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ERECTILE DYSFUNCTION IN CARDIOVASCULAR RISK POPULATION

Otto Ettala

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To Pia, Oiva and Helmi

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Erectile dysfunction in cardiovascular risk population
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ABSTRACT

The inability to achieve and to maintain erection, erectile dysfunction, is a bothersome symptom of elderly men. Moreover, there is a high comorbidity between cardiovascular diseases and erectile dysfunction. However, very little is known concerning the risk factors of ED in apparently healthy men without comorbidities affecting the arteries.

A cross-sectional population survey was conducted from August 2005 to September 2007 in two rural towns of Harjavalta and Kokemäki in Finland. Excluding those with previously diagnosed cardiovascular diseases, diabetes or chronic kidney disease, every community-dwelling inhabitant was invited to take part in the survey. Of the 2939 45- to 70-year-old men invited, 2049 responded. Selecting those at risk for cardiovascular diseases, 1000 eligible men were examined.

According to the International Index of Erectile Function short form 57% of the studied men reported erectile dysfunction. Increasing age, smoking, depressive symptoms, decreasing pulmonary function, sedentary lifestyle, non-marital status and low education level were associated with increasing risk of erectile dysfunction. However, hypertension, diabetes, obesity, hypercholesterolemia were not associated with erectile dysfunction, although these associations have been described in numerous previous studies. Moreover, erectile dysfunction was not associated with increasing risk of pre-diabetes.

In apparently healthy men, increasing age, smoking, depressive symptoms, decreasing pulmonary function, sedentary lifestyle, non-marital status, low education level but not hypertension, obesity, hypercholesterolemia, diabetes or pre-diabetes were associated with increasing risk of erectile dysfunction.

Keywords: erectile dysfunction, cardiovascular diseases, physical activity, pre-diabetes, hypertension, COPD

Otto Ettala
Erektiohäiriö valtimotautiriskiväestössä
Turun ylipisto, Lääketieteellinen tiedekunta, Kirurgia; Turun yliopiston kliininen tohtoriohjelma; Turun yliopistollinen keskussairaala, urologia; Satakunnan keskussairaala, Kirurgian klinikka; Keski-Satakunnan terveydenhuollon kuntayhtymä, Harjavalta
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TIIVISTELMÄ

Erektiohäiriö määritellään kyvyttömyydeksi saavuttaa ja ylläpitää erektio. Se on elämänlaatua haittaava oire, jonka tiedetään esiintyvän etenkin ikääntyvillä miehillä. Koska erektiohäiriön riski on selvästi suurentunut valtimotauteja sairastavilla miehillä, on mahdollista että tällä voi olla vaikutusta saatuihin tuloksiin. Itse asiassa erektiohäiriötutkimuksia, joissa tutkittavilla miehillä ei ole valtimotautia on vain yksittäisiä.

Harjavallassa ja Kokemäellä käynnistettiin väestöpohjainen, sydän ja verisuonitauteja selvittävä poikkileikkaustutkimus vuonna 2005. Kaikki 45-75 –vuotiaat kaupunkilaiset, joilla ei ollut aikaisemmin todettua valtimotautia, diabetesta tai munuaisten vajaatoimintaa, kutsuttiin tutkimukseen. Kun 2049 (70%) kutsuun vastanneesta miehestä valittiin ne, joilla oli korkea riski sairastua valtimotauteihin, yhteensä 1000 miehen tiedot analysoitiin.

Erektiokyky määritettiin International Index of Erectile Function short form –kaavakkeella, jonka mukaan 57% miehistä oli erektiohäiriö. Korkea ikä, tupakointi, masennusoireet, heikkenevä keuhkojen toimintakyky, liikkumattomuus, parisuhteen puuttuminen ja vähäinen koulutustaso liittyivät lisääntyneeseen erektiohäiriön riskiin. Vaikka useat tutkimukset ovatkin osoittaneet, että verenpaine, diabetes, lihavuus ja kolesteroliaineenvaihdunnanhäiriöt liittyvät erektiohäiriöön, tällaista yhteyttä ei tässä tutkimuksessa todettu. Erektiohäiriön ei myöskään todettu liittyvän lisääntyneeseen diabeteksen esiasteiden riskiin.

Miehillä, joilla ei ollut aikaisemmin todettua valtimotautia, diabetesta tai munuaistautia, korkea ikä, tupakointi, masennusoireet, heikkenevä keuhkojen toimintakyky, liikkumattomuus, parisuhteen puuttuminen ja vähäinen koulutustaso liittyivät lisääntyneeseen erektiohäiriön riskiin.

Avainsanat: erektiohäiriö, valtimotaudit, liikunta, sokeritauti, verenpainetauti, keuhkoahtaumatauti

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ABBREVIATIONS

2Q	questions two and three of IIEF-5 questionnaire
BDI	Beck's depression scale
BMI	body mass index
BP	blood pressure
CAD	coronary artery disease
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CVD	cardiovascular disease
DBP	diastolic blood pressure
ED	erectile dysfunction
eNOS	endothelial nitric oxide synthase
FEV_1	forced expiratory volume
FEV_1%	adjusted forced expiratory volume
GTP	guanosine triphosphate
HDL-C	high-density lipoprotein cholesterol
HPFS	the Health Professionals Follow-Up Study
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
IIEF-5	the International Index of Erectile Function short form
LDL-C	low density lipoprotein cholesterol
MetS	metabolic syndrome
MMAS	the Massachusetts Male Aging Study
NHSLS	the National Health and Social Life Survey
NIH	the National Institutes of Health
nNOS	nervous nitric oxide synthetase
NO	Nitric oxide
OGTT	2-hour oral glucose tolerance test
OR	odds ratio
OSA	obstructive sleep apnoea
RCT	randomized controlled trial
RR	relative risk
SBP	systolic blood pressure
SD	standard deviation
TAMUS	Tampere Aging Male Urological Study
TRT	testosterone replacement therapy
WC	waist circumference

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original publications, which are referred to in the text by the Roman numerals I-IV.

I **Ettala OO, Syvänen KT, Korhonen PE, Kaipia AJ, Vahlberg TJ, Boström PJ, Aarnio PT.** High intensity physical activity, stable relationship and high education level associate with decreasing risk of erectile dysfunction in 1000 apparently healthy cardiovascular risk subjects. *J Sex Med.* 2014; 9:2277-2284.

II **Ettala OO, Korhonen PE, Syvänen KT, Vahlberg TJ, Kaipia AJ, Aarnio PT, Boström PJ.** Erectile dysfuntion cannot be used in primary screening of pre-diabetes. *Diabetes Res Clin Pract* 2015; 108:e60-62.

III **Korhonen PE, Ettala O, Kautiainen H, Kantola I.** Factors modifying the effect of blood pressure on erectile function. *J Hypertens* 2015; 33:975-980.

IV **Ettala OO, Korhonen PE, Syvänen KT, Kaipia AJ, Vahlberg TJ, Aarnio PT, Boström PJ, Saaresranta T.** Decreased pulmonary function is associated with high risk of erectile dysfunction. *Submitted.*

1. INTRODUCTION

Erectile dysfunction (ED) is described as the inability to achieve and to maintain erection adequate for sexual activity and for penetration (National Institutes of Health, 1993). Although it is a benign condition, ED causes impairment in quality of life and discomfort not only to the affected man but also to his partner (Fisher et al, 2005). It is a sensitive issue to talk about and, therefore, not all men seek medical assistance (Nicolosi et al, 2006). Despite being a tabu, the worldwide prevalence of ED is projected to increase in coming decades and to reach 320 million by 2025 (Ayta et al, 1999).

ED *per se* does not cause morbidity or mortality and, therefore, it should rather be regarded as a symptom than a disease. At present ED is considered as a mixture of psychogenic factors and somatic causes (Hatzimouratidis et al, 2010) and regarded as a sentinel marker for underlying diseases, especially cardiovascular diseases (CVD). In fact, ED, coronary artery disease (CAD), peripheral arterial disease and cerebrovascular disease are suggested to be manifestations of the same disease affecting the arteries (Montorsi et al, 2003b). In fact, it has been demonstrated that ED is associated with silent CAD (Montorsi et al, 2003a) and becomes evident prior to major coronary artery events (Montorsi et al, 2006) and stroke (Ponholzer et al, 2005b).

Therefore, efforts to study ED are crucial not only for the condition itself but also to the investigation of its comorbidities. While there is a close interplay between CVD and ED, only few studies concerning ED have been conducted in healthy men devoid of comorbidities affecting the arteries (Grover et al, 2006). Therefore, the aim of this thesis was to investigate ED and its comorbidities in apparently healthy men without previously diagnosed CVD, diabetes or kidney disease.

2. REVIEW OF THE LITERATURE

2.1. Penile erection

The penis has basically two tasks: the passing of urine and the vaginal delivery of the semen. Urination usually involves the penis in the flaccid state but for the successful penetration the penis needs to be erect.

2.1.1. Anatomy of the penis

The penis is composed of three cylindrical structures, the paired corpora cavernosa and the corpus spongiosum, which houses the urethra. The basis of the corpora cavernosa, the crus, originate from each side of the lower margin of the inferior pubic ramus, meet and pair below the symphysis forming the dorsum of the penile shaft. The crus of the corpus spongiosum, in turn, originates from and attaches to pelvic floor muscles, traverses towards the symphysis to be attached to the groove below the two corpora cavernosa and then traverses to the tip of the penis forming the glans (Figure 1).

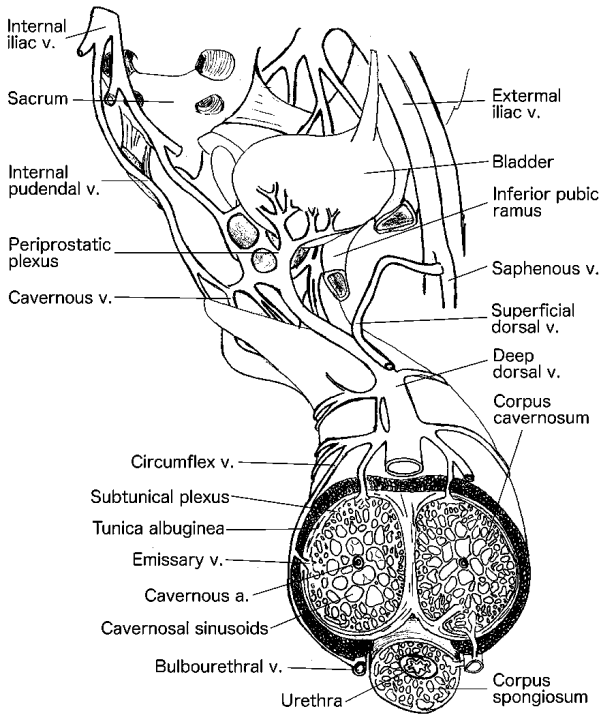

Figure 1. Venous anatomy of the penis Modified from Campbell-Walsh, Urology, 10th Edition.

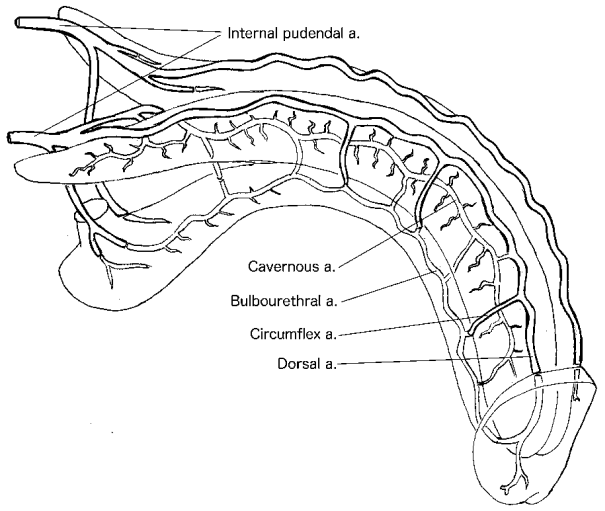

Figure 2. Arterial supply of the penis. Modified from Campbell-Walsh, Urology, 10th Edition.

The corpora carvernosa are covered with a thick bilayered fibrous capsule, the tunica albuginea. In the inner layer the fibres are oriented in circular fashion, whereas in the outer layers they form longitudinal structures. The thick tunica supports the spongy cavernosal sinusoids and enables the development of high intracavernosal pressure during erection. The thin capsule of corpus spongiosum, on the other hand, lacks outer fibres, ensuring a low-pressure compartment in the erected penis allowing the semen to traverse the urethra. In addition to these capsules, both the bases of the corpus spongiosum and the two cavernosa are covered with striated muscles, the bulbocavernosus and the ischiocavernosus, respectively (Hsieh et al, 2012).

Arteries of the penis originate from the internal pudendal artery a branch of the internal iliac artery. These paired arteries are divided into three branches, the dorsal, bulbourethral and cavernous arteries. The cavernous arteries traverse the corpora cavernosa giving rise to the helicine arteries, which directly supply the sinusoids. The dorsal arteries, in turn, travel to the tip of the penis to supply the glans, whereas the bulbourethral artery provides arterial blood to the corpus spongiosum. On reaching the tip, the three paired arteries all anastomose to form a vascular ring near the glans (Figures 1 and 2). Arterial blood is traversed through the trabecular tissue of the corpus, the cavernosal sinusoids, and drained into a subtunical venule plexus between the peripheral sinusoids and the tunica albuginea. The venous blood is further drained into the emissary veins piercing the tunica and then mainly to the deep dorsal vein joining the periprostatic venous plexus (Figure 1).

The innervation of the penis is both autonomic and somatic (Figure 3). The sympathetic efferent fibres from the 11^{th} thoracic to the 2^{nd} lumbar spinal segments travel in hypogastric and in the pelvic nerve, and to a lesser extent, in the pudendal nerve, whereas the parasympathetic efferent fibres from the 2^{nd} to the 4^{th} sacral spinal

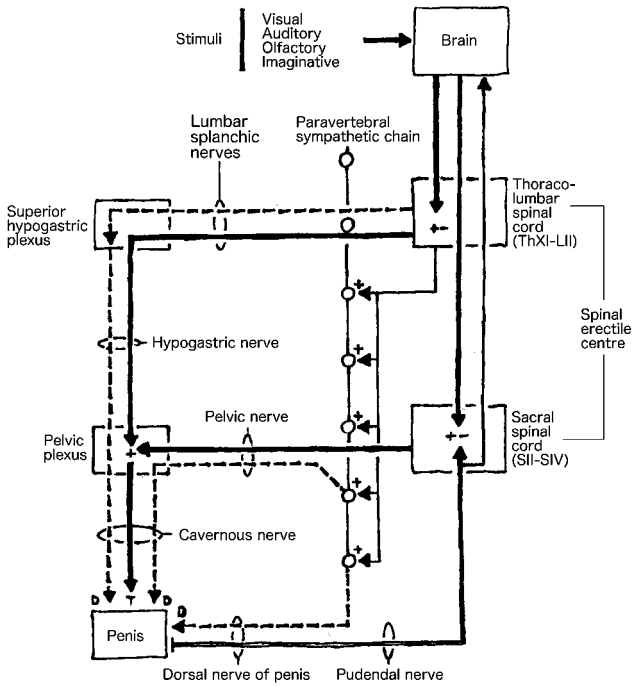

Figure 3. Autonomic and somatic innervation of the penis. Synaptic inhibitory and excitatory mechanisms are indicated by – and +, respectively. D, penile detumescence; T, penile tumescence. Modified from Steers. Neurosci Biobehav Rev. 2000; 24:507-516

segments travel in the pelvic nerve. The hypogastric and the pelvic nerve form the pelvic plexus located at the ventral margin of rectum. Originating from the plexus, the cavernous nerve, travels along the posterolater margin of the prostate, pierces the pelvic floor with the urethra and divides to the corpora cavernosa and corpus spongiosum to innervate the smooth muscle of helicine arteries and carvernous sinuses. Afferent somatosensory information from the penile skin, glans and urethra and a small fraction of the sympathetic fibres are carried in the dorsal nerve of the penis merging with the pudendal nerve. While the afferent somatosensory fibres enter the spinal cord via the 2nd to the 4th sacral roots, the efferent sympathetic fibres originate from the pelvic paravertebral sympathetic chain (Steers, 2000).

2.1.2. Physiology of penile erection and detumescence

Although it is a complex mechanism and nowhere nearly understood, it has been suggested that inhibitory and excitatory signals elicited from the brain and the periphery are integrated in the thoracic and sacral spinal cord referred to as the spinal erectile centre (Figure 3).

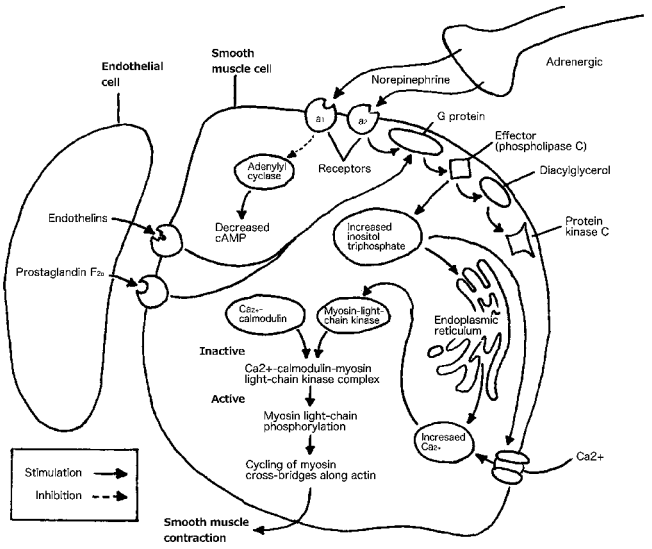

Figure 4. Schematic illustration of molecular mechanisms contributing smooth muscle contraction. cAMP, cyclic adenosine monophosphate. cAMP, cyclic adenosine monophosphate; a1 and a2, noradrenalin receptor subunits. Modified from Campbell-Walsh, Urology, 10th Edition.

The flaccidity of the penis is maintained by the semicontracted state of the intracorporeal smooth muscles lining the carvernosal sinusoids and the helicine arteries (Figure 4). Noradrenalin from the cavernous sympathetic nerve terminals, endothelins and prostaglandin F_{2a} from endothelial cells bind to receptors on smooth muscle cells lining the helicine arteries and sinusoids. After a cascade of cytosolic

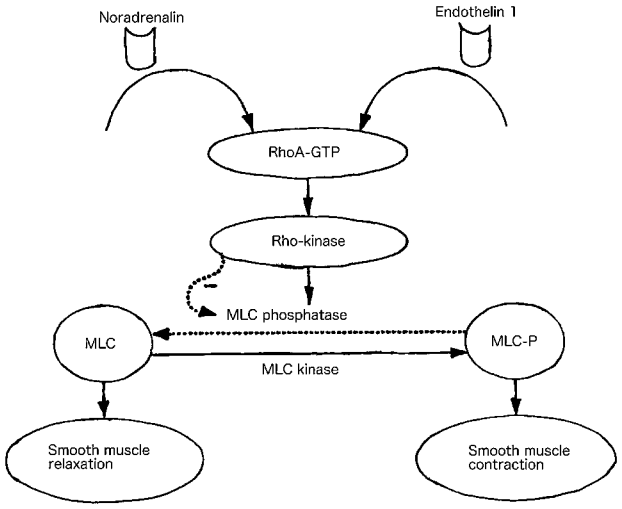

Figure 5. Schematic illustration of molecular mechanisms contributing smooth muscle relaxation by calcium-independent pathway. GTP, guanosine triphosphate; MLC, myosin light chain; MLC-P, phosphorylated myosin light chain. Modified from Campbell-Walsh, Urology, 10th Edition.

enzymatic reactions, Ca^{2+} is released from the sarcoplasmic reticulum and taken from the extracellular matrix leading to an increase in cytoplasmic Ca^{2+} and formation of calcium-calmodulin complex. This complex activates the myosin light chain kinase, which phosphorylates the myosin light chain, eventually leading to smooth muscle contraction. Theoretically, the contractile process is terminated by the de-phosphorylation of the myosin light chain by the myosin light chain phosphatase and, therefore, the pathway induces phasic smooth muscle contraction and only a short-term detumescence. However, a Rho-kinase pathway, also termed the calcium-sensitisation or calcium-independent pathway helps to maintain the flaccid state (Figure 5). Noradrenalin and endothelin induce Rho-GTP to activate Rho-kinase, which, in turn, inhibits myosin light chain phosphatase, leading to subsequent tonic smooth muscle contraction and long-term penile flaccidity.

In addition to excitatory impulses elicited in the brain by tactile, visual, imaginative or olfactory stimuli, the tactile excitation of the genitals can also induce a short-acting pro-erectile spinal reflex without involvement of supraspinal pathways (Figure 3). The excitatory signalling in the spinal erectile centre induces nonadrenergic noncholinergic terminals of the cavernous nerve to release nitric oxide (NO) produced by the neuronal nitric oxide synthase (nNOS) and parasympathetic nerve terminals to release

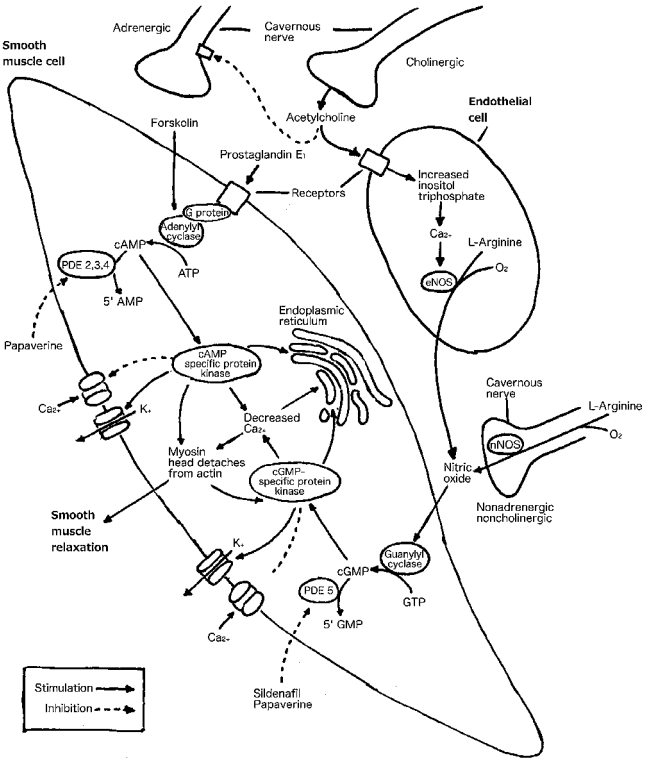

Figure 6. Schematic illustration of molecular mechanisms contributing smooth muscle relaxation. cGMP, cyclic guanosine monophosphate; cAMP, cyclic adenosine monophosphate; GTP, guanosine triphosphate; 5' GMP, guanosine monophosphate; PDE, phospodiesterase; eNOS, endothelial nitric oxide synthetase. Modified from Campbell-Walsh, Urology, 10th Edition.

acetylcholine. The increased cytosolic concentration of NO in the smooth muscle cell induces the formation of cyclic guanosine monophosphate. This leads to increased uptake of Ca^{2+} into the sarcoplasmic reticulum and its release out of the cytoplasm, eventually leading to a decrease in cytosolic Ca^{2+} (Mills et al, 2002). The mechanism is potentiated by the acetylcholine, which binds both the endothelial cells and the smooth muscle cells. It induces the formation of NO by the endothelial nitric oxide synthetase (eNOS), and actives the cyclic adenosine monophosphate pathway, both leading to further decrease in cytosolic Ca^{2+}. In addition to its action on Ca^{2+}-concentration, the increased NO concentration antagonises the RhoA/ Rho-kinase. Subsequently, the decreased cytosolic Ca^{2+} and the suppressed RhoA/ Rho-kinase activity lead to decreased calcium-calmodulin complex content and decreased myosin light chain kinase activity and smooth muscle relaxation. The cascade is further potentiated by the gap junctions, connecting the smooth muscle cells to one another, which allow a rapid spread of the generated electronic current and smooth muscle relaxation (Figure 6) (Christ, 2000).

The relaxation of the smooth muscle cells leads to vasodilatation of the helicine arteries and relaxation of the sinusoid walls and, eventually, to an increase in blood flow to the enlarged corporal sinusoids. The increase in sinusoid volume compresses the subtunical venular plexuses between the tunica and lateral margin of the cavernous sinusoids decreasing the venous drainage. Although the tunica albuginea allows a gradual enlargement of the penis, stretching to its maximum capacity, it further impairs the blood outflow by obstructing the emissary veins piercing the tunica. This increase in intracorporeal pressure and volume eventually leads to enlargement and erection of the penis. Finally the penile hardness is potentiated by the contraction of the ischiocavernosus muscles covering the bases of the two corpora cavernosa.

2.2. Erectile dysfunction

The terms impotence and erectile dysfunction were used interchangeably until the 1990s. However, the term impotence was abandoned due to its pejorative implications and lack of precision (Rosen & Leiblum, 1992). In 1993, the National Institutes of Health (NIH) defined ED as the inability to achieve and to maintain erection adequate for penetration (National Institutes of Health, 1993). It should be distinguished from sexual dysfunction, which is an umbrella term including ED, ejaculatory dysfunction, orgasmic dysfunction, low desire or performance and sexually related pain (Laumann et al, 1999a). The main focus of this thesis is on ED and, therefore, other aspects of sexual dysfunction are not discussed any further.

2.2.1. Pathophysiology of erectile dysfunction

Penile erection is a complex phenomenon including a delicate interplay of anatomical, vascular, neural and hormonal components. Although numerous classifications have been developed to categorise ED, in the most commonly used classification ED is divided into categories of vasculogenic, neurogenic, anatomical, hormonal, drug-induced, psychogenic, and traumatic ED (Hatzimouratidis et al, 2010). The main

interest of this thesis is to describe the association between cardiovascular risk factors and ED and, therefore, the review of the literature is mainly concentrated on vasculogenic ED.

During the past 30 years molecular mechanisms contributing the formation of ED have been under an extensive investigation. Although some studies have been conducted in humans, the pathophysiology and molecular mechanisms are mainly addressed in animal models (Chung et al, 2011).

NO-mediated relaxation is considered as one of the main mechanisms of penile erection (Burnett, 1997) and, in fact, one of the main targets of pathogenesis. Although it has been suggested that impaired signalling in eNOS (Hurt et al, 2002), endothelial dysfunction, is the main mediator in the pathogenesis, studies have shown that also impaired signalling of nNOS, the nitrenergic dysfunction, may lead to impaired erectile response. Endothelial dysfunction has been suggested to be due to either decreased eNOS expression (Bivalacqua et al, 2003), reduced eNOS activity (Musicki et al, 2005), increased expression of eNOS inhibitors such as the radical oxygen species (Bivalacqua et al, 2005), or hyperglycemia-induced factors such as N-acetylglucosamine (Musicki et al, 2005). Nitrenergic dysfunction, in turn, has been shown to be due to decreased expression of nNOS (Numao et al, 2007), although it is likely that also reduced nNOS activity and increased nNOS inhibitor content contribute to the condition as well. In addition to impairment in NO-induced vasodilatation, Reilly et al. demonstrated that castrated rats had enhanced reactivity to α_1–adrenoreceptor stimulation (Reilly et al, 1997), suggesting that sympathetic overactivity and subsequent increased smooth muscle contractility may facilitate impaired erectile function. Furthermore, it has been demonstrated that both the overactivity (Jin et al, 2006) or overexpression (Chang et al, 2003) of RhoA/ Rho-kinase resulted in increased smooth muscle contractility, suggesting its crucial role in the development of ED.

In addition to arterial and sinusoidal smooth muscle impairment, also venous leakage and impaired compression of the venous system have boon shown to lead to ED. This veno-occlusive dysfunction has been demonstrated to be due to decreased elasticity of the penile supportive structure and the endothelium by increased collagen synthesis (Moreland et al, 1995) and reduction in trabecular smooth muscle content (Nehra et al, 1998).

2.2.2. Methods to define erectile dysfunction

Basically, there are two categories of methods to define ED: self-administered questionnaires and objective tests and evaluations assessing erectile function. The most commonly used objective tests are the penile duplex doppler ultrasound and the penile tumescence and rigidity monitoring, of which the former has been shown to be the most reliable instrument to evaluate ED. Done using oral pharmacostimulation or intracavernosal injection of vasodilative agent and performed by a highly trained physician, penile duplex doppler ultrasound measures penile blood flow during erection (Lue et al, 1989). The penile tumescence and rigidity monitoring, in turn, was

developed to differentiate psychogenic ED from other causes of ED. It was designed to assess the number, the duration and the rigidity of nocturnal erections (Kessler, 1988). However at present, it is known that nocturnal erections may be absent in men with psychogenic ED as well (Thase et al, 1988). In adition to these two, there have also been several attempts to develop techniques to evaluate the neurophysiology of erectile function such as bulbocavernosus reflex latency time (Ertekin et al, 1985), pudendal somatosensory evoked potentials (Opsomer et al, 1986), and sympathetic skin response (Curt et al, 1996), none of which have gained clinical significance (Giuliano & Rowland, 2013). In fact, major limitations of all these techniques are the need for highly trained personnel and the fact that they are costly and time-consuming. Therefore, in clinical practice, they are used in selected patients only. In addition, they are not suitable for large-scale observational studies.

Questionnaires are criticised due to fact that they may not evaluate the aetiology (Blander et al, 1999) or the severity of the ED (Tokatli et al, 2006), and results are affected by the respondent's personal characteristics, i.e. literacy and cultural background. In addition, the results vary greatly if the data are collected via telephone, internet, or mail survey. However, questionnaires are non-invasive, easy-to-use and most often standardized and validated. Therefore, they are widely used in clinical practice and are the only rational options to be used in large epidemiological studies.

Several studies, including the Sildenafil trial (Goldstein et al, 1998), have used a single question concerning the penetration ability to define ED. However, the NIH Consensus Panel recommended the development of a quantitative and qualitative classification system for ED (National Institutes of Health, 1993). Partly due to this recommendation, several classifications have been developed. The most referenced questionnaires are the Brief Male Sexual Functional Inventory (O'Leary et al, 1995), the International Index of Erectile Function (IIEF) (Rosen et al, 1997), the Centre for Marital and Sexual Health Functioning Questionnaire (Glick et al, 1997), the Changes in Sexual Functioning Questionnaire (Clayton et al, 1997), and the Erectile Dysfunction Inventory of Treatment Satisfaction (Althof et al, 1999), of which the IIEF is the most widely used.

The IIEF was developed, cross-culturally and linguistically validated and psychometrically tested by Rosen et al. in 1996 to 1997 as an adjunct to the Sildenafil Clinical Trial Program. Although it was developed primarily to assess the efficacy endpoint in randomized controlled trials (RCT) of ED, it has been since widely adopted as a standard measure for assessing sexual function both in clinical and in research settings. The questionnaire consists of 15 items evaluating five different domains of sexual function, namely, erectile function, orgasmic function, sexual desire, intercourse satisfaction, and overall satisfaction. Each question is graded on a 5-point ordinal scale in which lower scores represent poorer erectile function.

Later, an abridged version concerning only erectile function was developed. The International Index of Erectile Function short form (IIEF-5) was constructed using four questions from the sexual function domain and a single question from the intercourse satisfaction domain of the original IIEF (Rosen et al, 1999). Although the

IIEF-5 was originally designed to assess erectile function over the past four weeks, later six months was adopted as the reference period due to recommendations published by the NIH (National Institutes of Health, 1993). Similarly to the IIEF, each question is graded on a 5-point scale. In addition, all questions but one, the confidence in having and maintaining erection, include a zero score denoting sexual inactivity.

2.3. Prevalence and incidence of erectile dysfunction

The first modern population-based study concerning ED, the Massachusetts Male Aging Study (MMAS), was conducted in 1987 in the US by Feldman et al. They described a prevalence of 52% among non-institutionalized 40- to 70-years-old men (Feldman et al, 1994). Since then, several studies have addressed the issue with substantial differences between the estimates. While Imai et al. reported in 2010 that as many as 89% of 280 Japanese men had ED of some degree (Imai et al, 2010), Andersen et al. found that among randomly selected men in Sao Paulo, Brazil, the prevalence was only 17% (Andersen et al, 2010). Additionally, two population-based studies in Finland have addressed the issue 15 years ago. In both studies, conducted in the Tampere region and published by Koskimäki et al. and Shiri et al., 75% of the men reported ED of some degree (Koskimäki et al, 2000; Shiri et al, 2003b). The heterogeneity of the prevalence estimates are suggested to be partly due to cultural differences (Nicolosi et al, 2003). However, equally important are the methods used to define the dysfunction, as well as the population characteristics of the selected participants such as age, comorbidities, and socioeconomic class.

While there are clearly fewer prospective than cross-sectional studies, similarly to prevalence, the estimates of incidence of ED vary considerably. In the MMAS follow

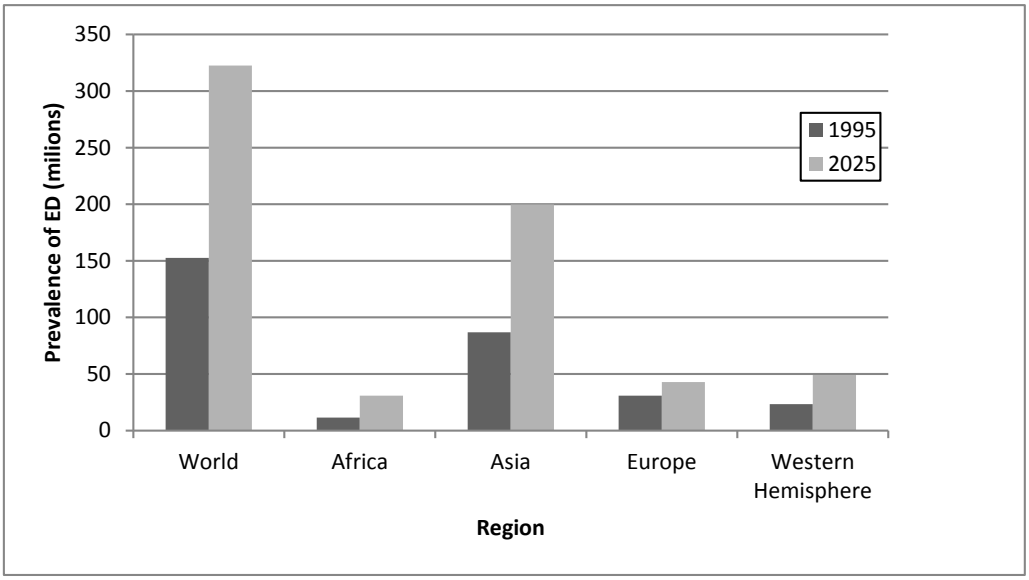

Figure 7. Worldwide increase in prevalence of erectile dysfunction by year 2025 predicted by Aytac in 1999. ED, erectile dysfunction. Modified from Aytac. BJU Int. 1999; 84:50-56.

up study, Johannes et al. reported that the incidence of ED was 25.9 /1,000 person years in 847 men 40 to 69 years of age [8], whereas Schouten at al. found an incidence of 87.1/ 1,000 person years in 50- to 75-years-old Dutch men (Johannes et al, 2000; Schouten et al, 2005).

Despite the heterogeneity of the studies, the worldwide prevalence of ED is predicted to increase considerably in 2025 (Figure 7) (Ayta et al, 1999). Interestingly, the increase is seen not only in developed countries but also in the developing world, in Africa, Asia, and Southern America. The trend has been postulated to be due to decreasing birth rate and increasing life expectancy, leading to global aging (Ayta et al, 1999; United Nations, 2013).

2.4. Risk factors for erectile dysfunction

The first large-scale observational studies were conducted in the US during the 1980s and the 1990s. In addition to the MMAS (Feldman et al, 1994), the Health Professionals Follow-Up Study (HPFS) (Bacon et al, 2003) and the National Health and Social Life Survey (NHSLS) (Laumann et al, 1999a), were also well known globally and considered to be the first valid studies to evaluate ED and its risk factors. However, the Tampere Aging Male Urological Study (TAMUS) (Shiri et al, 2003b) should not be forgotten, due to its impact on Finnish male health and urologic epidemiological research.

2.4.1. Age

The world population is aging, which leads to a profound increase in age-related conditions and ailments including ED. Age is, in fact, by far the most prominent factor affecting ED; and almost every observational study has demonstrated its detrimental effect on erectile function. It has been demonstrated to be associated with both the prevalence anf the incidence of ED. Chew et al. reported that 9% of 20- to 29-years-old and as high as 76% of 80-years-old or older Australian men had ED of some degree (Chew et al, 2000). In addition, it was evaluated that in 428 Brazilian men the incidence of ED was 33/ 1,000 person years among 40- to 49-years-old men and 190/ 1000 person years among 60- to 70-years-old men (Moreira et al, 2003). Furthermore, the association has been demonstrated to be dose-dependent: the older the man the poorer the erectile function. In a cross-sectional study of the 1982 Turkish men the prevalence of severe ED was 0.4% among 40- to 49-year-old men compared to 48.9% in 70 years old or older men (Akkus et al, 2002). Similarly, in those 1323 Finnish men attending the Tampere Aging Male Urologic Study, the incidence of complete ED was 5/ 1,000 person years among 50 to 55 years old men and it increased up to 52/ 1,000 person years in 70- to 75-years-old men (Shiri et al, 2003a).

However, what is worth of noting is the differences in experiencing and reporting ED among different age groups. In a telephone survey by Giuliano et al., younger French men (40 to 79 years of age) were more prone to over-estimate and older men (≥ 70 years of age) were likely to under-estimate their ED, when their self-reported ED was

compared to IIEF-scoring (Giuliano et al, 2002). In fact, this demonstrates the complexity of the phenomenon and the fact how ones perception greatly influences the outcome. Most probably this is partly due to fact that the older men have adapted to the situation by using other ways to get sexual gratification than penetration, or they consider impaired erectile function as a "normal for their age" (Perelman et al, 2005).

In addition to the increased prevalence of comorbidities affecting erectile function such as diabetes (Guariguata et al, 2014), late-onset male hypogonadism (Feldman et al, 2002), and CVD (Conroy et al, 2003), the effect of aging on ED has been suggested to be due to sensorial, hormonal and structural changes occurring in advancing age. Although veno-occlusive dysfunction is suggested to be the most prevalent factor in age-related ED (Kovanecz et al, 2007), impairments in nitrenergic relaxation (Numao et al, 2007) and elevated RhoA/Rho-kinase activity (Jin et al, 2006) have been demonstrated to be mediators of the age-realated ED as well. In addition to these, it has been demonstrated that age-related reduction in tactile sensitivity of the penis (Rowland et al, 1989) impairs the pro-erectile sacral spinal reflex and the excitatory signalling to the brain.

In summary, aging is closely related to ED in a dose-dependent manner and, therefore, should be considered a confounding factor in every epidemiological study evaluating ED.

2.4.2. Socioeconomic factors

Socioeconomic status is a composite measure incorporating measures of income, education and occupation (Dutton & Levine, 1989). The effect of low socioeconomic status and its different outcome measures have been studied extensively during the last 40 years and it has been linked with increased morbidity (Barnett et al, 2012) and mortality (Lantz et al, 1998).

Similarly, the association of low socioeconomic status and ED is well established (Kupelian et al, 2008). Although the majority of the studies have evaluated only the association between low education level and ED (Akkus et al, 2002; Grover et al, 2006; He et al, 2007; Mak et al, 2002; Nicolosi et al, 2003; Selvin et al, 2007), also low monthly income (Akkus et al, 2002; Ghalayini et al, 2010; Laumann et al, 1999b; Martin-Morales et al, 2001), and unemployment (Akkus et al, 2002) have been demonstrated to be associated with increased risk of ED. Although not officially included in socioeconomic status, marital status has also been demonstrated to be associated with ED (Grover et al, 2006; Laumann et al, 1999b; Mirone et al, 2004; Nicolosi et al, 2003). In more detail, men in a relationship tend to have better erectile function compared to men living alone.

Although the effect of low socioeconomic status on risk of ED has been addressed in numerous cross-sectional studies, only a few prospective studies have been published. Furthermore, the data have mainly concentrated on the effect of education level on incident ED. In 2000, Johannes et al. demonstrated that compared to men with a low education level, highly educated men were 0.64 (95% CI, 0.44 to 0.91) times less likely to develop ED in eight years of follow up (Johannes et al, 2000).

While it is evident that low socioeconomic status *per se* does not cause impairment in health, multiple studies have evaluated the underlying factors. Recently, a comprehensive review demonstrated the complexity of the association (Chen & Miller, 2013). The authors dealt with the issue on three levels: characteristics of the individual, of the family, and of the neighbourhood. They summarized that individuals with low socioeconomic status were more prone to live in violent neighbourhoods, which has been associated with increased risk for cardiovascular diseases and morbidity. In addition, subjects with low socioeconomic status more commonly came from families with psychiatric conditions such as depression or anxiety and with negative emotional and cognitive states such as hostility and pessimism contributing to a decline in one,s mental health. The review also concluded that that those with low socioeconomic status were prone to unhealthy behaviours such as smoking, physical inactivity, and poor diet (Chen & Miller, 2013).

2.4.3. Smoking

The first valid evidence to show the detrimental effect of smoking on ED was established in 1986 (Gilbert et al, 1986). Although not all studies have corroborated these results (Mak et al, 2002; Ponholzer et al, 2005a), the association has been shown in the majority of cross-sectional and prospective studies (Bacon et al, 2003; 2006; Blanker et al, 2001; Feldman et al, 2000; Grover et al, 2006; He et al, 2007; Nicolosi et al, 2003; Safarinejad, 2003). The evaluated increase in risk of ED in smoking men varies considerably among the populations studied. While Mirone et al. reported that smoking men had 1.2 (95% CI, 1.1 to 1.4) odds to have ED [20], Safarinejad evaluated that men who smoked were 2.4 (95% CI, 1.5 to 3.3) times more likely to have ED compared to non-smokers (Mirone et al, 2004; Safarinejad, 2003). In addition, was demonstrated that, smokers were 1.5 (95% CI, 1.3 to 1.7) times more likely to develop ED in 14 years of follow up (Bacon et al, 2006).

Although it is evident that smoking causes ED, the data concerning smoking cessation on erectile function are controversial and without long-term follow up. The majority of cross-sectional studies have shown that current and former smoking were associated with similarly increased odds to have ED, implying that there may be no risk reduction after smoking cessation (Bortolotti et al, 2001; Mirone et al, 2002; Safarinejad, 2003; Saigal et al, 2006). While no causality can be assessed in cross-sectional studies, Shiri et al. addressed the issue in a prospective setting (Shiri et al, 2005) and demonstrated that smokers, compared to non-smokers were less likely to recover from ED. However, due to the small sample size in the follow up, no significant effects could be found and the results were inconclusive.

Consistent with this, a few clinical trials have shown a moderate improvement in erectile function during a short-term follow up after smoking cessation. Guay et al. demonstrated that in ten currently smoking men nocturnal tumescence and rigidity improved after 24-hour smoking cessation (Guay et al, 1998), while Signinolfi et al. reported increased penile blood flow in 20 current smokers 24 to 36 hours after smoking cessation (Sighinolfi et al, 2007). In addition, Harte et al. evaluated that successful quitters showed a slight amelioration in penile tumescence but no

improvement in IIEF-5 scores in four weeks of follow up (Harte & Meston, 2012), whereas Pourmand et al. demonstrated an improvement in erectile function at least for one IIEF-5 score in one year of follow up (Pourmand et al, 2004).

Smoking has a profound effect on vasculature. It is well known that smoking impairs endothelium-dependent vasodilatation (Celermajer et al, 1993), induces an inflammatory response (Tracy et al, 1997), and prothrombotic effects (Fusegawa et al, 1999), all of which are proven to be essential for development of atherosclerosis. Although the pathophysiology is most likely similar also in smoke-induced ED, the impairment in the NO pathway has been demonstrated to be the most prominent (Imamura et al, 2007; Xie et al, 1997).

2.4.4. Physical activity

Similarly to the positive association between exercise and cardiovascular diseases (CVD) (Schuler et al, 1992), high physical activity has been shown to be associated with decreasing risk of ED. Although a few observational studies were not able to demonstrate the association (He et al, 2007), a meta-analysis published in 2007 by Cheng et al. showed that high physical activity was associated with lower odds to have ED, (OR: 0.53; 95% CI, 0.31 to 0.91) (Cheng et al, 2007b). Consistent with this, Bacon et al. demonstrated that in 14 years of follow up those men with a baseline physical activity level higher than 32.6 metabolic equivalent hours per week were 0.7 (95% CI, 0.7 to 0.8) times less likely to develop ED compared to sedentary men (<2.7 metabolic equivalent hours per week) (Bacon et al, 2006). Furthermore, the issue has been addressed in a few RCTs of which Esposito et al. were the first to demonstrate a positive effect of physical activity on erectile function. They compared 55 men receiving intensive guidance on reducing their calorie intake and increasing their level of physical activity, the intervention group, to 55 men receiving less intense bimonthly oral and written information concerning healthy diet and exercise, the control group. During two years of follow up, 31% of men in the intervention group regained their sexual function (IIEF-score $\geq$ 22) compared to 5% of those in the control group (Esposito et al, 2004). The increase seen in the IIEF-score was evaluated to be due to reductions in body mass index (BMI) and C-reactive protein, and an increase in physical activity level.

Although the molecular background concerning physical activity and ED has not been specifically addressed, studies conducted in subjects with CVD have suggested that the effect of physical activity is mediated through improving endothelial function. It has been demonstrated that increasing physical activity resulted in increased NO bioavailability (Matsumoto et al, 1994), a direct effect of increased eNOS activity (Hambrecht et al, 2003).

Although the results are confirmed also in men with type 2 diabetes (Rosen et al, 2009), in obese men (Khoo et al, 2013), in hypertensive men (Lamina et al, 2009), and in men with ischemic heart disease (Kalka et al, 2014), there are no data to show whether a high level of physical activity is also associated with improving erectile function in healthy men.

2.4.5. Obesity

The association between obesity and ED has been evaluated in numerous observational and clinical trials. Although many of the cross-sectional studies did not find any association (He et al, 2007; Mak et al, 2002; Ponholzer et al, 2005a; Selvin et al, 2007; Stranne et al, 2012), two large cross-sectional (Corona et al, 2010; Saigal et al, 2006) and two prospective studies (Feldman et al, 2000; Shiri et al, 2004a) did confirm the relationship. Because of the several definitions of obesity, namely, weight, BMI, waist circumference (WC), and other measures of visceral obesity such as the sagittal abdominal diameter and waist-to-hip index, and even more methods to define ED, the results are heterogeneous. Therefore, comparison of these studies should be made with caution.

Bearing this in mind, it seems that ED is associated with obesity but not with overweight (Blanker et al, 2001; Han et al, 2011). Comparing men with normal weight (BMI<25 kg/m^2) to overweight (25 kg/m^2 ≥ BMI < 30 kg/m^2) or obese (BMI ≥ 30 kg/m^2) men, the odds of ED were significantly increased in obese men (OR: 1.77; 95% CI, 1.39 to 2.25) but not in overweight men (OR 1.15; 95% CI, 0.84 to 1.57) (Han et al, 2011). Similarly, comparing men with normal WC (WC < 94cm) with less lean (94 cm ≥ WC <102 cm) or centrally obese men (WC ≥ 102cm) the odds of ED were significantly increased in centrally obese men (OR: 1.73; 95% CI, 1.50 to 1.98), whereas the association was not significant in less lean men (OR: 1.32; 95% CI, 0.99 to 1.75) (Han et al, 2011). In addition to this, Bacon et al. (Bacon et al, 2006) demonstrated that obese men (BMI ≥ 30 kg/m^2) compared to normal weight men (BMI<23 kg/m^2) were twice as likely to develop ED (relative risk (RR): 1.9; 95% CI, 1.6 to 2.2) in roughly 10 years of follow up.

In addition to observational studies, the association between obesity and ED has also been addressed in a few non-surgical and a surgical RCTs, all of which demonstrated an improvement in erectile function after weight loss. As described above, Esposito et al. demonstrated that after two years of intensive lifestyle changes, a third of the men with ED regained their erectile function, and that the increase seen in the IIEF-5 score was predicted by the decrease in BMI (Esposito et al, 2004). After this, a few non-surgical studies confirmed the data published by Esposito et. al. (Khoo et al, 2011; Wing et al, 2010). Although the results of one RCT (Reis et al, 2010) and two prospective studies (Dallal et al, 2008; Mora et al, 2013) have demonstrated a beneficial effect of bariatric surgery on erectile function, all three studies are rather small, and, therefore the results should be considered as preliminary.

While it has been well demonstrated that obesity is associated with decreasing testosterone (T) levels (Corona et al, 2008b; Derby et al, 2006), leading to subsequent impairment in erectile function, the specific pathogenic mechanisms of hypogonadism and obesity are complex and not fully understood (Diaz-Arjonilla et al, 2009; Giagulli et al, 1994). In addition to low T levels, while obesity is described as a condition of chronic oxidative stress and inflammation (Higdon & Frei, 2003), endothelial dysfunction is likely to be the most prominent factor in obesity-induced ED (Esposito et al, 2004).

2.4.6. Hypertension

It is generally thought and accepted that hypertension is associated with increased risk of ED, the odds ranging from 1.2 (95% CI, 1.1 to 1.3) (Bacon et al, 2003) to 2.2 (95% CI, 1.7 to 3.2) (Ghalayini et al, 2010). However, the evidence is anything but straightforward. Although the majority of the studies have demonstrated that hypertension is associated with ED (Akkus et al, 2002; Bacon et al, 2003; Braun et al, 2000; Ghalayini et al, 2010; Johannes et al, 2000; Mak et al, 2002; Martin-Morales et al, 2001; Marumo et al, 2001; Mirone et al, 2004; Moreira et al, 2003; Nicolosi et al, 2003; Ponholzer et al, 2005a; Rosen et al, 2003; Saigal et al, 2006; Shiri et al, 2004b), and only few claim otherwise (de Boer et al, 2004; Selvin et al, 2007; Wu et al, 2012), it should be noted that in the majority of the studies, hypertension was defined by patient self-report. In fact, only four of the studies mentioned above have used a proper means to define hypertension, i.e. performed BP measurement. Two of these clearly confirmed the association between hypertension and ED (He et al, 2007; Ponholzer et al, 2005a) and the other two did not (Selvin et al, 2007; Wu et al, 2012). Furthermore, it should be emphasized that participants in every study have included a considerable amount of men with diabetes (2-17%) and/or CVD (1-18%). The only study to address the issue in men without previously diagnosed diabetes or CVD was conducted in 2006 by Doumas et al. (Doumas et al, 2006).

In addition to hyperntension *per se*, it has been generally thought that the majority of cardiovascular and antihypertensive medications are associated with ED. However, these data are derived mainly from observational and prospective studies that, in fact, suggested that almost every group of cardiovascular and antihypertensive drugs was associated with decreasing erectile function (Bacon et al, 2003; Blanker et al, 2001; Derby et al, 2001; Shiri et al, 2007a). A recent systematic review evaluated 14 valid RCTs and concluded that the data are very limited, but it seems that thiazide diuretics and beta-blockers other than nebivolol may be associated with ED (Baumhäkel et al, 2011).

In addition to endothelial dysfunction (Vlachopoulos et al, 2008), also sympathetic overactivity has been suggested to mediate the effect of hypertension on ED (Carneiro et al, 2008b). Furthermore, Toblli et al. evaluated that, in rats, hypertension was associated with increased vascular and corporal smooth muscle cell content, perivascular and neural fibrosis, indicating a detrimental vascular, neural and corporeal changes leading to ED (Toblli et al, 2000).

Despite the rationale of pathogenesis, no valid epidemiological evidence concerning the association between hypertension and ED exists and, therefore, further studies, epidemiological in particular, are warranted.

2.4.7. Diabetes

Next to advancing age, diabetes is most likely the best known and documented risk factor for ED. Initial data concerning the association between diabetes and impotence dates back to the 1950s (Rubin & Babbott, 1958). Since then, the association has been shown in many cross-sectional as well as in prospective studies. In diabetic men, the

odds to have ED range from 1.2 (95% CI, 1.0 to 1.4) to 3.1 (95% CI, 2.4 to 4.2) (Grover et al, 2006; Mirone et al, 2004). In addition, it has been shown that men with baseline diabetes had a 2.49 (95% CI, 1.01 to 6.14) higher risk for developing ED (Moreira et al, 2003). Although the majority of the studies have not distinguished between the two types of diabetes, it is commonly accepted that both type 1 and type 2 diabetes are associated with ED. However, whether men with type 1 diabetes are more likely to have ED than those with type 2 diabetes (Bacon et al, 2002; Fedele et al, 1998) or whether the odds of having ED are similar in both groups (Kalter-Leibovici et al, 2005), is under debate.

Furthermore, it has been demonstrated that both the duration of diabetes and glycaemic control are associated with ED. In a cross-sectional study of 31,027 healthcare professionals, men with 6-10-year history of diabetes were 1.5 (95% CI, 1.2 to 1.9) times more likely to have ED, while men with a diagnosis of diabetes >20 years earlier were 1.7 (96% CI, 1.1 to 2.7) times more likely to have ED compared to those without diabetes (Bacon et al, 2002). In addition, in 9,868 Italian diabetic men, Fedele et al. demonstrated that men with fair (OR: 1.8; 95% CI, 1.5 to 2.1) and those with poor (OR: 3.0; 95% CI, 2.5 to 3.6) glycaemic control had increased risk for ED compared to men with good control of their condition (Fedele et al, 1998).

While the estimates in the Italian study were unadjusted and, therefore, should be interpreted with caution, Kalter-Leibovici confirmed the results five years later by demonstrating that every 1% increment in GHb-A1c was associated with a 9% increase in odds of ED (OR: 1.09; 95% CI, 1.01 to 1.18) (Kalter-Leibovici et al, 2005). Although this finding was not supported by the prospective study by Klein et al. (Klein et al, 2005), two clinical trials have corroborated the results of Fedele et al. and Kalter-Leibovici et al. In 41 diabetic men, Khatana et al. showed that in four months of follow up, the IIEF-5 score improved by two points in those meeting a risk-factor goal of HbA1c<7.0% compared to none in those not meeting the goal (Khatana et al, 2008). In addition, a sub-study of a large scale diabetes intervention, the Diabetes Control and Complications Trial, showed that in type 1 diabetic men with limited microvascular complications at baseline, intensive glycaemic control for 10 years resulted in significantly lower prevalence of ED (OR: 0.33; 95% CI, 0.18 to 0.60) (Wessells et al, 2011).

Consistent with this, also diabetic complications are shown to be associated with higher risk for ED. Retinopathy (OR: 2.06; 95% CI, 1.22 to 3.48) (Chew et al, 2013), microalbuminuria (OR: 2.5; 95% CI, 1.3 to 4.9) (Chuang et al, 2012), macroalbuminuria (OR: 4.5; 95% CI, 1.0 to 20.0) (Chuang et al, 2012) and neuropathy (OR: 2.04; 95% CI, 1.37 to 3.03) (Sasaki et al, 2005) have all been associated with increasing risk of ED.

Although it is generally accepted that diabetes precedes ED, the data are, in fact, controversial. As discussed above, in 31,027 healthcare professionals the risk of ED was not increased until 10 years after the diagnosis of diabetes (Bacon et al, 2002). However, in a study of 3,921 men without established diabetes, every 1mmol/L increase in fasting glucose was associated with a 14% increase in risk of ED (OR:

1.14; 95% CI, 1.04 to 1.24) (Grover et al, 2006). If this was the case, it would be meaningful to hypothesize, that men with ED may be at higher risk for pre-diabetes, namely, impaired fasting glucose (IFG) or impaired glucose tolerance (IGT).

Similarly to epidemiological data, pathogenesis of diabetes-induced ED has also been studied extensively. It has been demonstrated that all three pathways mediating erectile response are affected, the endothelial dysfunction (Bivalacqua et al, 2003), nitrenergic dysfunction (Vernet et al, 1995), as well as increased RhoA/Rho-kinase activity (Chang et al, 2003). Moreover, hyperglycaemia induces the formation of radical oxygen species, the advanced glycosylation end-products (Mullarkey et al, 1990), which are shown to decrease bioavailable NO leading to impaired erectile response (Usta et al, 2003). Furthermore, diabetes-induced ED has also been associated with veno-occlusive dysfunction (Kovanecz et al, 2006), hypogonadism (Corona et al, 2006), in type 2 diabetes in particular, and sympathetic overactivity (Carneiro et al, 2008a).

2.4.8. Dyslipidemia

The term dyslipidemia refers to impairments in concentrations of plasma total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), or triglycerides. While numerous studies have evaluated the association between diabetes and ED, the data concerning the association between ED and dyslipidemias are rather scarce and conflicting. Although there has been a similar number of observational studies demonstrating that dyslipidemias are associated with ED (Martin-Morales et al, 2001; Mirone et al, 2004; Ponholzer et al, 2005a; Rosen et al, 2003; Safarinejad, 2003) and those which do not (Bacon et al, 2003; He et al, 2007; Selvin et al, 2007; Wu et al, 2012), the studies not confirming the association have been, in fact, more valid. Only one of the positive studies and all but one of the negative studies have used a valid methodology to define dyslipidemia i.e. laboratory evaluation of cholesterol status. Moreover, in the majority of the positive studies the odds of ED were only age-adjusted, whereas in every negative study the effect of extensive co-founders was evaluated. Furthermore, the only longitudinal study to assess the effect of dyslipidemia on incident ED did not show any significant association (Feldman et al, 2000).

Consistent with this, the issue has been addressed in a meta-analysis concerning the effect of statin treatment on ED (Kostis & Dobrzynski, 2014). Combining 11 trials, Kostis et al. evaluated that men with statin treatment scored 3.4 (95% CI, 1.7 to 5.0) points more on the IIEF-5 compared to the control group. However, of those 11 trials only two reported a positive result and of these two only one evaluated the lipid lowering effect of statins on ED (Gokce et al, 2012). The other was designed to assess the pleiotropic, non-lipid lowering, effect of the statins on ED (El-Sisi et al, 2013), and suggested that the possible effect of statins on erectile function might not be mediated through improving lipid levels. In fact, of those 11 studies, the most valid trial did not report any effect at all (mean difference: 0.7; 95% CI, -2.3 to 3.7) (Trivedi et al, 2013).

Endothelial dysfunction has been suggested to be the major, and most studied, determinant in hypercholesterolemia-induced ED. In fact, it has been shown that impaired endothelium-dependent (Azadzoi & Saenz de Tejada, 1991), but not, neuronal (Azadzoi et al, 1998) relaxations are affected in hypercholesterolemic rabbits. Although the molecular biology behind the effect of hypercholesterolemia on erectile function is rather clear, the epidemiological evidence is controversial at present. Therefore, the results should be interpreted with caution and more studies are needed to address the issue conclusively.

2.4.9. Metabolic syndrome

Metabolic syndrome (MetS) is a clustering of cardiovascular and metabolic risk factors. Although several definitions have been developed (Alberti et al, 2005; Grundy et al, 2005), MetS is basically characterized as a combination of abdominal obesity, elevated blood pressure, impaired glucose metabolism and/ or dyslipidemia. Similarly to the notion that the presence of MetS is linked with incident CVD events and death (Gami et al, 2007), it has been suggested that men with Mets are more prone to have ED compared to those without (Esposito et al, 2005).

There are a few valid cross-sectional studies showing that MetS is also associated with ED. These eight studies have been summarized in a recent meta-analysis demonstrating that out of 12,067 men included in the analysis those having MetS had a 2.7 (95% CI, 1.80-3.97) increased risk for ED compared to those without the condition (Besiroglu et al, 2015). In addition, they demonstrated that all the components of MetS except HDL were associated with increasing risk of ED. However, concerning the association between MetS *per se*, and ED it should be noted that in only one of those eight studies was a comprehensive multivariate analysis performed. Furthermore, half of the studies reported unadjusted estimates of the association neglecting the evident and major effect of age on ED. Therefore, the results should be interpreted with caution.

In addition to cross-sectional surveys, the issue has been addressed in one prospective study. Kupelian et al. demonstrated that ED predicted MetS (RR: 2.09; 95% CI, 1.09 to 4.02) in 15 years of follow up. However, it is interesting that the effect was only seen in lean men (BMI<25kg/m^2), not in overweight men (BMI>25kg/m^2)(Kupelian et al, 2006). Thereore, the authors stressed the importance of screening CVD risk factors in those men with ED but not traditionally considered at risk for CVD.

Whether the effect of MetS on ED is only due to the combined effect of the components included in MetS or MetS *per se* is associated with ED is under debate. However, it has been demonstrated that men with ED and MetS presented hypogonadism three times more often than those with ED alone (Corona et al, 2008a), suggesting that low testosterone levels could serve as a link between MetS and ED. In addition, similarly to obesity, it has also been shown that low-grade inflammation is an important effector in MetS-related ED. Esposito et al. demonstrated that patients with MetS presented with increased prevalence of ED, reduced endothelial function score and more severe inflammatory-endothelial activation, compared to men without

the condition(Esposito et al, 2005). Interestingly, they also demonstrated an increase in the number of components of the MetS with a reciprocal decrease in prevalence of ED and a further increase in concentrations of markers of inflammation.

2.4.10. Hypogonadism

It has been evaluated that in representative samples, 23% to 36% of men with ED are hypogonadal (Tsertsvadze et al, 2009). However, the cut-off level adopted for the diagnosis of hypogonadism greatly affects the estimates. It has been demonstrated that as few as 7% and as high as 47% of men with ED are hypogonadal if cut-off levels of <7nmol/L and <14nmol/L are used respectively (Köhler et al, 2008). Although a lot of effort has been addressed to evaluate the association between low T and ED, the results are controversial. The main challenge is that the effect of hypogonadism on erectile function is extremely complex (Isidori et al, 2014). In addition, ED is only one of the sexual related symptoms seen in hypogonadal men (Wu et al, 2010), and distinguishing ED from decreased libido or overall sexual functioning may sometimes be challenging.

The European Male Aging Study demonstrated that while total T level correlated with overall sexual function, free T level was independently correlated with ED (O'Connor et al, 2011). In addition, the study also showed that the correlation was evident in total T levels below 8nmol/L, but not above. It is worth noting that the correlation was only evident between total T and overall sexual function and no data concerning ED were reported, However, the results corroborated the earlier notion that variation of total T levels within normal range (11nmol/L or 27nmol/L) had no effect on overall sexual function (Buena et al, 1993).

Several RCTs have addressed the effect of testosterone replacement (TRT) on ED, and recently three reviews/ meta-analyses combined these results (Boloña et al, 2007; Isidori et al, 2005; Tsertsvadze et al, 2009). Due to the fact that these meta-analyses gave conflicting results, Isidori et al. re-evaluated the results of the 20 RCTs in a comprehensive systematic review (Isidori et al, 2014). They concluded that no effect of TRT on ED was seen in men with T > 12nmol/L, and that in 14 studies conducted in hypogonadal men, a beneficial effect on erectile function was observed in ten studies. However, it should be emphasised that out of those 14 studies, hypogonadism was defined as T < 8nmol/L in nine studies, as T <10nmol/L in four studies, and as T < 14nmol/L in one study.

In the review it was also evaluated that TRT caused a mean improvement in the IIEF-5 score of 4.32 ± 2.01 points. Furthermore, they discussed that the effect of TRT on ED was rather slow. While it has been demonstrated that improvement in libido, ejaculation and sexual activity are seen in 2-3 weeks after TRT initiation, it may require as long as six months before the IIEF score is improved (Zitzmann et al, 2013). In fact, a gradual improvement in erectile function was observed beyond 12 months after open-label use of TRT (Hackett et al, 2013).

The effect of T on ED is extremely complex and far from understood. It has been shown that impaired erectile function in hypogonadal animal models is due to the

effect of T on both the central nervous system and the genital tissue. T modulates central nervous system nuclei mainly by stimulating the release of neurotransmitters such as dopamine, oxytocin, and NO, controlling not only ED but also the sexual behaviour (Hull et al, 1999). In genital tissue androgen deprivation, has been suggested to reduce both the nNOS activity and the density of NO excreting nerve fibres (Lugg et al, 1996), leading to decreased bioavailability of NO. The deprivation has also been postulated to increase the $alpha_1$ -adrenergic responsiveness of the smooth muscle cells (Reilly et al, 1997), and induce apoptosis of the smooth muscle cells and increase in extracellular matrix, eventually leading to fibrosis (Traish et al, 1999).

2.4.11. Alcohol usage

Moderate alcohol consumption has been demonstrated to have cardioprotective effects (Ronksley et al, 2011). Therefore, it has been also suggested that moderate alcohol consumption might improve erectile function due to its vasodilatory and anxiolytic effects (Miller & Gold, 1988). However, the data are controversial and scant. The few studies have demonstrated that alcohol worsens (Feldman et al, 1994), improves (Cho et al, 2006; Francis et al, 2007; Nicolosi et al, 2003) or does not have an effect at all (Shiri et al, 2004a) on erectile function. Due to this controversy, Cheng et al. summarized cross-sectional studies in a meta-analysis demonstrating that men consuming alcohol regularly had 0.79 (95% CI, 0.67 to 0.92) less likely to have ED compared to those non-regular users (Cheng et al, 2007a). However, the methodological differences between the studies were substantial and, therefore, the results should be interpreted with extreme caution. In addition, in the only prospective study baseline alcohol consumption did not increase the development of ED in 14 years of follow up (Bacon et al, 2006). In summary, the data is not conclusive and, therefore, both epidemiological and interventional studies are needed to address the issue.

2.4.12. Psychogenic factors

Although ED has been traditionally divided into two major categories, organic and psychogenic, there has recently been a debate concerning the classification (Jannini et al, 2010). The criticism has concentrated mainly on the fact that the majority of men with ED have a psychogenic component of some degree and, therefore, the dysfunction should rather be considered as psychosomatic (Jannini et al, 2010).

Although the majority of the studies have dealt with the association between depression or depressive symptoms and ED (Akkus et al, 2002; Mak et al, 2002; Nicolosi et al, 2003), there are data to show that ED is associated with anxiety (OR: 1.77; 99% CI, 1.15 to 2.72) (Sugimori et al, 2005), panic disorder (OR: 1.33; 95% CI, 1.17 to 1.51) (Blumentals et al, 2004), emotional stress (OR: 3.56; 95% CI, 2.00 to 6.34) (Laumann et al, 1999a), fear of failure (OR: 1.77; 95% CI, 1.08 to 2.90) (de Boer et al, 2004), as well as surmenage (OR: 1.37; 95% CI, 1.01 to 2.27) (de Boer et al, 2004). However, because of its predominance, special emphasis is given to depression.

In depressive men, the odds of ED ranges from 2.05 (1.43 to 2.93) (Mak et al, 2002) to 2.77 (1.83 to 4.21) (Kupelian et al, 2008). Although Martin et al. demonstrated that men with depressive symptoms at baseline were 2.5 (95% CI, 1.2 to 3.7) times more likely to develop ED in five years of follow up (Martin et al, 2014), it is reasonable to believe that the effect is, in fact, bi-directional. Consistent with this, Shiri et al. evaluated that in addition to increased incident ED in depressive men (incidence density range: 4.5; 95% CI, 2.2 to 9.2), those with baseline ED were more prone to develop depression (incidence density range: 1.9; 95% CI, 1.1 to 3.3) compared to men with normal erectile function (Shiri et al, 2007b).

It has been suggested that there are two distinctive mechanisms contributing to the development of psychogenic ED. The complex supraspinal pathways regulate the spinal erectile centre, and in psychogenic ED the inhibitory impulses are suggested to be overridden by the excitatory impulses (Steers, 2000). In addition to this, it has been demonstrated that in men with psychogenic ED, serum noradrenalin levels are higher compared to those with vasculogenic ED (Kim & Oh, 1992), suggesting that increased sympathetic activity plays a major role in this respect .

2.4.13. Impaired pulmonary function

Similarly to CVD, chronic obstructive pulmonary disease (COPD) is a systemic disease with low-grade inflammation(Agusti et al, 2010). Consistent with this, multiple studies have shown a close relationship between decreased pulmonary function and cardiovascular mortality (Sin et al, 2005). In addition, there are preliminary data to suggest that the majority (76%) of men with COPD suffer from ED (Köseoğlu et al, 2005). The only population-based study to address the issue demonstrated that men with self-reported COPD were 1.9 (95% CI, 1.1 to 3.6) times more likely to have ED compared to those with normal pulmonary function (Blanker et al, 2001). In addition, the few case-control studies addressing the issue have postulated that the association could be due to hypogonadism (Karadag et al, 2009), low oxygen levels (Kahraman et al, 2013), or comorbid conditions such as depression or anxiety (Maurer et al, 2008).

Similarly to COPD, obstructive sleep apnoea (OSA) has also been shown to predispose to fatal and non-fatal cardiovascular events (Marin et al, 2005). Bearing this in mind, comorbidity of OSA and ED has been suggested and a few cross-sectional and clinical trials have addressed the issue. It has been demonstrated that increased daytime sleepiness defined by the Epworth Sleepiness Scale and nocturnal decrease in transcutaneous oxygen saturation correlate with decreasing erectile function (Budweiser et al, 2009; Teloken et al, 2006). Additionally, it has been demonstrated in a large-scale registry survey that men with OSA were at increased risk of developing ED (HR: 2.0; 95% CI, 1.8 to 2.2) in four years of follow up (Chen et al, 2015). However, due to major limitations, of which the most prominent is lack of data concerning smoking, the results should be interpreted with caution.

It has been demonstrated that men with OSA present increased sympathetic activity (Ziegler et al, 1997), impaired diuarnal testosterone rhythm (Luboshitzky et al, 2001)

and oxidative stress, and subsequent decrease in NO levels (Yamauchi et al, 2005), all of which are postulated to serve as a pathophysiologic link between OSA and ED.

While asthma is also characterised as a low-grade inflammatory state, one survey suggests that asthma is associated with increasing risk of ED (Chou et al, 2011). However, in this survey, the data concerning smoking is also lacking and, therefore, more studies are needed to address the issue.

2.5. Cardiovascular diseases and erectile dysfunction

Similarly to the worldwide increase in the prevalence of ED, the global annual cardiovascular morbidity and mortality are projected to increase by 6 million in 2030 (WHO, 2014). In fact, it has been suggested that ED, CAD, peripheral arterial disease and cerebrovascular disease are all manifestations of the same disease affecting the arteries (Montorsi et al, 2003b).

The first reports concerning ED in men with coronary artery disease were published in the 1960s when Tuttle et al. reported that 10% of men with a history of myocardial infraction were impotent (Tuttle et al, 1964). However, it was not until 1994 that Feldman et al. published results from the MMAS cross-sectional cohort and demonstrated that of men with treated heart disease 39% were completely impotent compared to 10% in the entire sample (Feldman et al, 1994). During the next ten years a few cross-sectional studies corroborated the result of the MMAS (Blanker et al, 2001; Mak et al, 2002; Safarinejad, 2003) and it was suggested that perhaps ED would, in fact, precede CVD. In 2003, Montorsi et al. developed the "artery size hypothesis" suggesting that atherosclerosis is a systemic disorder, becoming symptomatic first in the small arteries of the penis (1-2mm), then in the intermediate coronary arteries (3-4mm), and finally in the large femoral arteries (6-8mm) (Montorsi et al, 2003b).

In fact, in the same year it was Montorsi et al. demonstrated that of the 147 men with both ED and CAD, 67% reported having ED symptoms approximately three years prior to detection of CAD (Montorsi et al, 2003a). Consistent with this, Vlachopoulos et al. demonstrated that of 50 asymptomatic men with ED, 19% had angiographically diagnosed CAD (Vlachopoulos et al, 2005). Moreover, Ponholzer et al. evaluated that compared to 2,495 Austrian men without ED, those with moderate to severe ED had 65% higher odds for a major CVD event within 10 years according to the Framingham risk score (Ponholzer et al, 2005b).

After these cross-sectional studies, Thompson et al. were the first to publish a prospective study demonstrating that in 8,063 US males attending Prostate Cancer Prevention Trial placebo arm, baseline or incidental ED, developing during the seven years of follow up, predicted cardiovascular events (hazard ratio: 1.45; 95% CI, 1.25 to 1.69) (Thompson et al, 2005). Consistent with these results, baseline ED was further demonstrated to predict all-cause mortality (OR: 1.84; 95% CI, 1.21 to 2.81) and cardiovascular mortality (OR: 1.93; 95% CI, 1.13 to 3.29) (Böhm et al, 2010) in

men with high risk for CVD. However, a study of 2,506 Austrian men was not able to show any association between ED and incident CVD events (HR: 1.2; 95% CI, 0.92 to 1.56) (Ponholzer et al, 2010), nor did Hotaling et al. demonstrate any independent association between ED and incident CVD mortality (hazard ratio: 0.93; 95% CI, 0.71 to 1.24) in 31,296 US males (Hotaling et al, 2012). Although these two studies cast a doubt on the hypothesis, the issue was addressed in a recent meta-analysis combining 14 prospective studies. It demonstrated that ED predicted cardiovascular events (RR: 1.44; 95% CI, 1.27 to 1.63) and all-cause mortality (RR 1.25; 95% CI, 1.12 to 1.39) (Vlachopoulos et al, 2013) but not cardiovascular death (RR 1.19; 95% CI, 0.97 to 1.46).

Although the association between ED and CVD has been explained by the mechanical obstruction of the penile arteries, the artery size hypothesis (Montorsi et al, 2003b), if true (Ponholzer et al, 2012), it is probably applicable only in specific cases of CVD-induced ED. More likely the effect of CVD on ED is mediated through low-grade inflammation and subsequent endothelial dysfunction (Azadzoi et al, 1998). In addition to this, it has been shown that also neurogenic NO-mediated relaxations, the nitrenergic dysfunction, were reduced and adrenergic contractions were potentiated in cavernous tissue of atherosclerotic animals (Azadzoi et al, 1998; Azadzoi et al, 1999).

2.6. General considerations concerning epidemiology

Epidemiology studies the patterns, causes, and effects of health and disease conditions in defined populations. Epidemiological study settings are classically divided into cohort or prospective studies, case-control studies and cross-sectional studies. RCTs and non-randomised controlled trials are referred to as interventional studies and, therefore, are not classically included in epidemiological studies (Mann, 2003).

While cohort studies are designed to study prognosis or incidence, cross-sectional studies are the best to assess associations at a given point of time. Although sometimes misinterpreted to demonstrate an effect, it should be noted that cross-sectional studies cannot assess causality. While they are rather easy and inexpensive to conduct and, therefore, usually a large population is studied, it is common that several variables and multiple outcomes are studied (Mann, 2003).

The most common biases in conducting and analysing cross-sectional studies are those concerning selection of the population, reporting the outcomes and those of cofounding factors (Yu & Tse, 2012). To achieve a representative population and to reduce selection bias, special attention should be paid on sampling and reducing the number of non-responders. If the entire target population cannot be studied, it is recommended that participants should be selected by random sampling in order to grant an equal opportunity for every subject to be included. In addition, meticulous measures i.e. reminder letters, phone calls, should be used to motivate the subjects selected to take part in the study (Mann, 2003).

While the probability of an event in prospective studies is defined using RR, in cross-sectional studies the magnitude of association is presented in OR. OR compares the odds of having the conditions among those with and without the risk factor. Ratios below one denote that the risk factor is associated with decreasing odds of the condition, while ratios above one denote the opposite (Uhari, 2012).

OR should always be accompanied by the CI and the *P*-value. In epidemiology, 95% CI and an alpha level of 0.05 are the most commonly used and should be defined during the study design. 95% CI denotes a range in which the observed parameter, the OR, falls in 95% of the time if the experiment is repeated several times. Generally speaking it describes the uncertainty associated with the observed measure. CI is affected by the sample size and the variability of the measure: the bigger the size and the smaller variability the narrower the CI and the more precise the estimate (Uhari, 2012).

The *P*-value is used for testing a statistical hypothesis. The null hypothesis is generated to oppose the research question. The *P*-value describes the probability of finding the observed results when the null hypothesis is true. If the statistical test yields a *P*-value lower or equal to the predefined alpha level, the null hypothesis is rejected and the observed result is considered statistically significant. It should be noted, however, that the alpha denotes the statistical significance level not the clinical significance or importance, which should be defined and interpreted by other means. Similarly to CI, bigger sample size results in smaller *P*-value. In addition, the precision of the effect estimate also affects the *P*-value: the greater the effect size the smaller the *P*-value (Uhari, 2012).

3. AIMS OF THE STUDY

This thesis was designed to clarify the association between various comorbidities and the odds of ED in an apparently healthy population of men. Specific aims of the study were the following:

1. To describe the prevalence of ED and CVD risk factors which are associated with ED, especially those associated with decreasing odds of erectile dysfunction
2. To study the association between ED and oral glucose tolerance test
3. To investigate whether hypertension *per se* is associated with erectile dysfunction
4. To evaluate the association between decreased pulmonary function measured by spirometry and erectile dysfunction

4. MATERIAL AND METHODS

4.1. Study population

The study subjects were drawn from a population survey, the Harmonica Project (Harjavalta Risk Monitoring for Cardiovascular Disease), conducted from August 2005 to September 2007 in the two rural towns of Harjavalta (7,646 inhabitants on 31.12.2007) and Kokemäki (8,217 inhabitants on 31.12.2007) in southwestern Finland (Korhonen et al, 2009). The aim was to study subjects at risk for cardiovascular diseases.

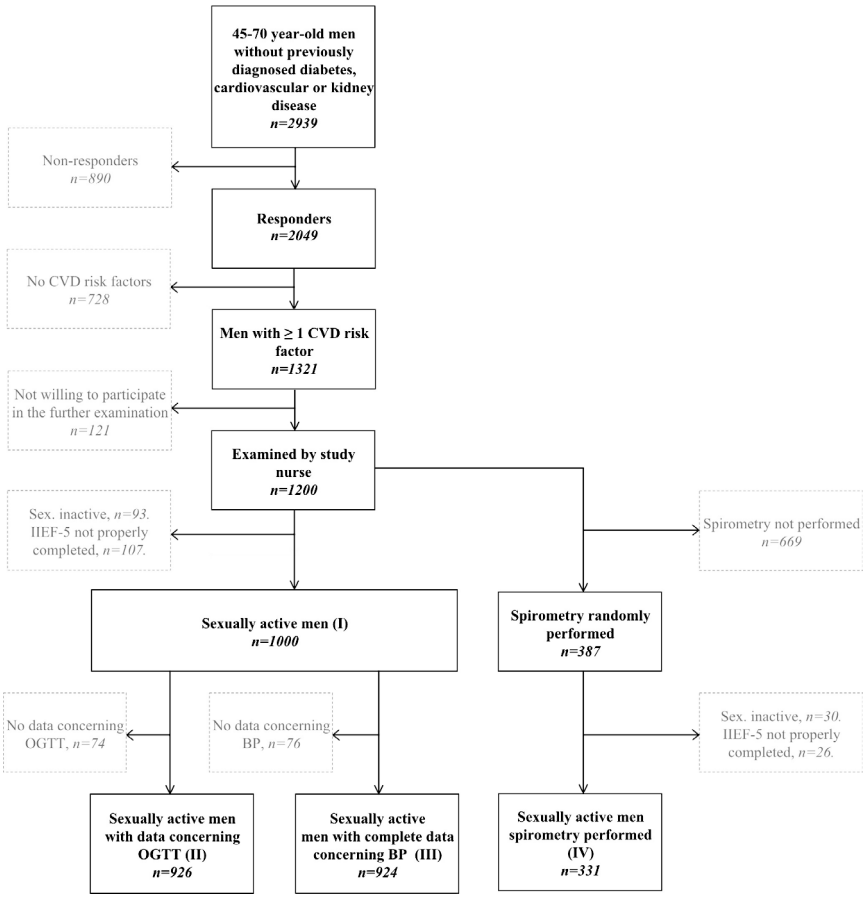

Figure 8. Selection of the study population for the different original publications marked in Roman numerals from I to IV. IIEF-5, CVD, cardiovascular diseases; International Index of Erectile Function short from; BP, blood pressure; OGTT, oral glucose tolerance test

Excluding those with previously diagnosed CVD, diabetes or chronic kidney disease, every community-dwelling inhabitant was invited to take part in the survey. Of the 6,013 invitees, 2,939 (49%) were men, of whom 2,049 (70%) responded. According to the questionnaire mailed with the invitation, men (n=1,321) having at least one of the

following cardiovascular risk factors, WC ≥ 94, high risk for diabetes in the Finnish Diabetes Risk Score (FINDRISC) questionnaire (Lindström & Tuomilehto, 2003), BP ≥ 140/90 mmHg or antihypertensive medication or family history of CVD, were invited to further examinations. During the visit participants were asked to complete a comprehensive self-administered questionnaire concerning their socio-demographic factors and lifestyle habits. In addition, thorough laboratory tests were performed.

In study I, men not willing to meet the study nurse (n=121), sexually inactive men (n=93), and those not fully completing the IIEF-5 questionnaire (n=107) were excluded and, therefore, 1,000 men were examined. Further exclusion of men without data concerning OGTT (n=74) yielded 926 men in study II. In addition, in study III, when men without complete data concerning BP and those using beta-blockers for cardiac arrhythmias were excluded (n=76), 924 men were analysed. Furthermore, in study IV, of the 1,200 men examined by the study nurse 387 men were randomly selected to perform minispirometry. After exclusion of sexually inactive men (n=30) and those without a properly completed IIEF-5 questionnaire (n=26), 331 eligible men were studied. (Figure 8)

4.2. Methods

4.2.1. Erectile dysfunction

ED was determined using the International Index of Erectile Dysfunction short form (IIEF-5) (Rosen et al, 1999). All men reporting a zero score on any of the questions or leaving any question unanswered were excluded. Cut-off levels were determined as defined by the original study. Scores >21 were considered as normal erectile function (I-III), and the severity of ED was determined as follows: mild, 17-21; mild to moderate, 12-16; moderate, 8-11 severe, 5-7 (I) (Rosen et al, 1999).

In study IV, a cut-off level of 16 was selected in order to study moderate to severe erectile dysfunction, and in study III, the two most severe categories were merged as follows: mild, 17-21; mild to moderate, 12-16; severe 5-11.

In order to compare the prevalence of ED with previously published Finnish prevalence data, a two-question composite was generated (I). ED was determined according to the NIH definition of ED (National Institutes of Health, 1993) by using IIEF-5 questions two and three (2Q), “Have you had trouble getting an erection before intercourse begins?” and “Have you had trouble maintaining an erection once intercourse has begun?” No trouble achieving or maintaining an erection was considered as normal erectile function and the severity of the dysfunction was determined accordingly: mild, a few times trouble achieving and/or maintaining; mild to moderate, sometimes trouble achieving and/or maintaining; moderate, most times trouble achieving and/or maintaining; severe almost always trouble achieving and/or maintaining an erection.

4.2.2. Anthropometric measurements

Height and weight were measured with the subjects in a standing position without shoes and outer garments. BMI was calculated by dividing weight in kilograms by the square of height in metres. WC was measured at the level midway between the lower rib margin and the iliac crest.

4.2.3. Blood pressure (III)

BP was measured by a trained nurse with a calibrated mercury sphygmomanometer with patients in a sitting posture, after resting at least 5 min. In each patient, the mean of the two readings taken at intervals of at least 2 min was used (European Society of Hypertension-European Society of Cardiology Guidelines Committee, 2003). Pulse pressure was calculated by subtracting the mean diastolic blood pressure (DBP) from the mean systolic blood pressure (SBP). Hypertension was defined as the use of antihypertensive medication (excluding those using beta-blockers for cardiac arrhythmias), or as the mean of home BP monitoring at least 135 mmHg for SBP or at least 85 mmHg for DBP (Parati et al, 2008). If study participants had no antihypertensive medication at enrolment, and the study nurse measured SBP at least 140 mmHg or DBP at least 90 mmHg, the participants were taught to use an automatic validated BP monitor (Omron M4 – 1, Japan) which was lent to them for home BP monitoring. The participants were instructed to take duplicate BP measurements in the seated position after 5 min of rest in the morning and evening for one week. The recorded measurements except those from the first day were used to calculate the mean home BP (Parati et al, 2008). Patients with 'white coat hypertension' (n=114), that is, office BP at least 140/90mmHg, but the mean of home BP measurements below 135/85 mmHg, were classified as normotensive individuals. Previously undetected hypertension was diagnosed if office SBP was at least 140mmHg or DBP was at least 90mmHg, and the mean of home BP monitoring was at least 135 mmHg systolic or at least 85 mmHg diastolic. The mean of the two office BP recordings was used in the analysis.

4.2.4. Lifestyle habits

Participants filled in the Alcohol Use Disorders Identification Test (AUDIT) (Babor et al, 2001). The frequency of consumption was defined using AUDIT question one.

The strain of physical activity and Leisure-time physical activity (LTPA) were determined using a detailed, non-validated questionnaire designed for the study. Participants were asked to report if they were getting sweaty or having shortness of breath during their usual physical activity on a three-point scale (I, IV). In addition to this, participants reported the frequency of at least 30 min of physical activity during their leisure time on a seven-point scale ranging from never to every day. LTPA was classified as follows: high, LTPA for at least 30 min at a time for six or more times a week; moderate, LTPA for at least 30 min at a time for four to five times a week; low, LTPA for at least 30 min at a time for a maximum of three times a week (III).

Similarly to physical activity, smoking habit was determined using a non-validated questionnaire. Participants were asked to report if they have ever smoked or are smoking on a regular basis (I, III) and, if yes, they further defined the duration of the smoking habit in years (IV).

4.2.5. Depressive symptoms

Beck´s depression scale (BDI) was used to screen depressive symptoms (Beck et al, 1961). Scores lower than 10 were considered as no depressive symptoms (Koponen et al, 2010), scores 10 to 16 as mild, 17 to 29 as moderate, and 30 or more as severe.

4.2.6. Oral glucose tolerance test (II)

Oral glucose tolerance test (OGTT) was performed by measuring capillary fasting plasma glucose and a 2-hour plasma glucose after ingestion of 75 g of anhydrous glucose dissolved in water. Glucose disorders were classified according to the updated World Health Organization (WHO)/ International Diabetes Federation (WHO, 2006) classification. On the basis of the 2-hour plasma glucose, men were divided into categories of newly diagnosed diabetes, IGT or normal plasma glucose if their 2-hour plasma glucose concentrations were ≥12.1, 8.9 to 12.1, and <8.9 mmol/l, respectively, and on the basis of fasting glucose into categories of newly diagnosed diabetes, IFG or normal blood plasma glucose using cut-off levels of ≥7.0, 6.1-6.9 and ≤6.0 mmol/l, respectively.

4.2.7. Other laboratory measurements

Laboratory tests were performed using blood samples, which were obtained after at least 12 h of fasting. Total cholesterol, HDL-C, triglycerides and plasma creatinine were measured enzymatically (Olympus® AU640, Japan). LDL-C was calculated according to the Friedewald formula. Plasma potassium and sodium were measured using the indirect ISE method (Olympus® AU640, Japan). Glucose values were measured from capillary whole blood using the HemoCue® Glucose 201+ system (Ängelholm, Sweden), which converts the result to plasma glucose values. Thyroid stimulating hormone (TSH) was measured by two-sided sandwich immunoassay using direct cheminoluminometric technology (Siemens Medical Solutions®, Germany)

4.2.8. Forced expiratory volume (IV)

FEV_1 was measured by hand-held spirometry (One Flow ®, Clement Clarke International, Essex, England) as instructed and monitored by the study nurse. Adjusted FEV_1 (FEV_1 (%)) was obtained by comparing the results with the Finnish reference values (Viljanen et al, 1982). FEV_1 (%) was divided into groups of normal, mildly and moderately to severely impaired pulmonary function using cut-off levels of >80%, 65% to 80% and <65% of predicted value, respectively.

4.2.9. Statistical analyses

Data were recorded on and analysed with SPSS for Mac 22 (SPSS, Inc., Chicago, IL, USA). Normality was studied visually using histograms (I, II, IV) or by Shaphiro –

Wilk W test (III). Continuous variables were tested using two-sample t-test or one-way ANOVA and presented with means (standard deviation). Categorical variables were compared with cross-tabulation and presented as numbers (%). Logistic regression was used to find significant independent associations between ED and selected variables and presented using odds ratios (OR) with 95% confidence intervals (CI). All the tests were two-sided. In each statistical analysis, an alpha level of 0.05 was selected and $P < 0.05$ was considered as statistically significant.

Study I
All continuous variables except weight, BMI, creatinine, triglycerides and HDL-C cholesterol were normally distributed. However, two-sample t-test yielded similar P-values in both cases, before and after a logarithmic transformation. Therefore, all the continuous and categorical variables were analysed as described above. Factors selected in the multivariate regression analysis were those with $P < 0.10$ in the univariate model.

Study II
Continuous variables were all normally distributed and therefore, comparison of un-adjusted glucose values between men with and without ED was performed as described above and comparison of age-adjusted glucose values was performed using analysis of covariance (Table 4). The results were presented as mean difference (95% CI). The categorical variable, the glucose disorders, was presented by crosstabulation. Age-adjusted comparison between glucose disorders and ED was analysed using multinomial logistic regression and presented as described above (Table 5).

Study III
To determine characteristics associated with ED severity, univariate and multivariate forward stepwise ordered logistic regression analyses were applied. To model the non-linear relationship between BP and ED, a restricted cubic spline (RCS) logistic model procedure was adopted. The associations between depressive symptoms and hypertension status in the risk of erectile dysfunction were analysed using general linear models, adjusted for age, WC, education level, and cohabiting. The U-shaped relationship between DBP and erectile dysfunction was tested using the Lind and Mehlum method (Figure 13) (Lind & Mehlum, 2010).

Study IV
All continuous variables except the IIEF-5 score, weight, BMI, HDL-C, and plasma triglycerides were normally distributed. Parametric continuous and categorical variables were presented as described above, whereas non-parametric variables were presented as medians (interquartile range) and analysed using Mann-Whitney U test (Table 6) or Kruskall-Wallis test **(**Figure 14). The covariates selected for the logistic regression analysis were those associating with ED, $P < 0.10$, and those that hypothetically could affect the association.

4.2.10. Ethical issues

The study was approved by the Ethics Committee of Satakunta hospital district on October 3rd 2005. All participants provided a written consent.

5. RESULTS

5.1. Population

Population characteristics of the 1,000 men studied are described in Table 1. Mean age and IIEF-5 score of the participants were 57 ± 7 years and 20 ± 4 points, respectively. Of the 1,000 men studied the majority had a low education level (71%) and were in a stable relationship (86%), whereas the minority were normal weight (15%) or had no central obesity (25%). In addition, 57% had hypertension, 33% were diagnosed with impaired glucose levels and 38%, 39% and 23% were never-smokers, previous smokers or current smokers, respectively.

Table 1. Population characteristics. SD, standard deviation; IIEF-5, International Index of Erectile Function short form; BMI, body mass index; WC, waist circumference

	mean (SD); n (%)
Age, years	57 (7)
IIEF-5, score	20 (4)
Education	
Primary school	689 (71)
Secondary school	176 (18)
High school	108 (11)
Marital status	
Single	51 (5)
Divorced or widowed	87 (9)
Married or cohabitation	837 (86)
BMI, kg/m^2	
<25	146 (15)
25-30	554 (56)
>30	290 (29)
WC, cm	
<94	251 (25)
94-02	349 (35)
>102	391 (40)
Smoke history	
Never	369 (38)
Previous	386 (39)
Current	220 (23)

The non-responders (mean ± standard deviation, 57 ± 7 years vs. 56 ± 7 years, p=0.0001) and men with incomplete IIEF-5 forms (60 ± 7yrs vs. 57 ± 7 years, p<0.0001) were older and had more central obesity (106 ± 11cm vs. 103 ± 10 cm, p=0.026) than the studied men. Otherwise, there were no statistically significant differences between the study sample and those excluded from the study.

5.2. Physical activity and erectile dysfunction (I)

The prevalence of ED was 57% using the IIEF-5 and 69% using the 2Q. Of the 1,000 men studied, 40% had mild, 11% mild to moderate, 4% moderate, and 2% severe dysfunction as defined by the IIEF-5. When the 2Q was used, the corresponding percentages were 44%, 8%, 12% and 5%, respectively (Figure 9).

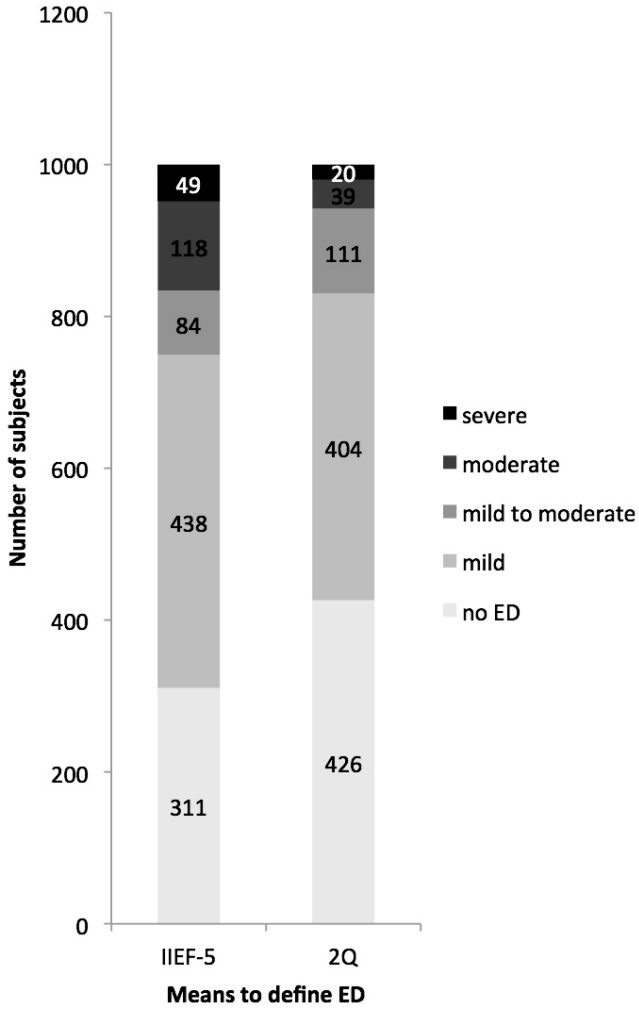

Figure 9. Rate and severity of ED among the study cohort using two definitions of ED. ED, erectile dysfunction; IIEF-5, International Index of Erectile Function short form; 2Q, questions two and three in the IIEF-5 questionnaire

Men with ED were older compared to men with normal erectile function (59±6 years vs. 55±6 years, $p<0.0001$). Although height and pulse pressure were associated with increased risk of ED in the univariate analysis, the association did not remain significant after adjustment for age. Otherwise, weight, BMI, WC, fasting glucose,

lipids, creatinine, haemoglobin, thyroid function, BP, sodium and potassium and blood leucocyte count were not associated with ED (Table 2).

Table 2. The association between clinical and laboratory measurements and ED. ED, erectile dysfunction; OR, odds ratio; CI, confidence interval; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; P-Krea, plasma creatinine; P-Hb, plasma hemoglobin; P-TSH, plasma thyroidea stimulating hormone; P-Na, plasma sodium; P-K, plasma potassium; B-Leuc, blood leucocyte count.

	ED	**no ED**		**AGE-ADJUSTED**	
	mean (SD)	mean (SD)	***P***	**OR (95% CI)**	***P***
Age,yrs	59 (6)	55 (6)	<0.001		
Height, cm	176 (7)	177 (8)	0.040	1.01 (0.98-1.03)	0.636
Weight, kg	89 (14)	89 (15)	0.642		
BMI, kg/m^2	28 (4)	28 (4)	0.821		
WC, cm	101 (11)	100 (4)	0.170		
SBP, mmHg	142 (19)	140 (19)	0.101		
DBP, mmHg	86 (11)	87 (9)	0.248		
PP, mmHg	56 (15)	53 (15)	0.005	0.99 (0.98-1.00)	0.157
fP-Gluc, mmol/l	5.8 (1.3)	5.6 (0.9)	0.055	1.09 (0.96-1.22)	0.175
total cholesterol, mmol/L	5.3 (1.1)	5.3 (0.9)	0.798		
HDL-C, mmol/L	1.4 (0.4)	1.4 (0.4)	0.369		
LDL-C, mmol/L	3.2 (0.9)	3.3 (2.2)	0.897		
Triglycerides, mmol/L	1.5 (1.5)	1.4 (0.7)	0.322		
P-Krea, μmol/L	82 (13)	81 (12)	0.225		
P-Hb, mg/L	148 (12)	149 (10)	0.056	0.99 (0.98-1.01)	0.378
P-TSH, mU/L	2.17 (1.02)	2.05 (1.39)	0.175		
P-Na, mmol/L	141 (1.8)	141 (1.5)	0.762		
P-K, mmol/L	4.0 (0.2)	4.0 (0.3)	0.139		
B-Leuc, nL	6.0 (1.6)	5.9 (1.6)	0.463		

In the multivariate logistic regression analysis, increasing age (p<0.001), smoking habit (p=0.028) and depressive symptoms (p=0.001) were associated with increased risk of ED, whereas high intensity physical activity (p=0.045), high level of education (p=0.013) and marriage (p=0.046) were associated with decreasing risk of ED. Although alcohol consumption was associated with ED in a u-shaped manner in the univariate analysis, it did not remain significant in the multivariate analysis (Table 3).

5.3. Erectile dysfunction and risk of pre-diabetes. (II)

Compared to the men studied (n=926) those without data concerning OGTT (n=74) were older (mean ± SD, 60 ± 6 years vs. 57 ± 6 years, *P*<0.001) and had more central obesity (106 ± 12 cm vs. 100 ± 10 cm, *P*<0.001). Otherwise, these two groups were alike.

Table 3. Association of lifestyle factors, socioeconomics, depression and age with ED. ED, erectile dysfunction; OR, odds ratio; 95% CI, 95% confidence interval. In multivariate analysis all the variables with p>0.1 in univariate analysis were included. * number of cases in the multivariate analysis

	UNADJUSTED		ADJUSTED	
	OR (95% CI)	***P***	**OR (95% CI)**	***P***
Age, years		<0.0001		<0.0001
<50	1		1	
50-54	2.04 (1.30-3.19)		1.90 (1.17-3.10)	
55-59	4.24 (2.74-6.57)		3.70 (2.28-6.00)	
60-64	5.85 (3.61-9.49)		4.98 (2.92-8.51)	
≥65	10.72 (6.27-18.31)		9.16 (5.00-16.79)	
Smoking		0.009		0.028
no	1		1	
yes	1.41 (1.09-1.84)		1.41 (1.04-1.91)	
Depression		<0.0001		0.001
no depression	1		1	
mild	2.13 (1.41-3.22)		2.07 (1.30-3.28)	
moderate-severe	3.52 (1.18-10.56)		4.04 (1.22-13.45)	
Education		<0.0001		0.013
primary school	1		1	
secondary school	0.52 (0.37-0.72)		0.74 (0.51-1.09)	
high school	0.41 (0.27-0.62)		0.52 (0.33-0.83)	
Marital status		0.089		0.046
single	1		1	
divorced or widowed	0.68 (0.33-1.43)		0.56 (0.24-1.32)	
married or cohabitating	0.53 (0.29-0.99)		0.43 (0.21-0.88)	
Physical activity		<0.0001		0.045
no sweating or shortness of breath	1		1	
some	0.63 (0.45-0.90)		0.77 (0.52-1.13)	
a lot	0.31 (0.19-0.50)		0.50 (0.29-0.86)	
Alcohol consumption		0.001		0.154
4 times or more a week	1		1	
2-3 times a week	0.65 (0.42-1.02)		0.71 (0.43-1.18)	
2-4 times a month	0.76 (0.49-1.18)		0.81 (0.49-1.33)	
once a month or less	1.36 (0.83-2.22)		1.14 (0.65-2.01)	

Of the 926 men studied, 114 (12%) had IFG, 115 (12%) IGT, 54 (6%) newly diagnosed type 2 diabetes mellitus, and 516 (56%) ED. After adjustment for age, fasting glucose (mean difference, 0.01 mmol/L; *P*=0.870) and 2-hour glucose (mean difference, 0.03 mmol/L; *P*=0.845) were similar in men with and without ED (Table 4).

Table 4. Association between ED and fasting glucose and 2-hour glucose in 926 men. ED, erectile dysfunction; SD, standard deviation; CI, confidence interval. [a] Two-sample t-test. [b] Analysis of covariance

	Fasting glucose, mean (SD), mmol/L	2-hour glucose, mean (SD), mmol/L		
no ED	5.57 (0.72)	7.01 (2.18)		
ED	5.62 (0.81)	7.41 (2.34)		
	UNADJUSTED, mean difference (95% CI), mmol/L			
	Fasting glucose	***P*[a]**	**2-hour glucose**	***P*[a]**
ED	0.04 (-0.14-0.06)	0.392	0.40 (0.10-0.69)	0.008
	AGE-ADJUSTED, mean difference (95% CI), mmol/L			
	Fasting glucose	***P*[b]**	**2-hour glucose**	***P*[b]**
ED	0.01 (-0.10-0.11)	0.870	0.03 (-0.27-0.33)	0.845

Although men with ED had 70% increased risk of having IGT and 49% higher odds of having type 2 diabetes mellitus compared to men with normal erectile function, after adjustment for age, the association did not remain significant, OR 1.18 (95% CI, 0.76-1.83) and OR 1.12 (95% CI, 0.61-2.04), respectively (Table 5).

Table 5. Association between erectile dysfunction and glucose disorders. ED, erectile dysfunction; IFG, impaired fasting glucose, IGT, impaired glucose tolerance; T2D, type 2 diabetes mellitus; CI, confidence interval. [a] Multinomial logistic regression

	Glucose disorders, n (%)				
	Normoglycemia	**IFG**	**IGT**	**T2D**	
no ED	299 (73)	52 (13)	39 (9)	20 (5)	
ED	344 (67)	62 (12)	76 (15)	34 (6)	
	UNADJUSTED, OR (95% CI)				
	Normoglycemia	**IFG**	**IGT**	**T2D**	***P*[a]**
ED	reference	1.04 (0.70-1.55)	1.70 (1.12-2.57)	1.49 (0.89-2.62)	0.054
	AGE-ADJUSTED, OR (95% CI)				
	Normoglycemia	**IFG**	**IGT**	**T2D**	***P*[a]**
ED	reference	0.94 (0.62-1.44)	1.18 (0.76-1.83)	1.12 (0.61-2.04)	0.847

5.4. Hypertension *per se* and erectile dysfunction. (III)

Among the 924 men studied, 483 (52%) were normotensive, 182 (20%) had previously undiagnosed hypertension, and 259 (28%) had medically treated hypertension. ED was detected in 245 (51%) of the normotensive men, 100 (55%) of the undiagnosed hypertensive men, and 166 (64%) of the treated hypertensive men (P=0.002). However, after adjustment for age, cohabiting status, WC, and education, the association was not statistically significant (P=0.31). The mean total IIEF-5 score was 21 ± 4 in normotensive men, 20 ± 4 in undiagnosed hypertensive men, and 19 ± 4 in treated hypertensive men (P=0.12 adjusted for age, cohabiting status, WC, and education). The distribution of IIEF-5 level according to BP groups is shown in Figure 10. After adjustment for age, cohabiting status, WC, and education, no significant differences were observed between the BP groups.

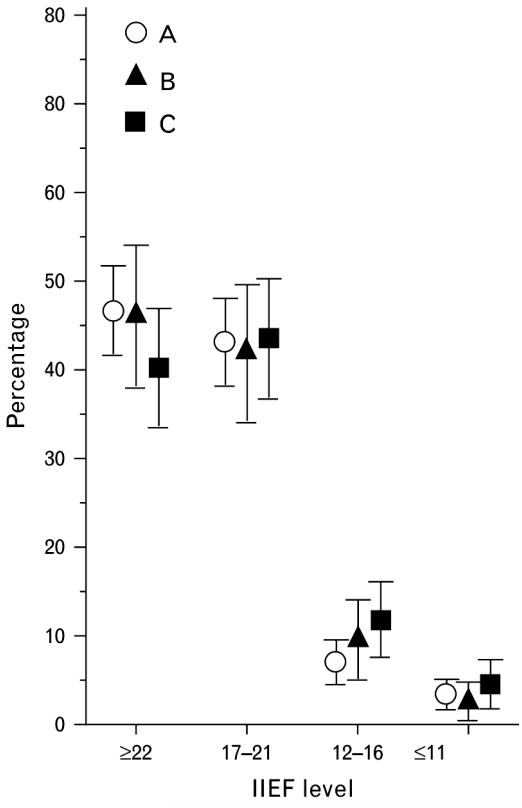

Figure 10. Distribution of IIEF-5 level in normotensive (A), previously undiagnosed hypertensive (B), and medically treated hypertensive (C) men, adjusted for age, cohabiting status, waist circumference, and education. Whiskers show 95% confidence intervals. IIEF, International Index of Erectile Function.

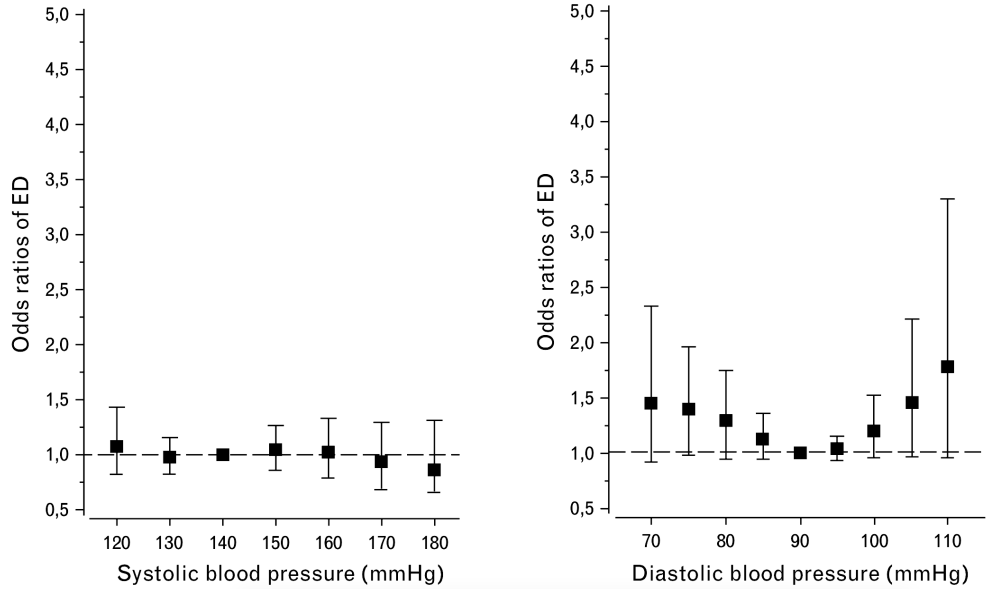

Figure 11. Odds ratios with 95% confidence intervals of erectile dysfunction according to blood pressure levels. Odds ratios were derived from a 5-knot restricted cubic spline logistic model with a blood pressure of 140 mmHg systolic and 90 mmHg diastolic as the reference values. The odds ratios were adjusted for age, waist circumference, education level, and cohabiting. ED, erectile dysfunction.

The curve relating DBP values and odds ratios (ORs) of erectile dysfunction was U-shaped (Figure 11). When the reference level was set at DBP 90 mmHg, the risk of erectile dysfunction rose with lower and higher DBP values. The overall test of presence of a U-shape is *P*=0.033. In regard to SBP values, no relationship with erectile dysfunction was observed (Figure 11).

Presence of depressive symptoms increased the adjusted OR of erectile dysfunction by 2.44 (95% CI 1.57 to 3.80) in normotensive men, by 7.62 (95% CI 1.89 to 30.65) in previously undiagnosed hypertensive men, and by 2.04 (95% CI 0.87 to 4.78) in medically treated hypertensive men. In the adjusted factorial design, the main effect of depressive symptoms was significant (*P*<0.001), while hypertensive status was not significant (*P*=0.31). In addition, the interaction between main effects was not significant (*P*=0.27) (Figure 12).

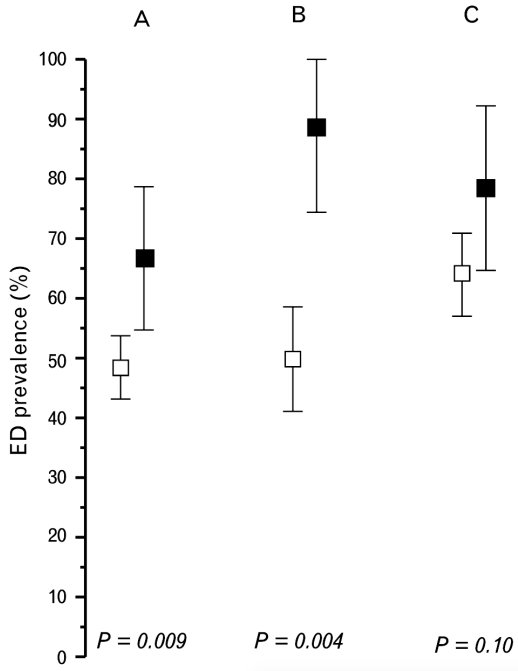

Figure 12. Prevalence of erectile dysfunction in normotensive (A), previously undiagnosed hypertensive (B), and medically treated hypertensive (C) men according to presence of depressive symptoms. P values adjusted with age, cohabiting status, BMI, and education (■ patients with BDI >10, □ patients with BDI <10). Whiskers show 95% confidence intervals. BDI, Beck's Depression Inventory.

5.5. Decreased pulmonary function and erectile dysfunction (IV)

The comparison of the men studied (n=313) and those without data concerning minispirometry (n=813) is presented in Table 6. The men studied were younger, had lower pulse pressure and high-density lipoprotein cholesterol, and had higher low-density lipoprotein cholesterol and triglycerides compared to the men not chosen to perform minispirometry.

Table 6. Poplation characteristics. SD, standard deviation; IQR, interquartal range; IIEF-5, International Index of Erectile Function short form. * Two-sample t-test, results presented as mean (SD). † Mann-Whitney U -test, results presented as median (IQR).

	MEANS (SD), MEDIAN (IQR)		
	no spirometry (n=813)	**studied (n=331)**	***P***
IIEF-5, score	20 (6)	21 (5)	0.142†
Age, mean (yrs)	58 (7)	56 (6)	<0.0001*
Clinical measurements			
Height, cm	177 (7)	177 (7)	0.435*
Weight, kg	90 (14)	89 (15)	0.243*
BMI, kg/m2	28 (5)	28 (5)	0.101†
Waist circumfenrence, cm	102 (11)	101 (11)	0.120*
systolic BP, mmHg	142 (22)	141 (19)	0.532*
diastolic BP, mmHg	86 (10)	88 (11)	0.005*
pulse pressure, mmHg	56 (19)	53 (14)	0.015*
Laboratory measurements			
fP-Gluc, mmol/l	5.76 (1.25)	5.74 (1.11)	0.833*
Total cholesterol, mmol/l	5.23 (1.07)	5.32 (0.95)	0.193*
HDL cholesterol, mmol/l	1.41 (0.44)	1.36 (0.32)	0.024*
LDL cholesterol, mmol/l	3.17 (0.89)	3.29 (0.84)	0.048*
Triglycerides, mmol/l	1.23 (0.90)	1.35 (0.91)	0.045†
Habits			
Smoking years, years	19 (12)	28 (13)	<0.0001*

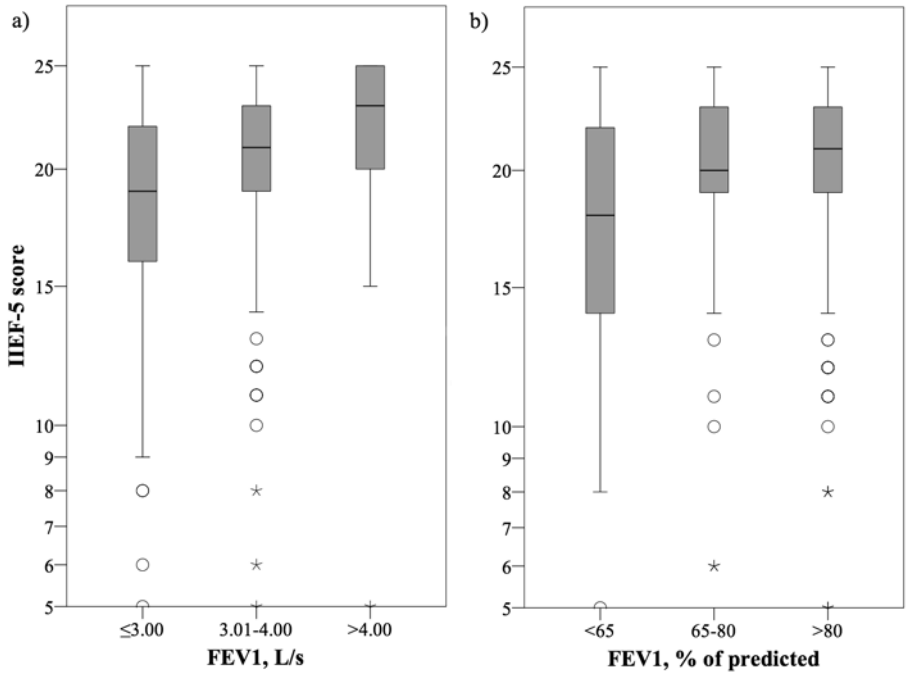

Figure 13. Mean International Index of Erectile Function short form (IIEF-5) score in different classes of forced expiratory volume in one second (FEV1). Whiskers show 95% CI. a) Association between FEV1 classes and mean score of IIEF-5. P<0.0001. b) Association of adjusted FEV1 classes and mean score of IIEF-5. P=0.002.

Of the 331 men studied, 219 (66%) had FEV_1 (%) within the reference range, 75 (23%) had mildly impaired, and 37 (11%) had moderately to severely impaired FEV_1 (%), whereas only nine (3%) reported a history of COPD or asthma. In addition, three men with and six men without a history of pulmonary disease reported the usage of inhaled corticosteroids or bronchodilators. Furthermore, 178 (54%) were current smokers, 74 (22%) were ex-smokers, and 59 (18%) had moderate to severe ED.

Table 7. Association between decreased pulmonary function and odds of moderate to severe erectile dysfunction. ED, erectile dysfunction; FEV1, forced expiratory volume in one second; OR, odds ratio; 95% CI, 95% confidence interval. * Binary logistic regression. † age, height, smoking years, depressive symptoms, education, marital status and exercise are used as covariates

			UN AJUSTED		**ADJUSTED†**	
	no ED	**ED**	**OR (95% CI)**	***P****	**OR (95% CI)**	***P****
FEV_1, n (%)				0.006		0.035
>80%	185 (68)	34 (57)	1		1	
65%-80%	64 (23)	11 (19)	0.87 (0.39-1.93)		1.01 (0.46-2.30)	
<65%	23 (9)	14 (24)	3.39 (1.54-7.48)		3.29 (1.30-8.44)	
FEV_1, L/s	3.4 (0.7)	2.9 (0.8)	0.42 (0.28-0.63)	<0.0001	0.49 (0.29-0.84)	0.009
FEV_1 %	86 (17)	79 (20)	0.98 (0.97-0.99)	0.020	0.98 (0.96-0.99)	0.020

The association between FEV_1, FEV_1 (%) and the IIEF-5 score is presented in Figure 13. While there was a clear dose-dependent positive association between FEV_1 and the IIEF-5 score, the IIEF-5 score was decreased only in men with moderately to severely impaired FEV_1 (%).

After adjustment for age, height, smoking years, depressive symptoms, education, marital status, and physical activity, men with moderately to severely impaired FEV_1 (%) were three times more likely to have moderate to severe ED compared to men with normal FEV_1 (%) (OR: 3.29; 95% CI, 1.28-8.44; *P*=0.013) or mildly impaired FEV_1 (%) (OR: 3.25; 95% CI, 1.14-9.30; *P*=0.028). Moreover, using identical covariates to those above, decreasing FEV_1 and FEV_1 (%), analysed as continuous variables, were independently associated with increasing risk of moderate to severe ED, (OR: 0.49; 95% CI, 0.29-0.84, *P*=0.009) and (OR 0.98; 95% CI, 0.96-0.99, *P*=0.020), respectively (Table 7).

The association between FEV_1 and risk of ED is depicted in Figure 14. The risk of moderate to severe ED was increased significantly in men with FEV_1 below 2.0 L/s and FEV_1 < 60% of predicted value. The odds of ED were 4.88 (95% CI: 1.39-17.12) and 16.33 (95% CI; 3.41-78.3) higher in men with FEV_1 1.0-2.0 L/s and FEV_1 < 1.0 L/s, respectively, compared to men with FEV1 > 4.0 L/s. In addition, the odds of ED were 4.07 (95% CI; 1.19-13.9) and 4.52 (95% CI; 1.06-19.18) higher in men with FEV_1 51-60% and FEV_1 < 50% predicted value, respectively, compared to men with FEV_1 91-100% of predicted value.

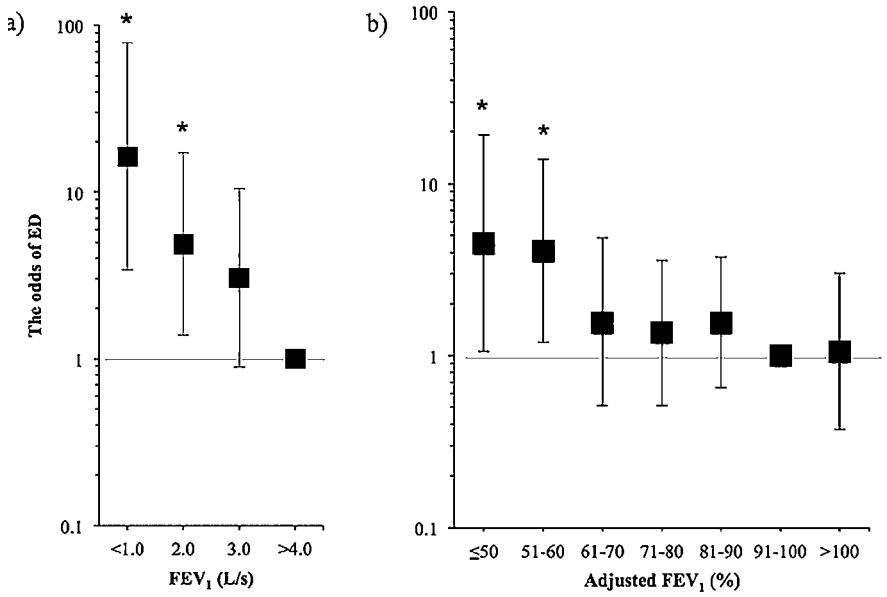

Figure 14. The risk of erectile dysfunction in different classes of forced expiratory volume in one second. Whiskers show 95% CI. a) The association between FEV1 and odds of erectile dysfunction. b) The assocation between adjusted FEV1 and odds of erectile dysfunction. ED, erectile dysfunction; FEV1, forced expiratory volume in first second. * *P*<0.05

6. DISCUSSION

6.1. Population

The men studied were drawn from a population survey. It covered every eligible inhabitant in two rural cities in southwest Finland at high risk for CVD but without previously diagnosed CVD, diabetes or kidney disease. The setting enabled us to study ED in a population at risk for CVD but without established comorbidities affecting the arteries.

Although several epidemiological studies concerning ED have been published during the past 20 years, response rate is reported only in some of the studies. The average response rate has been 64%, raging from as low as 20% (Giuliano et al, 2002) to 96% (Ghalayini et al, 2010). Therefore, the response rate of 70% in the current study may be considered as adequate and, in fact, rather good.

Although of the 2,939 men, 30% did not respond, 4% were not willing to see the study nurse, 3% were sexually inactive, and 4% did not complete the IIEF-5 questionnaire properly, the differences between the men excluded and those studied were minor. Similarly, only minor differences were observed between those 3% of men excluded from studies II and III and those who were studied. Therefore, the population may be considered as representative of white men of European origin.

However, in study IV, the men performing spirometry were more commonly current smokers compared to non-performers. Although the study nurse selected the performers randomly, it is understandable that smokers are more prone to be tested for their pulmonary function compared to non-smokers. Taking into account this limitation, the population may also be considered representative.

6.2. Methods

6.2.1. Erectile dysfunction

Although objective measurement of ED such as penile dupplex doppler ultrasound would have been the best way to avoid recall bias, its use in a large-scale epidemiological study is not possible. Although several questionnaires have been developed, the IIEF-5 has been the most commonly used in clinical as well as in research settings for the last 15 years. In addition to this, the questionnaire was originally cross-culturally validated in several languages including Finnish.

The major limitation of the questionnaire is its inability to assess erectile function in sexually inactive men (Yule et al, 2011). While there are several other reasons for sexual inactivity besides ED, some of those reporting sexual inactivity are likely to be completely capable of having an erection. Therefore, the questionnaire was originally

designed to exclude sexually inactive men by grading the total score from 5 to 25, which is not an optimal solution either.

Consistent with this, it was decided to exclude all men reporting a zero score in any of the questions in the current study. Although this led to a selection bias by including only men reporting sexual activity over the past six months, those excluded due to sexual inactivity were a minority (7%). Moreover, as discussed above, differences between sexually inactive and active men were minor.

6.2.2. Depressive symptoms

Although most psychogenic comorbidities have an effect on ED, depression is the most common. The diagnosis of depression needs a comprehensive psychiatric evaluation, which is not suitable for a large-scale epidemiological study and, therefore, a screening tool assessing depressive symptoms serves the study better. Similarly to IIEF-5, BDI (Beck et al, 1961) has been in clinical and in scientific use for decades and is also validated in Finnish. In addition, the questionnaire also provides a tool to assess the severity of the condition.

6.2.3. Oral glucose tolerance test

Glucose values were measured from capillary whole blood. Although the Finnish Current Care Guidelines 2013 recommends the use of venous plasma glucose in OGTT (the Finnish Medical Society Duodecim, 2013), the current WHO criteria states reference values also for capillary glucose measurement. Therefore, the glucose disorders were defined according to WHO criteria (WHO, 2006).

6.2.4. Anthropometrical measurements and blood pressure

The anthropologic measurements were made by trained medical staff and carried out according to the standard WHO MONICA procedures.(WHO, 1988) Furthermore, BP measurements done during the office visit and those at home were both performed using a standard procedure recommended by the guidelines of the European Society of Hypertension/ European Society of Cardiology (European Society of Hypertension-European Society of Cardiology Guidelines Committee, 2003; Parati et al, 2008).

6.2.5. Forced expiratory volume

The One Flow ® office spirometer met the American Thoracic Society 1994 updated recommendations (American Thoracic Society, 1995) when tested by a computer driven piston-pump. In addition to these tests conducted by the manufacturer, the One Flow ® was technically and functionally tested in a multi-centre study comparing 10 office spirometers to standard diagnostic spirometers (Liistro et al, 2006). Although the One Flow ® did not perform so well assessing forced vital capacity, it was as precise as a standard diagnostic spirometer in evaluating FEV_1.

6.3. Limitations

Although data concerning medication taken on a daily basis were comprehensively collected, those taken on demand were only randomly reported and collected. Therefore, no data concerning the use of PDE-5 inhibitors or other medication for ED are available. However, those using such medication would be more prone to report better erectile function leading to slight under- rather that overestimation of the prevalence of ED.

While it is known that lower urinary tract symptoms have an effect on ED (Blanker et al, 2001), not having a validated questionnaire to define such symptoms is clearly a deficiency. However, according to their medical history, only 25 (3%) men reported having prostate or bladder symptoms or use of alpha-blockers, 5-alpha-reductase inhibitors or anticholinergic drugs. Furthermore, these 25 men had only slightly higher but not statistically significant age-adjusted odds of having ED compared to those without these comorbidities or medications (OR: 1.35; 95% CI, 0.51-3.54; P=0.544).

Due to the cross-sectional nature of the study causalities, cannot be assessed. However, due to the representative sample, the study provides a valid estimation of prevalent ED. In addition, recording extensive variables enabled a comprehensive multivariate analysis to be performed, and allowed independent factors associating with ED to be evaluated.

6.4. Physical activity and erectile dysfunction. (I)

We demonstrated that 57% and 69% of apparently healthy men reported ED defined by the IIEF-5 and the 2Q, respectively. In addition, increasing age, smoking and depression associated with increasing risk of ED, whereas married, well-educated men exercising to a high intensity had a lower prevalence of ED than single, less educated men not exercising or exercising at a low intensity.

While the prevalence of ED varies greatly between different countries and different methods to define the condition, it is convenient to compare the prevalence rates obtained to previously reported results in Finland with a similar method of defining ED. Using the 2Q, 69% of the men studied were considered to have ED, which is in line with the 77% and 74% reported by Shiri et al. (Shiri et al, 2003b) and Koskimäki et al. (Koskimäki et al, 2000), respectively. The slightly older cohort in the previous studies may explain the noted difference, likewise the fact that ED is highly prevalent in men with diabetes or CVD, which were exclusion criteria in our sample.

The results corroborate the results of numerous population-based studies indicating that age, smoking, depressive symptoms, education, marital status and physical activity are independently associated with ED (Grover et al, 2006; Imai et al, 2010; Nicolosi et al, 2003). In addition, the current study proves that there is a clear dose-dependent association between age, depressive symptoms, physical activity and ED. However, in the majority of the populations studied there has been a substantial

amount of men with cardiovascular morbidity and only Grover et al. have studied a population without previous established CVD or diabetes (Grover et al, 2006). Similarly to our results they demonstrated that age, smoking, marital status and education are associated with ED. However, they also demonstrated that increase in fasting glucose levels associated with increasing risk of ED. Although men with ED had slightly higher fasting glucose levels compared to men with normal erectile function in the current study, the difference was not significant. This, in fact, corroborates the results of the HPFS study, in which it was demonstrated that diabetes precedes ED (Bacon et al, 2002).

The fact that high BP, obesity, lipid disorders or increasing fasting glucose alone were not associated with ED is likely partly due to the apparently healthy cohort devoid of previously diagnosed diabetes, CVD or kidney disease. Of course, it is possible that having more men with hypertension, high LDL-C or low HDL-C, the association between ED and these variables could have been significant. On the other hand, in the majority of the population-based cohorts the diagnosis of hypertension and also dyslipidemia, has been self-reported by participants. In the current study, the BP measurement was performed in controlled circumstances and by standard procedure, while the diagnosis of dyslipidemia was made according to laboratory measurements, making the diagnosis of hypertension and dyslipidemias more reliable.

Although there was a slight U-shaped association between alcohol consumption and ED, other comorbidities, mainly age, were the major determinants regarding this association. The result further strengthens the hypothesis that moderate alcohol consumption has no effect on erectile function. Of course it is possible that the positive effect of alcohol on ED is so modest that other variables override its effect.

6.5. Erectile dysfunction and risk of pre-diabetes. (II)

We demonstrated that ED was not independently associated with increased risk of IFG, IGT or newly diagnosed type 2 diabetes.

The only valid study to address this issue was conducted 30 years ago. Deutsch et al. (Deutsch & Sherman, 1980) demonstrated that in impotent men (n=58) the means of 1-hour and 2-hour glucose were significantly higher compared to control men (n=131). Although they failed to demonstrate that impotence was associated with an increased risk of IFG or IGT, 12% of the impotent men had previously unrecognized type 2 diabetes, while the control group did not show type 2 diabetes mellitus at all. It is worth noting that we are living in a totally different world compared to that of the 1980s. The prevalence of type 2 diabetes has doubled (Danaei et al, 2011). In addition, the introduction of effective pharmacotherapy for ED (Goldstein et al, 2002) has led to an increasing number of men seeking help for ED, not to mention the introduction of new methods to define ED and the updated method of OGTT (WHO, 2006).

Very recently, Skledon et al. demonstrated that in a sub-cohort of the National Health and Nutritional Examination Survey, ED was associated with newly diagnosed

diabetes (OR: 2.20; 95% CI, 1.10 to 4.37; $P<0.05$) (Skeldon et al, 2015). Although the results do not corroborate with our results, it should be emphasised that the methods to determine ED were different in the two studies. In the National Health and Nutrition Examination Survey cohort ED was determined by a single question originally described by the NIH compared to the IIEF-5 used in our study. More importantly, although it cannot be proved, it is probable that the duration of the newly diagnosed diabetes varies between the two studies.

Although there are no data concerning men with type 2 diabetes, the current data corroborate the HPFS study, which estimated that the risk of ED increases no sooner than 10 years after diagnosis of type 1 diabetes mellitus (Bacon et al, 2002).

6.6. Hypertension *per se* and erectile dysfunction. (III)

We demonstrated that hypertension *per se* was not associated with ED.

To the best of our knowledge, only the Greek study by Doumas et al. (Doumas et al, 2006) has investigated the prevalence of ED in hypertensive patients in a population without CVD, diabetes, renal failure, or liver disease. According to their results, the prevalence and severity of ED is greater in patients with essential hypertension compared to normotensive individuals. However, they did not perform a proper multivariate analysis. In addition, in the present study, we found that the adjustment for age, cohabiting status, education, and WC had a strong effect on the interaction between hypertension status and erectile dysfunction.

The present study also showed that a U-shaped curve relationship exists between DBP and erectile dysfunction with a nadir of DBP 90mmHg where the risk of erectile dysfunction was the lowest. Considering our substantially healthy study population, this phenomenon is probably not mediated by arterial stiffness and warrants further study.

The prevalence of depressive symptoms in the present study is similar to that of a recent population-based study of 4500 randomly selected Finnish individuals aged 45–74 years using the same instrument (Seppälä et al, 2012). Overall depression rates in Europe appear to be highest in central and eastern European countries and lowest in western and northern European countries including Finland (Van de Velde et al, 2010). Depression and ED frequently coexist. Men with depression have a nearly two-fold greater likelihood of ED compared with men with no depression. Thus, cultural differences in dealing with erectile dysfunction may explain the varying prevalence rates reported in studies concerning erectile dysfunction.

6.7. Decreased pulmonary function and erectile dysfunction (IV)

We demonstrated that both moderately impaired absolute FEV_1 and FEV_1 (%) were associated with increasing risk of moderate to severe ED in a rate-dependent manner

in apparently healthy men, regardless of smoking habit, physical activity, or depressive symptoms. Moreover, it is of interest that the majority of the men studied did not have previous history of doctor-diagnosed COPD or asthma.

The first valid study concerning the association between impaired pulmonary function and ED was conducted by Fletcher and Martin in 1982 (Fletcher & Martin, 1982). They demonstrated that nocturnal erections were absent in 30% of the 20 men with COPD. Later on, two small cross-sectional (Collins et al, 2012; Köseoğlu et al, 2005) and two case-control (Kahraman et al, 2013; Karadag et al, 2007) studies have confirmed these results. In a study of 95 men with COPD and 30 controls, those with COPD had significantly lower IIEF-5 scores (Karadag et al, 2007). Another study demonstrated a dose-dependent decrease in erectile function with decreasing FEV_1 (%) in 70 men with COPD (Kahraman et al, 2013). The only population-based study addressing the issue was the Krimpen study, which demonstrated that men with self-reported COPD had a 1.9 times increased risk for ED compared to men without a history of COPD (Blanker et al, 2001). In summary, the few studies describing the association of ED and COPD have all been rather small cross-sectional or case-control studies. Moreover, in the only population-based study, the definition of COPD was based on patient's self-report.

The study showed a clear association between impaired pulmonary function and ED. However, the aetiology of the association remains obscure. To test the hypothesis presented previously, androgen and blood oxygen levels should have been measured. However, depressive symptoms were included in the analysis with no effect on the association. When both ED (Giugliano et al, 2004) and low FEV_1 (Agusti et al, 2010) are demonstrated to be presented with low-grade inflammation, the subsequent endothelial dysfunction might serve as a link between these two conditions. Of course, to address the issue in more detail a study measuring markers of inflammation is needed.

6.8. Future aspects

Most of the large observational and prospective studies concerning ED were conducted ten to fifteen years ago. At present, only a few new epidemiological studies have been published. The main scientific interest has been focused on finding new ways to treat ED and to rehabilitate erectile function after radical surgery of the pelvic organs. However, some of the biggest, the MMAS and the HPFS are still collecting follow up data to unravel long-term effects of the known risk factors on ED.

A follow up study of the Harmonica project was established two years ago. Ideally all participants would have been re-invited to participate in the follow up but due to limited resources only a sub-population of the entire cohort was invited. However, it would be of interest to study the causal effect of ED and its risk factors, hypertension, hyperlipidemias, pre-diabetic conditions and impaired pulmonary function in particular.

Although ED was not shown to improve the prediction of incident CVD beyond traditional risk factors (Araujo et al, 2010), still it would be intriguing to hypothesise that by combining ED with other CVD risk factors, traditional or new, a more precise predictor for CVD events or cardiovascular death could be developed. With this in mind, a follow up study linking baseline data of the Harmonica to the registry data concerning the causes of deaths and cardiovascular interventions was conducted a year ago. These data should be ready for analysis after half a year.

While it is evident that smoking causes ED, very limited data exist concerning the effect of smoking cessation on ED, the long-term effect in particular. Although interventions for smoking cessation are hard to establish, it would be of interest to conduct and parallel comparative prospective study to compare ex-smokers to current smokers with long-term follow up.

The data concerning the effect of TRT on ED are vague at best. The studies conducted are mostly underpowered and without longer follow up. Therefore, a multi-centre RCT evaluating the effect of testosterone on sexual function and six other hypogonasidm-related symptoms was conducted in 2009 (ClinicalTrials.gov identifier NCT00799617). A total of 800 men are enrolled, and the results are expected in a year.

7. CONCLUSIONS

On the basis of this study and the analysis of the population-based data the following conclusions were drawn:

1. Depending on the method used to define ED, the prevalence of ED was 57% and 69%. Increasing age, smoking, and depressive symptoms were associated with increasing odds of ED, while high level of education, high level of physical activity, and living in a relationship, with decreasing odds of ED.

2. ED is not associated with increasing risk of newly diagnosed glucose disorders and, therefore, ED cannot be used as a marker of pre-diabetes.

3. Factors such as depression modify the effect of hypertension on ED and hypertension *per se* is not associated with increasing risk of ED.

4. Decreased performance in spirometry testing is associated with increasing odds of ED.

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