Henri Isojärvi

ASSOCIATION OF GLUCOSE METABOLISM, PHYSICAL ACTIVITY AND FITNESS WITH PERIPHERAL NERVOUS SYSTEM FUNCTION IN OVERWEIGHT PEOPLE
HENRI ISOJÄRVI

ASSOCIATION OF GLUCOSE METABOLISM, PHYSICAL ACTIVITY AND FITNESS WITH PERIPHERAL NERVOUS SYSTEM FUNCTION IN OVERWEIGHT PEOPLE

Academic dissertation to be presented with the assent of the Doctoral Training Committee of Health and Biosciences of the University of Oulu for public defence in the Auditorium of Kastelli Research Centre (Aapistie 1), on 9 June 2018, at 12 noon

UNIVERSITY OF OULU, OULU 2018
Type 2 diabetes causes impairment of peripheral nervous system (PNS) function, which can result in symptoms such as pain and numbness. Disturbances in glucose metabolism inflict negative alterations on PNS function even before diabetes occurs.

Forty non-diabetic overweight (BMI 25–30) or obese (BMI > 30) working-age adults without polyneuropathy were enrolled in this study. PNS function was measured at baseline and after 3 years by peroneal motor nerve and radial, sural, and medial plantar sensory nerve conduction studies (NCS). At baseline, serum insulin and glucose levels (fasting, 30 min, and 120 min) were measured with a 2-hour oral glucose tolerance test (OGTT), and fasting serum cholesterol and triglyceride levels were measured. Maximal oxygen uptake (VO2max) was measured with an incremental bicycle ergometer test. Physical activity at the age of 15, 30, and current age was defined by a questionnaire. Current physical activity was also measured with a pedometer. At 3-year follow-up, serum insulin and glucose values were measured with a 2-hour OGTT, and serum fasting cholesterol and triglyceride values were measured.

At baseline, a serum insulin level at 120 min was positively and statistically significantly associated with peroneus nerve F-wave minimum and maximum latency time, sural nerve latency and nerve conduction velocity (NCV), and medial plantar NCV. VO2max was positively associated with amplitudes of the distal and proximal peroneus nerve and medial plantar nerve. Physical activity at the age of 30 was positively and significantly associated with peroneus NCV, F-wave maximum latency, medial plantar latency, and NCV. At the 3-year follow-up study, all sensory nerve amplitudes decreased significantly, and a 120-min insulin change was positively associated with changes in peroneus NCV, F-wave average latency, sural NCV, and medial plantar NCV.

Serum 120-min insulin values were positively associated with NCVs. Physical activity and fitness were positively associated with PNS function. The significant decrease in all sensory nerve amplitudes during follow-up demonstrates that negative alterations may already occur in overweight and obese adults without diabetes. Overweight and obese adults should be encouraged to have an active lifestyle, as even a small increase in physical activity might have a positive effect on PNS function.

Keywords: cardiorespiratory fitness, hyperinsulinemia, insulin resistance, overweight, peripheral nervous system
Tyypin 2 diabetes aiheuttaa ääreishermoston toiminnan heikentymistä, mikä voi oireilla raajoissa kipuna ja tunnottomuutena. Sokeritasapainon häiriintyminen vaikuttaa haitallisesti ääreishermoston toimintaan jo ennen varsinaisen diabeteksen puhkeamista.


Alkutilanteessa 120 minuutin insuliiniarvo oli positiivisesti ja tilastollisesti merkitsevästi yhteydessä peroneushermon F-aallon minimi- ja maksimilatenssiaikaan, suralishermon latenssi- ja johtumisnopeuteen sekä mediaalisen plantaaarihernon johtumisnopeuteen. VO2max oli positiivisesti yhteydessä peroneushermon distaalisen ja proksimaalisen vasteen voimakkuuteen (amplitudi) sekä mediaalisen plantaaarihernon vasteen voimakkuuteen. Fyysinen aktiivisuus 30 vuoden äässä oli positiivisesti ja merkitsevästi yhteydessä peroneushermon johtumisnopeuteen, F-aallon maksimilatenssiaikaan, mediaalisen plantaaarihernon latenssi-ja johtumisnopeuteen. Seurantatutkimuksessa kaikkien sensoristen hermojen vasteet pienennivät merkitsevästi ja seerumin 120 min insuliiniarvon muutos oli positiivisesti yhteydessä peroneushermon johtumisnopeudeen, F-aallon maksimilatenssiaikaan, mediaalisen plantaaarihernon latenssi- ja johtumisnopeuteen.

Seerumin 120 min insuliiniarvot olivat positiivisesti yhteydessä ääreishermonjohtumisnopeuteen. Fyysinen aktiivisuus ja kunto olivat positiivisesti yhteydessä ääreishermoston toimintaan. Negatiiviset muutokset sensoristen hermojen vasteissa seurantatutkimuksessa osoittivat, että negatiivisia muutoksia ääreishermoston toimintaan voi tapahtua ylipainoissa, jonka vuoksi jopa ilman diabetetta. Ylipainoisista tulee kannustaa liikkumaan, sillä vähäiselläkin liikunnan lisäyksellä voi olla positiivinen vaikutus myös ääreishermostoon.
To my family
Acknowledgements

This study was carried out in 2007–2017 at the Center for Life Course Health Research, the Research Unit of Medical Imaging, Physics and Technology, University of Oulu, and the Department of Sports and Exercise Medicine, Oulu Deaconess Institute. First, I wish to warmly thank Kaisu Kaikkonen, the director of the Department of Sports and Exercise Medicine, Oulu Deaconess Institute, for calling me in the winter of 2006/2007 and suggesting this topic for my master’s thesis, which has eventually led to this dissertation.

I am deeply grateful to my supervisors, Professor Raija Korpelainen, Professor Sirkka Keinänen-Kiukaanniemi, and Professor Timo Jämsä. I want to especially thank Professor Raija Korpelainen for the kind, competent, and most of all, motivating guidance during all these years.

My sincere thanks go to Professor Sirkka Keinänen-Kiukaanniemi for sharing her vast knowledge in glucose metabolism and for encouraging me with this work over these years.

I would like to sincerely thank Professor Timo Jämsä for his expert guidance and for the opportunity to work for the Department of Medical Technology during 2007 and 2008 as a young researcher.

Experimental data regarding nerve conduction studies were performed by MD Mika Kallio and late Professor Uolevi Tolonen, to whom I would like to express my deepest gratitude. Thank you for guiding me into the fascinating world of the peripheral nervous system.

I am very grateful to Docent Juha Korpelainen for his valuable contributions and commitment to my work.

I wish to thank MSS Hannu Kaikkonen for the experimental data concerning maximal oxygen uptake. Furthermore, I wish to thank the competent personnel of the Department of Sports and Exercise Medicine, Oulu Deaconess Institute, for their valuable contribution of the experimental data. Also, thanks to Risto Bloigu, MSc, for his expertise with statistical consultations, and Docsents Timo Takala and Juha Oksa, members of the thesis follow-up committee.

I would like to thank my employers, Lea Karvala and Katja Niemi, and my dear colleagues and friends at Kir-Fix Oy for supporting this process.

I wish to thank my beloved parents, Ahti and Eeva-Liisa, for teaching me much about life and for always supporting me, also in this project. I want to also thank my stepfather, Jari, and stepmother, Auli, for their kindness and support all these years. To Petri, Tuulikki, Jarno, and Aaro, it is my pleasure to be your big brother.
My warmest and loving thanks goes to my beautiful girls: my wife Riitta and my daughters, Aamu and Seela. Thank you for your love and support all these years.

Many thanks to my mother-in-law, Päivi, and my father-in-law, Hannu. You’ve both been such valuable help in my family’s life. I’ve been lucky to have you both in my family.

Last but not least, thanks go to my friends—thank you for bringing so much joy into my life.

This work was supported by the Northern Ostrobothnia Hospital District.
Abbreviations

BMI       Body mass index
CMAP      Compound muscle action potential
CNS       Central nervous system
DSP       Diabetic sensorimotor polyneuropathy
IFG       Impaired fasting glucose
IGT       Impaired glucose tolerance
HDL       High density lipoprotein
LDL       Low density lipoprotein
MAG       Myelin associated glycoprotein
MBP       Myelin basic protein
NGT       Normal glucose tolerance
NCS       Nerve conduction studies
NCV       Nerve conduction velocity
OGTT      Oral glucose tolerance test
PAI       Physical activity index
PN        Peripheral neuropathy
PNS       Peripheral nervous system
SNAP      Sensory nerve action potential
QUICKI    Quantitative insulin sensitivity check index
VO₂max    Maximal oxygen uptake
Original publications

This thesis is based on the following publications, which are referred throughout the text by their Roman numerals:


# Contents

Abstract  
Tiivistelmä  
Acknowledgements  
Abbreviations  
Original publications  
Contents  

1 Introduction  
2 Review of the literature  
   2.1 Peripheral nervous system  
   2.1.1 Definition of peripheral nervous system  
   2.1.2 Function of peripheral nervous system  
   2.1.3 Structure of the peripheral nerve  
   2.1.4 Peripheral neuropathy  
   2.2 Nerve conduction studies  
   2.2.1 Motor conduction studies  
   2.2.2 Sensory nerve conduction studies  
   2.3 Overweight and obesity  
   2.4 Diabetes and the peripheral nervous system  
   2.5 Overweight and obesity and the peripheral nervous system  
   2.6 Physical activity, fitness, and the peripheral nervous system  
3 Purpose of the study  
4 Subjects and methods  
   4.1 Study design  
   4.2 Subjects  
   4.3 Methods  
   4.3.1 Nerve conduction studies (I, II, III)  
   4.3.2 Oral glucose tolerance test (I, II, III)  
   4.3.3 Cholesterol level (III)  
   4.3.4 Anthropometry (I, II, III)  
   4.3.5 Physical activity (II)  
   4.3.6 Maximal oxygen uptake (II)  
   4.3.7 Statistical methods (I, II, III)  
5 Results  
   5.1 Nerve conduction studies (I, II, III)  

9  
11  
13  
15  
17  
19  
21  
22  
24  
25  
27  
28  
31  
33  
35  
37  
39  
41
5.2 Insulin, glucose, and cholesterol levels and peripheral nervous system function (I & III) ................................................................. 42
5.3 Physical activity and fitness and peripheral nervous system function (II) ..................................................................................... 44

6 Discussion 47

6.1 Insulin, glucose, and cholesterol levels and peripheral nervous system function (I & III) ................................................................. 47
6.2 Physical activity, fitness, and peripheral nervous system function (II) ........................................................................................ 50
6.3 Methodology (I, II & III) ...................................................................... 51
6.4 Future perspectives/work ........................................................................ 52

7 Conclusion 53

References 55

Original publications 67
1 Introduction

Overweight and obesity are major global burdens that have significant economic and health-related impacts (Jensen et al., 2014). Overweight is highly related to insulin resistance and hyperinsulinemia. Insulin resistance and hyperinsulinemia are well-known precursors of impaired glucose tolerance (IGT) and type 2 diabetes. Overweight and insulin resistance are independent factors that both increase the risk of developing type 2 diabetes but are also highly inter-related (Sung, Jeong, Wild, & Byrne 2012).

Neuropathies are the most common complication of diabetes mellitus type 1 and type 2, affecting up to 50% of diabetes patients, and cause symptoms such as numbness, tingling, pain, and weakness (Pasnoor, Dimachkie, Kluding, & Barohn, 2013). Recent studies strongly suggest that overweight and its complications play a significant role in the development of peripheral neuropathy (PN). Namely, overweight individuals have significantly lower motor and sensory nerve action potential (SNAP) amplitudes compared with normal weight subjects (Buschbacher, 1998; Miscio et al., 2005). Higher waist circumference and body mass index (BMI) are independently associated with polyneuropathy (Tesfaye et al., 2005; Ziegler et al., 2008). In both children and adults, insulin resistance seems to be a significant neuropathy risk factor (Akın et al., 2016; Han et al., 2015). Furthermore, many clinical associations of obesity, such as raised triglyceride levels, BMI, and hypertension, are related to peripheral nervous system (PNS) function complications (Katzmarzyk et al., 2003; Tesfaye et al., 2005).

Being overweight or obese is inversely related to physical activity and maximal oxygen uptake (VO2max) (Ross & Katzmarzyk, 2003; Yang, Telama, Viikari & Raitakari 2006). Among type 2 diabetic patients, physical activity has been suggested to reduce the risk of neuropathy (Balducci et al., 2006). Previous studies have shown that hypertrophic changes occur in motor neurons due to periods of increased use (Eisen, Carpenter, Karpati, & Bellavance, 1973; Tomanek & Tipton, 1967), which may lead to faster motor nerve conduction velocities (Kamen, Taylor, & Beehler, 1984). These findings suggest that physical activity may also have direct beneficial effects on PNS function. There is also evidence suggesting that PNS function directly affects physical performance: A recent study indicated that poor peripheral nerve function has a direct, detrimental effect on physical performance, rather than an indirect effect through decreasing muscle function (Strotmeyer et al. 2008).
The pathogenesis of diabetic neuropathy is considered to be a complex process involving possibly one or more factors, such as hyperglycemia, dyslipidemia, impaired insulin signaling, and metabolic syndrome (Callaghan, Cheng, Stables, Smith, & Feldman 2012). Treatment of patients with diabetic neuropathy currently only includes improved glucose control and pain management (Tesfaye et al., 2005). Identification of modifiable risk factors for the development of diabetic neuropathy is crucial in light of the current limitations of clinical care. Top candidates for these risk factors are components of metabolic syndrome, such as obesity, hyperglycemia, hypertriglyceridemia, hypertension, and dyslipidemia (Callaghan et al., 2012b). Finding a causal relationship between these components, including prediabetes and the development of diabetic neuropathy, might lead to breakthroughs in disease-modifying therapies (Callaghan et al., 2012).

While overweight and obese people tend to be physically inactive and unfit, little is known about the association between VO2max, physical activity and fitness, and PNS function among overweight individuals. There is also a need to increase understanding of the changes in PNS function in the prediabetes stage due to possible hyperinsulinemia/insulin resistance, hyperglycemia, and dyslipidemia. The aim of this study was to examine the association of insulin and glucose levels, cholesterol levels, and physical activity and fitness with PNS function in overweight and obese adults without diabetes.
2 Review of the literature

2.1 Peripheral nervous system

2.1.1 Definition of peripheral nervous system

The human nervous system is divided into the central nervous system (CNS) and peripheral nervous system (PNS). Nerves range over the whole human body and connect the skin, muscles, joints, and internal organs to the brain and spinal cord (CNS). As “peripheral” implies, the peripheral nervous system consists of portions of motor nerves, autonomic nerves, and primary sensory nerves that extend outside the CNS. The separation of CNS and PNS is naturally artificial because they are interconnected. However, it is justified by the reality that many diseases affect only PNS; and in contrast to CNS, PNS has the ability to regenerate (Herskovitz et al., 2010; Latov, 2007).

2.1.2 Function of peripheral nervous system

Muscles and voluntary movements are controlled by motor nerves. Autonomic nerves involve involuntary functions such as blood pressure, heart rate, sweating, and the bladder and bowel. Sensory nerves transmit signals from specialized receptors in the skin, joints, and internal organs (Latov, 2007).

2.1.3 Structure of the peripheral nerve

The functional unit of the peripheral nerve is the axon, and a nerve consists of bundles of axons. Axons are long processes that extend from the nerve cell bodies, called neurons. They are specialized for conducting electrical signals (action potentials) and for bidirectional transportation of material to and from the parent neuron. The parent neuron is 50-100 µm in diameter and regulates an axon up to 1 meter. Motor neurons are located in the anterior part of the spinal cord, and they extend axons that connect to the muscles. Sensory neurons are clustered in dorsal root ganglia, and they extend axons that connect receptors in the skin and joints and to other neurons in the dorsal part of the spinal cord. Motor and sensory neurons’ axons exiting the spinal canal merge as they extend peripherally. Before fanning out to the upper and lower extremities, some of the axons converge in the brachial...
plexus at the armpit, or in the lumbosacral plexus in the pelvis (Brushart, 2011; Kimura, 2001; Latov, 2007).

Axons can be subdivided into small or large fibers. The unmyelinated small fibers are slow-conducting and transmit signals from pain receptors in the skin. Large fibers conduct motor signals to the muscles and sensory signals that transmit vibratory sensations or joint proprioception. Large fibers are myelinated by a series of Schwann cells, each devoted entirely to one axon (Figure 1). The myelin sheath is a multilamellar structure containing various lipids and proteins that stems from the spiral wrapping around the axons of the plasma membrane of myelin-forming glia (Schwann cells). The myelin sheath allows for more rapid conduction of the electrical signals via saltatory conduction. For the correct development of PNS and the formation and maintenance of the peripheral myelin sheath, a continuous and intimate communication between axons and Schwann cells is critical. Schwann cells participate in normal development, long-term survival of the axon, and the formation and organization of nodes of Ranvier, and in turn axons provide trophic and mitogenic factors to Schwann cells (Taveggia, 2016).

Fig. 1. A myelinated motor nerve. (Modified from "Weakness" (2006)).

Large myelinated axons can be divided into distinct domains, including nodes of Ranvier, paranodal junctions, juxtaparanodes, and internodes. Action potentials
traveling down the axon are regenerated at the regularly spaced nodes of Ranvier. The node of Ranvier is a short, $\sim 1$-μm gap in the myelin sheath in which voltage-dependent Na$^+$ - channels are expressed in high density and mediate the inward Na$^+$ currents responsible for the depolarization of the nodal membrane and the transmission of the action potential (Hille, 2001). The myelin sheath ends with a series of cytoplasmic loops (e.g., paranodal loops) near the nodes of Ranvier, creating a specialized junction with the axon (paranodal junctions). Paranodal junctions have functions that include attaching the myelin sheath to the axon, separating the electrical activity of nodal axolemma from internodal axolemma, and functioning as a boundary to limit the lateral diffusion of axonal membrane proteins (Rosenbluth, 2009). Juxtaparanodes are located beneath the compact myelin sheath at the interface between the paranodal junction and internode. A juxtaparanode is a 5–15-μm long domain characterized by the high expression of Shaker-type voltage-gated K$^+$ - channels (Wang, Kunkel, Martin, Schwartzkroin, & Tempel, 1993). These channels may stabilize conduction and help maintain the internodal resting potential, especially during myelination and remyelination (Rasband, 2010; Vabnick & Shrager, 1998). The fourth domain, the internodal axolemma, is located beneath the compact myelin sheath and is organized by the overlying myelin sheath. It is also considered a unique domain because distinct membrane proteins and structures comprise this region (Rasband & Peles, 2015).

2.1.4 Peripheral neuropathy

Peripheral neuropathy (PN) refers to any damage of the peripheral nerves (motor and/or sensory nerves), which may cause symptoms such as weakness, muscle atrophy, pain, insensitivity, or loss of sensation (Latov, 2007). When the autonomic nervous system is involved, symptoms may include abnormal heart rate and blood pressure and bowel dysfunction (Vinik, Nevoret, Casellini, & Parson, 2003). PN is a major cause of disability worldwide: In a prevalence study performed in the US, about 10% of normoglycemic adults and about 20% of type 2 diabetes patients had PN (Gregg et al., 2007). Pain is the most common symptom of PN, with an estimated prevalence in the general population of between 7 and 10% (Van Hecke, Austin, Khan, Smith, & Torrance, 2014). Typical causes of PN include diabetes and its early stages, B12 deficiency, and chronic alcohol misuse (Cioroiu & Brannagan, 2014; Martyn & Hughes, 1997).
2.2 Nerve conduction studies

Nerve conduction studies (NCS) comprise a diagnostic tool for disorders of the peripheral nerves. The tool aids in localizing the site or level of the damage and identifying whether the damage constitutes axonal loss or demyelination, or both. NCS are used for diagnosing mononeuropathies such as carpal tunnel syndrome or ulnar neuropathy. They are also used to diagnose more diffuse processes, such as PN caused by diabetes (Huynh & Kiernan, 2011).

NCS are performed by stimulating the nerves with small electrical impulses over several points (usually limbs) and measuring the resultant responses. Motor, sensory, and mixed nerves can be studied. The factors affecting the NCS parameters are height, age, and most importantly, temperature (Campbell, 2014). Both delivery and detection of the electrical impulses are achieved with surface electrodes. The test itself is safe but may cause minor discomfort. It has no long-term side effects. NCS is accomplished by clinical neurophysiologists in a normal outpatient room setting (Huynh & Kiernan, 2011).

There are several limitations of NCS. Most importantly, NCS tests large myelinated fibers, which represent only 10% of peripheral nerves, and thus small-fiber neuropathies that present with pain may demonstrate normal sensory nerve conduction (Huynh & Kiernan, 2011). In such cases, other diagnostic tools, such as quantitative sensory testing, may be needed. Furthermore, the changes in NCS may be subtle early in the course of disease and may therefore be missed. Reference values in the literature are derived from studies with neurologically “normal” subjects; thus, when interpreting the results, the relevant clinical information needs to be considered. Also, there are reproducibility problems with NCS, and a call for further standardization has been issued accordingly (Dyck et al., 2013). It has been suggested that when NCS are performed longitudinally, they should optimally be performed by a single examiner in order to minimize the degree of variability associated with different clinical neurophysiologists (Chaudhry et al., 1991).

2.2.1 Motor conduction studies

Motor conduction studies are performed by stimulating a motor nerve at one or more locations while recording the compound muscle action potential (CMAP) from a muscle belly innervated by that nerve. The active recording electrode is placed over the motor point on the belly of the muscle to be studied, and the reference or inactive electrode is placed distally over the relatively electrically
inactive tendon of the muscle. After the stimulation, the potential difference between the reference and the active electrodes is displayed, and the time between delivery of the stimulus and onset of the CMAP is determined. Stimulation is achieved by using supramaximal stimulation to activate all the fibers. An example of a motor nerve stimulation and response is shown in Figure 2 (Campbell, 2014).

Fig. 2. Motor median nerve CMAPs from different stimulus locations. (Modified from “Weakness” (2006)).

Variables determined in motor NCS are action potential amplitude (mV) from baseline to negative peak or peak to peak. This reflects the number of conducting axons, thus action potential amplitude is reduced in axonal loss. Distal and proximal latency (ms) represent the onset of evoked response by the stimulus. Duration (ms) of this response can also be determined. Furthermore, conduction velocity (m/s) is calculated from the distance between stimulation (usually 2 points, proximal and distal) and recording points and then divided by latency. Nerve conduction velocity (NCV) reflects the integrity of the myelin sheath, which is reduced in demyelinating processes (Brushart, 2011).

In motor NCS, late responses are detected and can be used to assess more proximal segments of the PNS, such as the plexus and nerve roots. With routine NCS techniques, it is impossible to study these proximal segments since positioning the electrodes in close proximity to the spinal cord or in its vicinity is not possible. These late responses provide useful information about longer nerve
pathways that would not be detectable in a shorter NCS range, particularly in diffuse or multisegmental disease processes. Late responses in lower limbs are called F-waves and, in upper limbs, H-waves. When a supramaximal stimulus is applied at a point along the motor nerve, the backfiring of activated motor neurons creates F-waves. From the stimulation point, the impulse travels to the spinal cord, where it activates a proportion of motor neurons and, as the impulse travels back down the motor nerve, it results in a delayed and smaller muscle response. F-waves usually occur approximately 45–60 ms after stimulation. Useful parameters in F-waves are their presence and minimum latencies (Huynh & Kiernan, 2011).

2.2.2 Sensory nerve conduction studies

Sensory conduction studies are conducted differently compared to motor NCS. Both stimulation and recording of the evoked nerve action potential is done directly by electrodes placed over the nerve. The stimulation is done supramaximally to activate all the axons. Furthermore, there are two methods for performing sensory NCS: antidromic or orthodromic. Orthodromic studies are performed by stimulating distally and recording proximally, in the direction of the normal impulse; in antidromic studies, stimulating and recording locations are the opposite. As long as the interelectrode separation is the same, latency will remain the same in both methods, but the amplitude is lower with the orthodromic method. Most clinical neurophysiologists prefer the antidromic method for all nerves except the palmar and plantar nerves (Campbell, 2014).

Variables measured in sensory NCS are latency, NCV, and SNAP amplitude. As in motor NCS, the slowing of NCV indicates demyelinating processes, and a reduction in amplitude reflects axonal degeneration. In order to obtain factual data on the amplitudes, the distance between electrodes should be about 3–4 cm, while the stimulation needs to be precisely over the nerve; otherwise, amplitudes will decrease due to technical mishap (Campbell, 2014).

Sensory NCS are affected by the temperature of the studied site even more than motor NCS. Low temperature causes a slowing of the latency, and hence NCV, but the amplitude remains unaffected. Other factors affecting sensory NCS are gender, height, age, and BMI. Sensory NCS tends to be more sensitive to nerve pathology than motor NCS, because changes usually occur earlier in sensory nerve conduction than in motor nerve conduction. Moreover, SNAP amplitude is considered to be the most sensitive indicator of nerve pathology (Campbell, 2014).
2.3 Overweight and obesity

According to the World Health Organization (WHO), overweight and obesity are defined as abnormal or excessive fat accumulation that cause risk to a person’s health. BMI is a crude population measure of obesity in which a person’s weight in kilograms is divided by the square of his or her height in meters. A person is considered overweight when his or her BMI is equal to or above 25, and obese when the BMI is above 30. Between 1980 and 2013, the worldwide prevalence of overweight and obesity combined increased by 27.5 % for adults and 47.1 % for children (Ng et al., 2014). In 2013, there were 2.1 billion overweight and obese persons, whereas in 1980 the figure was 857 million (Ng et al., 2014). The trend is increasing because the rate of obesity is increasing in both developed and developing countries. For example, obesity is associated with the risk of morbidity from hypertension, dyslipidemia, type 2 diabetes, coronary heart disease, gallbladder disease, stroke, osteoarthritis, sleep apnea, respiratory problems, and some cancers; and it is also related to increased risk of all-cause and cardiovascular disease mortality (Jensen et al., 2014). In the literature, there is a consensus that overweight and obesity place a significant financial burden on the healthcare system. In a recent review, obesity alone was estimated to account for between 0.7 % and 2.8 % of a country’s total healthcare costs; and when including overweight, the percentage can be as high as 9.1 % (Withrow & Alter, 2011).

2.4 Diabetes and the peripheral nervous system

Diabetes is a worldwide problem and a well-known factor causing axonal damage and segmental demyelination in the PNS; in fact, diabetes-associated neuropathy is the most common, costly, and disabling complication of diabetes (Pasnoor et al., 2013; Peltier, Goutman, & Callaghan, 2014). Most commonly, it causes distal symmetrical polyneuropathy, but it can also affect nerve plexus (plexopathy), nerve roots (radiculopathy), single nerves (mononeuropathy), and multiple individual nerves (mononeuritis multiplex) (Callaghan et al., 2012; Peltier et al., 2014; Vinik et al., 2013). Symptoms usually include numbness, tingling sensation, pain, or weakness, or it may be asymptomatic (Tesfaye et al., 2013; Truini et al., 2013; Truini et al., 2014; Van Hecke et al., 2014). These symptoms can cause disability in physical function, anxiety, and sleep disturbances (Galer, Gianas, & Jensen, 2000). Diabetes can also cause damage to the autonomic nervous system, leading to diabetic autonomic neuropathy, which is also a common complication of diabetes.
Diabetic autonomic neuropathy can be isolated, but it often coexists with other peripheral neuropathies. It causes symptoms such as heart rate and blood pressure abnormalities and gastrointestinal disturbances.

The most commonly encountered form of diabetic neuropathy is diabetic sensorimotor polyneuropathy (DSP), which is a distal and symmetrical form of neuropathy (Dyck et al., 1992). It has been demonstrated that 82% of newly diagnosed diabetes mellitus type 2 patients have negative alterations in PNS function (Rota et al., 2005). At the time of diabetes diagnosis, 8–20% had already developed diabetic neuropathy, and at least 30–50% of patients diagnosed with diabetes will develop diabetic neuropathy (Pop-Busui, Lu, Lopes, & Jones, 2009; Smith & Singleton, 2012; Sugimoto, Murakawa, & Sima, 2000). Furthermore, it has been estimated that neuropathic pain develops in more than 30% of these patients (Abbott, Malik, van Ross, Kulkarni, & Boulton, 2011; Bouhassira, Letanoux, & Hartemann, 2013).

The diagnosis of DSP relies on the interpretation of the combination of symptoms and signs as well as confirmatory testing. NCS are the most used diagnostic tool in clinical settings and longitudinal studies (Albers et al., 1996; Albers et al., 2010). The main findings in NCS in patients with DSP is reduced distal lower extremity SNAP amplitudes and reduced conduction velocities (Charles et al., 2010; Smith & Singleton, 2012). Multiple important mechanisms in the pathophysiology of DSP have been identified, but the pathophysiology itself is still not completely understood. In both type 1 and type 2 diabetes, hyperglycemia is generally accepted as the primary pathogenic element. However, especially in type 2 diabetes, other pathogenic factors, such as insulin signaling, hypertension, and dyslipidemia, might occur earlier than hyperglycemia (Callaghan et al., 2012b). Many studies have indicated that like diabetic nephropathy and retinopathy, diabetic neuropathy is a result of microvascular disease. However, microvascular damage is likely to be only one aspect of a more complex pathophysiology (Gonçalves et al., 2017).

Diabetic neuropathy is likely induced by a combination of axonal degeneration and demyelination owing to the metabolic consequences of hyperglycemia, insulin resistance, and toxic adiposity, as well as endothelial injury and microvascular dysfunction leading to nerve ischemia (Smith & Singleton, 2012). Furthermore, important metabolic pathways include the accumulation of advanced glycation end products, oxidative stress, and increased flux through the polyol pathway (Albers & Pop-Busui, 2014; Fernyhough, Roy, Chowdhury, & Schmidt, 2010). Also, the role of primary Schwann cell damage as a primary cause of diabetic neuropathy is
emerging, as the historical focus has mainly been on microvascular disease and axonopathy (Gonçalves et al., 2017).

The current treatment for painful diabetic neuropathy is mostly the management of the underlying diabetes and pain relief medication (Tesfaye et al., 2005). The enhanced glycemic control appears to be much more effective at preventing diabetic neuropathy in patients with type 1 diabetes than in those with type 2 (Boussageon et al., 2011; Callaghan et al., 2012a). It has been suggested that metabolic syndrome might account for this difference (Callaghan et al., 2012).

2.5 Overweight and obesity and the peripheral nervous system

It has been demonstrated that sensory and mixed nerve action potential amplitudes tend to decrease as BMI increases. Compared to lean subjects, obese subjects have approximately 20–40 % lower SNAP amplitudes (Buschbacher, 1998). This has been attributed to the fact that the nerves lie deeper, with more subcutaneous tissue dampening the electrical impulse (Buschbacher, 1998). In a more recent study, it was shown that BMI was associated with upper limb sensory SNAP amplitudes but not with lower limb SNAP amplitudes (Fujimaki et al., 2009). Miscio et al. (2005) compared NCS variables between non-diabetic obese subjects with hyperinsulinemia and age- and sex-matched normal weight subjects. They found that the CMAP amplitudes and SNAP amplitudes in both upper and lower limb nerves were lower in obese subjects, with the difference being greater in SNAP amplitudes. Both Buschbacher (1998) and Miscio et al. (2005) also found that in some nerves, latency was shorter and thus NCV was higher in obese patients, which Buschbacher suggested to be due to the subcutaneous fat’s thermal insulation maintaining a higher perineural temperature. Based on the abovementioned studies, BMI seems to have a negative effect on SNAP amplitudes and a neutral or positive effect on latencies and NCV.

Further on the obesity–diabetes spectrum, the evidence of peripheral nerve involvement increases. In a recent paper, it was discovered that peripheral nerve pathologies were present in obese children compared to normal-weight children (Akin et al., 2016). The damage to the PNS was more significant in obese children with insulin resistance. Also, with adult subjects, it has been shown that insulin resistance is independently associated with PN (Han et al., 2015).

Also, other factors related to obesity seem to have a direct relationship with PN. Metabolic syndrome has been associated with increased risk of PN (Callaghan & Feldman, 2013; Smith & Singleton 2006; Smith, Rose, & Singleton, 2008).
Elevated triglyceride levels have been associated with PN and the progression of diabetic neuropathy (Kassem et al., 2005; McManis, Windebank, & Kiziltan, 1994; Shankar, Shashikiram, Gopalraj, & Rathnabai, 2012; Wiggins et al., 2009); however, there is also evidence that hyperlipidemia is not independently associated with polyneuropathy (David, Mahdavi, Nance, & Khan, 1999; Rajabally & Shah, 2011; Tamer et al., 2006). Higher waist circumference, BMI, hypertension, and serum triglycerides are independently associated with neuropathy (Tesfaye et al., 2005; Ziegler et al., 2008). Furthermore, it has been demonstrated that prediabetes is related with similar risks of PN and severity of nerve damage as newly developed diabetes (Lee et al., 2015).

An association between IGT and PN has been reported (Hoffman-Snyder, Smith, Ross, Hernandez, & Bosch, 2006; Novella, Inzucchi, & Goldstein, 2001; Singleton, Smith, & Bromberg, 2001; Ziegler et al., 2009). There is also evidence that the PN associated with IGT is milder compared to diabetic neuropathy. The involvement of small fibers is more prominent in patients with IGT, while the involvement of large fibers is more prominent in patients with diabetes. Thus, the damage in small fibers might be the earliest detectable sign in PN associated with impaired glucose metabolism (Divisova et al., 2012; Sumner, Sheth, Griffin, Cornblath, & Polydefkis, 2003; Umapathi et al., 2007).

### 2.6 Physical activity, fitness, and the peripheral nervous system

There is a negative correlation between obesity and physical activity and VO2max (Ross & Katzmarzyk, 2003; Yang et al., 2006). Previous studies have shown that hypertrophic changes occur in motor neurons due to periods of increased use (Eisen et al. 1973; Tomanek & Tipton, 1967), which may lead to faster motor nerve conduction velocities (Kamen et al., 1984). Vice versa, evidence suggests that PNS function directly affects physical performance: A recent study indicated that poor peripheral nerve function has a direct, detrimental effect on physical performance, rather than an indirect effect through decreased muscle function (Strotmeyer et al., 2008).

Balducci et al. (2006) demonstrated that in type 2 diabetic subjects, long-term aerobic exercise training can prevent the onset or modify the natural history of diabetic neuropathy. Furthermore, a 12-week period of tai chi chuan (a Chinese martial art) resulted in significant improvement of the motor nerve conduction velocities of the bilateral median and tibial nerves, and of the distal sensory latencies of bilateral ulnar nerves in diabetic patients (Hung et al., 2009). A 10-
week aerobic and strengthening exercise program significantly reduced pain, neuropathic symptoms and increased intraepidermal nerve fiber branching from a proximal skin biopsy was noted in patients with diabetic neuropathy (Kluding et al., 2012).

Loprinzi, Hager, and Ramulu (2014) suggested, based on their findings, that proper physical activity together with good glycemic control is associated with less neuropathy in type 2 diabetic patients. In a recent systematic review about the role of exercise interventions in peripheral neuropathies, it was concluded that balance training appears to be the most effective type of exercise, while strength, or a combination of endurance and strength, seems to have a lower impact. However, they also concluded that endurance training plays an important role in metabolically induced neuropathies (Streckmann et al., 2014). Another recent systemic review stated that the literature on the role of physical activity and psychological coping mechanisms in the management of painful diabetic neuropathy is sparse and inconsistent, and that no firm conclusions can be drawn (Davies, Cramp, Gauntlett-Gilbert, Wynick, & McCabe, 2015).
3 Purpose of the study

The aim of this study was to examine the associations of insulin, glucose, and cholesterol levels and physical activity and fitness in PNS function in overweight and obese adults. The specific objectives of the study were:

1. To investigate the relationship between glucose and insulin levels and PNS function in overweight and obese adults (Study I).
2. To investigate the relationship between physical activity and fitness and PNS function in overweight and obese adults (Study II).
3. To identify the associations of glucose, insulin, and cholesterol levels and PNS in overweight and obese adults over a 3-year follow-up (Study III).
4 Subjects and methods

This study was carried out in Oulu, Finland during 2007–2017 in collaboration with the following institutions: Department of Sports Medicine, Oulu Deaconess Institute; the Center for Life Course Health Research, University of Oulu; and the Research Unit of Medical Imaging, Physics and Technology, University of Oulu. The Local Ethics Committee gave their approval for the study and written informed consent from all subjects was obtained.

4.1 Study design

The relationships between insulin and glucose levels, physical activity and fitness, and PNS function were investigated using a cross-sectional cohort study (Studies I and II). Prospective follow-up of the cohort was used to further investigate the associations of glucose, insulin, and cholesterol levels and PNS function (Study III).

4.2 Subjects

Forty overweight and obese adults (mean age 49 ± 11 years, range 28–68 years) were recruited from an exercise and obesity (LILA-tutkimus) study coordinated by the Department of Sports Medicine and Exercise, Oulu Deaconess Institute. The LILA data from 2-3 years earlier were also used in Study I. The inclusion criterion was a BMI ≥ 25. Subjects with type 2 diabetes, polyneuropathy, or chronic alcohol use were excluded from the study (Studies I and II). There were 11 drop outs in the follow-up study due to refusal to participate in either NCS or an oral glucose tolerance test (OGTT). Thus, the study population was reduced to 29 subjects (Study III).

4.3 Methods

4.3.1 Nerve conduction studies (I, II, III)

The peroneal motor nerve (Figure 3) and sural (Figure 4), radial (Figure 5), and medial plantar sensory (Figure 6) nerve conduction, with a standard distance, were measured bilaterally using Keypoint 4 and Keypoint portable devices (Medtronic, Skövlunde, Denmark). Using surface electrodes, sensory nerve conduction were
measured antidromically for the radial and sural nerves, and orthodromically for the medial plantar nerves. For the motor nerve conduction measurements, the distal and proximal latency, distal and proximal peak-to-peak CMAP, NCV, F-wave minimum, mean and maximum latency, and F-wave amount per 20 stimuli (persistence) were determined. For the sensory nerve conduction measurements, the onset latency, peak-to-peak SNAP, and NCV were determined. The skin surface temperature was measured immediately following the conduction measurements. The filter settings were 2 Hz to 10 kHz for the motor nerve conduction measurements and 20 Hz to 2 kHz for the sensory nerve conduction measurements. Follow-up measurements were conducted by the same investigators.

Fig. 3. Peroneal motor nerve measurement setting.
Fig. 4. Sural sensory nerve measurement setting.

Fig. 5. Sensory radial nerve measurement setting.
4.3.2 Oral glucose tolerance test (I, II, III)

Fasting and 30-min and 120-min glucose and insulin levels were measured with a standardized 75-g OGTT. Serum glucose levels were determined using a hexokinase assay (Konelab Analyzers, Thermo Electron Oy, Vantaa, Finland), and insulin levels were determined using a chemiluminescent microparticle immunoassay (Abbot Diagnostic, Abbot Park, IL, USA). For Study I, an OGTT had been performed 2-3 years earlier (baseline) and when the NCS were performed (current) in order to obtain more comprehensive information about the individual’s insulin and glucose levels in the near past. Insulin resistance was assessed with the quantitative insulin sensitivity check index (QUICKI). This method is significantly correlated with the hyperinsulinemic euglycemic glucose clamp and is considered a valid method for evaluating insulin resistance in obese patients (Katz et al., 2000). The QUICKI was calculated with the following equation: QUICKI = 1 / (log fasting insulin) x (log fasting plasma glucose).

4.3.3 Cholesterol level (III)

Serum total cholesterol, high density lipoprotein (HDL) cholesterol, and triglyceride analyses were performed using an auto analyzer (ADVIA 1650 Chemistry System, Siemens Medical Solutions, Tarrytown, USA), and low density
lipoprotein (LDL) cholesterol was calculated by the Friedwald equation (Warnick, Knopp, Fitzpatrick, & Branson, 1990).

### 4.3.4 Anthropometry (I, II, III)

Height was measured without shoes to the nearest 0.5 cm. Waist circumference was measured to the nearest 0.5 cm. Weight was measured on a calibrated scale to the nearest 0.1 kg. BMI was calculated as weight (kg) divided by height (m) squared.

### 4.3.5 Physical activity (II)

Self-rated physical activity was determined by a modified Paffenbarger questionnaire, where individuals were asked to recall their physical activity at the ages of 15, 30, and their current age. Individuals were classified by the highest level of exercise they performed for at least 15 minutes per session at least 3 times per week. Categories were strenuous (e.g., jogging), moderate (e.g., walking), mild (e.g., fishing) and less than mild (e.g., watching television). Physical activity indexes (PAI) were then scored, giving 1 point to less than mild exercise, 2 points to mild exercise, 3 points to moderate exercise, and 4 points to strenuous exercise (Greendale, Barrett-Connor, Edelstein, Ingles, & Haile, 1995; Korpelainen, Korpelainen, Heikkinen, Väänänen, & Keinänen-Kiukaanniemi, 2006). The amount of daily physical activity was measured by a wrist-held accelerometer (AW 200, Polar Electro Oy, Kempele, Finland), which also has a pedometer function (Brugniaux et al., 2008). Individuals were asked to wear the accelerometer for a 24-hour period. The sum and median of the intensity of the acceleration pulses, as well as the number of steps, were determined.

### 4.3.6 Maximal oxygen uptake (II)

All individuals underwent an incremental bicycle ergometer test (ERG 911, Schiller AG, Bitz, Germany) with VO₂ max analysis (Oxygen Pro Spirometer, Jaeger, Viasys Healthcare Inc., Hoechberg, Germany). The initial workload was 25 W, which was increased by 25 W every 2 minutes until the individual was exhausted.
4.3.7 Statistical methods (I, II, III)

Mean values and standard deviations were used as the descriptive statistics. Serum triglyceride and insulin levels were normalized by a logarithmic transformation. Univariate associations between the explanatory and response variables were analyzed using Pearson’s correlation coefficients. Student's *t*-test was used for the statistical comparisons (unpaired for the drop-out analysis and paired for the follow-up analysis). All variables significant in the univariate analyses were entered into the multivariate analyses. A multiple stepwise linear regression analysis was used to assess the relationship between changes in the insulin levels, cholesterol levels, weight, and nerve conduction measurements. Closely interrelated variables were entered separately in these models. All models in Studies I and II were adjusted for age, height, weight, and skin temperature. Models in Study III were adjusted for skin temperature and weight changes. The Bonferroni method was used in Study III to adjust *P* values for multiple comparisons. Otherwise, *p* < 0.05 was considered statistically significant. The SAS 9.1 (SAS Institute Inc. Cary, NC, USA) software program was used for all statistical analyses.
5 Results

The characteristics of the study population for Studies I and II are presented in Table 1. Mean age of the study participants was 49 years (SD 11). The study population consisted of 14 male and 26 female participants. According to WHO criteria, 36 had normal glucose tolerance (NGT), 2 had impaired fasting glucose (IFG), and 2 had IGT. Mean BMI was 34 (SD 5).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study I</th>
<th>Study II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/Women</td>
<td>14/26</td>
<td>14/26</td>
</tr>
<tr>
<td>Age, years</td>
<td>49 (11)</td>
<td>49 (11)</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.66 (0.09)</td>
<td>1.66 (0.09)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>93 (15)</td>
<td>93 (15)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34 (5)</td>
<td>34 (5)</td>
</tr>
<tr>
<td>NGT/IFG/IGT</td>
<td>36/2/2</td>
<td>36/2/2</td>
</tr>
<tr>
<td>*Insulin (µU/ml)</td>
<td>12.0 (10.3)</td>
<td></td>
</tr>
<tr>
<td>Fasting insulin (µU/ml)</td>
<td>9.4 (5.7)</td>
<td></td>
</tr>
<tr>
<td>*Insulin 120 min (µU/ml)</td>
<td>60.5 (48.5)</td>
<td></td>
</tr>
<tr>
<td>Insulin 120 min (µU/ml)</td>
<td>52.5 (51.5)</td>
<td></td>
</tr>
<tr>
<td>Mean insulin 120 min (µU/ml)</td>
<td>55.9 (44.5)</td>
<td></td>
</tr>
<tr>
<td>*Fasting glucose (mmol/l)</td>
<td>5.5 (0.6)</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.6 (0.6)</td>
<td></td>
</tr>
<tr>
<td>*Glucose 120 min (mmol/l)</td>
<td>6.9 (1.9)</td>
<td></td>
</tr>
<tr>
<td>Glucose 120 min (mmol/l)</td>
<td>6.2 (1.3)</td>
<td></td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.35 (0.03)</td>
<td></td>
</tr>
<tr>
<td>VO₂max (mL·min⁻¹·kg⁻¹)</td>
<td>25.84 (6.84)</td>
<td></td>
</tr>
<tr>
<td>High physical activity at the age of 30 years n (%)</td>
<td>12 (30)</td>
<td></td>
</tr>
<tr>
<td>Number of steps (24-h period)</td>
<td>6647 (4267)</td>
<td></td>
</tr>
</tbody>
</table>

SD, standard deviation; NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; *Values measured 2 to 3 years earlier.

The characteristics of the Study III population are presented in Table 2. The mean age of the individuals at the end of the follow-up was 52 (SD 11) years. In the beginning of the study, 6 participants were overweight (BMI 25–30) and 23 were obese (BMI > 30). At the end of the follow-up, 4 were overweight and 25 were obese. Two individuals had IFG and 2 had IGT in the beginning of the study, according to WHO criteria. At the end of the follow-up, 4 individuals had IFG, and
4 individuals had IGT; no one had developed diabetes. At the end of the follow-up, there was a slight increment in weight (NS) and waist circumference \((p = 0.04)\), along with the levels of fasting insulin \((p = 0.01)\) and glucose (NS). Total cholesterol \((p = 0.002)\), LDL cholesterol \((p = 0.003)\), and QUICKI \((p = 0.02)\) decreased significantly. The serum insulin level at 120 min (NS) and the glucose level at 120 min (NS) slightly decreased.

**Table 2. Characteristics of the study population (Study III). Values are given as mean (SD).**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>3 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 29)</td>
<td>(n = 29)</td>
</tr>
<tr>
<td>Men/Women</td>
<td>12/17</td>
<td>12/17</td>
</tr>
<tr>
<td>Age, years</td>
<td>49 (11)</td>
<td>52 (11)</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.70 (0.10)</td>
<td>1.70 (0.10)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>91 (15)</td>
<td>94 (13)</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>33 (4)</td>
<td>34 (4)</td>
</tr>
<tr>
<td>NGT/IFG/IGT</td>
<td>25/2/2</td>
<td>21/4/4</td>
</tr>
<tr>
<td>Fasting insulin (µU/ml)</td>
<td>8.8 (5.9)</td>
<td>10.2 (6.2)</td>
</tr>
<tr>
<td>Insulin 120 min (µU/ml)</td>
<td>44.5 (41.6)</td>
<td>43.2 (48.9)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.5 (0.7)</td>
<td>5.7 (0.7)</td>
</tr>
<tr>
<td>Glucose 120 min (mmol/l)</td>
<td>6.4 (1.8)</td>
<td>6.3 (1.5)</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.35 (0.03)</td>
<td>0.34 (0.03)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.54 (1.05)</td>
<td>5.00 (0.91)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.55 (0.40)</td>
<td>1.53 (0.42)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.38 (0.91)</td>
<td>2.91 (0.62)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.37 (0.62)</td>
<td>1.24 (0.79)</td>
</tr>
</tbody>
</table>

SD, standard deviation; NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance.
The main results of each study are presented in Table 3.

Table 3. The main results of Studies I–III.

<table>
<thead>
<tr>
<th>Study</th>
<th>Aim of study</th>
<th>Study design</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Association of glucose and insulin levels with PNS function</td>
<td>Cross-sectional study, 40 overweight or obese adults</td>
<td>Serum insulin level 120 min after an OGTT was positively associated with peroneal F-wave minimum latency, peroneal F-wave maximum latency, sural sensory latency, sural sensory NCV, and medial plantar sensory NCV.</td>
</tr>
<tr>
<td>II</td>
<td>Association of fitness and physical activity with PNS function</td>
<td>Cross-sectional study, 40 overweight or obese adults</td>
<td>VO2max was positively associated with peroneal CMAP amplitude, peroneal proximal CMAP amplitude. Physical activity index at the age of 30 years was positively associated with peroneal motor NCV, Peroneal F-wave maximum latency, medial plantar sensory latency, and medial plantar NCV.</td>
</tr>
<tr>
<td>III</td>
<td>Association of glucose and cholesterol levels with PNS function</td>
<td>Prospective 3-year follow-up, 29 overweight or obese adults</td>
<td>Serum insulin level change at 120 min after an OGTT was positively associated with changes in peroneal NCV and F-wave mean, sural NCV, and medial plantar NCV. Action potential amplitudes decreased consistently and significantly in all sensory nerves.</td>
</tr>
</tbody>
</table>

5.1 Nerve conduction studies (I, II, III)

The neurophysiological data of the studies are presented in Table 4. There were no significant differences between male and female study subjects’ neurophysiological variables. All SNAP amplitudes decreased significantly \( p < 0.001 \) during the follow-up. Significant changes in the peroneal motor nerve variables were seen, increases in distal CMAP amplitude \( (p = 0.02) \), proximal CMAP amplitude \( (p = 0.01) \), and F-wave min \( (p = 0.01) \), and decreases in distal latency \( (p = 0.03) \) and F-wave persistence \( (p = 0.03) \). Furthermore, the medial plantar latency increased \( (p = 0.02) \). There were no statistically significant changes in NCV during the 3-year follow-up.
Table 4. Neurophysiological variables. Values are mean (SD).

<table>
<thead>
<tr>
<th>Nerve</th>
<th>Neurophysiological variable</th>
<th>Studies I &amp; II (n = 40)</th>
<th>Study III Baseline (n = 29)</th>
<th>Study III 3-years (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Distal latency, ms</td>
<td>4.26 (0.63)</td>
<td>4.32 (0.61)</td>
</tr>
<tr>
<td>Peroneal</td>
<td></td>
<td>Proximal latency, ms</td>
<td>11.64 (1.22)</td>
<td>11.82 (1.22)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NCV, ms</td>
<td>49.75 (3.66)</td>
<td>49.20 (3.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Distal amplitude, mV</td>
<td>5.37 (2.62)</td>
<td>5.42 (2.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proximal amplitude, mV</td>
<td>4.86 (2.45)</td>
<td>4.87 (2.63)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F-wave min, ms</td>
<td>41.69 (4.08)</td>
<td>41.81 (4.22)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F-wave max, ms</td>
<td>47.74 (5.19)</td>
<td>48.34 (5.39)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F-wave mean, ms</td>
<td>44.58 (4.15)</td>
<td>44.94 (4.39)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F-wave persistence, x/20</td>
<td>11.26 (4.74)</td>
<td>11.66 (4.49)</td>
</tr>
<tr>
<td>Radial</td>
<td>Latency, ms</td>
<td>1.62 (0.10)</td>
<td>1.61 (0.09)</td>
<td>1.61 (0.09)</td>
</tr>
<tr>
<td></td>
<td>NCV, m/s</td>
<td>61.75 (3.61)</td>
<td>62.14 (3.31)</td>
<td>62.05 (3.18)</td>
</tr>
<tr>
<td></td>
<td>Amplitude, µV</td>
<td>42.91 (12.20)</td>
<td>42.57 (12.83)</td>
<td>34.34 (9.66)**</td>
</tr>
<tr>
<td>Sural</td>
<td>Latency, ms</td>
<td>2.11 (0.21)</td>
<td>2.10 (0.21)</td>
<td>2.07 (0.14)</td>
</tr>
<tr>
<td></td>
<td>NCV, m/s</td>
<td>47.93 (5.05)</td>
<td>48.36 (5.23)</td>
<td>48.40 (3.42)</td>
</tr>
<tr>
<td></td>
<td>Amplitude, µV</td>
<td>22.94 (8.92)</td>
<td>21.37 (6.51)</td>
<td>13.81 (5.47)**</td>
</tr>
<tr>
<td>Medial</td>
<td>Latency, ms</td>
<td>2.26 (0.30)</td>
<td>2.21 (0.31)</td>
<td>2.33 (0.31)</td>
</tr>
<tr>
<td>plantar</td>
<td>NCV, m/s</td>
<td>55.01 (4.72)</td>
<td>55.40 (4.69)</td>
<td>55.59 (5.22)</td>
</tr>
<tr>
<td></td>
<td>Amplitude, µV</td>
<td>12.53 (6.28)</td>
<td>12.46 (6.24)</td>
<td>5.73 (3.74)**</td>
</tr>
</tbody>
</table>

Comparison between baseline and 3 years; *p = < 0.05, **p = < 0.0001

5.2 Insulin, glucose, and cholesterol levels and peripheral nervous system function (I & III)

In Study I, in multiple stepwise linear regression analysis (models were adjusted for age, height, and skin temperature) baseline insulin levels measured 120 min after an OGTT explained 18 % of the variation in peroneal F-wave minimum latency, 8 % of peroneal F-wave maximum latency variation, 15 % of sural sensory latency variation, 13 % of sural sensory NCV variation, and 10 % of the variation in medial plantar sensory NCV (Table 5). The mean insulin level at 120 min for the two measurements explained 11 % of peroneal motor NCV variation, 13 % of F-wave minimum latency variation, and 11 % of the variation in medial plantar sensory NCV. The mean insulin level for the two measurements explained 6 % of the variation in radial sensory NCV almost significantly. Current insulin level at 120 min was a significant predictor of F-wave minimum latency variation with a
6 % coefficient of determination. No significant relationships between neurological variables and glucose levels and QUICKI were found.

Table 5. Neurophysiological data variation predicted by serum insulin levels in multiple stepwise regression analysis (Study I).

<table>
<thead>
<tr>
<th>Nerve</th>
<th>Dependent variable</th>
<th>Explanatory variable</th>
<th>β</th>
<th>CI 95 %</th>
<th>Partial R²</th>
<th>p^2</th>
<th>Model R²</th>
<th>p^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroneal</td>
<td>NCV</td>
<td>Mean insulin</td>
<td>3.23</td>
<td>0.44, 6.02</td>
<td>0.11</td>
<td>0.25</td>
<td>0.30</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>F-wave min</td>
<td>Mean insulin</td>
<td>-5.32</td>
<td>-8.07, -2.56</td>
<td>0.13</td>
<td>0.003</td>
<td>0.60</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>F-wave min</td>
<td>Baseline insulin</td>
<td>-4.95</td>
<td>-7.66, -2.22</td>
<td>0.18</td>
<td>0.0008</td>
<td>0.51</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>F-wave max</td>
<td>Baseline insulin</td>
<td>-2.50</td>
<td>-4.84, -0.17</td>
<td>0.06</td>
<td>0.036</td>
<td>0.58</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Radial</td>
<td>NCV</td>
<td>Mean insulin</td>
<td>2.45</td>
<td>-0.26, 5.17</td>
<td>0.06</td>
<td>0.08</td>
<td>0.26</td>
<td>0.0031</td>
</tr>
<tr>
<td>Sural</td>
<td>Latency</td>
<td>Baseline insulin</td>
<td>-0.21</td>
<td>-0.36, -0.07</td>
<td>0.15</td>
<td>0.005</td>
<td>0.38</td>
<td>0.0001</td>
</tr>
<tr>
<td>Medial</td>
<td>NCV</td>
<td>Mean insulin</td>
<td>4.30</td>
<td>0.80, 7.80</td>
<td>0.11</td>
<td>0.017</td>
<td>0.34</td>
<td>0.002</td>
</tr>
<tr>
<td>planter</td>
<td>NCV</td>
<td>Baseline insulin</td>
<td>3.90</td>
<td>0.52, 7.28</td>
<td>0.10</td>
<td>0.03</td>
<td>0.33</td>
<td>0.002</td>
</tr>
</tbody>
</table>

The results of the multivariate regression analyses (Study III) are shown in Table 6. Multiple stepwise regression analysis (models were adjusted for skin temperature change) was performed by adding the 120-min glucose and insulin levels, HDL cholesterol, weight and triglyceride level changes. Insulin level change at 120 min was a significant predictor of the changes in the peroneal NCV and F-wave mean, sural NCV, and medial plantar NCV with coefficients of determination of 15 %, 22 %, 10 %, and 25 %, respectively. Furthermore, HDL cholesterol level was a significant predictor of the change in the radial SNAP amplitude, and triglyceride level was a significant predictor of the change in the sural SNAP amplitude, with coefficients of determination of 15 % and 16 %, respectively. However, after Bonferroni p-value correction, insulin level change at 120 min remained a statistically significant predictor of the change in medial plantar NCV.
No significant relationships between neurological variables and glucose levels and QUICKI were found.

Table 6. Neurophysiological data changes predicted by serum insulin level changes in multiple stepwise regression analysis (Study III).

<table>
<thead>
<tr>
<th>Nerve Dependent variable</th>
<th>Explanatory variable</th>
<th>β</th>
<th>CI 95 %</th>
<th>Partial R²</th>
<th>pB</th>
<th>Model R²</th>
<th>pC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroneal NCV change</td>
<td>Insulin level change at 120 min</td>
<td>0.32</td>
<td>0.26, 6.54</td>
<td>0.15</td>
<td>0.04</td>
<td>0.33</td>
<td>0.07</td>
</tr>
<tr>
<td>F-wave mean</td>
<td>Insulin level change at 120 min</td>
<td>0.32</td>
<td>-5.73, -0.61</td>
<td>0.22</td>
<td>0.02</td>
<td>0.27</td>
<td>0.07</td>
</tr>
<tr>
<td>Radial Amplitude change</td>
<td>HDL cholesterol change</td>
<td>0.03</td>
<td>-0.02, 21.34</td>
<td>0.15</td>
<td>0.04</td>
<td>0.16</td>
<td>0.11</td>
</tr>
<tr>
<td>Sural NCV change</td>
<td>Insulin level change at 120 min</td>
<td>0.11</td>
<td>-0.28, 10.37</td>
<td>0.10</td>
<td>0.06</td>
<td>0.43</td>
<td>0.002</td>
</tr>
<tr>
<td>Amplitude change</td>
<td>Triglyceride change</td>
<td>0.37</td>
<td>-22.30, -1.22</td>
<td>0.16</td>
<td>0.03</td>
<td>0.26</td>
<td>0.02</td>
</tr>
<tr>
<td>Medial plantar NCV change</td>
<td>Insulin level change at 120 min</td>
<td>0.32</td>
<td>-0.51, 12.14</td>
<td>0.25</td>
<td>0.009</td>
<td>0.33</td>
<td>0.02</td>
</tr>
</tbody>
</table>

5.3 Physical activity and fitness and peripheral nervous system function (II)

The results of the multivariate regression analyses (Study II) are shown in Table 7. VO₂max explained 17 % of the peroneal distal CMAP amplitude variation and 16 % of the peroneal proximal CMAP amplitude variation. Additionally, VO₂max was a significant predictor of F-wave persistence, radial sensory NCV, and medial plantar SNAP amplitude variation with coefficients of determination of 18 % (0.35 increase/unit), 12 % (0.18 m/s decrease/unit), and 22 % (0.47 µV increase/unit), respectively. There was a significant positive association between PAI at the age of 30 years and nerve conduction measures. PAI at the age of 30 years was significantly associated with peroneal motor NCV, peroneal F-wave maximum latency, medial plantar sensory latency, and NCV variation with coefficients of determination of 9 % (0.98 m/s increase/unit), 8 % (1.23 s decrease/unit), 14 % (0.10 s decrease/unit), and 10 % (1.47 m/s increase/unit), respectively. A positive
An association between the number of steps measured by the accelerometer and medial plantar SNAP amplitude variation was found with a coefficient of determination of 10% (0.0005 µV increase/unit).

Table 7. Neurophysiological data variation predicted by VO_{2max}, PAI indexes, and number of steps in multiple stepwise regression analysis (Study II).

<table>
<thead>
<tr>
<th>Nerve</th>
<th>Dependent variable</th>
<th>Explanatory variable</th>
<th>β</th>
<th>CI 95 %</th>
<th>Partial R²</th>
<th>pB</th>
<th>Model R²</th>
<th>pC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroneal</td>
<td>Distal CMAP</td>
<td>VO_{2max}</td>
<td>0.18</td>
<td>0.05, 0.31</td>
<td>0.17</td>
<td>0.04</td>
<td>0.36</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Proximal CMAP</td>
<td>VO_{2max}</td>
<td>0.16</td>
<td>0.03, 0.29</td>
<td>0.16</td>
<td>0.05</td>
<td>0.34</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>NCV</td>
<td>PAI 30yr</td>
<td>0.97</td>
<td>0.07, 1.86</td>
<td>0.09</td>
<td>0.04</td>
<td>0.36</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>F-wave max latency</td>
<td>PAI 30yr</td>
<td>-1.23</td>
<td>-2.14, -0.32</td>
<td>0.08</td>
<td>0.01</td>
<td>0.67</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>F-wave persistence</td>
<td>VO_{2max}</td>
<td>0.35</td>
<td>0.12, 0.59</td>
<td>0.18</td>
<td>0.01</td>
<td>0.32</td>
<td>0.004</td>
</tr>
<tr>
<td>Radial</td>
<td>NCV</td>
<td>VO_{2max}</td>
<td>-0.18</td>
<td>-0.33, -0.03</td>
<td>0.12</td>
<td>0.03</td>
<td>0.27</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Latency</td>
<td>PAI 30yr</td>
<td>-0.10</td>
<td>-0.16, -0.04</td>
<td>0.14</td>
<td>0.01</td>
<td>0.55</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Medial plantar</td>
<td>SNAP</td>
<td>VO_{2max}</td>
<td>0.47</td>
<td>0.18, 0.76</td>
<td>0.22</td>
<td>0.05</td>
<td>0.41</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of steps</td>
<td>0.0005</td>
<td>0.0001, 0.001</td>
<td>0.10</td>
<td>0.01</td>
<td>0.41</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NCV</td>
<td>1.472</td>
<td>0.435, 2.51</td>
<td>0.10</td>
<td>0.01</td>
<td>0.43</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

PAI, physical activity index; VO_{2max}, maximal oxygen uptake
6 Discussion

Fig. 7. Main findings of Studies I–III. (Modified from “Weakness” (2006)).

6.1 Insulin, glucose, and cholesterol levels and peripheral nervous system function (I & III)

Figure 7 illustrates the main findings of Studies I, II, and III. In the cross-sectional study (I) and 3-year follow-up study (III), a positive association between serum insulin levels at 120 min after an OGGT and NCV in overweight and obese subjects was demonstrated. In the present studies, glucose levels and QUICKI were not related to neurophysiological variables. It seems that insulin is directly involved in the myelination process of large fibers. Miscio et al. (2005) found faster conduction velocities in peroneal motor nerves and ulnar and sural sensory nerves in obese subjects compared to the normal-weight controls. Landau et al. (2005) presented a similar finding with BMI, correlating positively with elbow ulnar motor NCV. It has been suggested that this phenomenon might be attributable to thicker subcutaneous fat’s thermal insulation maintaining a higher temperature around the
nerve (Buschbacher, 1998). The current study’s results contribute to an understanding of the phenomenon by suggesting that elevated insulin levels due to obesity might be a biological factor explaining why obese people without hyperglycemia demonstrate faster NCV in some nerves.

Insulin receptors are expressed in the CNS and PNS, as the highest densities of the insulin receptors occur at paranodal loops of Schwann cells, endothelial cells, and medium- and small-sized primary sensory neurons (Sugimoto, Murakawa, Zhang et al., 2000; Sugimoto et al., 2002). Insulin therapy seems to correct diabetes-induced disturbances in mitochondrial function in experimental diabetes (Huang et al., 2003) and elevate the production of ATP, which is an important energy source for efficient axon regeneration (Huang et al., 2005). The Na⁺/K⁺-ATPase is also regulated by insulin (Sweeney & Klip, 1998). Furthermore, insulin enhanced regenerative growth of neurons (Fernyhough et al., 1993) and partially reversed deficits in nerve blood flow and conduction in experimental diabetes (Biessels et al., 1996). Previous studies have shown that insulin is a neurotrophic factor responsible for regulating neuronal growth, survival, and differentiation (Toth et al., 2006; Xu et al., 2004).

A recent study has suggested that in the presence of hyperinsulinemia, the PNS can also become insulin resistant, and therefore cannot respond to the neurotrophic properties of insulin (Kim, McLean, Philip, & Feldman, 2011). Considering the important role of insulin as a neurotrophic factor, it was recently suggested that dysfunction in insulin signaling might result in neurodegeneration and thus might be an important factor in the pathogenesis of diabetic neuropathy (Kim & Feldman, 2012). Also, it has been shown that fasting hyperinsulinemia in the presence of normoglycemia and NGT was associated with deteriorated thermal sensations, suggesting a direct effect of insulin resistance on sensory nerve function (Delaney et al., 1994).

Recently, it has been discovered that Schwann cells express insulin receptors (Shetter, Muttagi, & Sagar, 2011). Insulin increased the expression of myelin basic protein (MBP) and myelin associated glycoprotein (MAG) in both diabetic rat sciatic nerves and Schwann cells grown in high glucose conditions (Rachana, Manu, & Advirao, 2016). MBP and MAG are related with the maintenance and formation of the myelin sheath (Readhead et al., 1990; Schachner & Bartsch, 2000). In diabetic rats, it was shown that the expression of these proteins is reduced and results in decreased myelination (Kawashima et al., 2007). Furthermore, Grote, Groover et al. (2013) suggested that the neuronal cell body and peripheral nerve are responsive to intrathecal insulin; and in another study, (Grote, Ryals et al., 2013),
they demonstrated that only the peripheral nerve is responsive to low doses of systemic insulin. Furthermore, Grote, Ryals et al. (2013) suggested that muscle, liver, and adipose tissue may be acting as a “sink” due to their quick utilization of insulin and could reduce available insulin to the PNS, thereby reducing the neurotrophic response. These recent findings may explain why a positive association was only found between insulin level at 120 min and NCV and not with amplitudes. It might be that the more insulin is produced systemically due to insulin resistance, the more insulin is available for the peripheral nerves to be utilized.

In the follow-up study, the SNAP amplitudes decreased consistently and significantly during the 3-year follow-up period. As known from the literature, SNAP amplitudes are the most sensitive indicator of peripheral nerve damage. None of the study participants developed type 2 diabetes during the follow-up, and only a few had IGT or IFG. However, QUICKI values decreased significantly, suggesting that insulin resistance increased among the study subjects. It has been suggested that lower SNAP amplitudes in obese subjects compared to normal-weight subjects are due to the dampening effect of subcutaneous fat tissues (Buschbacher, 1998; Miscio et al., 2005). In the present study, SNAP amplitudes decreased consistently and significantly even though the study subjects did not experience significant weight gain. This suggests that in addition to the dampening effect of the thicker subcutaneous fat, there might be metabolic factors that decrease nerve action potential amplitudes in overweight and obese people without hyperglycemia. This finding is supported by a recent study that compared nerve conduction variables between obese children with insulin resistance, obese children without insulin resistance, and normal-weight children and revealed that SNAP amplitudes were not lower in obese children without insulin resistance compared to normal-weight children. However, obese children with insulin resistance tended to have lower SNAP amplitudes compared to obese children without insulin resistance and normal-weight children, although a statistically significant difference was found only in the sural nerve (Akin et al., 2016).

In addition to insulin resistance, cholesterol levels also could have contributed to the decrease of SNAP amplitudes. In the follow-up study, it was found that increments in triglyceride levels reduced the sural SNAP and increments in HDL cholesterol levels increased the radial SNAP amplitude. However, after Bonferroni correction, these associations were no longer statistically significant. Pittenger et al. (2005) found a negative correlation between HDL cholesterol and sural SNAP amplitude, and they concluded that there might be a sensory neuropathy co-segregating with features of metabolic syndrome. Smith and Singleton (2013)
demonstrated that obesity and triglycerides were independently related to loss of small unmyelinated axons. Furthermore, cholesterol levels were found to be an independent risk factor of small-fiber neuropathy (Bednarik et al., 2009). These findings indicate that cholesterol levels might have a direct association with PNS function. In this study, no variable could be found that significantly explained the reduction of SNAP amplitudes. This might be because of the complex nature of peripheral nerve pathogenesis; hence, most likely, this study’s results could be explained by a combination of multiple factors, such as hyperlipidemia, alterations in insulin signaling in PNS, and oxidative stress.

6.2 Physical activity, fitness, and peripheral nervous system function (II)

In Study (II), an association between physical activity and fitness and PNS function in overweight and obese individuals was shown for the first time. Being overweight or obese is associated with various disorders, including hypertension, hyperlipidemia, insulin resistance, IGT, and type 2 diabetes. Cardiorespiratory fitness is associated with improved health and decreased morbidity, and is inversely related to chronic diseases, such as cardiovascular disease (Blair et al., 1996), high blood pressure (Sallis, Patterson, Buono, & Nader, 1988), metabolic syndrome (S. Lee et al., 2005), IGT, and type 2 diabetes (Wei et al., 1999). In the present study, VO\textsubscript{2}max was significantly associated with peroneal CMAP amplitudes and F-wave persistence.

PAI at the age of 30 years was significantly associated with both motor and sensory nerve conduction measures, further supporting this study’s hypothesis that physical activity is positively associated with PNS function. The number of steps measured by an accelerometer in a 24-hour time period was positively associated with medial plantar SNAP amplitude.

Several mechanisms are described in the literature that might explain the findings of the present study. According to a recent study, voluntary exercise increased axonal regeneration through a neurotrophin-dependent mechanism (Molteni, Zheng, Ying, Gomez-Pinilla, & Twiss, 2004). Another study observed that the concentration of Na+/K+-ATPase increased due to exercise (H. J. Green et al., 1993). In both large and small vessels, exercise augments endothelial, NO-dependent vasodilatation (D. J. Green et al., 2004). Evidence suggests that hypertrophic changes occur in motor neurons due to periods of increased use (Eisen et al., 1973; Tomanek & Tipton, 1967), helping explain the faster motor nerve
conduction velocities in athletes compared to nonathletes (Kamen et al., 1984). Furthermore, Fisher, Langbein, Collins, Williams, & Corzine (2007) demonstrated several physiological improvements after moderate exercise in individuals with diabetic neuropathy, including an increase in cardiorespiratory fitness, motor conduction velocities and CMAP amplitudes, sensory conduction velocities and SNAP amplitudes, and F-wave latencies. Furthermore, 8 weeks of moderate aerobic exercise (40–60 % of heart rate reserve) increased distal peroneal nerve and sural sensory NCV compared to the control group (Dixit, Maiya, & Shastry, 2014). Lifestyle intervention consisting of diet and exercise counseling for patients with neuropathy associated with IGT resulted in cutaneous reinnervation and improved pain (Smith et al., 2006).

6.3 Methodology (I, II & III)

In this study, the subjects consisted of 40 overweight and obese adults living in the Oulu region. The first two studies were cross-sectional, while the third study was a prospective 3-year follow-up. The number of drop outs was quite high—namely, the number of subjects dropped to 29 in the 3-year follow-up. There were some limitations in this study. First, the ability to draw definitive conclusions was limited by the relatively small sample size; in addition, the cross-sectional design in the first 2 studies prevented the provision of evidence of causality. The explanatory variables for glucose metabolism were limited to only insulin and glucose levels. The PNS function measurement was limited to the nerve conduction study, which did not reveal the status of small fibers. QUICKI values might not be a representative evaluation of possible insulin resistance in PNS.

A previous study has suggested that VO2max, assessed with maximal exercise testing, is a better measure of health outcomes than physical activity alone, as fitness assessment is less prone to misclassification (Blair et al., 2001). VO2max reflects cardiorespiratory fitness and does not reveal the type of physical activity engaged in by an individual. Furthermore, VO2max does not leave room to identify how much or what form of physical activity would be optimal when considering PNS function. Therefore, self-rated PAIs were also measured, and accelerometers were used to evaluate individuals’ physical activity. Subjective physical activity evaluation can be prone to misclassification, which should be taken into consideration. A 24-hour measurement period with an accelerometer represents only a sample of an individual’s average activity.
6.4 Future perspectives/work

In order to prevent PN in overweight and obese people without type 2 diabetes, a better understanding of the underlying pathomechanisms is needed as they seem to differ from the pathomechanisms of diabetic neuropathy. Insulin signaling and cholesterol levels are clearly becoming point of interests in these studies. Larger-scale studies are needed to identify the pathomechanisms, as are interventional studies for the prevention of PNS damage. From the prevention point of view, it would be of interest to study how a weight-loss, diet, and lifestyle change affects the PNS in overweight and obese people.
7 Conclusion

In this doctoral thesis, the associations between glucose, insulin and cholesterol levels, and PNS function in overweight and obese adults were investigated. Furthermore, the relationship between physical activity and fitness and PNS function in overweight and obese adults was also examined. Based on the findings, the following conclusions can be made:

1. Serum insulin levels at 120 min after an OGTT are positively associated with PNS function in overweight and obese adults.
2. Aerobic fitness and physical activity are positively associated with PNS function in overweight and obese adults. Overweight and obese people should be encouraged to increase their physical activity, as doing so is beneficial to PNS function.
3. Elevated serum insulin levels might have at least a temporary beneficial/compensatory effect on the myelination of large fibers. Negative alterations in NCS were seen in SNAP amplitudes and thus indicate that overweight and obese people without type 2 diabetes are at risk of PNS damage.
References


Original publications


Reprinted with permission from John Wiley and Sons and Wolters Kluwer.

Original publications are not included in the electronic version of the dissertation.
1449. Kajula, Outi (2018) Periytyvän rinnsyöpäalttiusmutaation (BRCA1/2) kantajamiesten perinnöllisyysneuvontamalli


1452. Capra, Janne (2018) Differentiation and malignant transformation of epithelial cells: 3D cell culture models

1453. Panjan, Peter (2018) Innovative microbioreactors and microfluidic integrated biosensors for biopharmaceutical process control

1454. Saarela, Ulla (2018) Novel culture and organoid technologies to study mammalian kidney development


1456. Vuollo, Ville (2018) 3D imaging and nonparametric function estimation methods for analysis of infant cranial shape and detection of twin zygosity


1461. Pasanen, Anu (2018) Genetic susceptibility to childhood bronchiolitis

1462. Kääriä, Juha (2018) Family history of mental disorders and long-term outcome in schizophrenia

1463. Xu, Qi (2018) Role of Wnt11 in kidney ontogenesis and development of renal organoid based models to identify candidate oncogenes

Henri Isojärvi

ASSOCIATION OF GLUCOSE METABOLISM, PHYSICAL ACTIVITY AND FITNESS WITH PERIPHERAL NERVOUS SYSTEM FUNCTION IN OVERWEIGHT PEOPLE