Hyperglycemia a risk factor for lower extremity muscle-tendon pathology? A prospective cohort study.

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Preface

I have struggled with tendons for the last 15 years. Initially from numerous injuries as a footballer and professionally on a daily basis through my clinical work with professional athletes. They say don't mix business with pleasure, but the more i struggle the more i realise that I really like tendons!

This thesis marks the end of two fantastic years of living and learning in Norway. Hopefully, it also marks the beginning of future investigations into this basic yet incredibly complex anatomical structure. It has been a pleasure to collaborate with co-advisors Christian Couppé, Dorthe Skovgaard and Volkert Siersma who share this passion for tendons and all have made great contributions to the field of research. I would like to express my gratitude to Christian and Dorthe for establishing contact with the registers and to Volkert for his help with the analysis. This project would not have been possible without your time and efforts. Also, I would like to thank thesis advisor Inger Haukeness her extensive knowledge and experience with epidemiological research has been of great value. Finally i have to thank my family for distant support and my girlfriend for local support and chocolate when needed.

Contents

Abstract

Introduction Tendon injury is a considerable problem affecting both physically active and sedentary people. The symptoms and reduction in performance may last for an extended period, potentially years and many never return to their previous activity level.

Background Emerging evidence associates hyperglycemia, hemoglobin A1c, to an increased risk of muscle- tendon injury. This emerging association is based on a little number of studies. Hence, there is a need for prospective studies that examines the relation between hyperglycemia and tendon pathology. A better understanding of how hyperglycemia might exacerbate a degenerative process could provide the basis for the prevention of injuries.

Method A prospective cohort study based on 6.238 individuals from the fourth wave (2001-2003) of the Copenhagen City Heart Study. Hyperglycemia (hemoglobin A1c) along with other baseline parameters was measured at baseline. Individuals were followed prospectively to the diagnosis of a muscle- tendon injury or for a maximum of 3 years using The Danish National Patient Register. Logistic regression models was used to explore if elevated levels of hemoglobin A1c lead to a greater risk of lower extremity muscle- tendon related pathology.

Results A statistically significant association was found between elevated hemoglobin A1c and lower extremity muscle- tendon injury (crude OR=2.85, 95% CI =1.06–7.64). When adjusted for sex, age and running habits, the OR among the elevated (hyperglycemia) group increased slightly (adjusted OR=3.04, 95% CI=1.13–8.19), and among the highly exposed group the OR turned significant (adjusted OR=3.41, 95% CI=1.01-11.55). Adjusting for all covariates, the OR among the elevated group changed marginally, whereas the association between the highly exposed group and the outcome were attenuated to a non-significant level.

Conclusions In this large−scale population study, it has been demonstrated that hyperglycemia is associated with an increased risk of lower extremity muscle- tendon injury. Due to the methodological limitations and low incidence of outcomes further investigations are needed to confirm this association and explore it in different populations.

Keywords Hyperglycemia, metabolic factors, tendinopathy, tendon injury, tendon pathology.

Abbreviations

AGEs	Advanced glycation end products
AT	Achilles tendon
BMI	Body mass index
CCHS	Copenhagen City Heart Study
CI	Confidence interval
CS	Cigarette smoking
CSA	Cross sectional area
CVD	Cardiovascular disease
DM	Diabetes Mellitus
DNPR	Danish National Patient Register
ECM	Extracellular matrix
EU	European Union
GC	Glucocorticoids
GP	General practitioner
HbA1c	Hemoglobin A1c
HDL-C	High-density lipoprotein cholesterol
HPLC	High−performance liquid chromatography
ICD-10	International Classification of Diseases
IQR	Interquartile range
LDL-C	Low-density lipoprotein cholesterol
MRI	Magnetic resonance imaging
NSAID	Nonsteroidal anti-inflammatory drug
OR	Odds ratio
RCT	Randomized controlled trial
SIF	Skin intrinsic fluorescence
TC	Total cholesterol
TG	Triglyceride
WHO	World health organization

Introduction

Tendon injury is a considerable problem affecting both physically active and sedentary people. This study is based on emerging evidence linking hyperglycemia, hemoglobin A1c (HbA1c), to an increased risk of muscle- tendon injury. A better understanding of how hyperglycemia might exacerbate the degenerative process in tendon tissue could contribute to a better understanding of the pathogenesis of tendinopathy and thus provide the basis for prevention of tendon injuries. The literature for this study was based on searches in: Research Gate, Google scholar, Cochrane Library and PubMed, with different combinations of the descriptors: HbA1c, hyperglycemia, glycated hemoglobin, hemoglobin A1c, metabolic factors, tendinosis, tendinitis, tendinopathy, tendon injury, tendon pathology. The search was conducted between August 2018 and April 2019.

Theoretical aspects and previous research

HbA1c

Hyperglycemia expressed by HbA1c is the main exposure for muscle- tendon injury in this study. Measurement of HbA1c is the prefered test for assessing chronic glycemia, it can be performed at any time of the day, is relatively insensitive to short-term lifestyle changes and does not require fasting. HbA1c is a valid indicator of long-term glycemic control with the ability to reflect the cumulative glycemic history of the preceding 8 to 12 weeks (Nathan, Turgeon, Regan, 2007; Sherwani et al., 2016).

Hyperglycemia and tendon injury

In recent years evidence has emerged for metabolic factors, such as hyperglycemia to play a role in the development of tendinopathy (Ranger, Wong, Cook, Gaida, 2016). The increasing attention to hyperglycemia as a risk factor for tendon pathology is based on research linking Diabetes Mellitus (DM) to tendon pathology. This association was already described in 1975 where Hamlin, Kohn and Luschin (1975) noted that individuals with DM had an accelerated aging of collagen by a factor of two. A recent systematic review by Ranger et al. (2016) reported that individuals with DM had a higher incidence of tendinopathy (OR=3.67), when compared to non-diabetic controls (Ranger et al., 2016; Zakaria, Davis, Davis, 2014). The most plausible explanation for this association is the biological pathway whereby hyperglycemia seems to have a negative effect on tendon tissue (Ranger et al., 2016). The rationale of the current study corresponds with the suggested biological pathway.

In hyperglycemia blood glucose attaches to collagen, in what is described as the Maillard reaction, resulting in an increased accumulation of non-enzymatic advanced glycation end products (AGEs) in the connective tissue. Progressive accumulation of AGEs are multifactorial and a natural part of the typical aging process however individuals with increased HbA1c are particular affected because higher levels of systemic glucose leads to an increased accumulation (Gautieri et al., 2016). Skin intrinsic fluorescence (SIF) is a non-invasive marker for the total accumulation of AGE. Cleary et al. (2013) showed a significant correlation between SIF and glycemic exposure measured with a 25 year mean HbA1c. The exact mechanisms by which AGEs contribute to connective tissue injury are still poorly understood. A key characteristic of AGEs is their ability to cross-link with collagen

fibers, once formed, AGEs can only be degraded when the collagen they are linked to is degraded (Abate, Schiavone, Salini, Andia, 2013). Consequently, the most extensive accumulation of AGEs will occur in tissues with low turnover, such as cartilage and tendon. This cross-linking has been shown to alter the mechanical properties of load bearing proteins such as collagen by increasing stiffness-brittleness and matrix disorganization (Couppé et al., 2016; Monnier et al., 2005). This leads to stiffer tendon tissue with a decreased viscoelasticity and decreased failure strain (Abate et al., 2013). This might explain the higher prevalence of tendinopathy in people with diabetes (Burner et al., 2012; Monnier et al., 2005; Gautieri et al., 2016). Several studies support the hypothesis that hyperglycemia may affect tendon structure and mechanics:

- Gautieri et al. (2016) found that AGEs reduce the viscoelasticity in tendon tissue by severely limiting fiber and fibril sliding.
- In line with the findings from Gautieri et al. (2016), Hansen et al. (2013) demonstrated how tendon viscoelasticity is negatively correlated to HbA1c levels, within the normal range in healthy female handball players.
- Otoshi et al. (2015) found that subjects with HbA1c $\geq$ 6.5% had a 3.37 times greater risk of lateral epicondylitis compared to individuals with a HbA1c level < 5.5%.
- Burner et al. (2012) demonstrated how hyperglycemia reduces proteoglycan levels in porcine patellar tendons. Proteoglycan relates to tendon synthesis and a reduction might contribute to tendon pathology. The authors suggest this reduction as an AGE-independent mechanism for tendon pathology.

The majority of these studies on tendinopathy in relation to hyperglycemia are for the most rather small, with some methodological flaws or conducted on animals. Hence, there is a need for prospective studies that examine the relationship between HbA1c and tendon pathology in humans.

Muscle- tendon injury

Terminology

Before the 1990s tendon related pain was diagnosed as tendinitis, the “-itis” suffix implying inflammation as the primary cause. The term tendinitis was widely accepted in the medical literature and adapted in clinical work where treatment strategies primarily consisted of trying to reduce the inflammation through medicines such as nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids (Rees, Stride, Scott, 2014). In the early 2000’s Khan, Cook,

Maffulli and Bonas (2002) posted a necessary and in the light of the understanding at the time relevant editorial advocating for a shift in terminology that should reflect a more complex diagnosis. The editorial criticised the deeply entrenched anti-inflammatory treatment strategies, related to the term tendinitis, that were still being deployed despite new evidence showing a non-inflammatory pathology in overuse tendon conditions. It was an important contribution emphasising that chronic tendon injury had a different and more complex etiology than other inflammatory conditions and that the treatment strategies often favored at the time was inadequate in addressing the non-inflammatory component of tendon injury. However, in light of recent evidence it would be wrong to consider all chronic tendinopathy as entirely non-inflammatory (Rees et al., 2014). A recent systematic analysis on the topic by Dean et al. (2016) reported an increased number of macrophages and mast cells in the pathological tendon. Earlier claims on absence of inflammatory cells in tendinopathy were primarily based on the absence of neutrophils. Today, tendinopathy is the preferred term and used to describe the same conditions that previously was identified with tendinitis or tendinosis. Tendinopathy is indicating a non-rupture injury in the tendon or paratendon and does not specify etiological factors or distinguish between the presence/absence of inflammation.

In this study the term “muscle- tendon injury” is used to cover both tendinopathy and muscle strain injuries. The muscle-tendon interface also referred to as the myotendinous junction is the weakest link of the muscle-tendon unit (Nikolaou et al., 1987). The pathology of most muscle injuries occur at a myotendinous junction and are by this not isolated to the muscle but the tissue damage will affect both the contractile muscle and the connective tissue of the tendon (Bayer et al., 2018). Improvements in magnetic resonance imaging (MRI) resolution and anatomical dissection studies has provided new evidence on how the tendon extends within the muscle belly providing support and attachment for the muscle fibres (Brukner & Connell, 2016). This questions the traditional view of a distinct proximal and distal delineation between the muscle belly and tendons. Studies on the myotendinous junction shows that the tendon extends the full length of the muscle belly in both the biceps femoris and semimembranosus (Brukner & Connell, 2016). Muscle strain injuries with intramuscular tendon involvement seem to have far longer recovery time. One study reporting a mean recovery of 72 days in comparison with 21 days for injuries without intramuscular tendon disruption (Comin et al., 2013).

Incidence

Tendon injury is a considerable problem in both elite and recreational athletes. Specifically, the incidence of tendon injuries has been estimated to be as high as 30% to 50% of all sports injuries and 6% of sedentary people will at some point experience tendon injury (Kujala, Sarna, Kaprio, 2005; Lopes et al., 2012). Moreover, the symptoms and reduction in performance may last for an extended period, potentially years (Kettunen, Kvist, Alanen, Kujala, 2002; Lian, Engebretsen, Bahr, 2005) and many never return to their previous activity level or even end their sports career (Panni, Tartarone, Maffulli, 2000; Cook et al., 1997). The highest prevalence of lower extremity tendon injuries are found in the Achilles tendon, tibialis posterior and patellar tendons (Wu, Nerlich, Docheva, 2017). Achilles tendinopathy is the most common running associated tendon disorder and patients with achilles rupture often have a previous history of tendinopathy. The majority, 80-90 % of achilles ruptures, occur in the hypovascular zone 2-6 cm proximal to the enthesis (Hess, 2010). In recent decades there has been a gradual increase in the prevalence of achilles tendinopathy and rupture. This is suggested to be a consequence of an increase in the elderly population as well as a higher participation in sporting activities among seniors, with 80% of achilles ruptures occurring with sporting activities (Kujala et al., 2005; Zafar, Mahmood, Maffulli, 2009).

Tendons basic biology

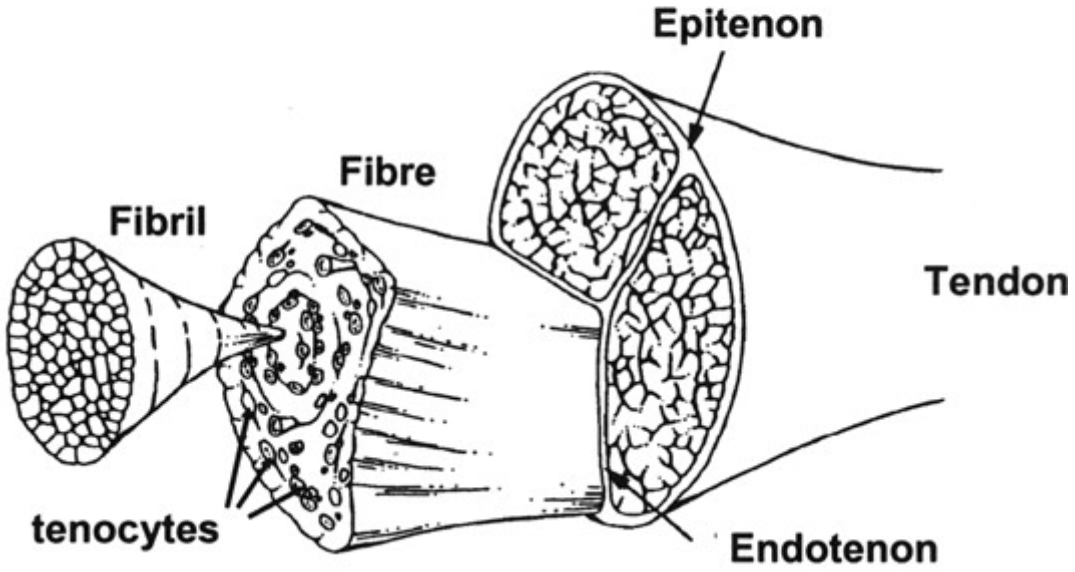
Anatomy and biology of tendon tissue

This study aims to evaluate a possible detrimental effect of a single biomarker on connective tissue. The following chapters will describe some basic properties of tendon tissue along with the etiology and pathogenesis for tendinopathy. This highlights the complexity and difficulties related to making assumptions about how isolated factors might contribute to a pathologic process in tendon tissue.

The primary function of tendons is to ensure movement and skeletal stability by transmitting forces from muscles to bones. The attachment of tendon to bone is labeled enthesis or osteotendinous junction and the attachment from muscle to tendon is called the myotendinous junction. Tendons are mainly composed of closely packed, parallel organized collagen fibres and cells within the extracellular matrix (ECM). The smallest structural unit is the fibril, these range from 10 to 500 nanometers, depending on age and location and largely consists of parallel packed collagen. The fibrils connect to form fibers and bundled fibers are

termed fascicles which are kept together by a thin layer of connective tissue known as the endotenon (Figure 1). Blood vessels, lymphatics and nerves stretch throughout the body of the tendon and are carried in the endotenon (Wu et al., 2017). Fascicles connect in bundles and are covered by the epitenon, a structure very similar to the endotenon (Riley, 2004).

Figure 1. The hierarchical structure of tendon



Bundles of fibrils form fibres, the fibres are bound together by a thin layer known as endotenon, and several fibre bundles are surrounded by an outer layer known as the epitenon. Reprinted with permission from author (appendix 1).

The fibers are primarily aligned with the long axis (the loading direction) as the tendon primarily responds to tensile forces, however a little part of the fibers run along a transverse and spiral axis providing resistance to transverse and rotational forces. This structure with individual fibres collected in bundles creates a safe mechanism where overall tendon strength can be sustained through failure of a few fibre bundles (Riley, 2004). Tendons are not a static tissue, it is capable of adapting to levels, frequency and direction of mechanical load (Riley, 2004). The ECM of tendon is predominantly composed by collagen type I, comprising 65% - 80% of the dry mass and 95% of the collagen, in addition to small levels of collagen type: III, V, XI, XII and XIV (Screen et al., 2015; Wu et al., 2017). In between the collagen units is a great variety of non-collagenous ECM components and these are an important part of the foundation for tendon function, homeostasis and repair. Recent research has contributed to a

better understanding of the nature and function of these ECM components. In short these non collagen proteins can be divided into: Proteoglycans, glycoproteins and glycoconjugates (Screen et al., 2015). The specific role and composition of the ECM is complex and beyond the scope of this study, however the presence of fibroblasts inside the fascicles are important to highlight as they are responsible for synthesising collagen and by that an important part of the natural healing process (Wu et al., 2017).

Vascularisation, innervation and healing capacity

This chapter outlines some of the structural changes related to the healing process and presents evidence for the exclusion criteria used in this study. Tendon tissue has a low metabolic rate, and the vascularity as well as healing capacity is inferior compared with many other tissues of the human body. The main source of vascularisation comes from the paratenon which are rich in blood vessels, lymphatics and nerves. This vascular network distributes blood to the deeper layers of the tendon through the endotenon and epitenon. In addition there are blood vessels originating from both the enthesis and the myotendinous junction (Kastelic, Galeski, Baer, 1978; Wu et al., 2017). Tendon tissue does not only have a low metabolic rate it is also considered hyponeural. Studies on the achilles tendon have shown how sensory nerves primarily are located on the surface, where the nerve endings connect to the paratenon. A small part of the sensory nerves enter the main body of the tendon following the vascular network of the endotenon, these nerves provide sensory information on pressure, tension and pain. Golgi tendon organs are specialised in providing information on tension and are primarily located at the muscle-tendon interface (Wu et al., 2017).

The physiology and mechanisms of tendon healing are complex, the following section will focus on some of the structural changes that have been related to the increased risk of subsequent tendon injury. In general terms tendon healing begins immediately after injury and it begins with the formation of a haematoma. The healing is composed by two overlapping mechanisms, an extrinsic and an intrinsic. The extrinsic is the initial healing response, facilitating an invasion of inflammatory cells to the site of injury, this promotes the repair process and initial synthesis of the collagen matrix. This is followed by an intrinsic healing mechanism responsible for the recruitment of local stem cells contributing to the repair process (Wu et al., 2017). It is important to highlight the structural changes this elicits

in tendon tissue. The scar tissue has a higher ratio of type III collagen, 20-30% in comparison with native tendon having <1%. Type III collagen has a smaller diameter, inferior strength properties and is more elastic than type I collagen. Microscopical observations of the diseased tendon has shown a thinning and disorientation of the fibres, collagen degradation, increase in vascularity and a more fibrocartilaginous composition (Aström & Rausing, 1995; Obaid & Connell, 2010). This larger amount of fibrous tissue in the diseased tendon, is to compensate for the mechanical insufficiency from the more elastic type III collagen, this results in a thickened but stiffened tendon (Obaid & Connell, 2010). The maturation of scar tissue is a slow process which may take 1-2 years (Obaid & Connell, 2010), this maturation improves the quality of the scar tissue through enlargement of fibril diameter and a decrease in the type III collagen ratio. However "the tissue remembers" and because of an inferior structural organization and poor matrix formation the mechanical properties of a healed tendon remain suboptimal compared to that of a native tendon (Obaid & Connell, 2010). A study on a norwegian population by Årøen, Helgø, Granlund and Bahr (2004) stated that an individual is 176 times more likely to have a contralateral tendon rupture following achilles tendon rupture. The authors proposed a multifactorial explanation with degenerative changes, genetic predisposition or atrophy from overall decreased physical activity following the injury resulting in this increased risk of an analogous injury (Årøen et al., 2004). Systemic factors such as hyperglycemia could be another relevant factor to consider as explanation for the increased risk of contralateral injury.

Etiology and pathogenesis of tendinopathy

This chapter presents some basic etiologic and pathologic properties of tendon tissue.
The etiology and pathogenesis of tendinopathy are multifactorial, complex and not fully elucidated. Risk factors are often divided into extrinsic (acting on the body) e.g. load related factors and intrinsic (acting from within the body) e.g. biomechanical or systemic factors. There is no doubt that load tolerance is an important factor for tendinopathy and evidence has shown a large individual variation in this (Gaida, Ashe, Bass, Cook, 2009). An explanation for this individual variation might be an intrinsic moderation where factors such as hyperglycemia might play an important role (Gaida et al., 2009). There is little knowledge about causal relations between specific risk factors and tendinopathy, since the majority of

the research is based on cross sectional or case control trials. However certain key factors are commonly associated with tendinopathy:

- Intrinsic - Age, previous injury, male gender, genes, biomechanics, metabolic and vascular factors, nutrition, body weight and systemic disease.
- Extrinsic - Physical load, occupation, sport and medication (Gaida et al., 2009; Riley, 2004).

Biomechanical factors and over-use most likely result in tendon degeneration and lead to cell phenotype changes and neovascularization (Riley, 2004). In general the consensus today acknowledges both the role of intrinsic and extrinsic factors. Tendinopathies are not identical, there is great variation in local anatomy of the tendon, designed to meet the functional demands of the specific location and a variety of factors can contribute to this "failure" of matrix adaptation and remodelling (Sharma & Mafulli, 2008). Histopathological assessments of the diseased tendon reveal that inflammatory and degenerative changes does not present in isolation but are found to coexist, the degenerative process in tendon tissue can be seen as an imbalance between tissue decomposition and synthesis (Dean et al., 2017).

Risk factors for muscle- tendon injury

The evidence for hyperglycemia as a risk factor for muscle- tendon injury has previously been presented. This chapter presents the evidence for the covariates selected for this study.

Medicine

Numerous medicines have been described in relation to muscle-tendon pathology. The specific pathophysiological mechanisms behind drug-induced tendon injury often remain elusive, however in recent years more drugs have been described in relation to muscle-tendon pathology. Drug induced tendinopathy is considered an underestimated problem and there is inadequate awareness about the possible severe adverse effects on tendon tissue in numerous drugs prescribed in everyday practice (Kirchgesner et al., 2014). A recently published case-control study on 1118 achilles tendon (AT) ruptures by Nyyssönen et al. (2018) reported the following odds ratios (ORs) for various drug treatments in relation to AT rupture:

- *Anti inflammatory and analgesic drugs* NSAIDs had an OR = 2.0 (p=0.000) and common analgesic drugs such as salicylic acid derivatives and paracetamol had an OR = 2.14 (p=0.037)

- *Lipid modifying agents* was associated with an increased risk for AT rupture, OR=1.54, however not statistically significant (p=0.465).
- *Systemic corticosteroids* showed a statistically significant association with OR 3.85 (p=0.000).

Anti inflammatory and analgesic drugs

There is a frequent use of analgesics in general and athletic populations. A Danish population study by Hargreave et al. (2010) on 45.000 individuals, reported that 27% of women and 18% of men had a regular monthly use of at least seven analgesic tablets during the last 12 months. Athletes usually have relatively unrestricted access to NSAIDs and there is reported alarmingly high intake in athletes and exercising individuals (Alaranta, Alaranta, Helenius, 2008). These drugs might be beneficial in the short term management of acute injuries with an inflammatory component (Ekman et al., 2006), but using analgesic drugs, in relation to muscle-tendon pathology, may contribute to mask pain during activity and thereby result in a progression of pathology. Studies have reported how these drugs seem to have a negative effect on the musculoskeletal system:

- A recent study by Lilja et al. (2018) on young adults (18-35 years) described how a maximal over-the-counter dose of NSAID (ibuprofen) attenuate strength and muscle hypertrophy after 8 weeks of resistance training.
- A study by Christensen et al. (2011) gave healthy runners indomethacin 72 hours before running a marathon. These runners showed a complete blunting of the exercise-induced collagen synthesis in their patella tendons when compared to their placebo-dosed counterparts. The authors concluded that the use of NSAIDs reduced prostaglandin production, which significantly decreased collagen synthesis in response to weight-bearing activity.

In addition, NSAIDs might also impair tendon healing. However, the majority of this evidence is based on animals and conflicting results have been reported - either an increase in tensile strength (Forslund, Bylander, Aspenberg, 2003) or a reduction in tendon breaking point (Shen et al., 2005; Cohen, Kawamura, Ehteshami, Rodeo, 2006). Studies reporting an increased tensile strength in rats, found an acceleration of cross-linkage between collagen fibers after treatment with NSAIDs (Forslund et al., 2003). However, these results are based on surgically divided achilles tendons in rats, which is a situation that does not reproduce the

pathogenesis or conditions of human tendinopathy. A rat study by Virchenko, Skoglund and Aspenberg (2004) reported that COX-2 inhibitors should be avoided in the early stages of tendon injury. They found that early inhibition of the inflammatory response with the administration of NSAIDs led to a decrease in tendon breaking point. In addition the available experimental and clinical evidence indicates that NSAID therapy can impair enthesis (tendon-to-bone) healing (Bailey & O'Connor, 2013).

Lipid modifying agents

Lipid lowering medication, statin, is frequently used all over the world. It is considered to have few side effects, however skeletal muscle pain and weakness are fairly common with the use of statin (Hoffman, Kraus, Dimbil, Golomb, 2012). The first reports on tendon injury in relation to statin was in the early 2000s, over 10 years after the first statins was introduced to patients in 1987 (Kirchgesner et al., 2014). During the last decade there has been an increasing attention to the potential side effects on tendon tissue with the use of statin. A retrospective trial by Marie et al. (2008) using a large pharmaceutical surveillance database over a sixteen-year period, tried to evaluate tendon manifestations occurring in patients treated with statin. They found that the majority of tendon pain or ruptures appear within a year after introduction of statin and that pain seems to disappear soon after treatment has been terminated. They described seven cases where statin treatment was reinstated and they all resulted in the recurrence of symptoms. In line with this Eliasson et al. (2017) recently reported how statin treatment had a detrimental effect on the mechanical properties (force and stiffness) on human tendon. Conflicting results are reported in rat studies; however, in human tendon statin treatment has been found to have detrimental effect on mechanical properties and induce biochemical changes (Kaleagasioglu, Olcay, Olgac, 2015).

Systemic corticosteroids

A systematic review by Dean et al. (2014) found that local injections with corticosteroid, glucocorticoids (GC), had significant negative effects and resulted in long-term reduction of the mechanical properties in tendon tissue. GC mainly affects the weight bearing tendons of the lower extremity and studies have reported how this association between GC and tendon damage is regardless of administration modalities (Kirchgesner et al., 2014). The time from treatment initiation to tendon rupture varies from 4 months to several years. The evidence is

primarily based on long-term oral GC therapy or locally injected GC. However prolonged use of inhaled GC therapy to treat chronic respiratory diseases was also incriminated in the early 90s in a 10-year retrospective study by Newnham, Douglas, Legge and Friend (1991). They found that the mean time from treatment initiation to tendon rupture was 4 years. This alleged association between inhaled GC and tendon injury is only confirmed on a case report level (Singh, Pandit, Doherty, 2009) and only sparsely described in literature. The pathophysiology behind corticosteroid induced tendon rupture is uncertain, it is described how it might deteriorate the collagen of the tendon via antimitotic effects and collagenase activation (Newnham et al., 1991). Another suggested hypothesis is an inhibition of tissue repair mechanisms accompanied with repetitive microtrauma (Kirchgesner et al., 2014).

Running

Under- and over stimulation in terms of mechanical loading has been established as important risk factors for tendinopathy (McCarthy & Hannafin., 2014). Running in the adult population is one of the most popular physical activities around the world and one of the most efficient ways to achieve physical fitness, which is linked with longevity (Fields, Sykes, Walker, Jackson, 2010). An issue with running is the high risk of injury as running is associated with a higher risk of overuse injury than other forms of aerobic exercise such as walking, swimming and cycling (Francis et al., 2019). A recent systematic review by Francis et al. (2019) found that about 70% of running injuries occur at or below the knee in both men and women and that achilles tendinopathy and patellofemoral pain syndrome is the two most common running related injuries. It seems that poorly perfused tissues such as ligaments, tendons and cartilage, are particularly at risk because they adapt slower, than muscles, to increased mechanical load. Moreover, 80% of the injuries in running are related to overuse and is proposed to be a mismatch between the resilience of the connective tissue and mechanical load (Van der Worp et al., 2015).

It is well established that mechanical loading such as running has beneficial effects on tendon morphology and is essential to maintain tendon homeostasis and promote the synthesis of proteoglycans and collagen (Heinemeier & Kjaer, 2011). This process is stimulated when the collagen fibers are stretched and a signal is transmitted inside the tenocytes releasing growth factors (Abate et al., 2009). When the mechanical loading is repeated and remains in the physiological window, anabolism prevails on catabolism: Both

synthesis and degradation of collagen are increased, but collagen synthesis prevails and persists longer than collagen degradation, and new extracellular matrix and collagen fibers are formed. Evidence shows that after several months of continuous exercise, the cross sectional area of the tendon increases and the biomechanical properties improve (Heinemeier & Kjaer 2011). There seems to be an upper threshold of mechanical loading that once exceeded reverses tendon adaptations from beneficial to degenerative (Abate et al., 2009).

Age

Age is an important covariate as the correlation between increasing age and the prevalence of tendon injury is well established (Wu et al., 2017). Moreover, HbA1c levels are found to be positively associated with aging in nondiabetic subjects (Pani et al., 2008). Human aging is associated with changes to the entire muscle-tendon unit with a reduction in muscle mass combined with structural changes to the tendon. The exact underlying mechanisms of tendon aging are not fully elucidated but aging is known to alter the mechanical properties and metabolism of tendon tissue (McCarthy & Hannafin, 2014). A degenerative process of tendon tissue related to biological aging has been explored in studies showing a decline in tensile strength, blood flow, the number and repair capacity of tissue-specific adult stem cells and increased lipid formation (Wu et al., 2017). Another factor that might contribute to the positive correlation between age and tendon injury is the progressive accumulation of advanced glycation end products (AGEs) that are a natural part of the typical aging process. As mentioned this accumulation of AGEs is also the best explanation, at the moment, for the increased prevalence of tendon injury in people with diabetes.

Sex

Differences in the health status of women and men are a subject of growing interest to medical researchers and a possible gender difference in tendon injury risk can be an important consideration when planning lifestyle interventions for patients with metabolic syndromes. A systematic review on amateur runners found that women in general seem to be at lower risk than men in terms of running-related injuries (Van der Worp et al., 2015; Taunton et al., 2002). Part of this gender difference is explained by sex hormonal differences. There is estrogen receptors in tendon tissue and it has been suggested that this might influence tendon structure and biomechanical properties (Hansen & Kjaer, 2016). The effects

of separate sex hormones on tendon tissue are complex and not fully elucidated. Research has shown that estrogen can enhance tendon collagen synthesis rate and that it might be beneficial for tendon load adaptations and recovery following an injury. On the other hand testosterone is known to increase tendon stiffness due to increased collagen content and collagen turnover and reduce the responsiveness to relaxin (Hansen & Kjaer, 2016). This results in men having a greater cross sectional area (CSA) of the tendon and decreased joint laxity when compared to women. It also seems that the ability to adapt to load in terms of tendon size is greater in men. Studies demonstrate how the CSA is increased in trained male runners when compared to untrained men, where no difference seems to exist between female runners and untrained females (Westh et al., 2008). Most likely, sex hormones influence tendon tissue and contribute to a gender difference in the risk tendon injury. This is supported by studies showing no difference in tendon tissue between prepubertal boys and girls (Quatman et al., 2007).

Body weight

Most likely, there is a positive correlation between bodyweight and HbA1c levels in both diabetic and nondiabetic individuals (Bae et al., 2016). With respect to overweight/obesity, a systematic review of observational studies found that elevated adiposity is frequently associated with tendon injury (Gaida et al., 2009). A mechanical hypothesis suggests that the increased risk of tendon injury, in obese individuals, may be due to tendon overload. This hypothesis is criticised for being overly simplistic, and recent evidence is in favor of a more systemic hypothesis stating that metabolic factors might have a direct detrimental effect on tendon tissue. A suggested direct systemic mechanism is that excessive fat promotes a release of cytokines that might influence tendon metabolism or response to microtrauma (Gaida et al., 2009). An indirect systemic mechanism might also be possible where metabolic factors associated with obesity such as hyperglycemia affects tendon structure. The systemic hypothesis is supported by evidence showing equal distributions of tendon injury in both upper and lower extremity in overweight people. If adiposity mainly increased the risk of tendon injury through excessive loading, there should be a stronger association with the weight bearing lower extremity tendons when compared to tendons of the upper extremity.

Dyslipidemia

The reported relationship between elevated adiposity and tendon injury has provided the basis for a closer examination of the abnormal lipid profile associated with obesity and the risk of tendon injury. Hypercholesterolemia might be an important factor for this association because cholesterol accumulates in tendons (Gaida et al., 2018). A recent systematic review by Tilley, Cook, Docking, and Gaida (2015) indicates an association between unfavorable changes in lipid parameters and tendinopathy. They found increased levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG), and decreased high-density lipoprotein cholesterol (HDL-C) in individuals with tendon pain or pathology. These unfavorable changes in lipid parameters associated with tendon injury are similar to those associated with cardiovascular disease (CVD). The pathology behind CVD and tendon injury is complex and a complete analysis of the pathophysiological similarities is beyond the scope of this study. However a brief explanation for this similar response to the metabolic environment is that both arteries and tendons are collagen based tissues capable of responding to load. It seems that particular areas of tendon and artery which are more exposed to shear and compression forces, combined with the accumulation of cholesterol in collagen, can cause a structural disruption of the collagen matrix and chronic low-grade inflammation (Tilley et al., 2015). With high proportions of patients with hypercholesterolaemia taking cholesterol-lowering medications, the relationship between dyslipidemia and tendon injury can be influenced by the use of statin. However, a longitudinal population-based follow-up study by Lin et al. (2015) with ≈ 500.000 participants found an increased risk of rotator cuff tendon pathology in patients with dyslipidemia with or without the use of statin.

Tobacco

Cigarette smoking (CS) has deleterious effects on the entire musculoskeletal system (Kanis et al., 2005; Kok, Hoekstra, Twisk, 2012). A recent systematic review by Al-Bashaireh et al. (2018) on the effects of smoking on musculoskeletal health identified 3 studies focusing on tendon tissue:

- A cross sectional study reported how smokers had more advanced degenerative changes in supraspinatus tendons (Lundgreen et al., 2014)

- A study by Carbone et al. (2012) on 408 patients who underwent arthroscopic rotator cuff tendon repair found a dose-response relationship between the amount of smoking and the severity of the tear.
- A case control study by Ağladioğlu et al. (2016) reported how CS led to significantly thinner achilles and patellar tendons and they found a negative correlation with patellar tendon strain ratio and amount of smoking.

Tobacco smoke has more than 7,000 harmful chemical compounds making the pathophysiology complex. In brief nicotine is the primary component of the toxic and addictive substance in cigarettes and the literature investigating the negative effects on tendon tissue with smoking is primarily based on nicotine (Ağladioğlu et al., 2016; Al-Bashaireh et al., 2018). Nicotine causes peripheral vasoconstriction and tissue ischemia, this is thought to be an important factor for the decreased tendon size and impaired healing of tendon tissue in cigarette smokers (Duygulu et al., 2006).

To sum up all of the above: The aim of this study is to explore a possible association between elevated HbA1c and muscle- tendon injury. As mentioned, emerging evidence of this association is based on rather small- or animal studies. Therefore, it still remains unknown if elevated HbA1c relate to tendon pathology in humans. The exact mechanisms of how hyperglycemia might interact with this myriad of both intrinsic and extrinsic factors is beyond the scope of this study.

Hyperglycemia and lifestyle diseases: a backdrop

Where the possible association between hyperglycemia and muscle- tendon injury is new and sparsely described in the medical literature, the evidence for an association between elevated HbA1c and the prevalence of lifestyle diseases such as DM and cardiovascular diseases (CVD) is extensive and well established. The positive association of HbA1c to CVD and DM is not the focus of this study, but it serves as a backdrop. Elevated HbA1c as a risk factor for both musculoskeletal injury and lifestyle diseases may create a vicious cycle were people who need exercise the most are the ones who are most prone to injury.

A HbA1c level above 6.5% is one of the diagnostic criterias for DM (WHO, 2011) and HbA1c is a well established and strongly associated risk factor for CVD in people with or without diabetes (Goto et al., 2015). A study by Eskesen et al. (2012) based on the same

database as this study, the Copenhagen City Heart Study (CCHS), confirmed the strong association between elevated HbA1c and CVD in otherwise healthy individuals. Structured exercise is considered a cornerstone in treatment and prevention of both CVD and DM. Long-term regular physical activity has been found to improve glycemic control and decrease the levels of HbA1c (Najafipour et al., 2017). However, numerous studies have reported how low compliance to exercise protocols is a big challenge (Schuler, Adams, Goto, 2013) and one study reported how a 50% drop out rate, in a lifestyle intervention for type 2 diabetes, was due to musculoskeletal symptoms (Praet et al., 2008). A position statement from the European Society of Cardiology stated how low adherence to exercise routines, in patients with increased risk of CVD, is the "Achilles heel" of exercise interventions and that solutions to overcome this barrier is warranted (Conraads et al., 2012).

The burden of CVD and DM can be measured in different ways, it carries a burden for the individual affected in terms of mortality and morbidity but it also carries a huge societal burden with economic costs to healthcare services and society as a whole. CVD is the biggest cause of death in the European Union (EU) responsible for over 2 million deaths annually, that is 42% of all deaths in the EU (Rayner, Allender, Scarborough, 2009). The economic costs of CVD was in 2009 estimated to €106 billion or 9% of the total healthcare expenditure across the EU (Nichols et al., 2012). In addition you have to consider non health care costs such as the expenses linked to informal care and production losses related to mortality and morbidity associated with CVD in the working age population. In 2006 the cost for production losses was estimated to €46 billion and the costs for informal care estimated to over €43 billion. Including these factors CVD was in 2009 estimated to an annual cost of over €195 billion across the european union (Nichols et al., 2012). The global epidemic of DM was in 2014 estimated to an overall health expenditure of $612 billion or 11% of the total spending on adults. In 2014 there was an estimated total of 52 million patients with DM in the EU, and an expected increase to 69 million by 2035 (Sherwani et al., 2016).

The Danish healthcare system

This study includes data from the Danish healthcare system. This chapter will describe some basic aspects of the handling and treatment of musculoskeletal injuries in Denmark.

Structure

In Denmark, the general practitioners (GP) act as gatekeepers to secondary care and patients need a referral from their GP to obtain specialist care in hospitals (Moth, Olesen, Vedsted, 2012). With growing pressure on the GP and with the role as gatekeeper being both time consuming and complicated this has become a point of stress within the healthcare system (Stochkendahl et al., 2019). Musculoskeletal illness accounts for 9.3% to 17% of all patient contacts in general practice in Denmark and 43% of these patients get referred by the GP to a physical therapist (Jørgensen, Fink, Olesen, 2001). In addition physiotherapists, chiropractors, and manual therapists are increasingly becoming the first point of contact and the principal provider of healthcare for individuals with musculoskeletal conditions (Stochkendahl et al., 2019).

Treatment of tendon injury

The literature presents different non-surgical treatment modalities for tendinopathy. This chapter will focus on the positive effects from progressive mechanical loading in achilles tendon rehabilitation. Progressive loading is considered first-line therapy for patients with tendinopathy and it highlights the paradox with mechanical loading being an important factor for both pathology and healing in tendon tissue.

It is well established that tendon treatment requires long periods of rehabilitation and that the biological and mechanical properties are difficult to restore (Wu et al., 2017). In the late 90's Alfredson, Pietilä and Jonsson (1998) published a study showing a positive effect of eccentric strength training for achilles tendinopathy. This was in many ways a defining study changing treatment modalities from inactive and passive strategies to active training. Inspired by the study from Alfredson researchers started investigating whether it was the eccentric training in isolation or the gradual increase in load, concentric or eccentric, eliciting the positive results. A randomized study on 44 patients with achilles tendinopathy by Mafi**,** Lorentzon and Alfredson (2001) showed better results for an eccentric than a concentric

exercise protocol. However, this study had a methodological flaw related to the mechanical loading - the eccentric group exercised with twice the load. Thus, focusing more on the role of mechanical loading than the significance of concentric versus eccentric training. This is confirmed in more recent research showing that both concentric and eccentric exercises can reduce pain and increase strength in pathologic achilles tendons (Malliaras, Barton, Reeves, Langberg, 2013; Allison & Purdam, 2009). Mechanical load, speed and frequency are important factors to consider for the exercise regimen when planning treatment for tendon injury. Current guidelines suggest slow movements, with heavy loads (8-15RM), 48 hours recovery and a minimum of 8-12 weeks (Malliaras et al., 2013). The research presented in this chapter is based on achilles tendinopathy and there are limitations related to these studies. As mentioned, tendons possess unique properties due to the fact that each tendon are structured in a certain way to meet the local demands.

Purpose

The primary aim of this study is to investigate the significance of hyperglycemia (HbA1c) in relation to muscle- tendon pathology. The exact injury mechanism remain elusive, but a better understanding of how hyperglycemia might exacerbate the degenerative process could contribute to a better understanding of the pathogenesis of tendinopathy and thus provide the basis for prevention of tendon injuries. HbA1c is a new and relatively undescribed risk factor in relation to muscle- tendon injury. It is only sparsely included in systematic reviews on the topic and usually under the term "metabolic factors". The findings from this study can potentially serve as a clinical tool, for primary healthcare practitioners, to help identify patients with an increased risk of future muscle-tendon injury. This is relevant not only in terms of injury prevention but also to increase compliance to exercise/lifestyle interventions for patients who need it the most.

In addition, this study wants to examine the incidence and distribution of lower extremity muscle- tendon injury and do a descriptive comparison between runners and non-runners with respect to HbA1c and the included covariates.

Research question

Are elevated levels of HbA1c associated with a greater risk of lower extremity muscle-tendon related pathology?

Method

Design

The design is a prospective cohort study that is appropriate when examining the association between baseline exposure (HbA1c) in a defined population (without the disease/injury of interest) and the outcome (muscle- tendon injury). In the current study the follow-up period was 3 years from baseline measures. This prospective design is chosen because of the higher likelihood of estimating causal relationships between exposure and outcome than for example in a cross-sectional study. To conduct this study data from two registers were linked: The Copenhagen City Heart Study (CCHS) and The Danish National Patient Register (DNPR).

Setting

The Copenhagen City Heart Study (CCHS)

This study is based on data from the CCHS, a large ongoing prospective cardiovascular population study of 19.329 men and women launched in 1975 by Dr Peter Schnohr, Dr Gorm Jensen, statistician Jørgen Nyboe and Prof. A. Tybjærg Hansen. The population was drawn from approximately 90.000 inhabitants aged 20 years or older living in 2 regions in Copenhagen, the entire Østerbro and the one third of Nørrebro closest to Rigshospitalet.

This study uses the fourth wave (2001 to 2003), where HbA1c was included in the examination for the first time. The sample was collected using the unique personal identification number and age-stratified to 5 year age groups, with a main focus on individuals aged from 35-70 years. Individuals selected for the study were invited according to their date of birth, converting the date to a six-digit number (day, month, year of birth). These numbers were used in ascending order, starting with individuals born on January 1st, February 1st etc. and ending with December 31th, ensuring that subsets of the sample examined during any period of time would constitute a random subsample. The response rate for the fourth wave was the lowest for the four examinations, 6.238 individuals (49.5%) of the 12.600 invited responded, compared to a response rate >70% of wave one and two. The CCHS has a follow-up completion rate at almost 100 percent (less than 0.1% have been lost to follow-up mainly through emigration), which is unusually high for large population studies (Aguib & Al Suwaidi, 2015).

Danish National Patient Register (DNPR)

The DNPR was established in 1976 and is a national register maintaining data on all patients who has been admitted for surgery or been diagnosed at Danish hospitals. ICD-10 codes were implemented in the danish healthcare system and included in the DNPR january 1st - 1994. Internationally the DNPR is considered to be the most comprehensive of its kind. It was established before hospital registers in the other Nordic countries and covers both psychiatric and somatic patients. The register is linkable to other registers due to the registration of a personal number unique to each resident in Denmark (Lynge, Sandegaard, Rebolj, 2011).

Linkage between data sources

Data linkage between CCHS and DNPR was made possible by the personal identification number. Participants enrolled in the fourth wave of the CCHS were followed-up for 3 years in the DNPR to register diagnoses of muscle- tendon injury (in accordance with the defined outcome). For each participant the follow-up period ran from the date of enrollment in the CCHS (where baseline measures were taken) and three years (36 months) ahead.

Participants

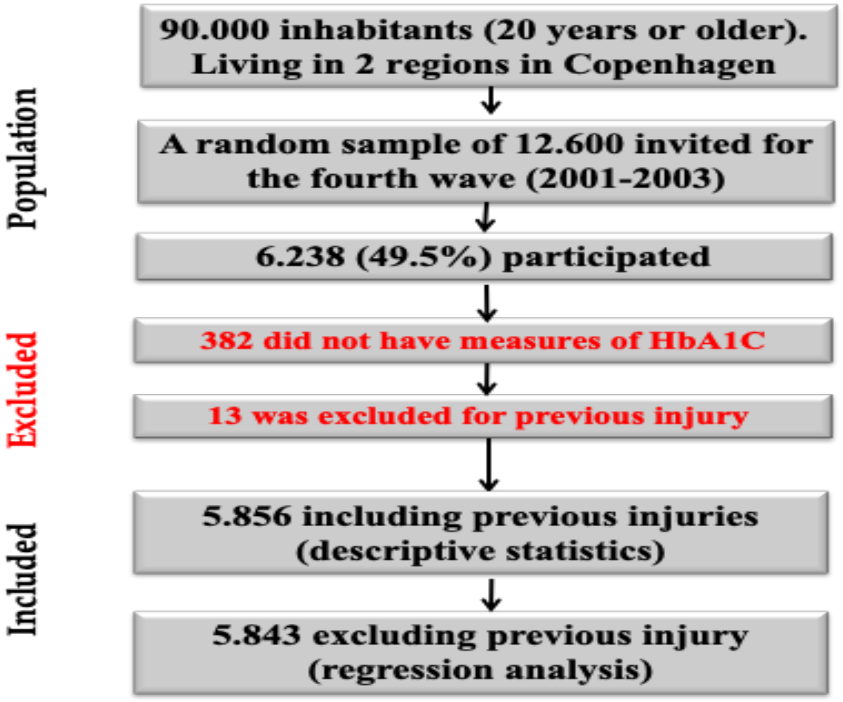
Inclusion

The population in the current study is based on the random sample of 6.238 individuals included in the fourth wave of the CCHS.

Exclusion criteria

Individuals who did not have measures of HbA1c or had a muscle- tendon injury registered in the DNPR (ICD-10 codes - appendix 2) one year prior to enrollment was excluded (Figure 2).

Figure 2. Flowchart



Flowchart of random sample, exclusion and final population.

Examination procedure: The Copenhagen City Heart Study

Established procedures and examinations for cardiovascular epidemiologic surveys described by Rose & Blackburn (1968) were used. Three weeks prior to the examination, the random sample of selected individuals were invited by letter to participate in the CCHS and the examination at Bispebjerg University Hospital. The letter held basic information of the CCHS including the main purpose of the study: Prevention and treatment of cardiovascular diseases. The person could confirm the appointment, alter the date or decline participation through a prepaid postage card attached to the letter. If the individual did not reply to the initial

invitation, a second attempt was posted one week prior to the examination date and finally a re-invitation was tried 6 months later for non-responders.

The fourth examination was conducted at Bispebjerg University Hospital and included questionnaires, clinical assessment and biomarkers and was done at 3 stations, lasting 6-8 minutes per station. Upon arrival at the examination participants completed a questionnaire regarding demographics, symptoms, diseases, medicine, familial disposition, socioeconomic status, smoking/drinking habits, physical activity at work and during leisure time and prior contact with the healthcare system.

- At the first station the questionnaire was reviewed by a staff member and a non fasting venous blood sample with measures of: TC, LDL-C, HDL-C and TGs was obtained. In *the fourth examination* many parameters were added and an almost total biochemical analysis of the blood sample was performed including measurements of HbA1c.
- At the second station information on height, weight, hip circumference and sagittal body diameter was measured.
- At the third station blood pressure was measured, the questionnaire was checked again and the results from the examination was explained to the participant.

Exposure

HbA1c is divided into three levels based on guidelines from the World Health Organization (WHO) where a HbA1c level <5.7% is considered normal, a level from 5.7 - 6.5 % is considered elevated and prediabetic, and a level >6.5 % is high and one of four diagnostic criterias for DM (WHO, 2011). Measurement of HbA1c was based on a turbidimetric inhibition immunoassay (Thermo Fisher Scientific) for haemolysed whole blood collected at the examination. Turbidimetric HbA1c assays are the most common assays in clinical laboratories and they have been found to have a good correlation ($r2 = 0.98$) with the high−performance liquid chromatography (HPLC) (Genc et al., 2012). HPLC has been appointed as the reference method for HbA1c assays by the American National Glycohemoglobin Standardization Program (Genc et al., 2012). The HbA1c testing method was standardized against the approved International Federation of Clinical Chemistry and Laboratory Medicine reference method (Jeppsson et al., 2002).

Outcome

Hospital contacts in the DNPR for lower extremity muscle- tendon related pathology was obtained from the following ICD-10 diagnoses and surgery codes (appendix 2):

ICD-10 diagnoses

S76 - Injury of muscle and tendon at hip and thigh level.

S76.0, **S76.1**, **S76.2**, **S76.3**, **S76.4** and **S76.7**.

S86 - Injury of muscle and tendon at lower leg level.

S86.0, **S86.1**, **S86.2**, **S86.3**, **S86.7**, **S86.8** and **S86.9.**

S96 - Injury of muscle and tendon at ankle and foot level.

S96.0, **S96.1**, **S96.2**, **S96.7**, **S96.8** and **S96.9**.

M66 - Spontaneous rupture of lower extremity tendon.

M66.2, **M66.3**, **M66.4** and **M66.5**.

M76 - Enthesopathies of lower limb, excluding foot.

M76.0, **M76.1**, **M76.3**, **M76.5**, **M76.6**, **M76.7**, **M76.8** and **M76.9**.

M77 - Other lower extremity enthesopathies.

M77.3, **M77.5** and **M77.8**.

Surgery codes

KH - Lower extremity muscle- tendon surgery.

KH39, KH49, KH69 and **KH99.**

Covariates

Tendon pathology is complex and as mentioned many intrinsic and extrinsic factors can contribute to the pathologic process. The association between HbA1c and muscle- tendon injuries that this study aims to explore, might be explained by another variable or covariate. A confounding variable is defined as a variable that is a risk factor for the outcome under study and also related to the exposure of interest (Grobbee & Hoes, 2008). This section describes the categories and source of data for the covariates included in this study.

Anti-inflammatory and analgesic drugs: Use of anti-inflammatory and analgesic drugs was measured in question 15 (appendix 3) with a yes/no answer to "a daily or almost daily use of painkillers" and used as a dichotomous variable (no=0, yes=1).

Lipid modifying agents: Use of lipid modifying agents was measured in question 4 (appendix 3) with a yes/no answer to "a daily or almost daily use of drugs for increased cholesterol" and used as a dichotomous variable.

Systemic corticosteroids: Use of systemic corticosteroids was measured in question 9 (appendix 3) with a yes/no answer to "a daily or almost daily use of medication to treat asthma/bronchitis (including spray or powder)" and used as a dichotomous variable.

Running: Running habits was measured in question 64 (appendix 4). Weekly average number of hours spent running was registered with 5 alternatives: No running, running < 0.5 hour/week, running 0.5 ≤ 1 hour/week, running ≤ 2 hours week and running > 2 hours/week.

Age: Age was measured as a continuous variable at the time of baseline assessment and divided into 8 categories: 20–24, 25–34, 35–44, 45–54, 55–64, 65–74, 75-84 and >85 years.

Sex: Sex was registered as either man or woman.

Body weight: Body weight and height was measured with one decimal at the second station in the examination. Bodyweight was calculated into body mass index (BMI) and divided into 5 groups: Underweight (<18.5 kg/m2), Normal weight (18.5-24.9 kg/m2), Overweight (25.0-29.9 kg/m2), Obese (30.0-34.9 kg/m2) and Severely obese (≥35.0 kg/m2).

Dyslipidemia: Cholesterol was measured at the first station in a non-fasting venous blood sample. Dyslipidemia was defined, using the guidelines from the European Society of Cardiology, with any of the following criterias: TC >240 mg/dL (6.206 mmol/l), TG >200 mg/dL (2.258 mmol/l), LDL-C >160 mg/dL (4.138 mmol/l), HDL-C <40 mg/dL (1.034 mmol/l) (Tilley et al., 2015).

Tobacco: Cigarette smoking was measured in question 31, 33 and 36 (appendix 5). Exposure to cigarette smoking/nicotine was divided into 4 categories: Never, former, former smoker using nicotine substitute or current smoker.

Statistical analysis

Baseline characteristics for the total population (5.856) are presented with median and interquartile range (IQR) for continuous variables. The analysis includes a descriptive comparison, of baseline characteristics, between individuals who identified as runners or non-runners. Chi-squared test (categorical variables) and Kruskal-Wallis test (continuous variables) are used to examine the distribution of independent variables across strata of the main exposure (HbA1c). Analysis on injury incidence and logistic regression models were based on 5.843 individuals (13 excluded for previous injury). Logistic regression models were performed to examine the relationship between lower extremity muscle- tendon injury and hyperglycemia (HbA1c) and results are presented as odds ratios (ORs) with 95% confidence intervals (CIs). P values of less than 0.05 were considered to indicate statistical significance. A crude value is presented for the main association and adjusted values when adjusted for potential confounders. The normal or unexposed category (HbA1c <5.7%) was used as a reference. All analyses were conducted using SAS®, Version 6.1 for Windows.

Ethical considerations

This study does not include any direct contact with participants and participants are not informed about the use of their data in this study. However, written statements of informed consent were obtained from all participants at baseline. The consent included that data from CCHS could be used for later studies and paired with information from hospitals by use of their personal identification number (appendix 6). The data extracted from the DNPR was performed in a closed system and once participants was linked to the CCHS the analysis was performed on a group level and could no longer be traced back to individuals. All sensitive information was kept in a closed system only accessible through a two factor security protocol. This project is not considered to harm any participants.

Results

A total of 5.856 subjects were included in the analysis. During the 3 year follow−up 30 muscle- tendon injuries were registered in the DNPR. Excluded participants (n=13), with injury one year before baseline, are included in the descriptive analysis (Tables 1 and 2) and excluded from the analysis on incidence, covariates association with outcome and regression models (Tables 4, 5 and 6).

Descriptive characteristics

Baseline characteristics for the runners (1.107), non-runners (4.749) and total population (5.856) are presented in Table 1. There are missing data for the following variables: Running habits (n=63), BMI (n=3) and smoking habits (N=4).

Total population

Median age for the total population was 62 years (IQR 48-72) and there was a slight overweight of men (57.2%). The majority (63.7%) of the total population were exposed to hyperglycemia (5.7% ≤ HbA1c), with 48.6% and 15.1% in the elevated and high category, respectively. The reference group with normal HbA1c (<5.7%) was 36.3%.

Runners versus non-runners

Median age for the non-runners was 64 years (IQR 53-74) compared to 43 years (IQR 30-54) for the runners. Non-runners had a slight overweight of men (60%), whereas women were in majority among runners (55.1%). The reference group with normal HbA1c <5.7%, consisted of 43% among runners, this proportion was lower for non-runners (34.7%). Among the runners 8,7% had HbA1c levels ≥6.5 % (high level), compared to 16.6% among non-runners. Individuals with HbA1c levels from 5.7 to < 6.5 % (elevated level) were the largest group among both runners (48.3%) and non-runners (48.6%).

In sum, the descriptive data indicate that the runners had lower age, lower HbA1c, lower body mass index, less use of tobacco, less use of medication and a lower prevalence of dyslipidemia when compared to non-runners.

Table 1. Baseline characteristics of runners, non-runners and total population

	Runners		Non-runners		Total population	
	n		n		n	
HbA1c (%), *median (IQR)*	**1107**	5.7 (5.4 - 6.1)	**4749**	5.9 (5.5 - 6.2)	**5856**	5.8 (5.5 - 6.2)
HbA1c, *n (%)*	**1107**		**4749**		**5856**	
Normal (HbA1c < 5.7%)		476 (43.0)		1648 (34.7)		2124 (36.3)
Elevated (5.7% ≤ HbA1c < 6.5%)		535 (48.3)		2313 (48.7)		2848 (48.6)
High (HbA1c ≥ 6.5%)		96 (8.7)		788 (16.6)		884 (15.1)
Running habits, *n (%)*	**1107**		**4686**		**5793**	
No running		-		4686 (100.0)		4686 (80.9)
<½ hour/week		400 (36.1)		-		400 (6.9)
½-1 hour/week		254 (22.9)		-		254 (4.4)
1-2 hours/week		286 (25.9)		-		286 (4.9)
>2 hours/week		167 (15.1)		-		167 (2.9)
Sex, *n (%)*	**1107**		**4749**		**5856**	
Men		497 (44.9)		2850 (60.0)		3347 (57.2)
Women		610 (55.1)		1899 (40.0)		2509 (42.8)
Age (years), *median (IQR)*	**1107**	43 (30 - 54)	**4749**	64 (53 - 74)	**5856**	62 (48 - 72)
Age, n (%)	**1107**		**4749**		**5856**	
20-24 years		95 (8.6)		52 (1.1)		147 (2.5)
25-34 years		263 (23.8)		175 (3.7)		438 (7.5)
35-44 years		239 (21.6)		345 (7.3)		584 (10.0)
45-54 years		255 (23.0)		710 (15.0)		965 (16.5)
55-64 years		170 (15.4)		1097 (23.1)		1267 (21.6)
65-74 years		64 (5.8)		1244 (26.2)		1308 (22.3)
75-84 years		20 (1.8)		944 (19.9)		964 (16.5)
≥85 years		1 (0.1)		182 (3.8)		183 (3.1)
BMI (kg/m2), *median (IQR)*	**1107**	23.8 (21.9-26.1)	**4746**	25.7 (23.1 - 28.7)	**5853**	25.3 (22.8-28.1)
BMI, *n (%)*	**1107**		**4746**		**5853**	
Underweight (<18.5 kg/m2)		10 (0.9)		67 (1.4)		77 (1.3)
Normal weight (18.5-24.9 kg/m2)		703 (63.5)		1959 (41.3)		2662 (45.5)
Overweight (25.0-29.9 kg/m2)		336 (30.4)		1888 (39.8)		2224 (38.0)
Obese (30.0-34.9 kg/m2)		51 (4.6)		628 (13.2)		679 (11.6)
Severely obese (≥35.0 kg/m2)		7 (0.6)		204 (4.3)		211 (3.6)
Smoking habits, *n (%)*	**1107**		**4745**		**5852**	
Never smoked		506 (45.7)		1421 (30.0)		1927 (32.9)
Former smoker		319 (28.8)		1621 (34.2)		1940 (33.2)
Current smoker		273 (24.7)		1649 (34.7)		1922 (32.8)
Former smoker using nicotine substitutes		9 (0.8)		54 (1.1)		63 (1.1)
Dyslipidemia, *n (%)*	**1107**	376 (34.0)	**4749**	2452 (51.6)	**5856**	2828 (48.3)
Daily/almost daily use of painkillers, *n (%)*	**1107**	39 (3.5)	**4749**	697 (14.7)	**5856**	736 (12.6)
Daily/almost daily use of drugs for cholesterol, *n (%)*	**1107**	18 (1.6)	**4749**	304 (6.4)	**5856**	322 (5.5)
Daily/almost daily use of medication to treat asthma/bronchitis, *n (%)*	**1107**	47 (4.3)	**4749**	408 (8.6)	**5856**	455 (7.8)

Table 2 shows the distribution of the covariates according to levels of HbA1c. Chi-squared test (for categorical variables) and Kruskal-Wallis test (for continuous variables) are used to examine the distribution of independent variables across strata of the main exposure (HbA1c). There were statistically significant differences (P-value <0.005) between the distribution of independent variables across strata of the main exposure (HbA1c), except for sex and use of painkillers (Table 2).

For the continuous variables age and BMI values increased with higher values of HbA1c. A similar trend was seen among measures of tobacco use, dyslipidemia and medication, i.e. more use of tobacco, more use of medication and higher prevalence of dyslipidemia was associated with higher values of of HbA1c. Whereas an opposite trend was seen for all levels of running.

Table 2. Clinical and demographic variables according to glycemic control

	Normal (HbA1c < 5.7%)		Elevated (5.7% ≤ 6.5%)		High (HbA1c ≥ 6.5%)		
	n		**n**		**n**		p-value[1]
Running habits, *n (%)*	**2104**		**2819**		**870**		<.0001
No running		1628 (77.4)		2284 (81.0)		774 (89.0)	
<½ hour/week		162 (7.7)		191 (6.8)		47 (5.4)	
½-1 hour/week		113 (5.4)		120 (4.3)		21 (2.4)	
1-2 hours/week		122 (5.8)		145 (5.1)		19 (2.2)	
>2 hours/week		79 (3.8)		79 (2.8)		9 (1.0)	
Sex, *n (%)*	**2124**		**2848**		**884**		0,9520
Men		1216 (57.3)		1630 (57.2)		501 (56.7)	
Women		908 (42.7)		1218 (42.8)		383 (43.3)	
Age (years), *median (IQR)*	**2124**	57 (44 - 68)	**2848**	62 (49 - 72)	**884**	68 (59 - 76)	<.0001
Age, n (%)	**2124**		**2848**		**884**		<.0001
20-24 years		71 (3.3)		71 (2.5)		5 (0.6)	
25-34 years		200 (9.4)		218 (7.7)		20 (2.3)	
35-44 years		283 (13.3)		265 (9.3)		36 (4.1)	
45-54 years		434 (20.4)		443 (15.6)		88 (10.0)	
55-64 years		434 (20.4)		624 (21.9)		209 (23.6)	
65-74 years		389 (18.3)		662 (23.2)		257 (29.1)	
75-84 years		264 (12.4)		471 (16.5)		229 (25.9)	
≥85 years		49 (2.3)		94 (3.3)		40 (4.5)	
BMI (kg/m2), *median (IQR)*	**2122**	24.7 (22.4 - 27.3)	**2847**	25.3 (22.8 - 28.2)	**884**	27.1 (24.1-30.5)	<.0001
BMI, *n (%)*	**2122**		**2847**		**884**		<.0001
Underweight (<18.5 kg/m2)		27 (1.3)		38 (1.3)		12 (1.4)	
Normal weight (18.5-24.9 kg/m2)		1099 (51.8)		1288 (45.2)		275 (31.1)	
Overweight (25.0-29.9 kg/m2)		776 (36.6)		1094 (38.4)		354 (40.1)	
Obese (30.0-34.9 kg/m2)		178 (8.4)		328 (11.5)		173 (19.6)	
Severely obese (≥35.0 kg/m2)		42 (2.0)		99 (3.5)		70 (7.9)	
Smoking habits, *n (%)*	**2123**		**2845**		**884**		0,0036
Never smoked		749 (35.3)		928 (32.6)		250 (28.3)	
Former smoker		668 (31.5)		936 (32.9)		336 (38.0)	
Current smoker		680 (32.0)		954 (33.5)		288 (32.6)	
Former smoker use nicotine substitutes		26 (1.2)		27 (1.0)		10 (1.1)	
Dyslipidemia, *n (%)*	**2124**	904 (42.6)	**2848**	1402 (49.2)	**884**	522 (59.1)	<.0001
Daily/almost daily use of painkillers, *n (%)*	**2124**	254 (12.0)	**2848**	351 (12.3)	**884**	131 (14.8)	0,0843
Daily/almost daily use of drugs for increased cholesterol, *n (%)*	**2124**	72 (3.4)	**2848**	139 (4.9)	**884**	111 (12.6)	<.0001
Daily/almost daily use of medication to treat asthma/bronchitis, *n (%)*	**2124**	139 (6.5)	**2848**	223 (7.8)	**884**	93 (10.5)	0,0010

[1]p-value of a Chi-squared test (categorical variables) or Kruskal-Wallis test (continuous variables)

Incidence

The 3 year incidence of lower extremity muscle- tendon injury are presented in Table 3. The overall incidence was low with 30 outcomes (0.51%) among the 5.843 individuals (13 excluded for previous injury). The greater part of injuries (83%) was at or below the knee and 14 (47%) of the diagnoses was related to the achilles tendon with 13 cases of tendinopathy/tendon injury and one achilles tendon rupture. The majority (86%) of the achilles tendon injuries occurred among exposed individuals. Overall, 25 of the 30 (83%) outcomes registered occurred to individuals exposed to hyperglycemia (elevated and high) and the majority (63%), of the total outcomes, occurred to individuals in the elevated category (Table 3). The majority of the outcomes registered, 27 of 30 (90.1%), occurred to non-runners and there was an almost equal outcome distribution among men and women (Table 4).

Table 3. Incidence of muscle- tendon injury according to glycemic control

	Normal (HbA1c < 5.7%)	Elevated (5.7% ≤ HbA1c < 6.5%)	High (HbA1c ≥ 6.5%)	**Total**
Psoas tendinitis	0	1	0	**1**
Iliotibial band injury	0	1	0	**1**
Achilles tendinitis	1	2	0	**3**
Peroneal tendinitis	0	0	1	**1**
Enthesopathy lower limb	1	0	1	**2**
Calcaneal spur	1	1	0	**2**
Other enthesopathy foot	0	2	0	**2**
Injury Patellar tendon	0	1	0	**1**
Injury achilles tendon	1	7	2	**10**
Rupture achilles tendon	0	1	0	**1**
Injury muscle/tendon low leg	1	1	0	**2**
Injury muscle/tendon ankle/foot	0	1	0	**1**
Tendon surgery hip/thigh	0	0	1	**1**
Tenodesis tendon hip/thigh	0	0	1	**1**
Muscle/tendon surgery hip/thigh	0	1	0	**1**
Total	**5**	**19**	**6**	**30**

Table 4 shows the association between outcome and the selected covariates. No significant association was found between any of the covariates and lower extremity muscle- tendon injury. The three covariates that showed the strongest association was age, 35-44 years (OR=2.64, 95% CI=0.88-7.88), severe obesity (OR=3.49, 95% CI=0.97-12.60) and use of drugs for increased cholesterol (OR=2.67, 95%CI=0.92-7.66) (highlighted variables, Table 4). However, not statistical significant with p-values ranging from 0.0565 - 0,0825 and large

95% confidence intervals as a result of the low number of outcomes detected within each strata of the covariate.

Table 4. Covariates association with outcome

	Outcome n(%)	OR(95%CI)	P-value
Running habits			0,3162
No running	27 (90.1)	(ref)	
<½ hour/week	0 (0.0)	0.00 (-)	0,9997
½-1 hour/week	1 (3.3)	0.68 (0.09 - 5.06)	0,7119
1-2 hours/week	1 (3.3)	0.60 (0.08 - 4.45)	0,6213
>2 hours/week	1 (3.3)	1.04 (0.14 - 7.66)	0,9714
Sex, *n (%)*			0,2448
Men	14 (46.7)	0.65 (0.32 - 1.34)	0,2453
Women	16 (53.3)	(ref)	
Age, n (%)			0,4074
20-24 years	1 (3.3)	1.49 (0.18 - 12.50)	0,7109
25-34 years	1 (3.3)	0.50 (0.06 - 4.13)	0,5165
35-44 years	**7 (23.3)**	**2.64 (0.88 - 7.88)**	**0,0825**
45-54 years	5 (16.7)	1.13 (0.34 - 3.71)	0,8422
55-64 years	5 (16.7)	(ref)	
65-74 years	6 (20.0)	0.86 (0.26 - 2.83)	0,8064
75-84 years	5 (16.7)	1.13 (0.35 - 3.72)	0,8368
≥85 years	0 (0.0)	0.00 (-)	<.0001
BMI			0,2524
Underweight (<18.5 kg/m2)	0 (0.0)	0.00 (-)	0,9997
Normal weight (18.5-24.9 kg/m2)	11 (36.7)	(ref)	
Overweight (25.0-29.9 kg/m2)	10 (33.3)	1.09 (0.46 - 2.57)	0,8436
Obese (30.0-34.9 kg/m2)	6 (20.0)	2.16 (0.79 - 5.85)	0,1315
Severely obese (≥35.0 kg/m2)	**3 (10.0)**	**3.49 (0.97 - 12.60)**	**0,0565**
Smoking habits			0,3559
Never smoked	12 (40.0)	(ref)	
Former smoker	12 (40.0)	0.99 (0.44 - 2.21)	0,9837
Current smoker	6 (20.0)	0.5 (0.19 - 1.33)	0,1656
Former smoker using nicotine substitutes	0 (0.0)	0.00 (-)	<.0001
Dyslipidemia, *n (%)*	13 (43.3)	0.82 (0.4 - 1.69)	0,5912
Daily/almost daily use of painkillers, *n (%)*	4 (13.3)	1.08 (0.37 - 3.09)	0,8937
Daily/almost daily use of drugs for increased cholesterol, *n (%)*	**4 (13.3)**	**2.67 (0.92 - 7.66)**	**0,0703**
Daily/almost daily use of medication to treat asthma/bronchitis, *n (%)*	2 (6.7)	0.85 (0.20 - 3.58)	0,8237

Multivariable analysis

The Tables 5 (a,b and c) express the association between hyperglycemia and muscle- tendon injury presented as odds ratios (ORs) with 95% confidence intervals. The normal or unexposed (HbA1c <5.7%) category was used as a reference group. A statistically significant

association was found between elevated HbA1c and muscle- tendon injury (crude OR=2.85, 95% CI =1.06–7.64) (Table 5a). When adjusting for sex, age and running habits, the OR among the elevated group increased slightly (adjusted OR=3.04, 95% CI=1.13–8.19), and among the highly exposed group the OR turned significant (adjusted OR=3.41, 95% CI=1.01 - 11.55) (Table 5b). In the final model, adjusting for all covariates, the OR among the elevated group changed marginally, whereas the association between the highly exposed group and the outcome were attenuated to a non-significant level (Table 5c). Thus, adjusting for all covariates only induced small changes to the association between the main exposure (HbA1c) and outcome.

Table 5a. Crude model - unadjusted.

	Injury after 3 years n (%)	**Unadjusted OR (95%CI)**	p-value
HbA1c			0,0591
Normal (HbA1c < 5.7%)	5 (0.2)	**(ref)**	
Elevated (5.7% ≤ HbA1c < 6.5%)	19 (0.7)	**2.85 (1.06 - 7.64)**	0,0377
High (HbA1c ≥ 6.5%)	6 (0.7)	**2.89 (0.88 - 9.50)**	0,0801

Table 5b. Multivariable analysis - Adjusted[1].

	Injury after 3 years n (%)	**Adjusted OR (95%CI)**	p-value
HbA1c			0,0369
Normal (HbA1c < 5.7%)	5 (0.2)	**(ref)**	
Elevated (5.7% ≤ HbA1c < 6.5%)	19 (0.7)	**3.04 (1.13 - 8.19)**	0,0279
High (HbA1c ≥ 6.5%)	6 (0.7)	**3.41 (1.01 - 11.55)**	0,0484

[1]Adjusted for sex, age and running habits.

Table 5c. Multivariable analysis - Adjusted[2].

	Injury after 3 years n (%)	**Adjusted OR (95%CI)**	p-value
HbA1c			0,0548
Normal (HbA1c < 5.7%)	5 (0.2)	**(ref)**	
Elevated (5.7% ≤ HbA1c < 6.5%)	19 (0.7)	**3.01 (1.11 - 8.17)**	0,0308
High (HbA1c ≥ 6.5%)	6 (0.7)	**2.88 (0.82 - 10.13)**	0,1002

[2]Adjusted for sex, age, running habits, BMI, smoking habits, dyslipidemia, use of painkillers, use of drugs for increased cholesterol, use of medication to treat asthma/bronchitis.

Discussion results

The results from this study express a detrimental effect of a single biomarker on the risk of lower extremity muscle- tendon injury. It appears that the present study, based on 5.856 individuals, is currently the largest report on the topic. To further elucidate the complex etiology and pathogenesis of tendinopathy, relevant risk factors must be evaluated one by one in a systematic manner. The information gained from ongoing studies will add to the knowledge base on tendon biology, and provide a better understanding of the intrinsic risk factors for tendon pathology.

Hyperglycemia and muscle- tendon injury

The main finding of this study was that elevated and high levels of HbA1c significantly increased the risk of lower extremity muscle tendon- injury after adjusting for sex, age and running habits. The crude model only demonstrated a statistically significant association for the elevated category. After adjusting for age, sex and running habits the elevated group had more than three times the odds of muscle tendon- injury compared with the reference (unexposed) group with normal levels of HbA1c. The relative risk was about the same among the highly exposed group. These findings are in line with emerging evidence on this field of research (Ranger et al., 2016; Gautieri et al., 2016; Hansen et al., 2013; Burner et al., 2012; Otoshi et al., 2015). Moreover, the 3 times greater risk among individuals with HbA1c ≥ 6.5% (adjusted for sex, age and running habits) is similar to the increase in risk of lateral epicondylitis (upper extremity), for individuals with HbA1c ≥ 6.5%, reported in a general population by Otoshi et al. (2015). As mentioned, a systemic process with an increased accumulation of advanced glycation end products (AGEs) in the connective tissue, is currently thought to be the main driver of tendon pathology in relation to hyperglycemia (Abate et al., 2013; Couppé et al., 2016; Monnier et al., 2005). A systemic process capable of affecting both upper and lower extremity tendons, is well in line with the findings from the current study and the study by Otoshi et al. (2015).

When interpreting these results it is important to consider the confounding potential from diabetes mellitus that has a well known positive association with tendon injury (Ranger et al., 2016; Zakaria et al., 2014). A HbA1c level >6.5% is one of four diagnostic criterias for DM and it was not included as a covariate because it would diminish the possibility of

exploring an effect of hyperglycemia on tendon tissue in HbA1c values >6.5%. Another argument for not including DM as a covariate is that the most plausible explanation for the increased risk among people with DM is the same as the rationale for this study, the biological pathway whereby hyperglycemia seems to have a negative effect on tendon tissue (Ranger et al., 2016). When adjusted for all possible confounders only the elevated category (5.7% ≤ HbA1c < 6.5%) showed a statistically significant increase in risk. The greater risk for tendon pathology found among individuals in the elevated category, is interesting because it might express a more "direct" effect from hyperglycemia with less interference from diabetes. Type 2 diabetes mellitus is associated with obesity and dyslipidemia (Cheung et al., 2009; Schofield et al., 2016) and the results from the highly exposed group, with an increased prevalence of DM, carries a greater risk of being confounded by factors associated with DM.

Prevalence and distribution of lower extremity muscle- tendon injury

The second finding from this study was the distribution of injuries with 83% occurring at or below the knee and 47% of the total injuries being to the achilles tendon. These findings are well in line with previous research on the distribution of lower extremity muscle- tendon injury (Francis et al., 2019; Wu et al., 2017). This study had a slight female dominance in the outcome distribution, men (14 injuries) and women (16 injuries). This finding is in disagreement with the majority of the literature on the topic reporting male gender as an important prognostic variable for muscle- tendon injury (Gaida et al., 2009; Riley, 2004; Van der Worp et al., 2015; Taunton et al., 2002). The female dominance found in the current study is not considered to be a significant finding due to the small number of outcomes detected. The overall incidence of lower extremity muscle- tendon injury for the 3 year follow up period was 0.51% or 1.7 per 1.000 person-years (incidence rate). The incidence rate found in this study is markedly lower compared to an incidence rate of 10.5 per 1000 person-years for lower extremity tendinopathy in a Dutch general population (Albers et al., 2016). This difference is probably related to the outcome register. The study by Albers et al. (2016) was based on information from general practice (primary care) and the present study is based on hospital contacts (secondary care).

A factor that could have led to an overestimation in the incidence of lower extremity muscle- tendon injury, was the inclusion of the diagnosis calcaneal spur. Two individuals were diagnosed with this, one in the unexposed group and one in the elevated group. The

diagnosis was included because it was hypothesized that people with plantar fasciitis (PF) would not be referred and registered in the DNPR (hospitals) before PF progressing to a calcaneal spur. This seems to be a valid hypothesis with zero incidents of plantar fasciitis detected even though this is a fairly common condition (Rathleff et al., 2015). In addition studies have reported an increased risk of calcaneal spurs in people with diabetes mellitus (Moroney et al., 2013), this is very likely to be linked to the same mechanisms as explored in this study. Calcaneal spur is etiologically linked to plantar fasciitis (Kirkpatrick, Yassaie, Mirjalili, 2017), and 89% of the time the two are found to co-exist (Johal & Milner, 2012). The pathology in plantar fasciitis is tendon related because the plantar fascia and the achilles tendon unite at the calcaneal origin (Shaw et al., 2008; Rathleff et al., 2015).

Descriptive comparison between runners and non-runners

This study is based on a general Danish population including both runners and non-runners. Runners had a lower injury incidence when compared to non-runners: 3 injuries among 1.107 runners (0.2%) compared to 27 injuries among 4.749 non-runners (0.5%). Running is associated with a higher risk of overuse injury than other forms of aerobic exercise (Francis et al., 2019) and the low injury incidence among runners was somewhat unexpected. However, it is complex to predict an expected injury incidence between runners and non-runners because both under- and over stimulation in terms of mechanical loading has been established as important risk factors (McCarthy & Hannafin., 2014; Heinemeier & Kjaer 2011). The lower injury incidence among runners corresponds with the descriptive comparison showing that runners had a lower proportion of individuals exposed to hyperglycemia (57%) when compared to non-runners (65.3%). In addition, the runners had more favourable values in all the potential confounders included in this study. This is in line with previous research reporting that running is associated with more favorable glycemic control (HbA1c), lipid parameters and BMI (Najafipour et al., 2017). However, it is not possible to determine causation when interpreting this because it might just reflect that running is an unusual habit among individuals with high levels of HbA1c.

It is possible that the positive systemic changes related to running also alters AGE accumulation or affect other mechanisms in the biological pathway between hyperglycemia and tendon injury. A separate analysis on the group of runners was not possible in the current

study due to the low outcome incidence among runners (3 injuries). However, this could potentially have provided additional information on this unique population and should be further addressed in future research.

Discussion method

Methodological strengths of the current study include a large and random population sample, a prospective cohort design, standardized HbA1c measurements, information about potential confounding variables, and the DNPR containing information on all participants.
However, there are some possible limitations to consider. In this chapter the validity and methodological issues related to *design*, *confounding variables*, *participants*, *exposure*, *outcome* and *statistical analysis* will be discussed. The terms bias and confounding are central, bias may be defined as any systematic error that results in an incorrect result and confounding is when the groups being compared differ with respect to another factor associated with the outcome (Evans, 1998). Both bias and confounding can be alternative explanations for the effects reported in this study.

Design

As mentioned, one of the strengths with a prospective cohort study is the higher likelihood of estimating causal relationships compared to a cross-sectional study design. A prospective cohort study measures exposure before the outcome occurs, providing a temporal framework with the potential to estimate causality (Song & Chung, 2010). In the current study individuals with muscle- tendon injury one year prior to enrollment were excluded due to the risk of continuous pathology at baseline. Ideally, all participants should have been examined at baseline to rule out pathology and we have to accept the fact that some individuals might present with various degrees of muscle- tendon pathology at baseline. Consequently, baseline HbA1c measurements might be affected by a potential reverse causality where outcome precedes exposure. The pathologic process in tendon tissue can develop over years and participants might tolerate this pathology for a long period before seeking medical care. This "tolerated pathology" with increasing pain could lead to a decrease in physical activity that is known to be related to HbA1c values (Najafipour et al., 2017). A related limitation to this design is the assumption that the results from the baseline examination represent a valid picture of present and to some extent future properties. Although HbA1c is relatively insensitive to short-term lifestyle change and is a test for chronic glycemia (Nathan et al., 2007), it is possible that different factors prior to the baseline examination have resulted in a HbA1c level that is not representative for the participants "habitual" value. Furthermore, participating in the CCHS, a study on cardiovascular diseases, and having the results

evaluated at the end of the examination, might promote a lifestyle change that could change levels of HbA1c.

A longer follow up period would probably have increased the number of outcomes but would at the same time increase the possibility of baseline HbA1c measurement no longer being representative. Thus decreasing the possibility of the outcome being related to the exposure of interest. As this study is based on a relatively new and sparsely described risk factor, exact evidence on the time from exposure to pathology is lacking. This study uses 3 years follow-up. The reasoning behind this 3 year follow-up consists of two factors: (i) The amount of time needed for the progression of tendon pathology, and (ii) a time frame where it is still possible to relate baseline HbA1c values to the outcome.

Confounding variables

Confounding variables is another issue to consider. In a prospective cohort study there is no guarantee that you have included all relevant covariates or confounding variables in your analysis. Tendon injury has a complex and not fully elucidated etiology and the association between muscle- tendon injury and HbA1c, found in this study, might be explained by an unknown confounding variable. The prefered design for limiting the role of confounding and exploring causal relationships, is the randomized controlled trial (RCT). The RCT design distributes both known and unknown confounders equally between two groups by its randomized allocation. In comparison to the RCT this study depends on measurement of relevant confounders and a statistical analysis whereby these are adjusted for. Statistical techniques may not always adjust for confounding adequately and a poorly measured variable might lead to an inadequate adjustment and residual confounding (Leon, 1993; Kamangar, 2012). The covariates selected for this study is based on certain key factors commonly associated with muscle- tendon injury. It was not possible to include a number of potentially associated factors such as: Genetics, biomechanics, nutrition, antibiotics, systemic disease, occupation and sport (Gaida et al., 2009; Riley, 2004). Not including factors with a known association with the outcome under study might result in residual confounding (Groenwold et al., 2011).

Limitations of the covariates included in this study

The majority of the covariates in this study was based on self reported baseline data obtained

from a questionnaire. Self reported data might be influenced by a *social desirability bias*, which is a type of *response bias* where participants answer questions in a manner that will be viewed favorably by others. Participating in a study on heart disease might elevate the risk of this bias. This can potentially have led to an over-reporting of the self-reported data on running and an under-reporting of the data on medicine and tobacco (Monyeki, Moss, Kemper, Twisk, 2018). In general, misunderstanding or poor interpretation of questions is another issue with self-reported data. However, this is not particularly relevant for the current study due to simple yes/no questions and the questionnaires being reviewed by a staff member upon completion. As mentioned, a common issue related to confounding variables is inadequate measurement and there are some limitations regarding the validity of the following variables:

Data on medicine: Participants was supposed to answer yes/no to all questions regarding medication, however the data had missing information and blank spaces was interpreted as a no. These blank spaces might reflect difficulties remembering different types of medication. Detailed information on type and dose of medication would have strengthened this study. The original plan was to include data from the Danish Medicines Agency, this could have provided detailed information on type, dose and changes in medicine throughout the study period. Unfortunately it was not possible to access this data in time for the current study.

Corticosteroids: This study defined the use of corticosteroids from a yes/no to a daily or almost daily use of medication to treat asthma/bronchitis (including spray or powder). But not all asthmatics use corticosteroids, some use inhaled $Beta_2$ agonists to treat acute symptoms. However, both inhaled and oral GC is considered the most effective and first- line therapy for the majority of people with asthma (Rai et al., 2011). These drugs are known to have a negative effect on tendon tissue regardless of administration modalities (Kirchgesner et al., 2014). But the risk of tendon pathology is increased with oral steroids and especially with locally injected GC (Dean et al., 2014). A confounding potential from corticosteroids can not be ruled out in this study with missing information on locally injected GC and oral corticosteroids being a common medicine for a number of different diseases besides asthma and bronchitis.

NSAID: The use of NSAIDs in relation to tendon pathology is always a point of controversy, because they might reflect a reverse causation where patients start taking NSAIDs because of the pain related to tendon pathology. The frequent use of these drugs with musculoskeletal conditions and a possible reverse causation is a great challenge for the current literature on the topic (Nyyssönen et al., 2018). The validity of the data on NSAIDs is affected by the question also including analgesic drugs, such as salicylic acid derivatives and paracetamol, that has no known detrimental effect on tendon tissue. However, both NSAIDs and analgesics may contribute to mask pain during activity and thereby result in a progression of pathology.

BMI: Bodyweight is included because of evidence linking it to tendon injury. This is thought to be a systemic mechanism where excessive fat promotes a release of cytokines that might influence tendon metabolism or response to microtrauma (Gaida et al., 2009). An issue with using BMI as a measure of excessive fat is that muscle mass modifies the association of BMI with adiposity (Abramowitz et al., 2018). Thus the overweight, obese and severely obese BMI categories might include individuals with great muscle mass who are not exposed to the metabolic changes related to adiposity.

Running: Self reported questionnaires on vigorous physical activity have shown acceptable reliability and validity for adults (Kurtze, Rangul, Hustvedt, Dana-Flanders, 2007). Unfortunately it is a challenge to get the same valid data for self reported measures of moderate and low levels of physical activity (Rangul et al., 2008). In terms of validity an objective measure of running would have been ideal (Monyeki et al., 2018), but objective measures are difficult to perform in large population studies like the CCHS. A study by Schnohr et al. (2013), based on the CCHS, showed that 64% who identified as runners in the third survey (1991–1994), were still runners in the fourth survey (2001–2003). Thus, running seems to be a fairly stable habit among participants of the CCHS. In terms of content validity you can argue that the data on running does not cover all aspects of mechanical loading. Ideally this should include a range of factors such as, work activity, sport and leisure activity, biomechanics, pace and frequency of running. The questionnaire included data on both pace and frequency and the original plan was to define the level of running from:

- Light runners - slow or average pace < 2.5 hours of jogging per week with a frequency of ≤ 3 times per week.

● Moderate runners - slow or average pace, and ≥ 2.5 hours of running per week with a frequency of >3 times per week. Moderate runners could also have a fast pace, ≤ 4 hours of running per week with a frequency of ≤ 3 times per week or fast pace, < 2.5 hours of running per week with a frequency of >3 times per week.
● Strenuous runners - fast pace and either > 4 hours of running per week or ≥ 2.5 hours of running per week with a frequency of > 3 times per week.
However, it is theoretically complex to decide whether pace, frequency or total hours has the biggest impact on tendon tissue and the data had inconsistencies on frequency and pace. Therefore, it was decided to reduce this to hours of running.

Participants

In large cohort studies response rate and missing data due to withdrawal, loss to follow-up, or death are factors to consider. The response rate for the fourth examination of the CCHS, was 49.5% and this relatively low response rate can affect the external validity of the study. The CCHS is dependent upon voluntary subject participation and this makes it particularly vulnerable to *sampling bias*. The non-response bias implies systematic differences between those who participate and those who chose not to participate. We were not able to assess non-responders in this study and we have to accept the fact that certain characteristics might be more or less prevalent with the 6.238 who participated compared to the 6.362 who did not participate. A study by Cheung et al. (2017) tried to evaluate the impact of *non-response bias* in public health studies. They compared differences between a mandatory sample and a voluntary sample and found that individuals in the voluntary sample had higher education levels, used less alcohol and tobacco, had better subjective health status and had a higher proportion of females.

A factor that could compromise the validity of the results and lead to an underestimation of injury incidence was that 229 individuals (3.9%) died and did not complete the follow-up period (3 years from baseline). The DNPR registered all relevant outcomes until the date of death, thus missing data is only from the date of death until the end of the follow-up period. A general rule of thumb requires that the loss to follow-up rate is below 20% of the sample (Song & Chung, 2010), hence the partially missing data on 3.9% of the total sample is not considered a major threat to the results presented. Considering the low incidence rate of the current study, it is safe to assume that only few, if any, additional

outcomes would have been detected within this group of individuals. Besides a potential *response-bias* the external validity to a Danish population should be fairly good due to the large and random sample of individuals and an almost complete follow-up through the DNPR.

Exclusion criterias

This study excluded patients with previous injury 1 year prior to enrollment because previous injury is a strong predictor for future pathology (Obaid & Connell, 2010; Årøen et al., 2004). The theoretical background for this exclusion criteria are challenging because the exact time frame for the increased risk of subsequent injury is uncertain. However, it is described that maturation does improve the quality of tendon scar tissue and that this occur within 1-2 years following injury (Obaid & Connell, 2010). This maturation timeline is supported by Williams, McCullagh and Silver (1984) who found that Type III collagen is less pronounced fourteen months after injury in animal tendon. The reasoning behind this 1 year exclusion criteria is to reduce the risk of an outcome being related to previous injury and at the same time not excluding relevant outcomes. In the present study you have to be aware that this exclusion criteria carries a risk of both.

Further exclusion criterias have been considered with different systemic diseases being linked to tendon pathology: Rheumatological diseases, endocrine or metabolic diseases and inherited disorders e.g. Ehlers-Danlos syndrome (EDS) (Riley, 2004). In addition, short term immobilization (days) in relation to illness and/or hospitalization have been found to markedly reduce muscle function, decrease tendon collagen content and reduce the biomechanical properties of the load-bearing connective tissues (Magnusson & Kjaer, 2018; Couppé et al., 2012). Hospital admissions for trauma and a long list of diseases that might affect tendon tissue could have been relevant as exclusion criterias. However, data from the DNPR was limited to outcome measures for muscle- tendon injury and it was not possible to obtain information on all ICD-10 codes. This is not considered a major threat to the present study with many of these diseases having a low prevalence in a Danish population, e.g. EDS with a national prevalence of 0.02% (Søborg et al., 2017) and you can also question if assessing the complete health records on all participants would have been within the ethical boundaries.

Exposure

Limitations of HbA1c

Despite the measurements of HbA1c being based on a valid assay and the testing method standardized against the approved reference method, measurement of HbA1c still has important limitations. Studies have found that HbA1c levels differ across ethnic and racial groups (Dagogo-Jack, 2010: Herman et al., 2007). The present study is using a homogenous Caucasian population, so ethnic and racial differences are not considered an issue for the present study. Furthermore, HbA1c can be affected by conditions that alter the lifespan of erythrocytes e.g. anemia or genetic variants including haemoglobinopathies and thalassaemia syndromes (Abass et al., 2017). Again, this is not considered a great issue in the present study, with a low prevalence of these conditions in Denmark compared to eastern Mediterranean and Asian regions (Nybo, Friis-Hansen, Felding, 2007).

Categorisation of continuous variables

HbA1c is divided into 3 levels based on guidelines from the World Health Organization: Normal (HbA1c < 5.7%), Elevated (5.7% ≤ HbA1c < 6.5%) and DM (HbA1c ≥ 6.5%). These categories are based on a level of HbA1c required to consider an individual as exposed to hyperglycemia, i.e. a value of <5.7% is considered normal and not exposed. Using the same categorical values, as for the diagnosis of DM, is for the convenience of interpreting the results in a clinical setting. These categories are often considered when planning lifestyle interventions for patients and the idea is that they might also be able to label an individual as having an increased risk of muscle- tendon injury. However, this categorisation is gained at some cost. Altmann & Royston (2006) has criticised the loss of information related to the categorisation of continuous variables. This is a valid critique for the approach used in this study, e.g. individuals with a value of 5.6% will be categorised as normal and individuals with 5.7% as exposed, where no categorical difference exists between individuals with a value of 5.7% and 6.5%.

Outcome

Basing the outcome on data from the DNPR only includes patients who has been admitted for surgery or been diagnosed at Danish hospitals. The exact ratio of patients with muscle-tendon injury treated/diagnosed in either primary care or secondary care (hospitals) is not

described in the literature (Lynge et al., 2011). However, knowing that the majority of treatment and diagnoses for individuals with musculoskeletal conditions are being provided by general practitioners, physiotherapists, chiropractors, and manual therapists (Stochkendahl et al., 2019; Jørgensen et al., 2001), it is safe to assume that a number of relevant outcomes are not included in this study. This is supported by the low incidence rate detected in the present study compared to studies using information from registers based on general practice (Albers et al., 2016). Basing the outcome on the DNPR can be viewed as a strength as well as a weakness. Patients referred from the GP to specialist health care/hospitals often have a poorly defined diagnose (Jørgensen et al., 2001) based on patient history and a brief physical examination (Scott et al., 2013). Diagnoses in the secondary care often includes MRI and/or ultrasound to confirm the pathology which increases the validity of the outcome measures included in this study (Titan & Andarawis-Puri, 2016). Basing the outcome on the DNPR is also thought to contribute to a greater proportion of intramuscular tendon involvement for the muscle strain injuries. Muscle injuries with intramuscular tendon disruption have a far longer recovery time (Comin et al., 2013), and a lack of progress increases the risk of patients being sent back and forth within the healthcare system (Wadmann, Strandberg-Larsen, Vrangbæk, 2009). The weakness is that potentially valid outcomes are not presented in specialist health care and thereby not in the register (DNPR). This may in turn contribute to a selection of outcomes with certain characteristics in the register. It can be speculated that the outcomes detected in the DNPR are patients who have not responded well to conservative treatment from their primary practitioner, which have lead to a referral. This might lead to a higher ratio of outcomes with severe tendon pathology, and a possible detrimental effect of hyperglycemia in the earlier stages of tendon pathology might not be detected in this study.

Statistical considerations

The multivariable analysis is based on a logistic regression model which is capable of analyzing the relationship between multiple independent variables and a dichotomous outcome (injury / no injury). Logistic regression corresponds well to data from prospective studies (Hsieh, Bloch, Larsen, 1998), and some of the advantages include; few assumptions are made, for example about the distribution of the outcome and that interpretable results are provided, because the regression coefficients represent odds ratios. It is recommended to

present both crude and adjusted estimates because it helps elucidate the extent to which the effect estimate is influenced by the hypothesized confounders (Schooling & Jones, 2018). The low number of outcomes detected in this study is a challenge to the regression model both for the main association, HbA1c and muscle tendon- injury (indicated by large 95% confidence intervals), and for the process of selecting which confounding variables to adjust for in the multivariable model. It is recommended to adjust for factors associated with the outcome, based on statistical tests or available knowledge, because a risk factor for the outcome that is evenly distributed among exposure groups can still be a confounding variable (Groenwold, Klungel, Grobbee, Hoes, 2011). Different strategies for selecting confounding variables have been proposed. A frequently applied strategy is a stepwise inclusion, testing the effect of a single variable on the main association, selection is usually based on a change-in-estimate criterion (e.g. 10% change in OR). However, adjusting for the three variables (age, statin and BMI), that showed the strongest relationship with the outcome only induced small changes in OR estimates. The same was found for the saturated model (adjusted for all confounders), compared to the adjusted (sex, age and running habits) and the unadjusted model. Thus, the present study used an *a priori* selection method of confounders with a known relation to the outcome. When available prior knowledge from the scientific literature is formally seen as the most important rationale for including or excluding covariates from a statistical analysis (Walter & Tiemeier, 2009). Covariate selection through subject-matter knowledge may play a particularly important role in studies with few outcomes, because statistical tests to assess whether a certain variable is associated with either exposure, outcome, or both, are typically insensitive in datasets with few outcomes (Patorno et al., 2014; Groenwold et al., 2011). This is also exemplified in the regression model with the covariates as exposure, the low number of outcomes within each strata of the covariate, provided non significant results with large 95% confidence intervals (Table 4). A limit of 10 events per included variable is suggested to increase the validity of the model (Peduzzi et al., 1996). This was applied in the adjusted model (Table 5b) including the variables sex, age and running habits that has been identified as strong prognostic variables for muscle- tendon injury.

Clinical perspectives

The findings of the current study might contribute to a better understanding of the pathogenesis of tendinopathy and thus provide the basis for prevention of tendon injuries. Furthermore, HbA1c as a risk factor for both musculoskeletal injury and lifestyle diseases, create a vicious cycle were people who needs exercise the most are the ones who are most prone to injury. The results from the current study provides additional information to the same categories of HbA1c commonly used as treatment goals or for the diagnosis of DM in clinical practice. This suggests that an increased risk of tendon pathology should be considered when planning exercise interventions for patients with hyperglycemia. Tailoring exercise interventions with respect to this might contribute to a greater adherence which is currently one of the biggest challenges to these interventions. Future intervention studies, comparing different tailored exercise interventions to the risk of tendon pathology in patients with hyperglycemia, might further improve the clinical value of this association.

Conclusion

This is the first time it has been demonstrated that hyperglycemia (elevated levels of HbA1c) is associated with an increased risk of lower extremity muscle- tendon injury in a large−scale population study. This study also indicates how runners appear to differ from non-runners regarding many aspects related to muscle- tendon pathology. Due to the methodological limitations and low incidence of outcomes further investigations are needed to validate this association and explore it in different populations.

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Appendix

Appendix 1

Figure 1 license

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Appendix 2

ICD 10-codes

S76 - Injury of muscle and tendon at hip and thigh level.

S76.0 Injury of muscle and tendon of hip, **S76.1** Injury of quadriceps muscle and tendon Patellar ligament (tendon), **S76.2** Injury of adductor muscle and tendon of thigh, **S76.3** Injury of muscle and tendon of the posterior muscle group at thigh level, **S76.4** Injury of other and unspecified muscles and tendons at thigh level and **S76.7** Injury of multiple muscles and tendons at hip and thigh level.

S86 - Injury of muscle and tendon at lower leg level.

S86.0 Injury of Achilles tendon, **S86.1** Injury of other muscle(s) and tendon(s) of posterior muscle group at lower leg level, **S86.2** Injury of muscle(s) and tendon(s) of anterior muscle group at lower leg level, **S86.3** Injury of muscle(s) and tendon(s) of peroneal muscle group at lower leg level, **S86.7** Injury of multiple muscles and tendons at lower leg level, **S86.8** Injury of other muscles and tendons at lower leg level and **S86.9** Injury of unspecified muscle and tendon at lower leg level.

S96 - Injury of muscle and tendon at ankle and foot level.

S96.0 Injury of muscle and tendon of long flexor muscle of toe at ankle and foot level, **S96.1** Injury of muscle and tendon of long extensor muscle of toe at ankle and foot level, **S96.2** Injury of intrinsic muscle and tendon at ankle and foot level, **S96.7** Injury of multiple muscles and tendons at ankle and foot level, **S96.8** Injury of other muscles and tendons at ankle and foot level and **S96.9** Injury of unspecified muscle and tendon at ankle and foot level.

M66 - Spontaneous rupture of lower extremity tendon.

M66.2 Spontaneous rupture of lower extremity extensor tendons, **M66.3** Spontaneous rupture of lower extremity flexor tendons, **M66.4** Spontaneous rupture of other lower extremity tendons and **M66.5** Spontaneous rupture of unspecified lower extremity tendon.

M76 - Enthesopathies of lower limb, excluding foot.

M76.0 Gluteal tendinitis, **M76.1** Psoas tendinitis, **M76.3** Iliotibial band syndrome, **M76.5** Patellar tendinitis, **M76.6** Achilles tendinitis, **M76.7** Peroneal tendinitis, **M76.8** Other enthesopathies of lower limb, excluding foot **M76.9** Enthesopathy of lower limb, unspecified.

M77 - Other lower extremity enthesopathies.

M77.3 Calcaneal spur, **M77.5** Other enthesopathy of foot and **M77.8** Other enthesopathies of foot, not elsewhere classified.

Surgery codes:

KH - Lower extremity muscle- tendon surgery.

KH39 Tendon surgery hip/thigh**, KH49** Reinsertion tendon ankle/foot**, KH69** Tenodesis tendon hip/thigh and **KH99** Muscle/tendon surgery hip/thigh**.**

Appendix 3

Medicine questionnaire

Øbus nr.: qbus4nr

(skal ikke udfyldes)

Navn: _____

	Tager De *daglig* eller *næsten* daglig		Ja	Nej	Hvis ja: Angiv præparatnavn, styrke og antal pr. dag	Antal år i behandling
1	Blodtrykspiller	m_01_01	☐	☐		t2 m_01_02
	" (flere)	m_01_03	☐	☐		t2 m_01_04
	" (flere)	m_01_05	☐	☐		t2 m_01_06
2	Hjertepiller	m_02_01	☐	☐		02_02
	" (flere)	m_02_03	☐	☐		04
	" (flere)	05	☐	☐		06
3	Vanddrivende piller	m_03_01	☐	☐		m_03_02
4	Medicin mod forhøjet kolesterol	04_01	☐	☐		04_02
5	Gigtpiller		☐	☐		OSV.
6	Sovepiller	OSV	☐	☐		
7	Nerve- eller beroligende piller		☐	☐		
8	Medicin mod mavesyre		☐	☐		
9	Medicin mod astma/bronkitis (inkl. Spray/pulver)		☐	☐		
10	Insulin		☐	☐		
11	Anden sukkersyge medicin		☐	☐		
12	P-piller		☐	☐		
13	Anden hormonbehandling		☐	☐		
14	Piller/dråber mod øjensygdom		☐	☐		
15	Smertestillende piller/medicin		☐	☐		
16	Slankepiller		☐	☐		
17	Andre piller		☐	☐		
18	Vitaminpiller		☐	☐		
19	Naturmedicin		☐	☐		
	" (anden)		☐	☐		
	" (anden)		☐	☐		

Praktiserende læges Navn	
Adresse	
Postnummer og by	

Appendix 4

Running habits questionnaire

64. Anfør **antal timer** De går, cykler og løber i gennemsnit, samt hvad Deres tempo er

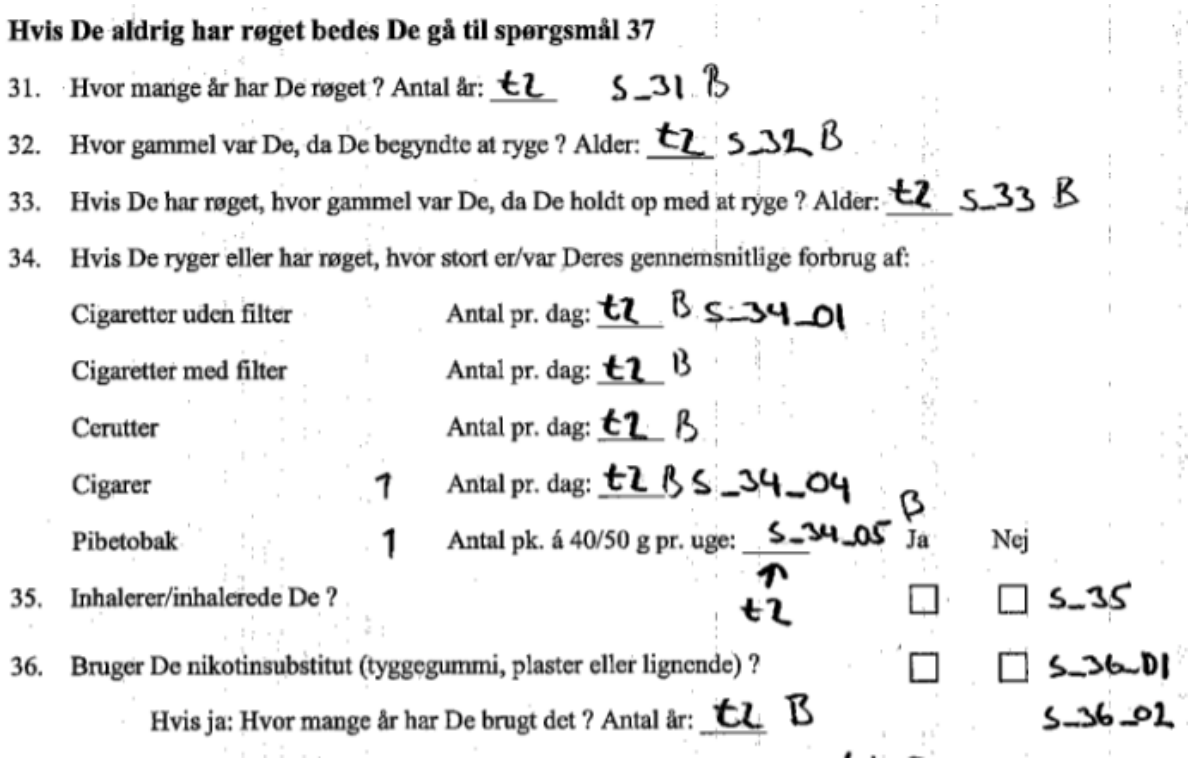
	GANG **pr. dag**		CYKLING **pr. dag**		LØB **pr. uge**	
	S_64_01 Sommer	S_64_02 Vinter	S_64_04 Sommer	05 Vinter	07 Sommer	08 Vinter
Aldrig	☐ 1	☐	☐	☐	☐	☐
< ½ time	☐ 2	☐	☐	☐	☐	☐
½ - 1 time	☐ 3	☐	☐	☐	☐	☐
1 - 2 timer	☐ 4	☐	☐	☐	☐	☐
3 - 4 timer	☐ 5	☐	☐	☐	☐	☐
> 4 timer	☐ 6	☐	☐	☐	☐	☐

	S_64_03 Mit gangtempo er	06 Mit cykeltempo er	09 Mit løbetempo er
Langsomt	☐ 1	☐	☐
Almindeligt	☐ 2	☐	☐
Hurtigt	☐ 3	☐	☐
Meget hurtigt	☐ 4	☐	☐

Hvis De løber: Hvor mange gange løber De i gennemsnit pr. uge ? Antal: ___ 11 S_64_10

Appendix 5

Use of tobacco questionnaire



Hvis De aldrig har røget bedes De gå til spørgsmål 37

31. Hvor mange år har De røget ? Antal år: t2 S_31 B

32. Hvor gammel var De, da De begyndte at ryge ? Alder: t2 S_32 B

33. Hvis De har røget, hvor gammel var De, da De holdt op med at ryge ? Alder: t2 S_33 B

34. Hvis De ryger eller har røget, hvor stort er/var Deres gennemsnitlige forbrug af:

Cigaretter uden filter		Antal pr. dag: t2 B	S_34_01
Cigaretter med filter		Antal pr. dag: t2 B	
Cerutter		Antal pr. dag: t2 B	
Cigarer	1	Antal pr. dag: t2 B	S_34_04
Pibetobak	1	Antal pk. á 40/50 g pr. uge: t2	S_34_05 B

35. Inhalerer/inhalerede De ? ☐ Ja ☐ Nej S_35

36. Bruger De nikotinsubstitut (tyggegummi, plaster eller lignende) ? ☐ Ja ☐ Nej S_36_01

Hvis ja: Hvor mange år har De brugt det ? Antal år: t2 B S_36_02

Appendix 6

Letter of consent

Erklæring om samtykke

I forbindelse med den 4. Østerbroundersøgelse, vil vi bede Dem om at afkrydse og underskrive en samtykkeerklæring:

ØBUS-nr.: ♀bus4nr _ _ _ _ _

Navn	
CPR.nr.	_ _ _ _ _ _ - _ _ _ _
Sæt kryds ☐ e_01	Jeg har modtaget skriftlig og mundtlig information om undersøgelsen
Sæt kryds ☐ e_02	Jeg giver tilladelse til, at Østerbroundersøgelsen må videregive resultater af undersøgelsen til min egen læge, hvis der ved undersøgelsen findes helbredsforhold, der kræver yderligere undersøgelse eller behandling.
Sæt kryds ☐ e_03	Jeg giver tilladelse til, at Østerbroundersøgelsen må indhente oplysninger om helbredsforhold fra min egen læge eller fra hospitalsindlæggelser
Sæt kryds ☐ e_04	Jeg giver tilladelse til, at Østerbroundersøgelsen må nedfryse min blodprøve og bruge den til senere undersøgelser
Sæt kryds ☐ e_05	Jeg giver tilladelse til, at Østerbroundersøgelsen må kontakte mig, hvis de skulle have yderligere spørgsmål.
Sæt kryds ☐ e_06	Jeg har modtaget en kopi af denne erklæring

Denne erklæring kan til hver en tid med øjeblikkelig virkning trækkes tilbage.

Dato: ....

Underskrift

Østerbroundersøgelsen's administration
Bispebjerg Hospital, Bispebjerg Bakke 23, 2400 København NV.
Tlf.: 3531 6095 E-mail: ma01@bbh.hosp.dk