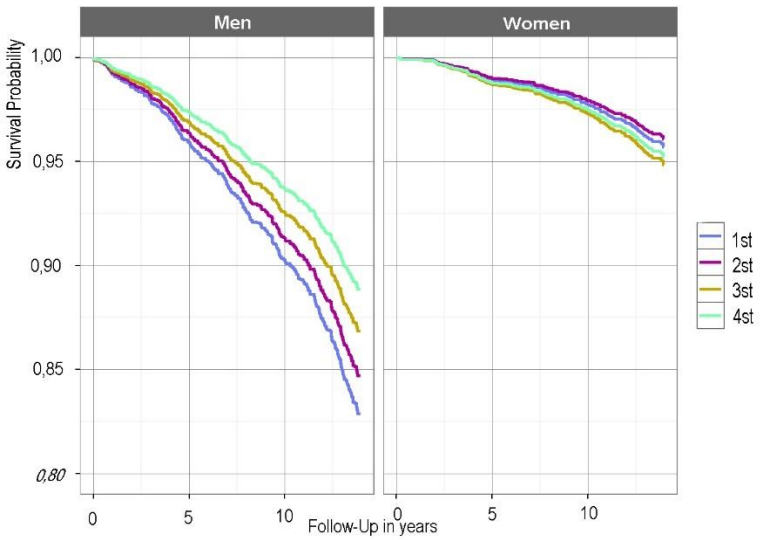

Figure 6: age-adjusted Kaplan-Meier-Survival-Curves (truncated for y-axis) for absence of AF and/or IST during observational time Categorized testosterone is shown. First quartile represents the lowest, the fourth quartile represents the highest quartile

3.5 Cox Regression for CHD and overall mortality

554 CHD cases (397 men 157 women) as well as 991 deaths (673 men, 318 women) resulted in 13.8 years. No difference could be found in TT levels between probands developing the endpoints and probands who did not reach the endpoint (men 16.00 vs 17.01 nmol/L; p=0.39; women: 1.17 vs. 1.15 nmol/L; p = 0.44). Linear TT did not show an association to future CHD or death for both sexes. Categorised TT did not show a significant association to the risk for future CHD or death. Results did not change after adjustment for cardiovascular risk factors and time of blood draw.

Table 10: Cox regressions: TT and CHD (Def. 4) for men and women

	Quartile 4 (highest)	Quartile 2	Quartile 3	Quartile 1 (lowest)	P for trend
			HR (95%CI)		
			Men		
Model 1	1	1.32 (0.92 –1.90)	1.25 (0.86 –1.80)	1.44 (1.00 –2.07)	0.075
Model 2	1	1.22 (0.84 –1.75)	0.96 (0.66 –1.40)	1.02 (0.70 –1.51)	0.79
			Women		
Model 1	1	0.69 (0.40 –1.16)	0.72 (0.44 –1.21)	0.87 (0.55 –1.40)	0.61
Model 2	1	0.79 (0.46 –1.35)	0.90 (0.54 –1.52)	1.13 (0.69 –1.85)	0.56

HR = hazard ratio. 95 % CI = confidence interval;
Model 1: *Region of Finland*
Model 2: *Region of Finland, log (Total cholesterol, log(HDL), log(Systolic BP), Hypertension medication, Current smoker, Waist-hip ratio, Prevalent T2D.*
Model 3*: Region of Finland, log (Total cholesterol), log(HDL), log(Systolic BP), Hypertension medication, Current smoker, Waist-hip ratio, Prevalent T2D, Time period of blood draw < 14:00.*

Figure 7 and **Figure 8** present the survival curves for the endpoint CHD distributed in quartiles of TT and separated for sex. The x-axis is used as observational time since baseline. Therefore, cox regression models of T2D have been adjusted only for age.

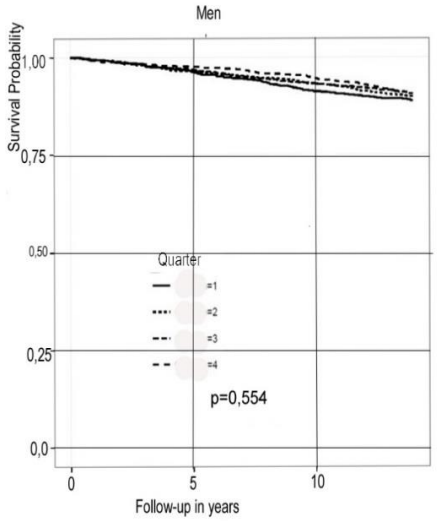

Figure 7: age-adjusted Kaplan-Meier-Survival-Curves for absence of CHD (Def. 4) during observational time in men. Categorized testosterone is shown. First quartile represents the lowest, the fourth quartile represents the highest quartile

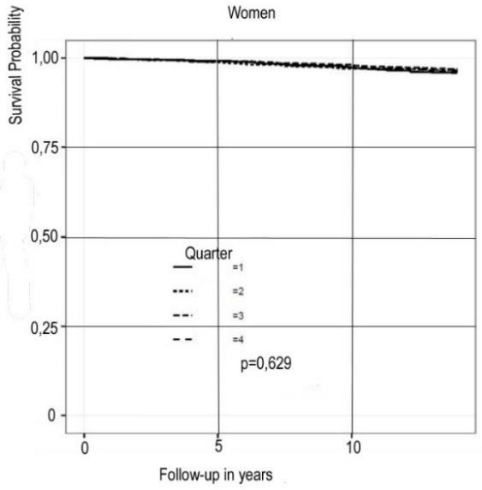

Figure 8: Age-adjusted Kaplan-Meier-Survival-Curves for absence of CHD (Def: 4) during observational time in women. Categorized testosterone is shown. The first quartile represents the lowest, the fourth quartile represents the highest quartile.

Table 11: Cox regressions: TT and mortality in men and women

	Quartile 4 (highest)	Quartile 2	Quartile 3	Quartile 1 (lowest)	P for trend
HR (95%CI)					
Men					
Model 1	1	0.95 (0.73-1.24)	1.06 (0.82-1.36)	1.15 (0.89-1.49)	0.23
Model 2	1	0.94 (0.73. 1.23)	0.98 (0.75. 1.28)	1.06 (0.80. 1.39)	0.67
Women					
Model 1	1	0.88 (0.63-1.23)	0.72 (0.51. 1.02)	0.88 (0.63. 1.21)	0.26
Model 2	1	0.89 (0.63. 1.25)	0.79 (0.56. 1.13)	0.99 (0.71. 1.39)	0.80

HR = hazard ratio. 95 % CI = confidence interval;
Model 1: *Region of Finland*
Model 2: *Region of Finland, log (Total cholesterol, log(HDL), log(Systolic BP), Hypertension medication, Current smoker, Waist-hip ratio, Prevalent T2D.*
Model 3*: Region of Finland, log (Total cholesterol), log(HDL), log(Systolic BP), Hypertension medication, Current smoker, Waist-hip ratio, Prevalent T2D, Time period of blood draw < 14:00.*

Figure 9 and **Figure 10** present the survival curves for the endpoint mortality distributed in quartiles of TT and separated for sex. The x-axis is used as observational time since baseline. Therefore, cox regression models of mortality were adjusted only for age.

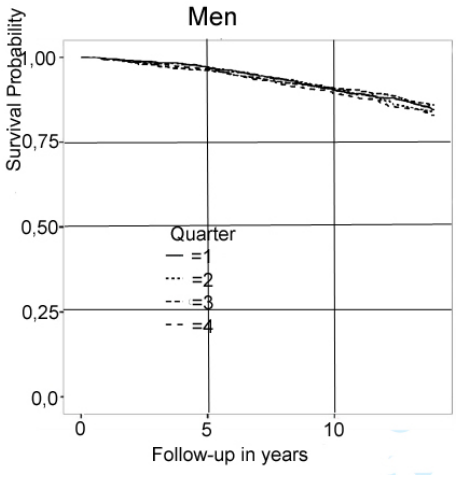

Figure 9: age-adjusted Kaplan-Meier-Survival-Curves for absence of mortality during observational time in men. Categorized testosterone is shown. The first quartile represents the highest, the fourth quartile represents the lowest quartile.

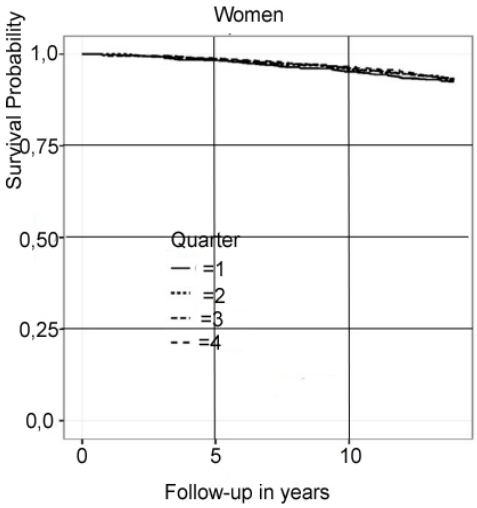

Figure 10: age-adjusted Kaplan-Meier-Survival-Curves for absence of mortality during observational time in women. Categorized testosterone is shown. The first quartile represents the highest, the fourth quartile represents the lowest quartile.

4. Discussion

The results of FINRISK 97 report several main findings of an European population.

I. Median total testosterone levels of both genders (men: 17.01 nmol/L; women: 1.15 nmol/L) are within the 50^{th} and the 75^{th} percentile of the CDC reference.

II. A small diurnal rhythm for TT can be reassured in both genders, but the influence on risk prediction was negligible.

III. In men, increasing TT is associated with a healthier cardiovascular risk profile (increased HDL and reduced adiposity), except for smoking men. Smoking is associated with higher TT in men. In women, no association can be found between TT and any cardiovascular risk factor. Total testosterone does not decrease in aging men but slightly falls in aging women. Men in FINRISK 97 offer a much more adverse cardiovascular risk profile, increased incidence of cardiovascular events, T2D as well as mortality compared to women. Body composition marker (WHR, BMI) are the most important risk factor for the development of T2D as well as AF/IST. Compared to adiposity, the effect of all other risk factors on risk prediction are trivial.

IV. In men, not only low but also low normal TT is associated with increased T2D independent from adiposity and other cardiovascular risk factors. High TT in women increases the overall risk for T2D, whereas the statistical significance gets lost after adjustment for cardiovascular risk factors.

V. Hypogonadal and eugonadal men show an increased risk for IST and/or AF compared with normal to supraphysiological TT. In women increased TT was associated with a higher risk for AF/IST. In hypogonadal women within quartile 1 and quartile 2 low TT shows a protective effect for AF and/or IST compared to women with physiological or supraphysiological TT.

VI. No association can be found between TT and CHD or overall mortality for both genders.

4.1 Testosterone and Type 2 Diabetes Mellitus

Men within quartile 1 and quartile 2 (0 –17.01 nmol/L) showed a significantly increased risk for the development of T2D compared to the highest levels of TT (21.99 –35.0 nmol/L). After the adjustment of cardiovascular risk factors the risk prediction remained significant for men within quartile 1 (0 –12.82 nmol/L). Considering the reference values for healthy young men due to the Endocrine Society (9.2 –31.8 nmol/L) (Travison et al, 2017), men in FINRISK 97 are already at increased risk of T2D, when their TT level is still "normal" and they do not have a high risk cardiovascular profile. This finding shows that not only hypogonadal but also eugonadal men have a higher risk for T2D, independent from cardiovascular risk factors.

It needs to be considered that TT within all quartiles except for quartile 1 represent values which can be seen in healthy young men.

The findings for linear TT and T2D in men were supported by several prospective population-based trials. While the meta-analysis of Eric L. Ding that included four European- and four American-prospective trials found an average of 42 % lower risk of T2D for those men in the upper dichotomy of TT (Yao et al, 2018) (Ding et al, 2006), the meta-analysis of Qiu-ming Yao found a 35 % lower risk for men with higher TT (Yao et al, 2018). In contrast, the results of FINRISK 97 supply a 51% reduced risk estimation for higher TT in men.

The two meta-analyses as well as the results of FINRISK 97 support the inverse association of TT and T2D in men. Possible explanations are still very heterogenous.

In FINRISK 97, adiposity plays a very important role in the development of hypoandrogenism as well as T2D. The results of FINRISK 97 promote a moderate association of TT and T2D, as well as a strong association of adiposity with T2D. Higher WHR predisposes for future T2D as the cox regression analysis reveals. Adipose men have a 10-fold higher risk for developing T2D compared to women and no other cardiovascular risk factor which has been considered in FINRISK 97, has as much influence on the development of T2D. The relationship between adiposity and TT is alternating. On the one side, weight gain is associated to lower TT (Derby et al, 2006), while low TT levels are associated to increased adiposity (Khaw and Barrett-Connor, 1992; Grossmann, 2018). Recent testosterone-application trials suspected a higher rate of the enzyme aromatase due to the increasing adipose tissue to be responsible for the increasing adiposity in low TT (Tan et al, 2015). Normally, TT is partly converted into estrogen by the enzyme aromatase. This enzyme can be found in the fat tissue as well. It has been hypothesized, that a higher activity of aromatase converts more androstenedione into estrogen, resulting in lower TT and higher estrogen (Karakas et al, 2018). Therefore, it was assumed that higher estrogen levels causes adiposity and further perpetuate the vicious circle of low TT and increasing adiposity (Tan et al, 2015). A recent trial of almost 200 men under

aromatase inhibiting therapy revealed, that low estrogen leads to increased fat mass, while TT modulated muscle mass (Finkelstein et al, 2013).

This corresponds with recent results of adipose men who not only have TT, but also low levels of estrogen (Grossmann, 2018). As adipose men show reduced levels of aromatase, it is assumed that the adipose tissue inhibits aromatase, leading to reduced levels of estrogen and increased adiposity. TT application in middle-aged men without aromatase inhibitor showed a growth in muscle mass as well as loss of fat-mass (Grossmann, 2018). Therefore, it is suggested that low TT is indirectly associated to adiposity due to the reduced aromatase activity followed by estrogen-insufficiency (Ghanim et al, 2018). An effective aromatase mainly protects from adiposity as well as from adiposity induced insulin resistance (Huhtaniemi et al, 2012; Dhindsa et al, 2011).

Consistently, weight loss especially after bariatric surgery leads to a strong increase in TT level as well as gonadotropins. (Leenen et al, 1994). This illustrates the much greater impact of adiposity on TT, compared to the modest fat reduction due to TT application (Corona et al, 2013). To understand why the adipose tissue reduces TT, it is necessary to imagine the adipose tissue as an organ which induces an immune-system-response. The adipose tissue discharges mass of proinflammatory cytokines and reactive oxygen species (ROS) which not only promote an acute-phase-reaction and a low grade inflammation in the body (Sjöholm and Nyström, 2005), but also prevents the hypothalamus from releasing GnRH (Jones, 2007). Without GnRH, the HPT-axis does not communicate and the testes do not get a stimulus to produce testosterone.

Besides this central hypogonadism, the adipose tissue produces the hormone leptin, which accelerates the central hypogonadism by inhibiting GnRH secretion. Additionally, leptin directly lowers the gonadotropin derived stimulation on testosterone synthesis in the testes (Pitteloud et al, 2005). The application of TT reduces the inflammatory reaction, upregulates several genes in the adipose tissue, skeletal muscles and heart muscles (Dhindsa et al, 2016). Adiposity reduces TT in men in different ways and promotes T2D. Therefore, low TT could be used as a marker for adiposity derived T2D.

So far, several prospective trials from (Vikan et al, 2010) as well as (Lakshman et al, 2010) and the existing prognostic population-based trials comprised in the meta-analysis by Yao (Yao et al, 2018) and Ding (Yao et al, 2018; Ding et al, 2006) were not able to maintain significant results after adjusting WHR. This suggests a complete adiposity derived effect of TT on T2D.

The results of FINRISK 97 revealed that developing T2D is not only a problem of hypogonadal and obese older men. The adjustment of cardiovascular risk factors in linear and categorized

TT left a significant association between lower TT and T2D independent from obesity and other cardiovascular risk factors. Similar results were found in several trials with androgen deprivation therapy (ADT). Very low levels of TT were achieved by ADT in men with prostate cancer. After 16 months, men within the group with ADT showed a higher incidence of T2D compared to prostate cancer patients without ADT independent from other risk factors. Men who already had T2D at baseline presented a worsened diabetic situation. (Shahani et al, 2008). Further evidence of TT protective effects against T2D have been found in TT application trials. Testosterone promotes signal pathways via insulin, decreases fasting glucose concentration, improves glycemic control as well as insulin sensitivity (Sjöholm and Nyström, 2005). Nevertheless, all TT induced improvements in T2D protection are small and inconsistent (Grossmann et al, 2015; Grossmann, 2018).

Testosterone has many effects on the adipose tissue, the skeletal system, the liver as well as the brain via a modulating effect of androgen receptors (AR). Testosterone derived effects on AR reduce insulin resistance, body fat and lipogenesis (Zitzmann et al, 2003; Fan et al, 2005). All effects are induced either centrally or peripheral. Different trials have proven an effect of testosterone on GLUT 4 transporter, an insulin dependent protein, which manages the glucose uptake in muscles and fat (Rao et al, 2013). Testosterone derived effects via AR not only protect against obesity but have also direct effects on glucose metabolism (Asih et al, 2017).

The results of women in FINRISK 97 showed an association between low TT and decreased risk for T2D. After adjustment of additional confounding factors, the statistical significance got lost. This could be due to the little number of T2D cases in FINRISK 97. Another reason could be the inappropriate measurement method for TT in women. The association of very high androgen levels and T2D is well known and has been described as “Achard Thiers syndrome” (Navarro et al, 2015). A meta-analysis of 36 cross-sectional studies comprising 4795 women by Ding et al revealed a significant association for higher TT and incident diabetes (Ding et al, 2006). While one prospective case control-trial of 359 women (average age 60 years) found an association between high TT and increased risk for T2D (Ding et al, 2007), the Rancho Bernardo Heart and Chronic Disease Study (589 women) as well as the Rotterdam Study of 3117 postmenopausal women were not able to find a significant association (Oh et al, 2002; Muka et al, 2017). One additional meta-analysis comprising four postmenopausal trials and one pre and postmenopausal women from Europe and America confirmed these results (Muka et al, 2017).

Pearson-correlation coefficients of TT and T2D in FINRISK 97 revealed an adiposity independent association between TT and T2D. Adipose women had a higher risk for T2D in FINRISK 97 but adipose women did not show higher testosterone levels. This findings are in

compliance to obesity independent association of TT and T2D in 845 healthy postmenopausal women of the PEPI trial (Kalish et al, 2003) and correlation coefficients from postmenopausal women of the Rancho Bernardo study (Oh et al, 2002), both with similar WHR and BMI at baseline. In contrast, a small trial with young healthy women revealed that higher WHR was associated with higher TT levels (Mondragón-Ceballos et al, 2015). Possibly, this association is age-related and gets lost in menopausal women. This could be the reason, why FINRISK 97 and other trials that included mostly menopausal and post-menopausal women could not find an adiposity related correlation. Another reason could be that WHR does not adequately reflect the fat distribution of women, who tend to have more subcutaneous fat as well as gluteal/femoral fat than visceral fat in men (Bouchard et al, 1993).

The number of trials examining TT in premenopausal women is sparse and focuses on women with polycystic ovary syndrome (PCOS). This endocrine disorder is characterized by multiple cystic alterations of ovaries which cause hyperandrogenemia and ovulatory dysfunction in young women. Its importance is often underestimated as one of six women is affected and it is often accompanied by compensatory insulin resistance, hyperinsulinemia and obesity. The expression of symptoms varies but only women with PCOS and hyperandrogenism develop T2D (Legro et al, 1999). Insulin stimulates the testosterone synthesis in ovarian theca cells through cognate receptors via the inositolglycan pathway of PCOS as well as non-PCOS (Nestler et al, 1998). Ovarian theca cells in PCOS are more sensitive for insulin than normal ovarian theca cells, causing higher TT values (Cadagan et al, 2016). Raised TT increases insulin-secretion of pancreatic β-cells via AR, which turns out in a vicious circle. Pancreatic β-cell- hyperplasia is followed by β-cell failure. While most tissues show increasing insulin resistance and glucose intolerance, the inositolglycan pathway maintains its effect on ovarian testosterone synthesis, possibly independent from age (Nestler et al, 1998).

Treating women with metformin, an antidiabetic drug, have proven a decrease in TT levels independent from BMI (Kolodziejczyk et al, 2000). This acknowledges an obesity independent association of TT with T2D in women. In healthy women hyperinsulinemia is also involved or may even be the reason for hyperandrogenism in premenopausal women (Dunaif, 1997). Chronic high values of insulin increase and regulate levels of TT by several direct effects on steroidogenesis (Barbieri and Ryan, 1983; Fox et al, 1993; Guo et al, 2001). However, in vivo trials are difficult to set up for non-diabetics by administering insulin for a longer time and considerations mostly derive from in vitro trials (Micic et al, 1988). Furthermore, trials with Diazoxin application, an insulin lowering drug, in diabetic women resulted not only in a reduced insulin secretion, but also in reduced TT.

Another hypothesis considers testosterone to be causal for T2D. Female to male transsexuals experience a lot of physical changes due to testosterone therapy. Besides they show a reduced glucose uptake as well as insulin resistance during ongoing TT supplementation (Polderman et al, 1994; Streed et al, 2017).

Furthermore, young healthy women who were treated with TT for several days exhibited a reduced insulin sensitivity in fat-cells and muscles-cells. Hepatic cells have not been affected (Diamond et al, 1998). The findings of that trial agree with a long-known fact, that adipose as well as muscle tissue in women show increased insulin-sensitivity compared to their male-counterparts. Releasing an identical amount of insulin in both men and women, causes a much higher insulin-stimulated glucose transport in the adipose- and muscle-tissue of women compared to men (Foley et al, 1984; Nuutila et al, 1995). Testosterone promotes the composition of those female muscle-fibers which are less insulin sensitive (Holmäng et al, 1992). Therefore, insulin sensitive tissues like muscles or fat can lose the ability to answer arriving insulin leading to hyperinsulinism and decreased glucose-utilization (Ye, 2013). With its effect on skeletal muscles, TT promotes insulin-insensitivity, leading to T2D with increasing or continuous elevated TT.

The impact of TT on health in women could be even more extensive. Testosterone application in prenatal rhesus monkeys of pregnant females causes insulin resistance and obesity as soon as the offspring is grown-up (Eisner et al, 2000). The question needs to be asked, if TT in adults could be used as a predictive marker not only for T2D and adiposity but also for the unborn descendants.

4.2 Testosterone and Cardiovascular Diseases

4.2.1 Association between Atrial Fibrillation and/or Ischemic Stroke

15 to 20 % of all ISTs are referred to AF and those with previous stroke. An underlying diagnosis of AF show an increased risk for a second ischemic brain event within the first year after stroke. The risk of developing an IST is five times larger in probands with AF compared to those without this specific rhythm disorder at baseline (Wolf et al, 1991). Neurological damages especially in the insula-cortex can cause AF after stroke (post-stroke AF) and raises the prevalence of AF to 39% in patients with IST (Sposato et al, 2015). Considering that post-stroke AF increases the risk for IST alike pre-stroke, the relevance of AF for ischemic-stroke-aetiology would be even higher. Vitamin K antagonists as well as anticoagulants are proven to reduce the risk for further cardiogenic embolic strokes by approximately 64 % (Sposato et al, 2015). While the age-adjusted prevalence of AF maintains stable in Caucasian population over the last 30 years, the rate of AF increases due to the aging population. Besides more efficient diagnostic methods, a higher expectation of life is mainly responsible for the increasing relevance. This cardiac rhythm disorder is three-times more presented in 85 years old, compared to 65 to 70 years old probands. This illustrates the relevance of AF for incident IST and the close link between AF and IST. Consequently, it is reasonable to combine and examine AF and IST in one endpoint for FINRISK 97.

4.2.2 Testosterone and Atrial Fibrillation and/or Ischemic-Stroke

The overall effect of TT reveals, that it is protective in men and deleterious in women.

While hypogonadism protects women from AF and/or IST, hypogonadal men have an increased risk for the specific endpoint.

In men, smoking as well as adiposity independently increase the risk for AF/IST as well as the TT level. Accordingly, adipose non-smokers would have a reduced risk for AF/IST and lower TT levels compared to adipose smokers. Higher TT in smoking men would therefore possibly be associated to an increased risk for AF/IST. So higher TT in a subgroup of men would be associated to an increased risk for AF/IST. This thesis contrasts with the general belief, that low TT is associated to an increased risk for AF/IST.

However, smoking and adiposity have much bigger impact on AF/IST than low levels of TT. The administration of testosterone would only derive little improvement in risk prediction if smoking and adiposity would not be changed by lifestyle modifications. This hypothesis needs to be validated in further trials.

In FINRISK 97, T2D did not increase the risk for AF and/ or IST for both genders. This finding contrasts with a recent meta-analysis published in Lancet. The authors demonstrated that T2D is an independent risk factor for IST in patients with AF (Group, The Stroke Risk in Atrial Fibrillation Working, 2007).

It is known that high cholesterol is a risk factor for IST and lowering cholesterol level decreases the risk for CVD. In contrast to that, men in FINRISK 97 with high cholesterol levels showed a reduced risk for AF/IST. The inverse correlation for cholesterol and AF has been described before (Magnussen et al, 2017). The severity of IST was inversely associated to total cholesterol levels (Olsen et al, 2007). In women of FINRISK 97, the inverse correlation between cholesterol and AF/IST did not reach statistical significance.

In women, no cardiovascular risk factor showed a simultaneous association to TT and the endpoint. There is need for confounding factors like SHGB which is proven to affect TT as well as the endpoint and is subject to fluctuations (Brand and Schouw, Y T van der, 2010).

To include the relevant state of studies the combined endpoint needs to be separated into AF and IST and considered independently. No other trial has examined both diseases as one endpoint.

Most of population-based trials like the prospective Copenhagen City Heart Study are summarized in the meta-analysis of Holmegard et al. It confirms that TT below the tenth percentile in men (median: < 6 nmol/L) is associated with a higher incidence of stroke

compared to men above the 90th percentile (median: 14 nmol/L). No statistical significance between TT and incident stroke could be found in women (Holmegard et al, 2016).

The Copenhagen City Heart Study as well as the meta-analysis only focus on borderline TT concentrations and did not show an overall effect of TT on stroke.

On the other side, the Honolulu Asia Aging Study could not find an association between TT and stroke in 2197 elderly men (Abbott et al, 2007). A large prospective trial of 2914 female patients with a median age of 58 revealed that very low TT predicts stroke in women (Sievers et al, 2010).

All existing trials have either found no level or low level of TT to be associated with incident stroke in men. Existing trials in women have found an association for incident stroke in low levels, no levels and very high levels of TT.

Taking a closer look on comparable trials, it is possible to see important differences compared to FINRISK 97. Most of the mentioned trials comprise older men and women with an average age over 58 years (median age > 70 years). As aging is often accompanied by increasing comorbidities and geriatric manifestations these participants are affected by an increased risk for non-adjusted health conditions which could falsify the correlation.

Besides, existing trials differ regarding the endpoint stroke. The Health in Men Study (HIMS) of older community dwelling men (median age: 77) included all stroke related events (haemorrhage, transient ischemic attack, subarachnoid bleeding) (Yeap et al, 2009). This approach only focuses on stroke related symptoms and neglects the different pathogeneses of strokes.

The points of criticism question the informative value regarding the effect of TT for all men and women. It seems that existing trials are only valid for a subgroup of all men and women. Stroke pathologies are very heterogenous. The combination of all kind of strokes could be considered as a marker for cardiovascular health rather than a marker for stroke.

There are several possible hypothesises how TT can increase the risk for IST.

Based on the TOAST criteria, there are several reasons for ISTs. Besides AF, 20 % of all ISTs are derived from carotid or intracranial vessels atherosclerotic stenosis (Flaherty et al, 2013). The increasing degree of stenosis is presented as increasing Intima-Media-Thickness (IMT) and can be measured via ultrasound. The IMT have been proven to be an independent risk factor for IST as well as myocardial infarction (Ludwig et al, 2003; Hollander et al, 2003) Several trials have shown that IMT is inversely associated with TT (SVARTBERG et al, 2006).

The impact of TT on AF could be one of the reasons why high TT in women is associated to a higher risk of IST. Women with premature menopause have an increased risk for IST (Archer, 2009). High TT could also be a modulator of atherosclerosis through indirect effects on atherosclerosis, hyperlipidemia and T2D (Kaufman and Vermeulen, 2005). Therefore, an early onset of menopause with an early loss of estrogen and a longer period of predominant TT could be another explanation for the increased risk for IST in women. In other studies, TT was associated with protective effects as oophorectomized women with low TT and low estrogen levels had a reduced cardiovascular risk when additionally treated with testosterone (Barrett-Connor, 2013). In general, TT seems to have both deleterious and protective effects on the cardiovascular system and especially on the development of IST. The overall effect may depend on the estrogen/testosterone ratio as well as comorbidities. Therefore, TT in women is rather a marker for cardiovascular health than a specific marker for stroke or AF.

In a group of middle aged to older men of a community-based cohort in FRS, the longitudinal analysis over ten years showed inconsistent results in different age groups regarding TT and AF (Magnani et al, 2014). While low TT in men between 55 and 65 as well as men over 80 years showed a significant higher risk for AF, this association could not be reproduced for men between 70 and 79 years. In contrast to the FRS, the male population in FINRISK 97 is much younger, has less prevalent T2D and higher median TT levels.

Another cohort of 1019 older men (>76 years) in the Cardiovascular Health study with a higher prevalence of T2D and lower TT levels could not find an association between TT and incident AF (Rosenberg et al, 2018). The multi-ethnic study of Atherosclerosis of 4883 men and women with an average age of 62 in men and 65 in women revealed that lower levels of bioavailable testosterone in men predict an increased risk for AF. An association for TT could not been found (O'Neal et al, 2017).

So far, several gender-differences in AF regarding electrophysiologic pathology (Staerk et al, 2017; Ko et al, 2016), stroke incidence (Yarnoz and Curtis, 2008), risk factors (Magnussen et al, 2017) and age at onset (Schnabel, 2012) have been described before (Ravens, 2018). A possible explanation for men could be found in a trial with gonadectomized rats from (Tsuneda et al, 2009). The authors demonstrated that TT deficient rats showed an increased rate of atrial rhythm disorders compared to testosterone treated rats. This finding could explain the association of low TT in young adults with the onset of lone AF (Lai et al, 2009). Lone AF only represents one part of AF and the main part of AF follows structural heart disease. Therefore, low TT indirectly increases the risk for AF by worsening the risk factors like hypertension and T2D, which are independently associated to structural heart disease (Magnussen et al, 2017).

4.2.3 Testosterone and Coronary-Heart-Disease

Due to the results of FINRISK 97, TT has no predictive impact on CAD. Neither very low nor did very high TT altered the risk. The adjustment of age and classical cardiovascular risk factors did not change these associations.

The existing literature regarding major CV -event as well as death from fatal CV -events is very heterogenous offering observational and narrative reviews. One part of them supports TT to be protective while another part could not find an effect on incident CVD (Ruige et al, 2011; Liu et al, 2003; Wu and Eckardstein, 2003).

No known epidemiological trial up to the year 2009 could find an association between TT level and incident CHD in men. In 2011, the MrOS study of 2416 participants (Ohlsson et al, 2011) proved that men within the highest quartile of TT (> 19 nmol/L) had an reduced risk for CVD, death including CHD events as well as cerebrovascular events. In contrast to all previous epidemiological trials as well as FINRISK 97, gas chromatography/mass spectrometry was used for testosterone evaluation. Although using gas chromatography/mass spectrometry, Shores et al 2014 could not confirm an association between TT and CVD/mortality among 1032 men (Shores et al, 2014). This confirms, that testosterone measurement is not the reason for an insufficient association found in FINRISK 97. A case-control study by Soisson et al. revealed an increased risk for incident CHD among French men within the lowest quintile (< 13.7 nmol/L) and within quintile three to five (> 16.9 nmol/L) (Soisson et al, 2013). Interestingly the trial of Soisson as well as the MrOS study had an observational time between four to five years but obtained as many major CV -events as FINRISK 97 did in ten to 15 years. With a median age of 73 and a high rate of comorbidities at baseline like hypertension and T2D, these trials do not apply to the average male section of the population but rather to elderly men. TT could be a marker for health in older people (Ruige et al, 2011). Only the results of FINRISK 97 can prove the predictive impact of TT on CHD as they represent all male adults.

In women the results of FINRISK support the current state of trials comprising the Study of Health in Pomerania (Schaffrath et al, 2015) as well as a meta-analysis by Holmegard et al. combined with the Copenhagen City Heart Study (Holmegard et al, 2016). TT is not an independent risk factor for CHD as well as mortality. Previous trials have shown very contradictory results. Postmenopausal women of the Rancho Bernardo Study with low TT levels and an average age of 73 showed an highly increased risk for CHD with extremely low levels of TT within the lowest quintile (0.04 nmol/L– 0.28 nmol/L) versus all other quintiles (0.28 nmol/L– 2.61 nmol/L). With an average level of 0.49 nmol/L these women had much lower levels of TT compared to FINRISK 97 (1.15 nmol/L) and they were below the reference values for normal TT in postmenopausal women (Eisenhofer et al, 2017). Besides, they have a higher

rate of hypertension (79 %) compared to FINRISK 97 (13 %), higher rate of T2D (16 %) compared to FINRISK (4.9%) and a lower BMI (24) compared to FINRSIK 97 (26). Additional to the much higher rate of comorbidities and a high rate of mortality within 12 years (21%), these women represent unphysiological low TT. As the testosterone synthesis in women maintains after menopause (Miller, 2008), medication, tumours and other diseases (Miller, 2008) can significantly decrease TT in women. Further on, it is possible that selection bias influenced the results of the Rancho -Bernardo -Study. In general, these results regarding TT and CHD may apply to a restricted part of women. But this finding cannot be validated for all women. In contrast, the Copenhagen City Heart Study showed that women at the 95% percentile or above have a higher risk for CHD and death compared to women within the eleventh and 89th percentile. The blood has been taken in the years 1981 –1983. TT was stored at -20°C for 30 years until the measurement was done with CMIA in 2009-2011. Several trials have proven an increase in TT with prolonged measurement (see more in section 4.2.4). Therefore, a falsification could be the reason for the association (Gislefoss et al, 2012; Holl et al, 2008). Another trial of 108 postmenopausal women attending coronary angiography showed that lower TT was associated with the onset of CHD (Kaczmarek et al, 2003). Despite differences in cardiovascular risk factors between both groups especially regarding T2D, only age was considered as confounding factor. It is possible that selection bias as well as neglection of confounding factors caused the association in this cross-sectional analysis. In general, the results of the existing literature regarding CHD and endogenous TT are very contradictory and cannot sufficiently prove the impact of TT on CHD as the results of FINRISK 97 show.

4.2.4 Testosterone and Overall Mortality

The results of FINRISK 97 reveal TT has no predictive impact on overall mortality. Summarizing all comparable and existing studies, the result of one meta-analysis of 2011 revealed an increased risk for overall mortality in men with lower TT (Meyer and Wittert, 2018; Araujo et al, 2011). Although there have been several trials which could not find an association between TT and the overall mortality, these meta-analyses are not valid for all men due to several limitations. Although all trials are large, observational and adjusted for age, they differ in many details which mostly are a side -effect of aging. Comparing baseline results Finish men were not only younger (48 years vs. 61 years) but also had higher TT levels (17.15 nmol/L vs 16.9 nmol/L), smoked less (26.6 % vs. 28 %) and had been observed for a longer time (14 years vs. 9.7 years). The protective effect of TT only applies for a part of men in advanced years and with several comorbidities at baseline but cannot be accepted for the general population. Due to findings of FINRISK 97 low TT within the range of hypogonadism is not an independent risk factor for mortality but probably a good risk marker for the general health in men.

Results of FINRISK 97 regarding TT and overall mortality in women are in line with the present state of literature. The Study of Health in Pomerania (SHIP) (n=2192) with an identical median age (49 years) and an equal number of women before and after menopause could not find a predictive value for TT and mortality (Schaffrath et al, 2015). Interestingly, median TT was much higher in FINRISK 97 although there were less women in FINRISK 97 with hypertension (14 % vs. 39 %), smoking (17.4 % vs 27 %) or T2D (5% vs 10%). A possible explanation for the difference in median TT could be explained in the well-known difficulty to correctly measure very low TT levels in women (see section 4.2.1). While FINRISK 97 uses competitive-immuno-assay, gas chromatography/mass spectrometry were the measurement method in SHIP.

4.3 Discussion of cardiovascular risk factors in FINRISK 97

Several cardiovascular risk factors were proven to alter the risk for CVD and T2D. Some are also associated to testosterone, which is discussed in the following.

4.3.1 Lipids

The linear correlation analysis of HDL and testosterone revealed that men with a higher TT have higher HDL values. Results for men are in line with several population based trials (Vikan et al, 2009; Khaw and Barrett-Connor, 1991; Haffner et al, 1993). Surprisingly, the correlation of total cholesterol and TT in men did not reveal statistical significance in contrast to earlier trials (Mäkinen et al, 2008; Haffner and Valdez, 1995).

TT in women did not reveal a statistically significant association neither to HDL levels nor to total cholesterol. These results contrast with several population-based trails of women which demonstrated lower TT to be associated with higher HDL and lower total cholesterol (Laughlin et al, 2010; Sievers et al, 2010; Brand and Schouw, Y T van der, 2010). A possible explanation for the missing correlation can be the age of included women. Women in FINRISK 97 are much younger (median age: 47 years) than the comparable population-based trials mentioned before. The average age of the onset of menopause is 51 years (te Velde and Pearson, 2002). During menopausal transition, women are affected by ovarian insufficiency which changes the hormone balance and worsens the lipid profile. This leads to an increased cardiovascular risk in (post)menopausal women (Muka et al, 2016). Most population-based trials include postmenopausal women with an average age much older than 50 years. Large trials of younger women mostly comprise women with polycystic ovary syndrome (PCOS) or transsexuals. The inclusion of younger healthy and perimenopausal women could be the reason for the missing statistical significance between TT and total cholesterol in FINRISK 97.

4.3.2 Smoking

Almost 50 % of TT variability is assumed to be attributed to environmental factors for both genders (Harden et al, 2014). In FINRISK 97, Pearson-correlation coefficients revealed that smoking men have higher TT, whereas no association between TT and smoking could be found in women.

A recent meta-analysis examined the influence of smoking on TT in men among 22 trials and 13317 men with the age of 18 to 61 years. The TT levels in smokers were significantly higher compared to the group of non-smokers. A meta-analysis of young to middle aged women could not prove an association between TT and smoking (Zhao et al, 2016 Apr). Prenatal influences

could have an impact on later TT level, as experiments with rats demonstrated. Female exposure to nicotine raised the mother's TT level as well as the TT level in female offspring, but not in male offspring (Harden et al, 2014).

4.3.3 Body composition marker

In FINRISK 97, TT and body composition markers (WHR and BMI) show a strong inverse correlation in pearson-correlations throughout all age groups. Men with higher BMI or WHR have lower TT levels, whereas TT in women did not alter WHR or BMI (see table 5). Interestingly, the correlation between BMI and TT is stronger than between WHR and testosterone (0.23 vs 0.20). A possible explanation is described in the following. Additionally, it is figured out why WHR but not BMI is used for cox-regression analyses.

BMI represents the relationship of height and weight, including subcutaneous fat, visceral fat, muscle mass and hydration status. Especially the last two factors can fluctuate in aging men. Loosing muscle -mass and increasing fat -mass is associated with an increased risk for metabolic disease, although the BMI does not necessarily change significantly (Kelly and Jones, 2015). In contrast, WHR considers the fat disposition and represents the visceral fat. WHR is assumed to be stronger associated to adiposity derived comorbidities than BMI (Haslam and James, 2005). Several trials which used computed tomography and magnetic resonance image to determine fat mass could demonstrate that only visceral fat shows an inverse correlation with TT during an observation period of several years (Tsai et al, 2000; Couillard et al, 2000; Garaulet et al, 2000). The results of FINRISK show, that changes in TT not only have an influence on visceral fat but additionally influences the body composition including muscle mass.

In contrast to the missing correlation in FINRISK women, literature regarding the association between TT and body composition markers among premenopausal women is mostly based on hyperandrogenism and obesity in women with PCOS or male-to-female transsexuals (Gambineri et al, 2002; Elbers et al, 1997). A small trial of 30 young healthy women used dual energy x-ray absorptiometry and computed tomography to measure fat mass and muscle mass. It revealed that young women (median age: 27) with higher physiologic TT had higher overall adiposity but the TT did not correlate with visceral adiposity, subcutaneous fat and skeletal muscle mass (Keller et al, 2011). These data suppose that fat distribution and body composition in FINRISK women as well as other epidemiological trials (Muka et al, 2017) were poorly determined with BMI and WHR.

Despite the strong correlation of TT and BMI/WHR in men it is debatable if these results can be used globally as body composition varies between different races/ethnicities (Seo et al, 2017) and TT and obesity are strongly associated.

4.3.4 Testosterone and age

The Pearson-correlation coefficient of FINRISK 97 demonstrates that the TT does not decrease in aging men. Aging women show increasing TT levels. Almost stable estimated median TT levels for over 10000 healthy men between 50 and 99 years from 13 heterogenous trials have been confirmed by (Kelsey et al, 2014) as well as by (Travison et al, 2017). This result contrasts to the general opinion of age-related decline after the age of 50 years, which was merely set up by the results of larger cross-sectional studies as well as one longitudinal study of the Massachusetts-Male-Aging-Study (MMAS) with 1532 male probands (Travison et al, 2007; van Anders et al, 2014). Differences between these similar prospective trials and cross-sectional trials derive from varying TT measurements and calibration methods as well as differences in an adjustment of confounding factors and the increasing variance in older male. For instance, no lipid profile was added to the MMAS (Travison et al, 2007). Finally, FINRISK 97 does not answer the question if an unknown environmental factor lowers TT transiently or persistently in all men. This would partly explain the age-related decline in TT of the MMAS. There is only one additional known study in women, which documented an age-related decline in TT levels in women (Zumoff et al, 1995).

4.4 Strengths of FINRISK 97

4.4.1 Study Design

The existing literature comprises cross-sectional and prospective trials. The following discusses the advantages of FINRISK as a prospective population-based trial.

4.4.1.1 Population Based Advantages of FINRISK

The significance of the results of FINRISK are highlighted by the quantity of 8000 participants, an observational range of 13 years, a wide age range of 25 -74 years and an equal distribution between sexes. FINRISK 97 is one of the largest population-based trials regarding testosterone and CVD and mortality in healthy individuals.

Due to history, geography and mixture of habitants, the Finish population shows a very homogenous genetic pool and a stable environment. Therefore, it is ideally suitable for population-based studies of diseases in healthy individuals. Finland is representative as a genetically, environmentally and ethnically homogenous isolate with good linkage disequilibrium (Traglia et al, 2009; Varilo et al, 2000).

Recently, the NHANES (2011–2012) study demonstrated differences in TT among race/ethnic groups of unknown reason (Vesper et al, 2015). Several trials even assign 55 % to 60 % for testosterone heritability in men (Harden et al, 2014; Harris et al, 1998) and similar familial similarity in women (Koenis et al, 2013). Several twin and family studies have confirmed testosterone inheritance to be a modest to very good cause of variability in testosterone levels (Panizzon et al, 2013). Several genes are responsible for synthesis, regulation and action of testosterone. Beside a single-nucleotide polymorphism (SNP), which is related to base testosterone levels (Chen et al, 2016), most effort has been invested in research of androgen receptor (AR) and SHGB (Vandenput and Ohlsson, 2014). The length of CAG triplets as well as GGN triplets on two different AR genes has been positively associated with the amount of testosterone in men (Travison et al, 2010; Bogaert et al, 2009). The length of GGN triplets varied between ethnic groups (Kaufman et al, 2019). Not only testosterone is subject to genetic variability, but also cardiovascular risk factors. The Genome-wide association studies (GWAS) have mapped several genes to be involved in the development of CVD (Giral et al, 2018) (Siitonen et al, 2011; Benjamin et al, 2017). The homogenous genetic pool of the finish population offers less genetically derived confounding factors and is therefore beneficial to determine the predictive impact of TT on cardiovascular disease.

Beside ethnical differences, the geographical location is of great importance for the testosterone level (Harden et al, 2014; Ellison et al, 2002). The multicenter study MrOS (Osteoporotic Fractures in Men), which combines older men from Hong-Kong, Sweden and

the United-States, demonstrated lower testosterone to be associated with a higher cardiovascular risk in men (Ohlsson et al, 2011). The results stay in contrast with FINRISK 97. A possible explanation could be the negligence of ethnic as well as geographical origin to be an important confounding factor in the MrOs study. Even within Finland, there are geographical differences regarding the cardiovascular risk prediction (Vartiainen et al, 2000). 4.4.1.2 Population Structure of FINRISK

FINRISK 97 subjects have an average age of 47 to 49 years. According to the Framingham risk score (FRS), even subjects between the age of 45 to 49 are already at increased risk. However, only one trial examines younger subjects between 45–59 years (Smith et al, 2005). The main part of trials summarized in the meta-analysis from Araujo (Araujo et al, 2011) examines aging probands (>59 years). This form of selection bias reduces the validity of predictive value for testosterone on CVD.

Probands in FINRISK 97 are between 25 to 74 years old and were randomly chosen. Involving children as seen in NHANES would lead to misinterpretation, as TT within male aged 6 to 12 years show completely different prepubertal values (Vesper et al, 2015). In contrast to several other population-based cohorts, Finish probands are not only younger but also healthier (Araujo et al, 2010) (Vikan et al, 2010). Additionally, FINRISK 97 has a good linkage to centrally managed registers. External validation of the FINRISK biomarker study took place in other prospective trials like in the Southall And Brent REvisited study (SABRE with n=2622) as well as in the British Women's Heart and Health Study (BWHHS with n=3563) (Tillin et al, 2013; Lawlor et al, 2003; Würtz et al, 2015).

4.4.1.3 Categorization of Testosterone

The use of categorized variables is ambivalent. To simplify the statistics, testosterone levels are divided into quartiles. This allows to compare extreme values with each other and uncovers associations between sub cohorts, which would disappear within the overall association. Comparing these quartiles leads to several problems since two probands with very similar testosterone levels can be in different quartiles. This leads to the assumption that the two probands could show a very different risk prediction, despite similar testosterone level. The imprecision is therefore intensified by implicating a step-function assuming the results of CVD risk estimation within the same quartile show homogenous results and do not vary within each quartile. However, there is no proof for a constant risk within each quartile.

Except for FINRISK 97, quartiles are often distributed not due to the median cutoff-point, but due to statistical significance. Quartiles show ranges of different amplitude and every amplitude shows a different number of participants. Ranges of quartiles differ between studies

(Brand et al, 2014) allowing only restricted comparability between studies. Improvement in risk estimation can be done by adding splines and fractional polynominals to multivariable regressions (Bennette and Vickers, 2012). 4.4.1.4 Multiple Imputation:

Missing data are common in large trials. This influence on results increases with the percentage of missing data (Moons et al, 2012). In FINRISK 97, missing data have not been excluded, but multiple imputations were performed to prevent false estimates by a subsample with completely observed data. The studies as well as metanalysis covering the same objective regarding testosterone and cardiovascular disease did not mention the use of missing data (Ding et al, 2006). The analyses regarding AF/IST and T2D only minimally affected the number of individuals using hypertensive medication as well as changed a bit the median testosterone values. The entire baseline-table with available cases is not shown.

4.5 Limitations of FINRISK 97

Despite many advantages mentioned before, FINRISK 97 also reveals limitations, which need to be considered before drawing possible conclusions.

4.5.1 Testosterone measurement

The chemiluminescent microparticle immunoassay (CMIA) (Abbott ARCHITECT 2nd Generation Testosterone; Abbott Diagnostics) is a one-step direct immunoassay, with several disadvantages. The measurement accuracy is affected by positive falsification when the analyte decreases. The assay quantifies additional and increasing competing compounds besides testosterone (cross-reactivity) (Rowe and Rabet, 2018). It took decades to reduce this so called cross-reactivity between different androgens. The negative bias is generated by the incomplete isolation of SHGB from TT as well as the higher affinity of special substances to the antibody. Additionally, the within-assay inaccuracy is diverse at low concentration being smaller than < 8% at concentrations of > 24 nmol/L and between 6.1 % and 22 % at concentrations of < 3nmol/L (Taieb et al, 2003). The perpetual bias is made by different calibration methods.

In 2001, Taieb et al. compared the Architect i2000 as well as other eleven immunoassays on the basis of 116 men, children and women with an average age of 44.7 years and with a range of 19 –71 years regarding testosterone comparability. The internal reference was an isotope-dilution gas chromatography-mass spectrometry (ID/GC-MS), which does not have the feature of cross-reactivity or matrix -effect and therefore is the gold standard for androgen measurement especially in probands with low levels (Siekmann, 1979). In accordance with earlier results, all participating assays clearly differed by over- or underestimation from the gold standard especially in women and children. Although the correlation coefficient was between 0.86 and 0.97, the overall underestimation was 12% on average below gold standard (Taieb et al, 2003). The results of the Architect i2000 overestimated testosterone for women twofold but convinced with accurately results for men. Another direct immunoassay called Immulite 2000 have been used for testosterone measurements in the Tromsø Study by Vikan et al. (Vikan et al, 2009), as well as in the EPIC-Norfolk Trial by Khaw et al. (Khaw et al, 2007). In both trials the age-adjusted average testosterone level in men were much lower and showed fivefold diffuse values for women compared with the gold standard (Taieb et al, 2003). Using the Immulite 2000, both trials found a significant association between lower TT as well as fT and a raised mortality in men. The study design these two trials (EPIC Norfolk and the Tromsø study) are similar to FINRISK but differ in terms of testosterone measurement. It could be

possible that the different results are based on the inaccuracy of the Immulite 2000 compared to the Architect i2000 in FINRISK 97.

From all eleven participating immunoassays, the Architect I2000 was the only one to receive satisfying results for men regarding testosterone levels at the lower end of the range (Taieb et al, 2003).

In FINRISK 97, male participants with an average age of 49 years had a median TT level of 17.01 nmol/l. A very similar prospective population-based trial of Laaksonen et al. comprising Finish men with an average age of 51.3 years showed a median TT of 18.0 to 20.6 nmol/l (Laaksonen et al, 2004). As similar trials regarding healthy men with comparable age show ambivalent results regarding testosterone and T2D, the question raises if immunoassays are an acceptable method to quantify androgens. Based on the cross-sectional Study of Health in Pomerania (SHIP) (age-range: 40-74 years), Haring et al proved, that immunoassays and ID/GC-MS did only differ regarding three of ten cardiovascular risk factors comprising BMI, serum glucose levels and waist circumference), but not for total testosterone (Haring et al, 2013).

To compare TT around the world, the Endocrine Society sets up harmonized age-adjusted reference ranges for TT in men. Therefore, several community-dwelling cohort studies (FHS, EMAS, MrOS, SIBLOS) with all together over 9000 men were combined and the arithmetic median values for TT were assessed. Different assays have been calibrated by using a reference method at Centers for Disease Control and Prevention (CDC) (Travison et al, 2017). Compared to the CDC reference range, the median testosterone level of 17.01 (not adjusted for obesity) with an average age of 49 in FINRISK 97 is in between the 50th and the 75th percentile of the CDC reference. Although the architect I2000 is a sufficient tool for testosterone measurement, it remains a challenge to accurately determine testosterone levels at the lower borderline of testosterone. To improve the results, the testosterone assays should be standardized for gender, age as well as comorbidities (Travison et al, 2017). Besides there is need for a testosterone standard in calibration for all laboratories (Rosner et al, 2007) as well as more than just a single testosterone measurement for each patient. In FINRISK 97 all endpoints have been associated to a single baseline TT measurement. No data is given about the intraindividual variability of TT within the observational time.

4.5.2 Time of testosterone measurement

Blood in FINRISK 97 has been taken throw-out the day. Pearson-correlation-coefficients of TT and time of blood draw revealed that the earlier the blood has been taken during the day, the higher the testosterone level has been in both genders. This finding is in line with a diurnal rhythm of TT, showing high levels in the morning and lower levels in the afternoon (Diver et al, 2003). It has been proven, that the acrophase of TT is between 07:00 and 07:30 AM for young and middle aged men (Diver et al, 2003). 30 minutes after waking, TT is supposed to drop by 32% to 39% of diurnal alteration (Panizzon et al, 2013). Therefore, cox-regression analyses have been adjusted in model 3 for time of blood draw before 2 AM. Considering, that model 3 differs from model 2 due to the confounding factor time of blood draw before 2 PM, the results were not as expected.

Although the association between TT and T2D for men increased in FINRISK 97, the risk estimations for all other endpoints (AF/IST, CHD/mortality) did not change in men and no endpoint changed for women. This finding relativizes the importance which diurnal rhythm exerts on time of testosterone measurement in FINRISK 97. A meaningful diurnal effect would have affected all endpoints. Testosterone rises in healthy young men during daytime for the same amount as for an equivalent span of time during the night. Testosterone falls while awaking and during sleeping disturbances (Axelsson et al, 2005). This small trial with only seven participants shows TT to be regulated by sleeping patterns, making it possible to present similar TT during day and nighttime after long-time-sleeping. Sleeping less than 5 hours during five following nights lowers testosterone levels around 10% -15% in young adults (Leproult and van Cauter, 2011). A daily sleep of less than four hours is associated with significantly reduced (28%) levels of testosterone (Goh et al, 2007).

In another trial, blood was taken every 2.5min from 10 young and 8 old men, while an EEG recorded their sleeping status. The depth of sleeping showed a positive relationship to TT, although none of them used to be hypogonadal. While deep sleep raised TT in young probands, this association could not be found in the older probands. This raises the question, if age has an influence on diurnal rhythm of TT due to the increasing sleeping disturbances in aging men and women.

Sleeping quality and sleeping quantity release over lifetime (Oh et al, 2012). As disturbance of sleeping patterns as well as sleep length have an impact on TT in young healthy men, it is possible to assume an age-related consequence on lower TT due to reduced sleep efficiency. Several trials with men aged from 45 –74 were able to show, that reduced night time sleep is associated with lower TT in the morning (Lord et al, 2014). Sleep quality could be even used as an independent indicator for TT (Penev, 2007). The results in coordinated secretion of

testosterone as well as their regulatory hormones (GnRh/LH) could be embed in a regulatory sleep/wake pathway of the central nervous system, whose function is restricted due to the process of aging (Veldhuis et al, 2000; Garcia-Falgueras et al, 2011).

In summary, the number of sleeping hours in middle aged to older men in FINRISK 97 is important for preserving a diurnal rhythm of TT with high levels in the morning and lower levels in the afternoon. Diminishing diurnal rhythm could be a sign of disturbed nigh sleep or vise verse. Fragmented sleep as well as short time sleep is supposed to have a lowering effect on TT (Axelsson et al, 2005). Upper airway resistance mostly appearing as sleeping apnea (OSAS) is a popular disease especially in aging men (Luboshitzky et al, 2002) as well as an independent risk factor in the finish population for CHD and T2D (Strausz et al, 2018). Due to the upper airway resistance, the oxygen intake is reduced, followed by a disturbed night sleep. OSAS could possibly be a reason for reduced testosterone in the morning as well as diminishing diurnal rhythm. Treating patients suffering from OSAS with continuous positive airway pressure (CPAP) showed ambivalent amelioration of testosterone levels (Wittert, 2014). Future research should consider OSAS as a possible confounding factor. The very small effect of diurnal variation in TT could be caused by sleep disturbances, as sleep shows great influence on TT and sleep quality decreases with age.

On the other side, probands who came in for assessment before 2 PM showed a reduced risk for incident T2D. It could be a possible explanation that older probands with a higher risk for T2D were invited later the day for clinical examination and blood draw. Another explanation could be found in diurnal variations of fasting glucose-levels. The Dawn phenomen as well as the Somogyi effect can cause hyperglycemia in prediabetic as well as unknown diabetic probands (Rybicka et al, 2011). Both syndromes are the result of diminishing glycemic control and show hyperglycemia in the morning, while fasting glucose levels in the afternoon appear to be normal (Bolli et al, 1984). In FINRISK 97, T2D was diagnosed as having a fasting glucose level above 126 ng/dl at baseline. Therefore, probands in the morning would be preferentially diagnosed with T2D and excluded from further analyses compared to probands in the afternoon. The possibility of developing T2D during the next years would be higher in the afternoon group. This hypothesis however assumes that asymptomatic prediabetes and T2D distributed evenly throughout the day.

4.5.3 Confounding factors

4.5.3.1 Medication

Medication can interfere with testosterone as well as with the endpoint. Aging is associated with increasing comorbidities and consistently increasing medication use. In FINRISK 97, only blood pressure medication was taken into account. Popular conventional drugs with an impact on testosterone are explained in the following to point out, whether this consideration is sufficient or if additional confounding factors should be included in future population-based trials.

Oral contraception (COC) therapies are widespread around the world but so far not included in any population-based study. They reduce the TT level by hampering androgen synthesis and increasing SHGB. A meta-analysis of 1495 healthy young women (between 18–40 years) found an average decrease of 31 % in TT during combined oral contraception (Zimmerman et al, 2014). The COC probably constrains testosterone synthesis by inhibiting the ovarian (Kuhl et al, 1985) as well as the adrenal androgen synthesis (Fern et al, 1978). Further analyses regarding testosterone in women should consider COC as well as other hormone therapy as confounding factor.

Due to the Narcotics Control Board (INCB), the consumption of morphine increased seven-fold from 1970 to 1998. While opioid consumption in the United States, Australia, New Zealand and many European countries extensively raised during the last decades, many low income countries as well as Finland, France, Ireland and Switzerland showed a declining or low level remaining use of opioids untill 2016 (Bosetti et al, 2019). The WHO assumes, that 66 % of the world population is not able to get opioid medication to release pain and only 7.5 % is able to benefit from opioids. Opioids are more and more consumed by patients suffering pain from non-malignant origin. (Duthey and Scholten, 2014). A meta-analysis in 2015 including 17 trials with 800 probands using opioids as well as 1969 controls demonstrated approximately 50 % lower testosterone levels in men, but not in women (Bawor et al, 2015). The opioid-induced androgen deficiency (OPIAD) is well known (O'Rourke and Wosnitzer, 2016), but there is an ongoing debate, which daily morphine-dose induces hypogonadism and which opioid is more likely to develop hypogonadism. Morphine can down regulate the hypothalamic-pituitary-axis and therefore reduces the testosterone secretion (Gabriel et al, 1985; Vuong et al, 2010). But chronic abuse does not have to be causal for low TT. Testosterone is supposed to have an antinociceptive effect on the temporomandibular joint (Fischer et al, 2007), as well as modulating the μ-opioid receptor (MOR) and therefore to reduce pain. By influencing the endogenous opioid system, low TT may be a marker for pain and hyperalgesia (Coluzzi et al, 2018). In summary, the use of opioids in an aging society with chronic pain is increasing and therefore the impact of opioids on TT raises. No data about opioid use have been collected in

FINRISK 97. However, it can be assumed, that the impact of opioid on the testosterone level is small due to the low prevalence of opioid use in Finland compared to other European countries as well as the USA. Additional population-based trials are required.

Cholesterol is indispensable for the synthesis of testosterone. Statins and HMG-CoA reductase inhibitors lower cholesterol levels by reducing the de-novo synthesis of cholesterol in the liver. Considering that cholesterol is ingested sufficiently, no impact on testosterone level would be assumed. However, a recent meta-analysis of Schooling et al. reported a significant average decline in TT in men by about 4 % and by about 11 % in women (Alemao et al, 2006). Probands took statins mostly in a dose-rate ranging from 20 to 40 mg per day (Schooling et al, 2013). There is no information about statin use in FINRISK 97, but it is suggested. that "[…] nearly all of the decrease in serum cholesterol levels was explained by dietary changes" (Alemao. 2006. p353; (Alemao et al, 2006). Statin therapy receives an increasing importance in primary and secondary prevention of atherosclerotic CVD and as first line therapy for hypercholesterinemia (Cao and Devaraj, 2019). It is very likely, that the continuous decline in median cholesterol level since the beginning of FINRISK in 1972 (Alemao et al, 2006) will continue as well in the future for both genders (Cao and Devaraj, 2019). It is therefore necessary for future trials to take statins as potential confounders into account. Due to the linear regression analysis of FINRISK, TT and total cholesterol-levels are not associated in both genders. This could be partly caused by the negligible amount of statin-users in 1997 as well as the interindividual difficulty to achieve total cholesterol level < 5mmol/l despite moderate statin therapy (Alemao et al, 2006).

Although alcohol-use has not been examined in FINRISK 97, it can have an impact on the TT. While acute alcohol intoxication reduces TT in healthy men (Välimäki et al, 1990) and raises TT in women (Sarkola et al, 2001), the influence of chronic alcohol intake on TT is ambivalent. While most trials were not able to find an effect of moderate alcohol use on TT, a large trial among European and American men reported young and old men frequently drinking more than 24 units alcohol within a week having higher (Alemao et al, 2006) TT (1.0 nmol/L) compared to men drinking 1–10 units a week (Jensen et al, 2014). These results have been confirmed for men and women by a meta-analysis of three population-based cohorts from Finland (Würtz et al, 2015). This specific confounder should be included in further examinations.

In Finrisk 97 no information is collected about the use of secondary prevention therapy in CVD except for antihypertensive medication. Although the overall benefit of Aspirin as primary prevention in the aging population is not certain (Baigent et al, 2009; Gaziano et al, 2018), Aspirin has been proven to reduce the risk of recurrent vascular events for about 25%

(Antithrombotic Trialists' Collaboration, 2002). The benefit of oral anticoagulation for secondary prevention has been demonstrated in patients with AF (López-López et al, 2017).

4.5.3.2 Stability of testosterone in serum

Blood has been stored for 20 years until it was analyzed (Zeller et al, 2018). This long span of time raises concerns, if testosterone values are falsified due to decomposition processes during storage and conservation. A study measured TT of four different periods ranging from one month, four years, 17 years to 29 years, using liquid chromatography. Before measurement, blood has been stored at -25°C. Interestingly, the oldest samples showed the highest TT (Gislefoss et al, 2012). But this trial could not prove if TT increases due to storage time, or if men had higher TT 29 years ago. Another trial analyzed blood samples in pregnant women, using the Immulite 2000, a chemiluminescent EIA. TT could not be associated to the storage time (Holl et al, 2008). Stability of male testosterone levels for 10 years at -70°C has also been described in another study (Cauley et al, 1987). Significant alterations in TT of blood, which has been stored for 20 years, are possible, but there is lack of evidence. Therefore, TT of FINRISK measured in 1997 are valid after 20 years of storage. It would be interesting, if the average TT of Finish probands has decreased during the last years.

4.5.3.3 Hyperthyreoidism

Hyperthyroidism in the Finish population is prevalent in 2.6 % of women and in 0.6 % of men with increasing tendency in aging. The estimated number of undiagnosed cases is much higher (Bjoro et al, 2000). Furthermore, the overall incidence of hyperthyroidism has raised within the last years (Leese et al, 2008). Hyperthyroidism is associated to a 20 % increased risk of severe CVD events as well as mortality in subclinical hyperthyroidism as well as 65 % increased risk for CVD in patients with known hyperthyroidism (Dekkers et al, 2017; Brandt et al, 2013). The risk for CVD morbidity remains higher compared to the healthy population even decades after thyroidectomy (Ryödi et al, 2014). The functionality of the thyroid gland is tightly connected to TT as triiodothyronine (T3) stimulates SHGB synthesis and SHGB correlates with the functionality of the thyroid gland in the peripheral tissue (La Vignera et al, 2017). Considering that SHGB tightly binds 20 –40 % of all testosterone depending on sex, TT varies with SHGB level (Goldman et al, 2017). Therefore, it can be assumed, that not only hyperthyroidism but also the thyroid function has an influence on TT as well as the cardiovascular endpoint. Further epidemiologic trials should take this specific confounding factor into account.

4.5.3.4 Subfractions of testosterone and cardiovascular risk

The FRS found SHGB independently associated to an increased risk for metabolic syndrome. Even after adjustment of cardiovascular risk factors, the association remained significant in contrast to TT (Bhasin et al, 2011). Another trial found lower SHBG to be independently associated to incident T2D even after adjustment of TT or fT (Lakshman et al, 2010). In contrast, the increased risk for T2D and metabolic syndrome lost statistical significance after adjustment of SHGB (Bhasin et al, 2011).

Similar inverse relation between SHBG and T2D have been found in women as well (Fenske et al, 2015; Muka et al, 2017). Besides, lower SHGB levels have been associated to an adverse cardiovascular risk profile in men (Canoy et al, 2014) as well as in women (Goodman-Gruen and Barrett-Connor, 1996) and is even sometimes stronger associated to increased CVD risk profile compared to TT (Brand and Schouw, Y T van der, 2010). The results regarding low free testosterone and CVD are heterogenous, but several epidemiological trials found a positive association between lower free testosterone and higher risk for overall mortality and stroke (Hyde et al, 2012; Yeap et al, 2014; Yeap, 2015). In another trial fT lost statistically significance, while lower TT was associated with T2D (Lakshman et al, 2010). Due to the strong link between TT and protein-bound testosterone, further analyses of FINRISK should consider SHBG and albumin as confounding factors.

5. Summary and Conclusion

In contrast to most existing population-based trials, FINRISK compares both genders, comprises a substantial number of healthy participants, offers a long observation time of 13 years as well as the inclusion of young probands (median age 47– 49 years) at baseline.

The results of FINRISK 97 reveal the sex-specific risk prediction based on TT to suffer from T2D or a cardiovascular disease (AF/IST) within the observation time of 13 years. Not only hypogonadal men but also men with very low to normal values of TT have an increased risk for T2D and for AF/IST. These results are independent from cardiovascular risk factors, especially adiposity. On the other side, women with very low to low TT have a decreased risk of developing T2D and AF and/or IST compared to women with high TT. However, the statistical significance gets lost after the adjustment for cardiovascular risk factors. No predictive value of TT could be found in both sexes for CHD and mortality.

All cardiovascular risk factors are significantly associated to all endpoints. Except for total cholesterol and HDL, every included cardiovascular risk factor is positively associated to all endpoints. Interestingly, these risk factors had a much greater influence on risk prediction in men than in women. Obese men had a tenfold increased risk for T2D compared to obese women. In contrast to the literature, T2D was not determined as a risk factor for IST in patients with baseline AF. Total cholesterol showed an inverse correlation not only to AF and/or IST. Geographical differences between western and eastern Finland in disease distribution for men still existed. Men in Finland faced a much higher risk for CHD, IST, AF, T2D and mortality compared to women.

Despite many important results of this study, FINRISK has several limitations. Important confounding factors such as SHGB, blood-thinners, alcohol, OCT, opioids, statins as well as individuals under testosterone supplementation, which have an influence on TT or on one of the endpoints, were not considered. In women, no cardiovascular risk factor showed a statistically significant association to the endpoint or to TT. Body composition markers (BMI, WHR) did not correlate with TT although changes in body composition due to testosterone alterations were proven. Therefore, future research should consider additional confounding factors especially for women.

Diurnal rhythm of TT had a negligible effect on TT in FINRISK 97. A possible explanation could be the diminishing diurnal rhythm of aging men due to increasing sleep disturbances. Sleep quality or sleep quantity should be involved in future research.

Additionally, it is questionable if the TT values are valid due to the insufficiency of chemiluminescent immunoassay for TT measurement in women and men with borderline values. Future studies should prefer liquid chromatography-tandem mass spectrometry. All

participating laboratories should implement standardized calibration methods for TT measurement.

A further limitation is that the blood sampling in FINRISK 97 is based on only one serum and one measurement from each patient. Intraindividual changes in TT during the observation time were not considered. The blood was stored for 20 years. Falsifications of measurement cannot be excluded as the impact of long-term storing on testosterone cannot be regarded as certain.

The risk prediction beyond 13 years is unclear. Considering the Kaplan-Meier survival curves for AF and/or IST as well as T2D however, it is possible to see a trend within the observation time. Over 13 years the survival curves for each quartile become more and more segregated. Assuming a further linear development, the differences in risk prediction among different quartiles would grow leading to a higher risk for men with low TT compared to men with high TT beyond 13 years.

More than 70 % of all CVD deaths occur in low- and middle-income countries of Asia and Africa. Nevertheless, most of trials like FINRISK were set up in Europe and in the USA. Therefore, all prediction models are only suitable for Northern Europe and not validated in African and Asian populations. There is a trend of developing new models, repeating the same trials as well as finding new biomarkers instead of validating and improving the common prediction models to receive generalizability between different populations and continents (Damen, Johanna A A G et al, 2016 May 16).

The results of FINRISK 97 regarding TT and the association with CVD and overall mortality can be summarized in two possible hypotheses. Firstly, TT modifies cardiovascular risk factors like adiposity or lipids and therefore increases the risk for T2D and AF/IST. Secondly, TT influences the endpoint with indirect effects on vascular tone, lipid and glucose metabolism (Kaufman and Vermeulen, 2005; Sievers et al. 2010). TT has also an influence on cardiovascular risk factors as well as T2D and ischemic events. Although the impact is small, it illustrates the importance of TT for the general health. In general, high TT in men and low TT in women could be seen as a good marker for cardiovascular health and as an independent risk marker for T2D. Due to reciprocal relationship between TT and the endpoints AF and/or IST as well as T2D, it is not possible to find a causal correlation.

The results of FINRISK 97 confirm the sex-specific predictive value of testosterone for both genders and especially draws attention to healthy and middle-aged individuals. Due to the impact of TT on the cardiovascular health, TT should be tested for further existing cardiovascular risk prediction models (Chock et al, 2012) and should be used in the clinical setting for both genders to evaluate the individual risk.

However, low TT in men should not be substituted to raise the value and reduce the statistical risk for future cardiovascular events or T2D since the impact of substituted testosterone on a hormonal imbalance are still not clear. The increased rate of cardiovascular events due to testosterone therapy emphasize the danger of the allegedly harmless testosterone substitution. A focus should be put on adiposity. Reducing WHR in men can not only reduce the risk for CVD and T2D but also increase TT.

6. Zusammenfassung

Im Gegensatz zu den meisten bestehenden bevölkerungsbasierten Studien vergleicht FINRISK beide Geschlechter, bezieht eine erhebliche Zahl an gesunden Probanden ein und bietet einen langen Beobachtungszeitraum von 13 Jahren sowie vergleichsweise junge Probanden (Durchschnittsalter von 47– 50 Jahren).

Die Ergebnisse von FINRISK 97 zeigen den geschlechtsspezifischen Vorhersagewert des Gesamt-Testosterons in Bezug auf das Risiko T2D oder kardiovaskuläre Erkrankungen innerhalb des Beobachtungszeitraums von 13 Jahren zu erleiden. Nicht nur hypogonadale Männer, sondern auch Männer mit geringen bis normalen Werten von TT haben ein erhöhtes Risiko für T2D und AF/IST. Diese Ergebnisse sind unabhängig von kardiovaskulären Risikofaktoren, speziell Fettleibigkeit. Auf der anderen Seite haben Frauen mit niedrigen TT ein kleineres Risiko, an T2D und AF/IST zu erkranken, als Frauen mit hohem TT. Diese statistische Signifikanz geht allerdings verloren, sobald die Ergebnisse an kardiovaskuläre Risikofaktoren angepasst werden (WHR, BMI). Für beide Geschlechter konnte kein Vorhersagewert von TT bezogen auf CHD oder Sterblichkeit bestimmt werden.

Unter Berücksichtigung von kardiovaskulären Risikofaktoren vergrößerte sich das Risiko für alle Endpunkte. Diese Faktoren hatten einen viel größeren Einfluss auf die Risikovorhersage bei Männern als bei Frauen. Adipöse Männer hatten ein zehnfach größeres Risiko für T2D als adipöse Frauen. Abweichend von der Literatur wurde T2D bei Patienten, die bereits AF hatten, nicht als Risikofaktor für das Auftreten von zerebralen Ischämien erkannt. Das Gesamt Cholesterin zeigte eine negative Korrelation sowohl zu AF als auch zu IST.

Geographische Unterschiede in der Krankheitsverteilung existierten nach wie vor bei Männern zwischen dem Westen und Osten Finnlands. Hierbei zeigten die finnischen Männer ein höheres Risiko für CHD, IST, AF, T2D und die Gesamt-Mortalität als finnische Frauen.

Trotz vieler wichtiger Ergebnisse dieser Studie hat FINRISK einige Limitationen: Wichtige Faktoren wie SHGB, Blutverdünner, Alkohol, OCT, Opioide, Statine sowie einzelne Personen mit supplementärem Testosteron, welche einen Einfluss auf TT oder auf einen der Endpunkte haben, wurden nicht berücksichtigt. In Frauen wurde kein statistisch signifikanter Zusammenhang zwischen kardiovaskulären Risikofaktoren und einem Endpunkt oder TT nachgewiesen. Marker der Körperbeschaffenheit (BMI, WHR) korrelierten nicht mit TT obwohl Veränderungen der Fettmasse und Muskelmasse hervorgerufen durch Veränderungen des

Testosterons in Frauen bereits nachgewiesen wurden. Daher sind weitere Studien notwendig, um den prädiktiven Wert mittels zusätzlicher Störfaktoren präzise vorherzusagen.

Darüber hinaus ist es aufgrund der Insuffizienz der Chemilumineszenz-Immunoassays für TT Messungen bei Frauen im Allgemeinen und Männern mit niedrigen Grenzwerten fraglich, ob die TT Werte gültig sind. Zukünftige Studien sollten bevorzugt Flüssigkeitschromatographie/Tandem-Massenspektrometrie für die Messungen verwenden. Alle teilnehmenden Labore sollten standardisierte Kalibrierungsmethoden für TT Messungen verwenden.

Eine weitere Limitierung ist, dass die Blutentnahme in FINRISK 97 auf nur einer Blutentnahme und einer Messung pro Teilnehmer basiert. Intraindividuelle Veränderungen in TT wurde damit nicht mit einbezogen. Das Blut wurde für 20 Jahre gelagert. Verfälschungen der Messungen können nicht ausgeschlossen werden, da der Einfluss auf eine Langzeit-Lagerung bisher nicht ausreichend untersucht worden ist.

Der prädiktive Wert über 13 Jahre hinaus ist unklar. Unter Berücksichtigung der Kaplan-Meier Überlebenskurven für AF und IST sowie für T2D ist es aber möglich, einen Trend innerhalb der Beobachtungszeit festzustellen. Über 13 Jahre teilte sich die Überlebenskurve für jedes Quartile mehr und mehr auf. Wird eine weitere lineare Entwicklung vorausgesetzt, würden die Unterschiede der Risikovorhersage innerhalb der verschiedenen Quartile weiter wachsen, was zu einem höheren Risiko für Männer mit niedrigem TT verglichen mit hohem TT führen würde.

Mehr als 70 % aller CVD Tode betreffen Menschen in asiatischen und afrikanischen Ländern mit geringem oder mittlerem Einkommen. Dennoch stammen die meisten großen epidemiologischen Studien wie FINRISK aus Europa oder den USA. Alle Vorhersagemodelle sind daher lediglich für Nordeuropa oder die USA gültig und können nicht auf Afrika oder Asien bezogen werden.

Die Ergebnisse von FINRISK bezüglich TT und der Assoziation zu CVD und Sterblichkeit können in zwei Hypothesen zusammengefasst werden. Erstens modifiziert TT kardiovaskuläre Risikofaktoren wie Fettleibigkeit oder Lipide und verändert damit das Risiko an Diabetes Mellitus Typ 2 oder Vorhofflimmern mit oder ohne zerebrale Ischämie zu erkranken. Zweitens beeinflusst TT das Entstehen kardiovaskulärer Erkrankungen durch indirekte Auswirkungen auf den Gefäßtonus, Lipid- und Glucose-Metabolismus. TT hat Einfluss auf kardiovaskuläre Risikofaktoren, Diabetes mellitus Typ 2 und ischämische Ereignisse. Trotz eines geringen Einflusses auf kardiovaskuläre Risikofaktoren wird die Wichtigkeit von TT auf die Gesundheit deutlich. Generell konnte ein hoher TT Wert in Männern und ein niedriger TT Wert in Frauen als ein guter Marker für die kardiovaskuläre Gesundheut und als ein unabhängiger Risikomarker für T2D betrachtet werden. Aufgrund der wechselseitigen Beziehung zwischen

TT und den Endpunkten AF und IST sowie T2D ist es nicht möglich eine kausale Korrelation festzustellen.

Die Ergebnisse aus FINRISK 97 bestätigen Testosteron als prädiktiven Marker für beide Geschlechter und konzentriert die Aufmerksamkeit auf die gesunde Bevölkerungsschicht mittleren Alters, die ein zunehmendes Risiko für kardiovaskuläre Erkrankungen und Diabetes Mellitus Typ 2 entwickelt. Neben der Anwendung im FRS sollte Testosteron in weiteren Risikoprädiktionsmodellen getestet werden (Chock et al, 2012) und zunehmend auch im klinischen Setting der Primärprävention genutzt werden, um das individuelle Risiko für kardiovaskuläre Erkrankungen besser einschätzen zu können. Jedoch sollten niedrige Testosteron Werte nicht mittels Substitution ausgeglichen werden, um damit das statistische Risiko für kardiovaskuläre Erkrankungen zu reduzieren. Die Auswirkungen von substituiertem Testosteron auf ein hormonelles Ungleichgewicht sind immer noch unklar und eine erhöhte Anzahl an kardiovaskuläre Ereignissen nach Testosteron Substitution verdeutlicht die Gefährlichkeit der vermeintlich harmlosen Testosteron Substitution. Ein Schwerpunkt sollte auf die Reduktion von Fettleibigkeit gelegt werden, da hiermit das Risiko für Diabetes Mellitus Typ 2 und kardiovaskuläre Erkrankungen effektiv reduziert werden und gleichzeitig der Testosteron Spiegel bei Männern erhöht werden kann.

7. Liste der aus der Dissertation hervorgegangenen Vorveröffentlichungen

1. Zeller T, Schnabel RB, Appelbaum S, Ojeda F, Berisha F, Schulte-Steinberg B, et al. Low testosterone levels are predictive for incident atrial fibrillation and ischaemic stroke in men, but protective in women - results from the FINRISK study. Eur J Prev Cardiol. 2018;25(11):1133–9. doi:10.1177/2047487318778346.

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8. Table of Figures

9. List of Tables

10. List of Abbreviations

AF/IST: Atrial Fibrillation and/or Ischemic Stroke 23, 24, 36, 38, 52, 57, 59
AIDS: Acquired Immune Deficiency Syndrome 6
AM: ante meridiem 38
AR: Androgen receptor 34, 49, 50
BMI: Body-Mass-Index 14, 15, 16, 17, 37, 45, 46, 49, 50, 56
BWHHS: British Women's Heart and Health Study 35
CAD: Coronary artery disease 9, 55
CDC: Centers for Disease Control and Prevention 38
CHD: Coronary heart disease 3, 9, 14, 15, 29, 30, 38, 55, 56, 57, 60
CMIA: Chemiluminiscent Microparticle Immunoassay 12, 36
CVD: Cardiovascular disease 3, 4, 5, 8, 9, 10, 15, 35, 44, 55, 57, 58, 59
EMAS: European Male Aeging Study 7, 38
EPIC: European Prospective Investigation of Cancer study 37
FDA: Food and Drug Administration 8
FHS: Framingham Heart Study 4, 38
FRS: Framingham risk score 4, 5, 9, 10, 35;
Framingham Risk Score 4
fT: free testosterone 6
GWAS: Genome-wide association study 34
HR: Hazard Ratio 17, 18, 19, 20, 21, 23, 25, 26, 27, 29, 31
ID/GC-MS: isotope-dilution gas chromatography-mass spectrometry 37
LOH: Late-onset hypogonadism 7
MI: Myocardial Infarction 4, 15
MrOS study: Osteoporotic Fractures in Men study 35, 38, 55
NHANES: National Health and Nutrition Examination Survey **34**, **35**
SABRE: Southall And Brent REvisited 35
SHGB: Sex hormone-binding globulin 34, 36, 40, 52, 58
SHIP: Study of Health in Pomerania 37, 57
SIBLOS: Longitudinal Extension Phase of Sibling Pair Linkage Analysis 38
SNP: single-nucleotide polymorphism 34
T2D: Type 2 Diabetes Mellitus 3, 4, 9, 10, 14, 15, 17, 18, 19, 20, 21, 22, 23, 25, 26, 27, 29, 31, 36, 37, 38, 39, 44, 46, 47, 48, 49, 50, 51, 52, 54, 55, 56, 57, 59, 60
T3: Triiodothyronine 43
TIA: Transient ischemic attack 9

11. References

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13. Lebenslauf

„Lebenslauf wurde aus datenschutzrechtlichen Gründen entfernt“

14. Eidesstattliche Erklärung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.

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