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ASSOCIATIONS OF CHRONOTYPE WITH DIETARY HABITS, OBESITY AND GENETICS

A population-based study in Finnish adults

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ABSTRACT

Chronotype refers to preferences in timing the daily activities; accordingly, individuals can be divided from extreme morning to extreme evening types. The evening type has been associated with unhealthier behavior and higher morbidity and mortality risk than the morning type. Furthermore, twin studies have suggested genetic underpinnings behind chronotype trait. However, chronotype associations of dietary habits, obesity and genetics have not been thoroughly examined.

The aim of this thesis was to study the associations between chronotype, dietary habits (overall diet quality, energy and macronutrient intake timing) and obesity (weight, body mass index [BMI], waist circumference, body fat percentage) and the interrelationships between these factors. This thesis additionally aimed to clarify the genetic basis of chronotype (clock gene analysis, genome-wide association study [GWAS] of chronotype, developing a genetic risk score [GRS] for chronotype).

The study population included participants from the population-based National FINRISK 2007 (n=9958) and 2012 (n=9905) studies and the following sub-studies of FINRISK 2007: DIetary Lifestyle and Genetic determinants of Obesity and Metabolic syndrome (DILGOM) 2007 (n=5024), DILGOM 2014 (n=3735, follow-up) and National FINDIET 2007 (n=2054) conducted at the Finnish Institute for Health and Welfare. Chronotype was assessed with a shortened morningness–eveningness questionnaire. Overall diet was assessed with a validated food frequency questionnaire and measured with the Baltic Sea Diet Score (BSDS), which illustrates adherence to the healthy Nordic diet. Energy and macronutrient intake timing was assessed with 48-hour dietary recalls and 3-day food records. Anthropometric measures were based on measured and self-reported values. Statistical analyses were conducted with analysis of covariance and with linear and logistic regression.

The evening type was associated with lower adherence to the healthy diet and with lower energy and macronutrient intake (except for sucrose [E%]) in the morning (by 10:00 a.m.) and higher energy, sucrose (E%), fat (E%) and saturated fat (E%) intakes in the evening (after 8:00 p.m.). Differences between morning and evening types in energy and macronutrient intake timing were even more pronounced at the weekend.

Those with a higher tendency towards eveningness more likely had a lower baseline BMI in men but not in women. A higher percentage of evening-typed women had at least a 5% increase in weight and BMI than did morning-typed women during a seven-year follow-up period. These associations, however, attenuated after excluding participants with depression.

When interrelationships between chronotype, dietary habits and obesity were examined, no evidence was found that the BSDS would mediate the association between chronotype and obesity or that chronotype would modify the association between the BSDS and obesity. Instead, higher evening energy intake was associated with a higher obesity risk independent of chronotype.

Clock gene analysis revealed a novel association between chronotype and the *NR1D2* gene. No genome-wide significant associations were found, but the genetic risk score based on 313 single nucleotide polymorphisms (SNPs) that have previously been associated with chronotype predicted the chronotypes in the present study population.

In conclusion, despite unhealthier dietary habits (lower adherence to the BSDS, later energy intake timing) of evening chronotypes, evening types were not significantly more prone to obesity nor did chronotype play a role in the association between healthy diet/energy intake timing and obesity. Furthermore, a novel clock gene association was found with the *NR1D2* clock gene, which has previously been demonstrated to have a role in carbohydrate and lipid metabolism. A GRS based on GWAS studies of chronotype may be a useful tool for capturing the genetic aspect of chronotype in different populations.

Keywords: Baltic Sea diet, Chronotype, Diet, Diet quality, Epidemiology, Genetics, Morningness-Eveningness, Obesity, Timing of food intake

TIIVISTELMÄ

Kronotyypillä viitataan yksilöiden välisiin eroihin päivittäisten toimien ajoittumisessa. Kronotyypin mukaan yksilöt voidaan luokitella jatkumolla aamutyypeistä iltatyyppeihin. Kaksostutkimuksissa on havaittu, että kronotyyppiin vaikuttavat sekä perimä että ympäristötekijät. Iltatyyppisyys on yhdistetty aamutyyppejä epäterveellisempiin elämäntapoihin sekä suurempaan riskiin sairastua tai kuolla ennenaikaisesti. Kronotyypin geneettisistä yhteyksistä sekä kokonaisruokavalion laadusta ja energiansaannin ajoittumisesta tiedetään kuitenkin vasta vähän. Lisäksi kronotyypin yhteydet lihavuuteen ovat epäselviä.

Tämän tutkimuksen tavoitteena oli tutkia kronotyypin yhteyttä ravintoon (kokonaisruokavalion laatu, energian ja energiaravintoaineiden saannin ajoittuminen) ja lihavuuteen (paino, painoindeksi, vyötärönympärys, kehon rasvaprosentti) sekä näiden tekijöiden keskinäisiä yhteyksiä. Lisäksi tutkimuksen tavoitteena oli tutkia kronotyypin geneettistä taustaa (yhteydet vuorokausirytmiä säätteleviin kellogeeneihin, genomin laajuinen assosiaatio tutkimus sekä kronotyyppiä ennustavan geneettisen indeksin kehittäminen).

Tutkimuksissa käytettiin Terveyden ja hyvinvoinnin laitoksen koordinoimia väestötutkimuksia: Kansallinen FINRISKI 2007 (n=9958) ja FINRISKI 2012 (n=9905). Näiden lisäksi käytettiin kahta FINRISKI 2007 -tutkimuksen alaotosta: DIetary Lifestyle and Genetic determinants of Obesity and Metabolic syndrome (DILGOM) 2007 (n=5024, alkutilanne), DILGOM 2014 (n=3735, seuranta) sekä kansallista FinRavinto 2007 -tutkimusta (n=2054). Tutkimuksissa käytettiin validoituja menetelmiä; kronotyyppi arvioitiin lyhennetyllä aamu- ja iltatyyppisyyskyselyllä, kokonaisruokavalion mittaamiseen käytettiin frekvenssityyppistä ruoankäyttökyselyä (FFQ) ja ruokavalion laadun mittarina käytettiin Itämeren ruokavalioindeksiä (BSDS), joka perustuu terveelliseen Pohjoismaiseen ruokavalioon. Energian ja energiaravintoaineiden ajoittuminen mitattiin 48-tunnin ruoankäyttöhaastatteluilla sekä 3-päivän ruokapäiväkirjalla. Käytetyt lihavuusmittarit perustuivat mitattuihin sekä raportoituihin arvoihin. Tilastollisissa analyyseissä käytettiin kovarianssianalyysia sekä lineaarista ja logistista regressiota.

Iltatyyppisyys oli yhteydessä huonompaan kokonaisruokavalion laatuun sekä pienempään energian ja energianravintoaineiden (lukuun ottamatta sakkaroosi [E%]) saantiin aamulla (klo 10 mennessä) ja suurempaan energian, sakkaroosin (E%), rasvan (E%) sekä tyydyttyneen rasvan (E%) saantiin illalla (klo 20 jälkeen) verrattuna aamutyyppeihin. Viikonloppuna erot energian ja energiaravintoaineiden saannin ajoittumisessa korostuivat.

Miehillä iltatyyppisyys oli yhteydessä pienempään painoindeksiin, mutta naisilla ei tätä yhteyttä löytynyt. Sen sijaan seitsemän vuoden seurannan aikana niiden naisten osuus, jotka lihoivat vähintään 5% oli iltatyypeissä

suurempi kuin aamutyypeissä. Nämä yhteydet kuitenkin heikkenivät, kun ne tutkittavat, joilla oli diagnosoitu masennus, poistettiin analyyseistä.

Tutkittaessa kronotyypin, ruokavalion ja lihavuuden keskinäisiä yhteyksiä havaittiin, että ruokavalion laatu ei selittänyt kronotyypin ja lihavuuden välistä yhteyttä. Kronotyyppi ei myöskään modifioinut ruokavalion laadun ja lihavuuden välistä yhteyttä. Lisäksi energian kokonaissaannissa ei ollut eroja kronotyppien välillä, mutta sen sijaan energiansaannin painottuminen iltaan oli yhteydessä lihavuuteen kronotyypistä riippumatta.

Tutkittaessa kellogeenien ja kronotyypin välistä yhteyttä löydettiin uusi yhteys *NR1D2* geenin ja kronotyypin välillä. Genomin laajuisessa assosiaatiotutkimuksessa ei merkitseviä yhteyksiä löytynyt. Sen sijaan aiemmin kronotyyppiin yhdistettyihin geneettisiin variaatioihin perustuva geneettinen indeksi oli yhteydessä kronotyyppiin tässä aineistossa.

Yhteenvetona voidaan todeta, että iltatyyppien epäterveellisemmistä ruokatottumuksista (huonompi ruokavalion kokonaislaatu, myöhäisempi energiansaannin ajoittuminen) huolimatta lihavuuden riski ei heillä ollut merkittävästi muita suurempi. Iltapainotteinen energiansaanti sen sijaan oli yhteydessä lihavuuteen kronotyypistä riippumatta. Kronotyyppi oli yhteydessä *NR1D2* kellogeeniin, joka on aikaisemmin yhdistetty hiilihydraatti- ja rasva-aineenvaihduntaan. Kronotyypin geneettistä indeksiä voisi hyödyntää kronotyypin tunnistamisessa eri väestöissä.

Avainsanat: Aamu- ja iltatyyppisyys, Epidemiologia, Itämeren ruokavalio, Kronotyyppi, Ruokavalio, Ruokavalion kokonaislaatu, Genetiikka, Lihavuus, Ravinnon saannin ajoittuminen, Väestötutkimus

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications and some unpublished results from additional analyses:

I Maukonen M, Kanerva N, Partonen T, Kronholm E, Konttinen H, Wennman H, Männistö S (2016). The associations between chronotype, a healthy diet and obesity. *Chronobiol. Int.* **33**:972–981.

II Maukonen M, Kanerva N, Partonen T, Kronholm E, Tapanainen H, Kontto J, Männistö S (2017). Chronotype differences in timing of energy and macronutrient intakes: A population-based study in adults. *Obesity* (Silver Spring) **25**:608–615.

III Maukonen M, Kanerva N, Partonen T, Männistö S (2019). Chronotype and energy intake timing in relation to changes in anthropometrics: a 7-year follow-up study in adults. *Chronobiol. Int.* **36**:27–41.

IV Maukonen M, Havulinna AS, Männistö S, Kanerva N, Salomaa V, Partonen T (2020). Genetic associations of chronotype in the Finnish general population. *J. Biol. Rhythms.* **35**:501–511.

The publications are referred to in the text by their Roman numerals.

ABBREVIATIONS

%TEI	Percentage of total energy intake
ANCOVA	Analysis of covariance
ARNTL1-2	Aryl hydrocarbon receptor nuclear translocator-like gene (aka *BMAL1-2*) encoding ARNTL1-2/BMAL1-2 proteins
BF%	Body fat percentage
BH	Benjamini-Hochberg false discovery rate method
BHLHE40-41	Basic helix-loop-helix family member E40-41 genes encoding BHLHE40-41 proteins
BMAL1-2	Brain and Muscle ARNT-Like 1-2 genes (aka *ARNTL*1-2) encoding BMAL1-2/ARNTL1-2 proteins
BMI	Body mass index
BSDS	Baltic Sea Diet Score
BY	Benjamini-Yekutieli false discovery rate method
CI	Confidence interval
CLOCK	Clock circadian regulator gene (aka Circadian Locomotor Output Cycles Kaput) encoding CLOCK proteins
CRP	C-reactive protein
CRY1-2	Cryptochrome circadian regulator 1-2 genes encoding CRY1-2 proteins
CSM	Composite scale of morningness
CSNK1D	Casein kinase 1 delta gene encoding CSNK1D protein
CSNK1E	Casein kinase 1 epsilon gene encoding CSNK1E protein
DILGOM	DIetary Lifestyle and Genetic determinants of Obesity and Metabolic syndrome
DLMO	Dim light melatonin onset
E%	Percentage of energy intake
FFQ	Food frequency questionnaire
GRS	Genetic risk score
GWAS	Genome-wide association study
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
HWE	Hardy Weinberg equilibrium
IL-6	Interleukin-6
INFO	Imputed information score
LD	Linkage disequilibrium
LDL	Low-density lipoprotein cholesterol
MAF	Minor allele frequency
MCTQ	Munich ChronoType Questionnaire
ME	Morningness-eveningness
MEQ	Morningness-Eveningness Questionnaire
MSF	Midpoint of sleep on free days

NFIL3	Nuclear factor interleukin-3-regulated gene encoding NFIL3 protein
NPAS2	Neuronal PAS domain protein 2 gene encoding NPAS2 protein
NR1D1-2	Nuclear receptor subfamily 1 group D member 1-2 genes (aka *REV-ERBα-β*) encoding NR1D1-2/REV-ERBα-β proteins
OR	Odds ratio
REV-ERBα-β	Nuclear receptor Rev-Erb-alpha-beta gene *(*aka *NR1D1-2)* encoding REV-ERBα-β/NR1D1-2 proteins
RORα/β/γ	Retinoic acid receptor-related orphan receptor alpha/beta/gamma genes encoding RORα/β/γ proteins
PER1-3	Period circadian regulator 1-3 genes encoding PER1-3 proteins
PUFA	Polyunsaturated fatty acids
SCN	Suprachiasmatic nucleus
SAFA	Saturated fatty acids
SE	Standard error
SNP	Single nucleotide polymorphism
TNF-α	Tumor necrosis factor alpha
THL	Finnish Institute for Health and Welfare
WHO	World Health Organization
WC	Waist circumference

1 INTRODUCTION

A fundamental feature of life on Earth is its capacity to adapt to the daily changes in the surrounding environment. To adapt to these changes different life forms have developed internal time-keeping systems that anticipate changes in light and temperature and help them to optimize their physiology and behavior to the Earth's rotation (Vitaterna et al. 2001). This time-keeping system in human beings consists of approximately 24-hour cycles that create the circadian rhythm.

Circadian rhythms are created by circadian clocks (Takahashi 2017). Circadian clocks have an important role in regulating metabolism, and many metabolic processes follow the 24-hour light and dark cycle (Bass and Takahashi 2010). Chronotype refers to individual preferences in the timing of daily activities that reflect the underlying intrinsic circadian rhythms (Horne and Östberg 1976). Epidemiological studies have shown that later chronotypes (evening types) have a higher risk of morbidity and premature mortality than earlier chronotypes (morning types) (Merikanto et al. 2013b, Broms et al. 2014, Patterson et al. 2018, Knutson and von Schantz 2018). However, findings regarding chronotype and obesity have been inconsistent (Johnsen et al. 2013, Celis-Morales et al. 2017, Sun et al. 2020).

Several observational studies have further shown that evening types have a higher consumption of unhealthier foods than morning types (Sato-Mito et al. 2011a, Kanerva et al. 2012, Patterson et al. 2016). However, the associations between chronotype and dietary habits have not been thoroughly studied. Population-based studies on overall diet quality of chronotypes and differences in timing of food intake are lacking.

Studying an overall diet quality may reveal stronger diet-health associations than studying the role of single nutrients and foods alone (Hu 2002). For example, the Baltic Sea Diet score (BSDS) based on the healthy Nordic diet has been associated with a lower risk of abdominal obesity (Kanerva et al. 2013) and weight change (Kanerva et al. 2018).

The timing of food intake may also have a crucial effect on metabolic health. Animal models have suggested that eating the same amount of calories during the rest period versus the active period may lead to weight gain (Arble et al. 2009). Observational studies in humans have suggested a link between evening energy intake and obesity, whereas morning calories have been associated with a lower obesity risk (Fong et al. 2017, Beccuti et al. 2017), though evidence from trials is controversial (Fong et al. 2017).

Furthermore, chronotype has genetic underpinnings with twin and family studies that estimate about 50% heritability (Koskenvuo et al. 2007, Barclay et al. 2010). Previous studies aiming to identify genetic polymorphisms associated with chronotype have been either small-scale studies or larger-scale studies in which chronotype assessments have been based on a single

self-evaluation question on chronotype (Barclay et al. 2011, Parsons et al. 2014, Hu et al. 2016, Jankowski and Dmitrzak-Weglarz 2017, Jones et al. 2019).

The aim of this population-based study was to examine the associations of chronotype with overall diet quality (assessed with the BSDS), energy and macronutrient intake timing, obesity and weight change. Furthermore, this study aimed to extend the knowledge of the genetic basis of chronotype by conducting a clock gene analysis, a genome-wide association study (GWAS) of chronotype, and by developing a genetic risk score for chronotype.

2 REVIEW OF THE LITERATURE

2.1 CIRCADIAN RHYTHM AND CHRONOTYPE

2.1.1 CIRCADIAN RHYTHM IN HUMANS

Circadian rhythms are endogenous and entrainable oscillations of approximately 24 hours (Takahashi 2017). Circadian rhythms regulate the sleep-wake cycle, enabling individuals to anticipate and prepare for the environmental changes as well as coordinate behavioral and metabolic processes with the environment.

Circadian rhythms are generated, maintained and controlled by circadian clocks (Takahashi 2017). The central circadian clock located in the hypothalamic suprachiasmatic nucleus (SCN) of the brain acts as a master pacemaker synchronizing peripheral clocks (Figure 1). Peripheral clocks are present in nearly all cells and tissues, including those important in terms of metabolism such as the liver, pancreas, gastrointestinal tract, and adipose tissue (Bass and Takahashi 2010, Ribas-Latre and Eckel-Mahan 2016, Reinke and Asher 2019). Circadian rhythms consequently have an important role in controlling metabolism, in particular energy, lipid and glucose metabolism and, for example, insulin sensitivity and secretion, cholesterol synthesis, energy expenditure and blood pressure follow the 24-hour rhythms (Bass and Takahashi 2010, Reinke and Asher 2019). This rhythmicity can also be seen in many processes related to digestion, for example, gastric emptying seems to peak in the morning (Goo et al. 1987), postprandial glucose response tends to decrease towards evening (Van Cauter et al. 1989, Qian et al. 2018) and diet-induced thermogenesis seems to be lower in the evening than in the morning (Romon et al. 1993, Morris et al. 2016). Other physiological processes that follow the 24-hour cycle include body temperature and the secretion of many hormones such as melatonin and cortisol, for example (Rivkees 2007).

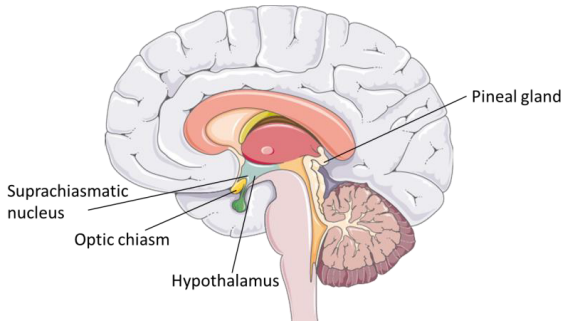

Figure 1 The location of the suprachiasmatic nucleus (SCN) in the anterior hypothalamus of the brain. Modified from Smart Servier Medical Art (http://smart.servier.com) image with license CC-BY 3.0.

At the cellular level, the central and peripheral clocks form similar clock gene machinery consisting of interconnected transcriptional and translational feedback loops in which the expression of clock genes is regulated by the proteins they produce (Figure 2).

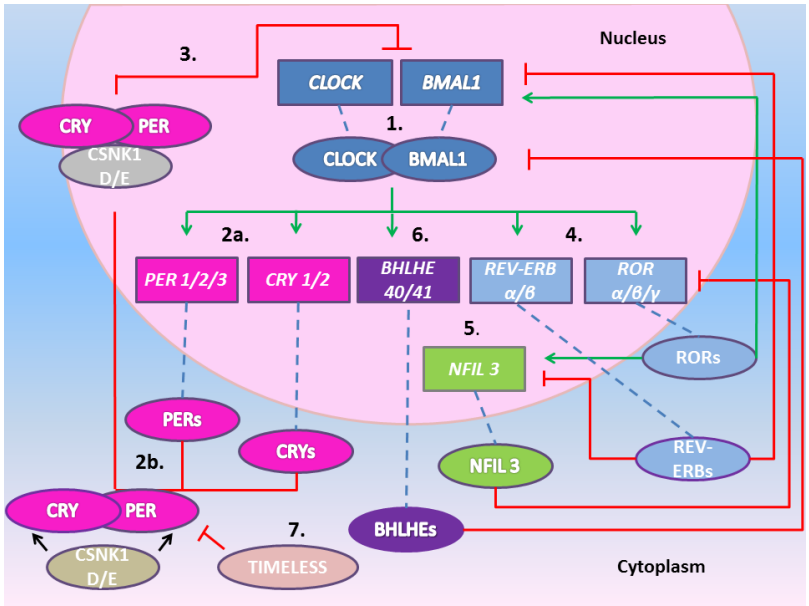

Figure 2 A simplified molecular mechanism of the circadian clock machinery in humans. **1.** The 'core' clock genes Brain and Muscle ARNT-Like 1 (*BMAL1*) (also known as *ARNTL1*) or its interchangeable *BMAL2* and Circadian Locomotor Output Cycles Kaput (*CLOCK)* (or its paralog, *NPAS2*) encode activator proteins that form a heterodimer, which drives the rhythmic expression of the clock-controlled genes (DeBruyne et al. 2007, Shi et al. 2010, Bass and Takahashi 2010). **2a**. The CLOCK/BMAL1 heterodimer activates a feedback loop consisting of repressor clock genes *Period* (*PER1, PER2, PER3*) and *Cryptochrome* (*CRY1*, *CRY2*) at the beginning of the day (Takahashi 2017). **b.** During the day the PER and CRY proteins form a heterodimer that interacts with the Casein Kinase 1 Delta (CSNK1D) and Casein Kinase 1 Epsilon (CSNK1E) proteins in the cytoplasm. **3.** During the night this complex translocates to the nucleus and represses the transcription of *PER* and *CRY* genes by directly inhibiting the transcriptional activity of the CLOCK/BMAL1 heterodimer. **4.** The second CLOCK/BMAL1 activated feedback loop consist of the nuclear receptors genes *REV-ERBα* (also known as *NR1D1*) and *REV-ERBβ* (also known as *NR1D2*) and competing retinoic acid-related orphan receptor genes (*RORα, RORβ*, *RORγ),* which both peak in the daytime. REV-ERBs act as transcription repressors for *BMAL1* gene and in this way inhibit their own transcription, whereas RORs are transcription activators for the *BMAL1*. **5.** In an additional short feedback loop, REV-ERBs and RORs compete in repressing or activating the transcription of interleukin-3-regulated protein (NFIL3). NFIL3 in turn acts as a repressor and represses the transcription of RORs. **6.** Another additional feedback loop consists of CLOCK/BMAL1 activated basic helix-loop-helix family members (*BHLHE40*, *BHLHE41)*. BHLHE40 and BHLHE41 inhibit their own production by repressing the CLOCK/BMAL1-induced transactivation by binding to the BMAL1 protein (Sato et al. 2018). **7.** The role of the *TIMELESS* clock gene in the mammalian circadian clock machinery has been unclear, but it seems that TIMELESS acts as another negative regulator of CLOCK/BMAL1-induced transactivation of PER1 possibly by destabilizing PER2/CRY2 complex in the cytoplasm (Kurien et al. 2019). *Red lines*: repression, *Green lines*: activation. Figure by the author modified from Ramsey et al. (2007) and Takahashi (2017).

The timing (phase) of the circadian rhythm is produced by the circadian clock but influenced by external time cues called Zeitgebers (German for "time giver" or "synchronizer") (Roenneberg and Merrow 2007, Andreani et al. 2015). These entrain the circadian rhythms to the local environment and the 24-hour cycle on light/dark transitions by modulating the temporal expression of the clock genes. In the absence of external time-cues, circadian rhythms will start to follow an endogenous length of the day (period), which is close to 24 hours (called the free-running period) (Vitaterna et al. 2001, Roenneberg and Merrow 2007, Rivkees 2007). The anticipation of external cues in the environment aims to ensure that the organism's physiological and behavioral processes occur at the optimum time of the day at the habitat. The central clock's rhythm is primarily entrained by light, whereas food intake is the most potent entrainer for peripheral clocks, although the integrated inputs from the central clock and other external factors, such as physical activity, also play a role in entraining the peripheral clocks (Roenneberg and Merrow 2007, Reinke and Asher 2019). When circadian rhythm is entrained to a 24-hour cycle of light/dark transitions, it is called a diurnal rhythm for day-active organisms such as humans and a nocturnal rhythm for night-active organisms (Vitaterna et al. 2001).

Disruptions in circadian rhythms may have detrimental consequences on our metabolic health (Baron and Reid 2014). Circadian misalignment refers to a state in which circadian rhythms are disrupted, resulting from inappropriately timed sleep and wake behavior with biological time (Wittmann et al. 2006, Baron and Reid 2014). Circadian misalignment may also lead to misalignment between central and peripheral clocks. Modern lifestyles give rise to different forms of circadian misalignment, including rotating shift work and social jetlag. Shift workers are exposed to chronic circadian misalignment, and shiftwork has consequently been associated with a higher risk of obesity (Liu et al. 2018) and cardiovascular diseases (Torquati et al. 2018). Social jetlag refers to a situation in which social schedules (e.g., work and school) interfere with biological time such that the individual accumulates a considerable sleep debt during work days that they compensate for on free days (Wittmann et al. 2006). Social jetlag has also been associated with obesity and metabolic dysfunctions (Roenneberg et al. 2012, Koopman et al. 2017, Islam et al. 2018). Furthermore, laboratory studies with a forced desynchrony protocol (participants are scheduled to a day length that is much longer or shorter than the 24-hour day) on otherwise healthy humans have demonstrated that eating and sleeping out of sync with intrinsic circadian time may result in metabolic dysfunctions, such as reduced glucose tolerance and insulin resistance, decreases in the satiety hormone leptin, and increased blood pressure (Scheer et al. 2009, Buxton et al. 2012, Morris et al. 2016).

Furthermore, it has been suggested that mutations in circadian and clock-controlled genes may also lead to circadian misalignment (Baron and Reid 2014). Many studies have consequently shown that single nucleotide

polymorphisms (SNPs, the most common type of genetic variation among individuals, referring to a single base-pair difference in DNA) in many of the clock genes may play a role in metabolic dysfunctions, obesity and even in dietary intake (Woon et al. 2007, Garaulet et al. 2009, 2010, 2014, Goumidi et al. 2013, Garcia-Rios et al. 2014, Dashti et al. 2014, Corella et al. 2016).

2.1.2 CHRONOTYPE

Definition

Chronotype refers to individual preferences in the timing of daily activities such as sleeping, eating, and exercising that reflect the underlying intrinsic circadian rhythms (Horne and Östberg 1976). Individuals can, accordingly, be divided from extreme morning to extreme evening types. Morning types prefer waking up relatively early and their peak alertness is in the morning hours, whereas evening types prefer staying up relatively late and their peak alertness is in the evening hours.

Chronotype is considered to be a rather stable trait, although it may be subject to age and gender differences. Cross-sectional data (e.g., Roenneberg et al. 2007) have shown that evening types tend to be younger than morning types with the peak of eveningness reached during the period of adolescence, whereas the longitudinal data on the association between chronotype and age are scarce (Broms et al. 2014, Didikoglu et al. 2019). The first longitudinal study was based on the data from the Male Former Top Athletes study from Finland (n=567, 23.4 years follow-up) (Broms et al. 2014). It showed a shift towards morningness over time, but only a very few shifted from the clearly evening to clearly morning type or vice versa. These findings were supported by a recent longitudinal study from the UK based on the University of Manchester Longitudinal Study of Cognition in Normal Healthy Old Age data (n=6375, 35.5 years follow-up), which showed a shift towards earlier sleep timing with age (Didikoglu et al. 2019). The shift towards an earlier chronotype with age may at least be partly explained by findings indicating a higher premature all-cause mortality risk among evening types, which has also been found in both aforementioned studies and among the UK Biobank data (n=433, 268) (Broms et al. 2014, Knutson and von Schantz 2018, Didikoglu et al. 2019). Furthermore, evening types seem to be men more often than women, although these differences seem to diminish with age (Randler and Engelke 2019).

Chronotype assessment methods

Chronotype is usually assessed in observational studies with validated questionnaires, of which the most widely used one is Horne & Östberg's (1976) Morningness-Eveningness Questionnaire (MEQ). The original MEQ has 19 items on the individual's preferences to time the daily activities, including a self-evaluation question on chronotype. Based on the responses

to the items, a sum score ranging from 16 to 86 is calculated that indicates a respondent's tendency towards eveningness versus morningness. The MEQ has been validated against daily changes in core body temperature and the dim light melatonin onset (DLMO) (Horne and Östberg 1976, Bailey and Heitkemper 2001, Kantermann et al. 2015). Different shortened versions have been further developed from the MEQ, such as a reduced five-item (items 1, 7, 10, 18, 19) version (rMEQ) (Adan and Almirall 1991). The Composite Scale for Morningness (CSM) is a questionnaire with 13 items, in which nine items are from the MEQ (Smith et al. 1989). Another widely used questionnaire for assessing chronotype is the Munich ChronoType Questionnaire (MCTQ) (Roenneberg et al. 2007), in which chronotype assessment is based on the midpoint of sleep on free days (MSF) calculated from sleep onset and offset times on free days. Both the MCTQ and the CSM have been found to correlate with the MEQ (Zavada et al. 2005, Di Milia et al. 2013).

Chronotype and health

Numerous studies have shown that the evening chronotype associates with unhealthier behaviors and a higher morbidity risk. Evening types are more often smokers and physically inactive compared to morning types (Nakade et al. 2009, Wennman et al. 2015, Patterson et al. 2016, Hisler et al. 2017). Furthermore, evening types more often report unhealthier sleeping patterns with insomnia, nightmares and insufficient sleep (Merikanto et al. 2012). Evening types are more prone to misalignment of circadian rhythms (social jetlag), because they are more likely to live against their biological time due to societies' social structures (e.g., Wittmann et al. 2006). They are also more prone to psychological disorders such as depression (Merikanto et al. 2015, Vetter et al. 2018) and have a higher risk of cardiovascular disease, hypertension, type 2 diabetes and metabolic dysfunctions with poorer glycemic control among individuals with diabetes (Merikanto et al. 2013b, Reutrakul et al. 2014, Yu et al. 2015, Patterson et al. 2018, Knutson and von Schantz 2018).

2.2 GENETICS AND CHRONOTYPE

2.2.1 HERITABILITY

Heritability is defined as the degree of individual genetic variation that accounts for phenotypic variation seen in a population at a particular time and age (Visscher et al. 2008). Genetic effects can be further divided into additive or non-additive effects (Visscher et al. 2008, Mayhew and Meyre 2017). Additive genetic effects are the sum of the individual effects of each allele at two or more loci that influence the phenotype. Non-additive genetic

effects include interactions between alleles at the same locus (dominance) or interactions between alleles at different loci (epistasis). Narrow-sense heritability (h^2) refers to the additive genetic effects, and it measures how much of a variation among the parent's phenotype is passed to their offspring. Broad-sense heritability (H^2) is the ratio of total genetic variance to a phenotype, and it includes all genetic aspects of heritability.

The classic way to determine heritability is by using twin or family studies. Regarding the genetic basis of chronotype, twin and family studies have shown heritability estimates for chronotype ranging from 37% to 54% (Hur et al. 1998, Vink et al. 2001, Hur 2007, Koskenvuo et al. 2007, Barclay et al. 2010, Watson et al. 2013, von Schantz et al. 2015). In Finland, the overall heritability estimate was 50% (broad-sense heritability) with estimates of 12% for additive genetic effects and 38% for non-additive (dominant) genetic effects (Koskenvuo et al. 2007). Genetic effects on chronotype may attenuate with age, particularly during the period from 36 to 64 years (Barclay et al. 2014). This may be driven by environmental entrainers (e.g., work and family responsibilities) playing a more important role during that age period.

2.2.2 GENETICS

An increasing interest has been previously focused on searching for identifying genetic polymorphisms associated with chronotype. Most of the studies have been conducted using a candidate gene approach, but four genome-wide association studies of chronotype have also been published (Hu et al. 2016, Lane et al. 2016, Jones et al. 2016, 2019).

Candidate gene approach studies

Candidate gene studies focus on selecting potential candidate genes in relation to a phenotype of interest; prior knowledge of the mechanisms and genes underlying the certain phenotype is needed to do that (Kwon and Goate 2000). Clock genes pose a strong candidate gene group for chronotype associations not only because they control the circadian rhythms but also because of their important role in regulating metabolism (see section 2.1.1 on page 16). The first reported genetic association of chronotype was with *CLOCK* gene SNP rs1801260 on middle-aged US citizens (n=410) (Katzenberg et al. 1998). Since then this finding has been replicated in one study on Japanese adults (n=421) (Mishima et al. 2005), but, despite several attempts, more recent studies over the last decade have been unable to replicate the finding (Choub et al. 2011, Chang et al. 2011, Barclay et al. 2011, Etain et al. 2014, Kripke et al. 2014, Parsons et al. 2014, Song et al. 2016, Kim et al. 2016). Findings involving SNPs in other key clock genes (see Figure 2 for key clock genes on page 18) have also been inconsistent and mostly small-scale studies (Table 1), which points to the need for further research in various and larger population-based samples.

Table 1. *Candidate gene approach studies on associations between chronotype and clock genes SNPs published over the past decade.*

SNP	Gene	Association found	n	Age	Chronotype measure	Population	Study, country
rs11824092	*ARNTL*	yes (controls)	111 + 126	22-83	MEQ	Mood disorder patients and controls	Dmitrzak-Weglarz et al. 2016, Poland
rs1481892	*ARNTL*	yes (controls)	111 + 126	22-83	MEQ	Inpatients with mood disorders and controls	Dmitrzak-Weglarz et al. 2016, Poland
		no	338	18-32	CSM	University students	Jankowski and Dmitrzak-Weglarz 2017, Poland
rs12363415	*ARNTL*	yes (MI patients)	200 + 200	64 ± 13	MEQ	Myocardial infarction (MI) patients and controls	Škrlec et al. 2019, Croatia
rs922270	*ARNTL2*	yes	952	18-27	MEQ	Population sample of young adults	Parsons et al. 2014, UK
rs1268271	*CLOCK*	yes (controls)	111 + 126	22-83	MEQ	Mood disorder patients and controls	Dmitrzak-Weglarz et al. 2016, Poland
		no	925	36 ± 12	MEQ	Individuals with sleep disorder and controls	Hida et al. 2014, Japan
rs2292912	*CRY2*	yes (controls)	200 + 200	64 ± 13	MEQ	Myocardial infarction (MI) patients and controls	Škrlec et al. 2019, Croatia
rs2482705	*NFIL3*	yes	45	22-82	MEQ	Individuals with delayed phase or bipolar disorder	Kripke et al. 2014, USA
rs934945	*PER2*	yes	299	18-32	CSM	Medical college students	Lee et al. 2011, Korea
		yes	499	18-33	CSM	Healthy volunteers	Song et al. 2016, Korea
		no	925	36 ± 12	MEQ	Individuals with sleep disorder and controls	Hida et al. 2014, Japan
		no	952	18-27	MEQ	Population sample of young adults	Parsons et al. 2014, UK
		no	200 + 200	64 ± 13	MEQ	Myocardial infarction (MI) patients and controls	Škrlec et al. 2019, Croatia

Table 1 continues

SNP	Gene	Association found	n	Age	Chronotype measure	Population	Study, country
rs35333999	*PER2*	yes (controls)	200 + 200	64 ± 13	MEQ	Myocardial infarction (MI) patients and controls	Škrlec et al. 2019, Croatia
rs335789	*PER2*	yes	335 789	40-69	single item chronotype[1]	The UK biobank study participants	Chang et al. 2019, UK
rs228697	*PER3*	yes	925	36 ± 12	MEQ	Individuals with sleep disorder and controls	Hida et al. 2014, Japan
rs10462020	*PER3*	yes	952	18–27	MEQ	Population sample of young adults	Parsons et al. 2014, UK
rs3828057	*RORC*	yes	45	22-82	MEQ	Individuals with delayed phase or bipolar disorder	Kripke et al. 2014, USA
rs774045	*TIMELESS*	yes	100 + 72	mean >50	CSM	Individuals with bipolar disorder and controls	Etain et al. 2014, France
rs2291738	*TIMELESS*	yes	338	18-32	CSM	University students	Jankowski and Dmitrzak-Weglarz 2017, Poland

CSM; Composite Scale of Morningness, MEQ; morningness-eveningness questionnaire

[1] Chronotype was assessed with a single self-evaluation question on chronotype: “There are so called morning people and evening people, which are you?”

GWASs of chronotype

GWASs, unlike candidate gene approach studies, do not require a priori knowledge of genes, which could underlie the phenotype of interest (Tam et al. 2019). Instead, GWAS searches the entire genome for genetic variations that occur more commonly in individuals with that particular phenotypic trait of interest.

The previous GWASs of chronotype have identified 351 independent loci associated with chronotype (Hu et al. 2016, Lane et al. 2016, Jones et al. 2016, 2019). Clock genes are also among these loci, among which *PER2* was identified by all four GWASs, thus supporting the findings from the candidate gene approach studies related to *PER2* (e.g., Lee et al. 2011, Song et al. 2016). Other loci among those identified are, for example, enriched for genes involved in insulin signaling pathways (Jones et al. 2019).

The four GWASs are based on two large cohorts, the 23andMe (Hu et al. 2016) and the UK Biobank Study (Lane et al. 2016, Jones et al. 2016). The most recent and the largest of the GWASs is a meta-analysis of these two cohorts (n=697,828) (Jones et al. 2019). Chronotype assessment of both cohorts was based on a single self-evaluation question. The 23andMe questionnaire included two identically worded questions ("Are you naturally a night person or a morning person?"), whereas the question in the UK Biobank study ("There are so called morning people and evening people, which are you?") was a modification from the original MEQ item 19 ("One hears about 'morning' and 'evening' types of people. Which ONE of these types do you consider yourself to be?"). Thus far, there are no published GWASs of chronotype with a validated, questionnaire-based chronotype assessment.

2.3 DIETARY HABITS AND CHRONOTYPE

Dietary assessment methods

Measuring dietary intake is challenging, and all the specific methods (food frequency questionnaires [FFQ]), diet records and dietary recalls) have limitations. A dietary assessment method should be selected with consideration of research aims, hypothesis, design, and available resources.

The FFQ, the main method used for assessing diet in epidemiological studies, was developed to assess the habitual diet, usually from the previous 6-12 months (Willett 2013). The FFQ aims to rank individuals along the distribution of intake by separating those with low intakes from those with high intakes, thus making it a convenient method for epidemiological studies. It also may be more accurate than the other methods for estimating average intake of those nutrients/foods having large day-to-day variability and that are consumed occasionally or seasonally. The semi-quantitative FFQ includes portion-size estimates, but inclusion of portion sizes is not

mandatory. Overall, the FFQ is fast, practical and cheap to administer. However, the FFQ must be validated among the population of interest before being used.

Dietary recalls and records are often used in population surveys to monitor nutrient intake levels and dietary habits. They give more detailed intake data over a short period of time and can also be used for assessing food intake timing (Baranowski 2013). A trained reviewer of a dietary recall gathers detailed information about all foods and beverages consumed and their consumption time and place by the participant during the previous day (24-hour recall) or over multiple, preferably non-consecutive days. Recalls are relatively easy to conduct and do not affect the participant's dietary intake. However, they are somewhat more burdensome for participants because they rely greatly on the participant's memory. In diet records, the participant reports all foods and beverages consumed and their consumption time and place preferably over at least three days (including one weekend day) for a population-level intake estimation and for at least seven days for estimating individual-level intakes. A downside is that recording their dietary intake is burdensome for participants, and it may also change what they eat during a recording period.

2.3.1 OVERALL DIET QUALITY AND THE BALTIC SEA DIET SCORE

Overall diet quality

In nutritional epidemiology, the traditional way to conduct a nutritional study has been by focusing on single dietary components and their role in health and disease (Hu 2002). However, a more holistic approach has emerged of capturing the diet as a whole instead of single nutrients or foods. The rationale behind this approach is that people do not consume single nutrients or foods but combinations of foods with different nutrients. Foods and nutrients are often correlated, and they may have cumulative effects and interactions that may be difficult to take into account when studying associations of single nutrients and foods. Therefore, taking overall diet quality into account may capture the diet's complexity better and produce stronger associations between diet and health/disease than focusing on a single nutrient or food alone.

There are two main methods for capturing an overall diet quality: *a priori* and *a posteriori* (Hu 2002, Waijers et al. 2007). The *a priori* method is based on diet quality scores that reflect adherence to the diet under investigation. Diet scores are defined beforehand and are generally based on the current nutritional knowledge (Waijers et al. 2007). Several predefined scores reflecting an overall diet quality have been developed such as the Healthy Eating Index (based on the dietary guidelines for Americans) (Krebs-Smith et al. 2018), the Mediterranean Diet Score (based on the

traditional diet typical for Mediterranean region) (Trichopoulou et al. 2003) and the Baltic Sea Diet Score (based on the healthy Nordic diet) (Kanerva et al. 2014b). The *a posteriori* method is based on dietary patterns that are derived afterwards from dietary data with the use of statistical methods such as factor analysis (Hu 2002).

Healthy Nordic Diet and the Baltic Sea Diet Score

The healthy Nordic diet includes typical, locally grown and culturally accepted foods of the Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden) (Adamsson et al. 2012). It is characterized as being rich in Nordic berries, roots, cereals (rye, oat and barley), fatty fish, low-fat dairy and rapeseed oil and low in red and processed meat. The Baltic Sea Diet Pyramid (Figure 3) has been created to illustrate the healthy Nordic Diet (by the University of Eastern Finland, the Finnish Diabetes Association and the Finnish Heart Association). Foods recommended to be eaten plentifully and on a daily basis (such as vegetables, fruits and berries) are at the bottom of the pyramid; foods recommended to be eaten seldom (such as sweets) are at the top of the pyramid.

Figure 3 The Baltic Sea Diet Pyramid (created by the University of Eastern Finland, the Finnish Diabetes Association and the Finnish Heart Association). Source: the Finnish Diabetes Association

The health effects of the healthy Nordic diet have been examined in several randomized, controlled trials conducted in the Nordic countries. The largest of these is a Scandinavian multicenter trial (Sysdiet) (n=200, duration 18-24 weeks), a collaboration between Finland, Denmark, Iceland and Sweden that included participants with overweight/obesity and features of metabolic syndrome (Uusitupa et al. 2013). Several smaller local trials have also been conducted, for example, in Finland (Sysdimet, n=106) (de Mello et al. 2011), Sweden (Nordiet, n=88) (Adamsson et al. 2012) and in Denmark (New Nordic Diet, n=181) (Poulsen et al. 2014). Findings from these trials have shown positive effects from the healthy Nordic diet on cardiovascular risk factors (e.g., cholesterol profile, blood pressure) (Adamsson et al. 2012; Uusitupa et al. 2013; Poulsen et al. 2014) and on inflammation (C-reactive protein [CRP]) (de Mello et al. 2011; Poulsen et al. 2014). Furthermore, in terms of body weight and glucose control, two meta-analyses (conducted by the same study group) have been conducted to evaluate the evidence from these trials (Ramezani-Jolfaie et al. 2020, Zimorovat et al. 2020). They concluded that a higher adherence to the Nordic diet may have positive effects on body weight (the meta-analysis included five trials, among which two studies included participants from the same main study, n=647) (Ramezani-Jolfaie et al. 2020) and may improve serum insulin and the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) levels (the meta-analysis included four trials, n=359) (Zimorovat et al. 2020).

The nine-item BSDS has been developed for epidemiological research and is one of the dietary scores based on the healthy Nordic diet (Kanerva et al. 2014b) (Table 2). Other scores based on the healthy Nordic diet include the Healthy Nordic Food Index (including rye bread, oatmeal, root vegetables, cabbages, fish, apples and pears) developed in Denmark (Olsen et al. 2011) and the New Nordic Diet Score (e.g., Nordic fruits and berries, root vegetables, cabbages, potatoes, whole grain breads, oatmeal, game, fish and seafood, consumption of unsweetened milk relative to fruit juice and consumption of water relative to sweetened beverages and a question on meal frequency) from Norway (Hillesund et al. 2014).

Higher adherence to the BSDS has been associated with, e.g., lower risk of abdominal obesity (Kanerva et al. 2013) and better weight maintenance (Kanerva et al. 2018), lower risk of low-grade inflammation (high-sensitivity-CRP) (Kanerva et al. 2014c, a) and lower disease mortality (Tertsunen et al. 2020).

Table 2. *Components of the Baltic Sea Diet Score (BSDS) (Kanerva et al. 2014b).*

Component	Contents	Scoring[1]
Fruits and berries	Apples, pears and berries (such as bilberries and lingonberries)	0-3 (positive)
Vegetables	Tomatoes, lettuce, cucumbers, different cabbages, peas and roots (excluding potatoes)	0-3 (positive)
Cereals	Rye, oat and barley	0-3 (positive)
Low-fat milk	Fat-free milk and milk with fat content ≤1.5 %),	0-3 (positive)
Fish	Salmon and freshwater fish (such as, Baltic herring)	0-3 (positive)
Red and processed meat	Beef, pork, lamb, processed meat products and sausages	0-3 (negative)
Total fat, E%	Total fat intake	0-3 (negative)
Fat quality	PUFA/SFA + trans-fatty acids	0-3 (positive)
Alcohol	Ethanol intake	0-1 (negative)
Total		0-25

E%; percentage of energy intake, SFA; saturated fatty acids, PUFA; polyunsaturated fatty acids
[1]Scoring was based on fourths (except for alcohol) of consumption calculated separately for men and women. For alcohol, men who consumed 20g or less a day and women who consumed 10g or less a day were given 1 point; otherwise, 0 points were given.

Chronotype, diet and overall diet quality

Evidence gathered from observational studies on the associations between chronotype and dietary habits suggest that the evening type is associated with unhealthier food and nutrient intakes compared to morning types (Mazri et al. 2020). For example, in a Finnish population-based study (n=4493), evening types had a lower consumption of fruits, vegetables, whole grain and fish and a higher consumption of sweets and chocolate compared to morning types (Kanerva et al. 2012). Of the macronutrients, evening types had lower intakes of carbohydrates (as a percentage of energy intake [E%]), sucrose (E%), protein (E%) and fiber and higher intakes of fat (E%) and saturated fat (E%). Furthermore, several studies have associated evening types with a higher alcohol intake (Nakade et al. 2009, Sato-Mito et al. 2011a, Kanerva et al. 2012, Whittier et al. 2014).

The overall diet quality of chronotypes, assessed with dietary scores, has been examined in four very recent cross-sectional studies, among which one assessed adherence to the Brazilian version of the Healthy Eating Index (Gontijo et al. 2019), while three assessed adherence to the Mediterranean Diet (De Amicis et al. 2020, Muscogiuri et al. 2020, Rodríguez-Muñoz et al.

2020). Adherence to the Brazilian Healthy Eating Index-Revised was assessed among pregnant Brazilians (n=100) in which chronotype was assessed with MSF (Gontijo et al. 2019). The total scores of index were not associated with chronotype but, of the individual components, a higher intake of total grains was associated with a later chronotype, whereas a higher consumption of total fruits was associated with an earlier chronotype. Evening type was associated with a lower adherence to the Mediterranean Diet in an Italian study (*n*=416) of adults in which chronotype was assessed with a reduced five-item MEQ (De Amicis et al. 2020). Associations were not examined between chronotype and individual components of the diet score. The evening type was similarly associated with a lower adherence to the Mediterranean Diet in another Italian study that included middle-aged adults (n=172) and which assessed chronotype with the MEQ (Muscogiuri et al. 2020). Of the individual score items, evening type was associated with a lower consumption of vegetables, fruits, fish, poultry, nuts, olive oil and wine and a higher consumption of soda drinks, red meat, butter, cream, margarine and sweets. A Spanish study on university students (n=457) in which chronotype was assessed with a reduced five-item MEQ also showed that evening types had a lower adherence to the Mediterranean Diet (Rodríguez-Muñoz et al. 2020). Of the single score items, evening types had a lower consumption of fruits, pulses, cereals and olive oil.

In summary, previous literature indicates that evening types have unhealthier food and nutrient intakes. Furthermore, studies indicated lower overall diet quality, as assessed with the Mediterranean Diet score, among evening types compared to morning types (De Amicis et al. 2020, Muscogiuri et al. 2020, Rodríguez-Muñoz et al. 2020). No association occurred with the Brazilian Healthy Eating Index (Gontijo et al. 2019); however, the selected study population that included pregnant women (which may affect their dietary habits) may at least partly explain why no association was observed between chronotype and dietary score in that particular study. Large population-based studies on chronotype associations with overall diet quality are lacking. Furthermore, chronotype adherence to the healthy Nordic diet has not been examined before.

2.3.2 TIMING OF FOOD INTAKE

In addition to overall diet quality approach, another emerging area in the field of nutritional epidemiology is related to the temporal aspect of eating (Asher and Sassone-Corsi 2015). This approach is based on the idea that it is not just *how* we eat but also *when* we eat that could be critical for an individual's metabolic health. Our energy metabolism is tightly linked to the circadian clocks, discussed earlier (see section 2.1.1 on page 16), and food intake is the most potent external factor that can entrain the peripheral clocks, just as light is for the central circadian clock (Reinke and Asher 2019). Therefore, *when* in addition to *how* we eat may have the potential to

modulate the circadian clocks and even disrupt the synchronization between the central and peripheral clocks, leading to circadian misalignment (Asher and Sassone-Corsi 2015, Ribas-Latre and Eckel-Mahan 2016, Reinke and Asher 2019).

One of the first studies demonstrating the importance of the timing of food intake to metabolic health was an experimental rodent study that showed that mice fed during the rest period gained more weight than mice fed during the active period with an equal amount of energy and physical activity (Arble et al. 2009).

In humans, the role of morning energy intake in obesity has probably been studied the most. A recent meta-analysis of breakfast skipping on obesity (including 36 cross-sectional and nine cohort studies) concluded that skipping breakfast was associated with increased risk of overweight/obesity and weight change (Ma et al. 2020), whereas evidence from randomized controlled trials indicate that eating breakfast could contribute to weight gain (Sievert et al. 2019, Bonnet et al. 2020). A recent systematic review and meta-analysis of randomized controlled trials (including seven trials, n=425, duration from 4 to 16 weeks) evaluated breakfast skipping compared with breakfast consumption on weight change and cardiometabolic risk factors (Bonnet et al. 2020). They concluded that breakfast skipping modestly but significantly reduced body weight but also increased low-density lipoprotein cholesterol (LDL). Another systematic review and meta-analysis evaluated the role of breakfast on total daily energy intake (including 10 trials, n=930, duration from 2 days to 6 weeks) in addition to weight change (seven trials, among which six overlapped with Bonnet et al. (2020), n=486, duration from 2 to 16 weeks) (Sievert et al. 2019). Similar results were found for weight change but, regarding differences in total daily energy intake, they found that breakfast consumers had a higher daily energy intake (~260 kcal/d) than breakfast skippers, which probably at least partly explains these findings from the trials.

The evidence related to distribution of energy intake over the course of the day on metabolic health has been evaluated by a systematic review of four cohort studies (n=270 to 4243, follow-up from 20 weeks to 6 years) and four clinical trials (n=6 to 60, duration from 1 day to 12 weeks) (Beccuti et al. 2017). It concluded that consuming more calories at the beginning of a day may be protective against overweight/obesity and have a beneficial effect on glucose metabolism. A meta-analysis of four cross-sectional studies (n=6685) and five trials (n=420, duration from 6 to 16 weeks) evaluated the role of evening energy intake on BMI and weight change (Fong et al. 2017). They concluded that the evidence from the observational studies showed a borderline significant positive association between evening energy intake and BMI, whereas the trials showed no difference between low versus high evening energy intake on weight change. The authors declared that high heterogeneity between studies makes it difficult to draw a definitive conclusion from the associations. They further pointed out that one of the

main limitations among studies was the lack of a consistent approach to define timing of meal intake.

Other aspects closely related to the temporal aspect of eating are frequency and regularity of eating, which will be discussed only shortly. The role of high versus low meal frequencies in body weight and composition seem to be slightly in favor of higher meal frequency, although the difference seems to be negligible (Schoenfeld et al. 2015, Paoli et al. 2019). Irregularities in meal patterns, such as high variability in day-to-day timing and frequency of meals, may also have unfavorable effects on weight management (St-Onge et al. 2017). Furthermore, an emerging view of the temporal aspect of eating is to restrict the eating within a consistent 8- to 12-hour window during the individual´s active period, which may have beneficial effects in terms of weight management, although thus far the studies are few and have been relatively small scale (Gill and Panda 2015, Zarrinpar et al. 2016, Manoogian et al. 2019).

None of these reviews/studies have evaluated the role of individual differences in circadian timing in humans in the association between energy intake timing and obesity.

Chronotype and food intake timing

Several cross-sectional studies have shown that evening types skip breakfast more often than morning types (Nakade et al. 2009, Sato-Mito et al. 2011a, Meule et al. 2012, Reutrakul et al. 2014, Teixeira et al. 2018, Mirghani et al. 2019).

Five studies have examined chronotype differences in clock timing of meals, and the evening type was associated with later timing of meals (breakfast, lunch and dinner) in all of them (Baron et al. 2011, Sato-Mito et al. 2011b, Lucassen et al. 2013, Nimitphong et al. 2018, Xiao et al. 2019) (Table 3). Differences in clock timing were most pronounced in a US study (n=52) that included evening and intermediate types (morning types were excluded from the study due to low numbers). The evening types in the study had their breakfast on average almost three hours later (2 h 46 min) than the intermediate types (Baron et al. 2011). As for lunch and dinner, the differences between evening and intermediate types were a little over an hour on average (lunch: 1 h 19 min, dinner 1 h 6 min). Another US study on obese and short-sleeping (<6.5 hours a night) participants (n=119) distinguished working days from non-working days (Lucassen et al. 2013). They found that evening types had later timing of the first eating occasion (1 h 21 min) on working days but no significant difference emerged on non-working days.

Three small-scale studies have examined the distribution of evening energy or macronutrient intakes (Baron et al. 2011, 2013, Lucassen et al. 2013) (Table 3). The US study (n=52) found that evening types had twice as many calories (754 kcal vs. 376 kcal) in the evening (after 8:00 p.m.) than intermediate types (Baron et al. 2011). They also reported that evening types had higher percentages of their total daily fat, protein and carbohydrate

intakes in the evening (Baron et al. 2013). Another US study on obese and short-sleeping participants (n=119) found that evening types had a higher percentage of their total energy intake (%TEI) in the evening on working (30 %TEI vs. 14% TEI) and on non-working days (24%TEI vs. 14%TEI) than morning types, but no differences emerged in macronutrient intakes after 8:00 p.m. (Lucassen et al. 2013).

In summary, these findings indicated later clock timing of meal intakes among evening types and also that evening types have higher energy intakes in the evening (after 8:00 p.m.). Morning distribution of energy and macronutrients has not been examined among chronotypes. Furthermore, population-based studies on these associations are lacking.

Chronotype, food intake timing and obesity

It is unclear whether eating at a later clock time is equivalent to eating at a "wrong time" of day for evening types whose circadian phase is delayed compared to other chronotypes. Thus far, only few studies have circulated this question and examined the interrelationships between circadian rhythms, energy intake timing and obesity (Table 3).

A small-scale US (n=52) study found that a higher energy intake after 8:00 p.m. (Baron et al. 2011) and a protein intake four hours before sleep (Baron et al. 2013) were associated with a higher BMI independent of sleep timing or duration.

A Spanish study of university staff (n=171) suggested that the association between chronotype and meal timing may differ according to BMI (Muñoz et al. 2017). They found that normal-weight evening types tended to have a higher %TEI at breakfast and at dinner, whereas evening types with overweight/obesity had a higher %TEI at lunch. Normal-weight morning types had a higher %TEI at breakfast and lunch, and morning types with overweight/obesity had a higher %TEI at dinner.

A study from Thailand of participants with type 2 diabetes (n=210) suggested that breakfast timing could mediate the association between chronotype and BMI because they found that morning type was associated with an earlier breakfast time, and an earlier breakfast time was associated with a lower BMI (Nimitphong et al. 2018).

A recent US study (n=872) on middle-to-older aged participants found that, overall, a higher %TEI consumed in the morning (defined as intake appearing within two hours after getting out of bed) was associated with a lower risk of being overweight or obese (Xiao et al. 2019). This association was stronger among morning than evening types, whereas a higher %TEI consumed in the evening (defined as intake within two hours before bedtime) was associated with a higher risk of being overweight or obese. This association was stronger among evening types.

Finally, another US study touched the subject by examining energy intake timing relative to a clock hour and to endogenous circadian time (as assessed by DLMO) on college-aged individuals (n=110) (McHill et al. 2017). Their

findings suggested that higher body fatness was associated with the circadian timing of eating rather than the clock timing of eating, because participants with a high body fat percentage (BF%) had a higher %TEI closer to their biological night than did participants with low BF%, whereas no differences were found in clock hour distribution of energy intake between those with a low or high BF%.

In summary, the role of intrinsic circadian rhythms in the association between energy intake timing and obesity seems unclear based on these studies. One study found that higher energy intake in the evening associates with obesity despite chronotype (Baron et al. 2011). Another study suggested to the contrary that later eating would be beneficial for evening types (Muñoz et al. 2017). However, two of the studies suggested that eating closer to sleep time/biological night may be more harmful in terms of obesity than the clock hour of eating (McHill et al. 2017, Xiao et al. 2019) and that this would be even more harmful for the evening types (Xiao et al. 2019). Regarding the morning intake, it seems that it would be beneficial to eat earlier in the morning/closer to wake-up timing for all chronotypes (Nimitphong et al. 2018), or at least morning types would benefit from early eating (Xiao et al. 2019). However, again, larger scale population-based studies on these associations are lacking.

Table 3. *Main findings from the studies examining association between chronotype, energy intake timing and obesity.*

Study	Population	n, (age)	Chronotype measure	Dietary intake assessment method	Clock timing of meals	Morning/evening intake as percentages (%) of total daily intake.		Interrelationships between, energy intake timing, chronotype and obesity
						Energy	Macronutrients	
Baron et al. 2011, USA	Adults recruited via advert	52, w+m (18-71)	MEQ[1], MSF based on actigrafy	7-day diet records	Evening types had later breakfast (mean 11:53 a.m. vs.9:07), lunch (mean 2:26 p.m. vs 1.07 p.m.), dinner (mean 8:13 p.m. vs.7:07) and last meal times (mean 10:17 p.m. vs 8:25 p.m.)	Evening types had a higher energy intake (754 kcal vs. 376 kcal) after 8:00 p.m.	-	Higher energy intake after 8 p.m. was associated with a higher self-reported BMI independent of chronotype (β =0.44, *P* =0.03)
Sato-Mito et al. 2011 (a), Japan	University students	3304, w (18-20)	MSF	A questionnaire on meal times	Evening types had later breakfast (mean 9:19 vs. 6:35), lunch (mean 12:42 vs. 12:20) and dinner (mean 19:19 vs. 18:51)	-	-	-
Baron et al. 2013, USA	Adults recruited via advert	52, w+m (18-71)	MEQ[1], MSF based on actigrafy	7-day diet records	-	-	Evening types had a higher % of total carbohydrate (33% vs. 19%), fat (35% vs. 19%) and protein (37% vs. 21%) intakes after 8 p.m. and a higher % of fat (33% vs. 9%) 4 hours before bed.	Protein consumed 4 hours before sleep was positively associated with self-reported BMI independent of chronotype (β=0.31, *P*=0.03).

Table 3. continues

Study	Population	n, (age)	Chronotype measure	Dietary intake assessment method	Clock timing of meals	Morning/evening intake as percentages (%) of total daily intake		Interrelationships between, energy intake timing, chronotype and obesity
						Energy	Macronutrients	
Lucassen et al. 2013, USA	Short- sleeping (<6.5 h) adults with obesity	119, m+w (18-50)	MEQ	3-day food records	Evening types had a later timing of first eating occasion (8:38 a.m. vs. 7:17 a.m.) on working days but no difference emerged on non-working days. Clock timing of other eating occasions were not reported.	Evening types had a higher energy intake after 8 p.m. on working days (30 %TEI vs. 14% TEI) and on non-working days (24%TEI vs. 14%TEI).	No chronotype differences were found on macronutrient intakes after 8 p.m. on working or non-working days.	-
McHill et al. 2017,USA	Students	110 m+w (18-22)	Endogenous circadian time (assessed by DLMO)	7-day food records via MealLogger application	-	-	-	Participants with a higher BF% had consumed 50% of their total daily energy intake 1.1 h closer to DLMO than those with lower BF% (HR: 1.7; 95% CI: 1.1, 2.6). No difference in clock time was found (16:14 vs. 16:41; HR: 0.9; 95% CI: 0.6, 1.4). Participants who consumed a greater percentage of their daily calories between 4 h before DLMO and sleep onset had a higher BF% (P = 0.006).

Table 3. continues

Study	Population	n, (age)	Chronotype measure	Dietary intake assessment method	Clock timing of meals	Morning/evening intake as percentages (%) of total daily intake		Interrelationships between, energy intake timing, chronotype and obesity
						Energy	Macronutriens	
Muñoz et al. 2017, Spain	University staff	171, m+w (30-60)	MEQ	FFQ, 24-h dietary recall	-	-	-	Normal-weight evening types tended to have higher %TEI at breakfast (14% vs. 12%) and at dinner (32% vs.31%), whereas evening types with overweight/obesity at lunch (43% vs.40%). Normal-weight morning types had a higher %TEI at breakfast (17% vs. 15%) and lunch (46% vs. 45%), whereas morning types with overweight/obesity at dinner (31% vs. 28%).
Nimitphong et al. 2018, Thailand	Subjects with Type 2 Diabetes	210 m+w (58.6±11)	CSM	24-h dietary recall	Evening type was associated with a later timing of breakfast (08:00(07:30–09:00) vs. 08:00 (07:00–08:30)), lunch (12:15 (12:00–13:23) vs. 12:00 (12:00–13:00)), dinner (18.30 (18:00–19:00) vs 18:00 (17:30–19:00)) and last meal (19:00 (18:00–19:38) vs. 18:20 (17:53–19:00)).	-	-	Morning type was associated with an earlier breakfast time, and lower BMI by 0.4 kg/m^2 (B = 0.37, 95% CI: 0.88, 0.07),

Table 3. continues

Study	Population	n, (age)	Chronotype measure	Dietary intake assessment method	Clock timing of meals	Morning/evening intake as percentages (%) of total daily intake		Interrelationships between, energy intake timing, chronotype and obesity
						Energy	Macronutrients	
Xiao et al. 2019, USA	The Interactive Diet and Activity Tracking in American Association of Retired Persons (AARP) study participants	872 m+w (50-74)	MSF	6 x 24-h dietary recalls	Evening types had a later timing of breakfast (-1 h), lunch (-12 min), dinner (-18 min) and shorter time between waking up and breakfast (36 min vs. 60 min) and longer time between dinner and bedtime (5 h vs. 4 h)	-	-	Morning energy intake (%TEI) associated with lower odds of being overweight or obese (odds ratio (95% confidence intervals), 0.53 (0.31, 0.89)), particularly in morning types (OR 0.32, 95% CI 0.16, 0.66). Evening energy intake was associated with higher odds of being overweight or obese (OR 1.82, 95% CI: 1.07, 3.08), particularly in evening types (OR 4.94, 95% CI: 1.61, 15.14).

%TEI; percentage of total energy intake, CI; confidence interval, CSM; Composite Scale for Morningness, DLMO; dim light melatonin onset, FFQ; food frequency questionnaire, h; hour, m; men, min; minutes, MSF; midpoint of sleep on free days, w; women

[1] Study included only evening and intermediate types.

2.4 OBESITY AND CHRONOTYPE

Definition

The World Health Organization (WHO) defines overweight and obesity as excess fat tissue that has accumulated to an extent that it presents a risk to health (WHO 2000). Obesity is associated with increased risk of developing a number of health conditions, including metabolic syndrome, coronary heart disease and premature mortality (WHO 2000). The most-used tool for measuring obesity and overweight is the BMI, calculated by dividing weight (kg) by the square of height (m^2) (WHO 2000). In general, a person with a BMI≥25 is considered overweight, and a person with a BMI≥30 is considered obese. Another widely used measure of obesity is waist circumference (WC) (WHO 2011). WC is a good indicator of abdominal fat, which is associated with a greater risk of chronic diseases than fat located in other areas. A WC>102 cm for men and >88 cm for women indicates an increased risk of chronic diseases.

Prevalence

Obesity is a worldwide public health concern whose prevalence has nearly tripled during the last 40 years (WHO 2020). Globally, 39% of adults were overweight and 13% were obese in 2016. In Finland, the latest National FinHealth 2017 Study reported that 72% of men and 63% of women over 30 years of age were at least overweight, and 26% of men and 28% of women were obese (Koponen et al. 2018). Furthermore, nearly half (46%) of the adults had abdominal obesity. Obesity has also become more prevalent in Finland, and the trend is on the increase after a steadier period in the early 2000s (Laatikainen et al. 2019).

Chronotype and obesity

Six cross-sectional studies (n>500) that have examined the association between chronotype and measured anthropometrics in adults have shown inconsistent findings (Table 4). In a UK Biobank study (n=119 679), evening types were more likely overweight (OR 1.12, 95% CI: 1.04, 1.21) or obese (OR 1.15, 95% CI: 1.04, 1.27) and had abdominal obesity (OR 1.08, 95% CI 1.01, 1.16) than morning types (Celis-Morales et al. 2017). Similar findings emerged in a community-based US study of middle-aged men and women (n=1197) in which evening types had 1.67 odds (95% CI 1.08-2.56) for being obese compared to morning types (Sun et al. 2020). A study from Thailand on pre-diabetic patients (n=2133) also reported a higher BMI among evening types (Anothaisintawee et al. 2018). However, a Norwegian population-based study (Johnsen et al. 2013) and a study of Americans with Hispanic/Latino origins (Knutson et al. 2017) reported no association between chronotype

and BMI. Furthermore, no association between chronotype and BMI was found in the Korean population-based study, but their findings indicated that evening types had an unhealthier body composition (e.g., higher body fat mass and more subcutaneous fat) than morning types (Yu et al. 2015).

Two small-scale studies have examined the longitudinal association between chronotype and obesity. In a US study on college freshmen (n=54), evening type (assessed with MEQ) was associated with an increase of 0.50 (95% CI 0.04–0.95) BMI points (self-reported) during an 8-week follow-up period (Culnan et al. 2013). A Spanish study (n=252) on severely obese bariatric surgery patients found that evening types (assessed with the MEQ) had a higher measured BMI and WC in the baseline than morning types (Ruiz-Lozano et al. 2016). Evening types were also less likely to lose more weight during a six-year follow-up after the surgery.

In summary, evidence from the cross-sectional studies seems inconsistent. While some studies suggest higher obesity prevalence among evening types (Celis-Morales et al. 2017, Anothaisintawee et al. 2018, Sun et al. 2020), the same number of studies report no association (Johnsen et al. 2013, Yu et al. 2015, Knutson et al. 2017). Longitudinal data indicate that evening types were more prone to weight gain and less likely to lose weight; however, the extremely short follow-up period in Culnan et al.'s study (2013) and the selected study populations in both longitudinal studies limit the findings' generalizability. Thus, large-scale, population-based studies on longitudinal associations of chronotype are needed.

Table 4. *Cross-sectional studies (n>500) on the association between chronotype and obesity.*

Study, country	Population	n, (age)	Chronotype measure	Outcome	Main findings (evening vs. morning type)
Johnsen et al. 2013, Norway	Tromsø Study participants	6412, m+w (30-65)	MSF	measured BMI, BMI ≥25, WC ≥88 cm women and ≥102 cm men	No associations.
Yu et al. 2015, Korea	Korean Genome Epidemiology Study (KoGES) participants	1620, m+w (47-59)	MEQ	measured BMI, WC, SFA, VFA, body fat mass, body lean mass	Evening type: SFA ↑ (208 vs.185 cm^2), body fat mass ↑ (19 vs.17 kg), body lean mass ↓ (41 vs. 44 kg)
Celis-Morales et al. 2017, UK	UK Biobank participants	119 679, m+w (37-73)	single-item chronotype	measured BMI ≥25, ≥30, WC ≥88 women and ≥102 men	Evening type: overweight ↑ (OR 1.12, 95% CI: 1.04, 1.21), obesity ↑ (OR 1.15, 95% CI: 1.04, 1.27), abdominal obesity ↑ (OR 1.08, 95% CI 1.01, 1.16).
Knutson et al. 2017, USA	Hispanic Community Health Study / Study of Latinos (HCHS/SOL) participants	13 429, m+w (18-74)	MSF	measured BMI	No association.
Anothaisin-tawee et al. 2018, Thailand	Prediabetic patients	2133, m+w (32-92)	CSM	measured BMI	Evening type: BMI ↑ (*B* −0.08, *P*<0.001)
Sun et al., 2020, USA	Bogalusa Heart Study participants	1197, m+w (48±5)	rMEQ	measured BMI ≥30	Evening type: obesity ↑ (OR 1.67, 95% CI 1.08-2.56)

BMI; body mass index (kg/m^2), CI; confidence interval, CSM; Composite Scale for Morningness, m; men, MEQ; morningness-eveningness questionnaire, MSF; midpoint of sleep on free days, OR; odds ratio, rMEQ; reduced 5-item MEQ, SFA; subcutaneous fat area ($cm^{2)}$, VAF; Visceral fat area ($cm^{2)}$, w; women, WC; waist circumference (cm)

3 AIMS OF THE STUDY

The general aim of this doctoral thesis was to examine associations of chronotype on dietary habits and anthropometric measures and further the interrelationships between these factors. Additionally, this thesis aimed to explore the genetic background of chronotype to clarify the underlying mechanisms behind the dietary behavior and health of chronotypes. The more specific aims were:

1. To examine associations of chronotype on dietary habits (overall diet quality, energy and macronutrient intake timing) in a cross-sectional design (I and II).

   Hypothesis: Evening types have poorer overall diet quality and delayed timing of energy and macronutrient intakes compared to morning types.

2. To examine associations of chronotype with anthropometric measures in cross-sectional (I) and longitudinal (III) designs.

   Hypothesis: Evening types have higher anthropometric measures and are more likely to gain weight over a seven-year follow-up than morning types.

3. To examine the association between dietary habits (overall diet quality and energy intake timing) on anthropometrics by chronotypes in cross-sectional (I, III) and longitudinal (III) designs.

   Hypothesis: Evening types with poorer adherence to a healthy diet/higher energy intake in the evening hours are more likely with obesity than morning types.

4. To examine the genetic associations of chronotype (clock genes, GWAS of chronotype and to develop a genetic risk score (GRS) for chronotype (IV).

   Hypothesis: Clock gene SNPs and GRS associate with chronotype, whereas GWAS analysis of chronotype is hypothesis free.

4 METHODS

4.1 STUDY SAMPLES

This thesis consists of the population-based National FINRISK 2007 and 2012 studies and the following three substudies of FINRISK 2007: DIetary Lifestyle and Genetic determinants of Obesity and Metabolic syndrome (DILGOM) 2007, DILGOM 2014 (follow-up) and the National FINDIET 2007 Study conducted at the Finnish Institute for Health and Welfare (THL) (Table 5).

4.1.1 THE NATIONAL FINRISK 2007 (II, IV) AND 2012 (IV) STUDIES

The FINRISK studies monitored trends in risk factors of non-communicable diseases in the Finnish population and were conducted every five years from 1972 until 2012 (Vartiainen et al. 2010, Borodulin et al. 2015). This thesis includes data from FINRISK 2007 (Vartiainen et al. 2010) and 2012 (Borodulin et al. 2015). A random sample (9958 in 2007; and 9905 in 2012) of men and women were invited to participate in the study (Table 5). They were selected from the National Population Register covering the age groups between 25 and 74 years in five large geographical regions: 1) Helsinki and Vantaa (the capital area), 2) Turku and Loimaa, 3) North Savo, 4) North Karelia, and 5) Northern Ostrobothnia and Kainuu. Both studies included a health examination (e.g., anthropometric measures and blood samples) and self-administered questionnaires (e.g., questions on medical history and socio-economic factors, including questions on preferences to time the daily activities and socioeconomic status), which occurred between January and March for FINRISK 2007 and between January and April for FINRISK 2012. Of the invited, 6258 participated in 2007 (participation rate 63%) and 5827 in 2012 (participation rate 59%).

4.1.2 DIETARY LIFESTYLE AND GENETIC DETERMINANTS OF OBESITY AND METABOLIC SYNDROME (DILGOM) 2007 AND 2014 STUDIES (I, III)

The DILGOM 2007 Study is a substudy of FINRISK 2007 with a focus on obesity and metabolic syndrome (Konttinen et al. 2010) (Appendix I). All the participants of FINRISK 2007 were invited to DILGOM 2007, which was conducted between April and June. The participants underwent a more detailed health examination and completed questionnaires on food consumption, sleep and other health-related behavior. Of the invited, 5024 participated (participation rate 80%) (Table 5).

The DILGOM follow-up study was conducted between April and June 2014 (Kanerva et al. 2018). In all, 4581 participants were invited to the follow-up after excluding those who had died or moved outside of Finland during the follow-up period or whose contact information was unavailable (n=443) (Table 5). Of the invited, 3735 participated (participation rate 82%). The follow-up was carried out in two groups: Group 1 included participants of the capital area and southwestern Finland who had a health examination and completed questionnaires (n=1312); Group 2 included participants from the other three study areas who completed questionnaires (n=2423).

4.1.3 THE NATIONAL FINDIET 2007 STUDY (II, III)

One third (n=3286) of FINRISK 2007 participants were invited to FINDIET 2007, another substudy of FINRISK 2007 (Paturi et al. 2008) (Table 5) (Appendix I). FINDIET focuses on dietary habits and nutrient intakes of the Finnish population and has been conducted every five years since 1982. Dietary intake was collected using 48-hour dietary recall and 3-day food records. Of the invited, 2054 participants participated in the recalls and 2038 (62%) recalls were accepted. Approximately half of the participants (n=1646) invited to the 48-hour dietary recall were also asked to complete the 3-day food records at home starting from the day following the 48-hour dietary recall (Paturi et al. 2008). A total of 935 food records were returned and 912 accepted.

Table 5. *General characteristics of study samples used in the thesis.*

	FINRISK 2007	DILGOM 2007	FINDIET 2007	FINRISK 2012	DILGOM 2014
Data collection year	2007	2007	2007	2012	2014
Invited, n	9958	6258	3286	9905	4581
Participated in the health examination or completed questionnaires, n	6258	5024	2038	5827	3735[1]
Age range, years	25-74	25-74	25-74	25-74	25-74
Women, %	53%	54%	66%	52%	55%
Chronotype data, n	5696	x	x	4496	3093
Dietary data used by substudies					
Returned FFQ, n (I)	x	4874	x	x	x
Accepted 48-hour dietary recalls, n (II and III)	x	x	2038	x	x
Accepted 3-day dietary records, n (II)	x	x	912	x	x
Genetic data, n (IV)	5330	x	x	3439	x

[1]Group 1: n=1312 health examination and questionnaires, Group 2: n=2423 questionnaires.

4.1.4 STUDY DESIGN AND INCLUSION CRITERIA

Table 6 presents data, final sample size, study design and inclusion criteria by each substudy.

Table 6. *Inclusion criteria and number of participants of the substudies.*

Substudy	Data	n	Design	Inclusion criteria
I	DILGOM 2007	4421	cross-sectional	Men and non-pregnant women with chronotype information, FFQ and anthropometric measurements
II	FINRISK 2007, FINDIET 2007	1854	cross-sectional	Men and women with chronotype information and 48-hour dietary recalls
III	DILGOM 2007/2014, FINDIET 2007	1097	longitudinal	Men and non-pregnant women with baseline chronotype information, 48-hour dietary recalls and anthropometric measurements from baseline and follow-up
IV	FINRISK 2007/ 2012	8433	cross-sectional	Men and women with chronotype and genetic information

FFQ; food frequency questionnaire

4.2 ETHICAL CONSIDERATIONS

The FINRISK 2007 and 2012, DILGOM 2007/2014 and FINDIET 2007 adhered to the guidelines of the Declaration of Helsinki. The Ethics Committee of the Hospital District of Helsinki and Uusimaa approved the research protocols (FINRISK 2007/FINDIET2007/DILGOM2007: HUS 229/EO/2006, FINRISK 2012: HUS 162/13/03/11 and DILGOM 2014: HUS 332/13/03/00/13). All participants signed the informed consent.

4.3 STUDY MEASURES

4.3.1 CHRONOTYPE

Chronotype was assessed with six questions from the self-administered questionnaire. These six questions (items 4, 7, 9, 15, 17, 19) were derived from the original 19-item MEQ (Horne and Östberg 1976, Hätönen et al. 2008) (Appendix II). The six questions accounted for 83% of the total variance of the original MEQ with Cronbach's α of 0.80 (Hätönen et al. 2008). The scoring of these questions was based on the scoring of the

original MEQ (Horne and Östberg 1976). Thus, the shortened sum of the morningness-eveningness (ME) score could vary from 5 (extreme eveningness) to 27 (extreme morningness). In all substudies, the ME score was used as a continuous variable and as a categorical variable. In substudy I the ME score was categorized into study-specific thirds (morning type, intermediate type and evening type) to obtain larger group sizes for studying associations by BSDS fifths. In substudies II and III the ME score was categorized in a way that corresponded to the scaling of the original MEQ: (definitely or moderately) evening (5-12 points), intermediate (13-18 points) and (definitely or moderately) morning type (19-27 points) (Merikanto et al. 2012). Analyses in substudy IV required a dichotomized response variable; therefore, chronotype was used as a binary variable (median cut-off) evening type (5-15 points) and morning type (16-27 points). In substudy IV chronotype was also assessed based on the single self-evaluation question on chronotype (single-item chronotype) (question 6) for comparison in which "rather a morning than an evening type" and "definitely a morning type" composed a morning type group and "rather an evening than a morning type", and "definitely an evening type" composed an evening type group.

4.3.2 DIETARY HABITS

In substudy I overall diet quality was measured with the BSDS and dietary intake was assessed with an FFQ. In the substudies II and III timing of energy and macronutrient intakes were examined using dietary intake information from 48-hour recalls and 3-day food records (II).

FFQ

Habitual diet over the previous 12 months was measured with a 131-item semi-quantitative FFQ that was developed and updated at the THL (Männistö et al. 1996, Paalanen et al. 2006, Kaartinen et al. 2012). The FFQ has been validated against dietary records (Männistö et al. 1996, Paalanen et al. 2006, Kaartinen et al. 2012). Participants completed the FFQs at the study site of DILGOM 2007, and trained study nurses reviewed the FFQs after their completion. The average consumption of each FFQ food-item was assessed with a scale of nine frequencies ranging from "never or seldom" to "six or more times a day." The portion sizes in the questionnaire were predefined and appeared as common household units, such as a glass or a slice. The average portion sizes of each FFQ item separately for men and women were based on the FINDIET 2007 (dietary recalls). The average daily intakes of foods, nutrients and energy were calculated by the Finnish National Food Composition Database (Fineli®) developed at the THL (Reinivuo et al. 2010).

The Baltic Sea Diet score

The overall diet quality was assessed with the nine-item BSDS, which illustrates a healthy Nordic diet (see Table 2 on page 29) (Kanerva et al. 2014b). The scoring of these nine items was based on fourths of consumption of each item separately for men and women. For fruits and berries, vegetables, cereals, low-fat milk, fish and the fat ratio, the lowest fourth of consumption was given 0 points, the second 1 point, the third 2 points and the highest fourth of consumption 3 points. The opposite scoring was used for red meat and processed meat and total fat. For alcohol, men who consumed 20g or less a day and women who consumed 10g or less a day were given 1 point; otherwise, 0 points were given. The resulting BSDS could range from 0 to 25 with higher scores indicating higher adherence to the Baltic Sea diet.

The 48-hour dietary recalls and the 3-day food records

The 48-hour dietary recalls (II, III) and the 3-day food records (II) were used to assess the mean daily energy intake by clock hours. In FINDIET 2007, trained interviewers recorded all foods and beverages consumed and time and place of consumption from the two preceding days of the interview (Paturi et al. 2008). The interviews occurred between Mondays and Fridays; therefore, the 48-hour recalls did not cover Fridays (Reinivuo et al., 2010). The 3-day food records were asked to be completed at home starting the day following the 48-hour dietary recalls (Paturi et al. 2008). Similar to the recalls, participants were asked to record all foods and beverages consumed and the time and place of consumption during those days in the dietary records. Participants estimated portion sizes with a food photograph booklet in both dietary assessment methods (Paturi et al. 2006). Daily intakes of energy and macronutrients were calculated with Fineli® (Reinivuo et al., 2010).

Energy and macronutrient intake timing

The morning energy intake refers to energy intake appearing from 3:00 a.m. till 9:59 a.m., whereas the evening intake refers to the energy intake appearing from 8:00 p.m. till 2:59 a.m.. These time periods were based on the previous literature (Baron et al., 2011) and on average timing of morning and evening meals of the Finnish population (Helldán et al., 2013). Morning and evening energy intakes were expressed as the mean %TEI, which were calculated as the energy consumed during each period divided by the total daily energy intake. Morning and evening macronutrient intakes were expressed as E% during each period in order to take energy intake into account.

Energy and macronutrient timing analysis in substudy II and III were based on the 48-hour dietary recalls, including all days of the week (except

for Fridays). However, additional analyses were conducted in substudy II to examine differences in the timing of energy and macronutrient intakes between weekdays (Monday to Friday) and weekends (Saturday and Sunday). The combined data from the 48-hour dietary recalls and 3-day food records were used for these analyses, because the 48-hour dietary recalls did not include Fridays. Differences between the 48-hour dietary recalls and the 3-day food records in average total daily macronutrient intakes (carbohydrates, fat, protein) have been found to be rather small (<1E%) but slightly larger for energy (298 kJ) and alcohol (2.9 g) intakes (Maukonen 2015). Therefore, in substudy II, effect sizes for practical significances were also estimated with Cohen´s d (Cohen 1992). Effect sizes were ≤0.2 for energy and for all macronutrient intakes (except for alcohol d=0.24). The difference between two means can be considered practically insignificant when d≤0.2 and small when d=0.2-0.3. Small effect size regarding alcohol intake can be at least partly explained by the fact that the 48-hour dietary recalls did not cover Fridays. Thus, based on these findings, the dietary data from the 48-hour dietary recalls and 3-day food records were combined to analyze the differences in the timing of energy and macronutrient intakes between weekdays and weekends.

The weekday analysis yielded results similar to the analysis based solely on the 48-hour dietary recalls (including all days of the week, except for Fridays); therefore, the results from the weekday analysis will not be reported. The results based solely on the 48-hour dietary recalls, together with findings from the weekend analysis, will be reported instead.

4.3.3 ANTHROPOMETRIC MEASURES

Anthropometric measures were used as outcome variables in substudy I (BMI, WC, BF%) and in substudy III (weight, BMI, WC).

At the DILGOM 2007 and 2014 (group 1) sites, trained study nurses measured weight, height, WC and BF% of participants who wore light clothing and were barefooted according to the standardized international protocols (Tolonen et al. 2008). Height was measured to the nearest 0.1 cm with a wall-attached stadiometer and weight to the nearest 0.1 kg with a beam balance scale. BMI was calculated as weight (kg) divided by squared height (kg/m^2). Obesity was defined as BMI ≥ 30 kg/m^2 according to the WHO guidelines (WHO 2000). WC was measured to the nearest 0.5 cm with a soft measuring tape that was placed around the waist to the mid-point of the lowest rib and iliac crest. BF% was measured using an electric bioimpedance scale (Tanita TBF-300MA; Tanita Corporation of America, Arlington Heights, IL, USA).

The anthropometric measurements were based on self-reports in DILGOM 2014 Group 2 (Appendix I). Therefore, substudy III included both self-reported and measured anthropometrics. Self-reported weight and height were queried in the questionnaire as “What is your current weight? (in

kg)" and "What is your current height? (in cm)." The self-reported BMI was calculated based on these questions. A measurement tape was sent with written instructions that included illustrations for the self-reported WC. The instructions advised participants to place the measuring tape around the waist to the midpoint between the lowest rib and iliac crest on bare skin or on light clothing in front of a mirror to help with the correct placement.

Anthropometric measures are subject to reporting biases that may lead to differences between self-reported and measured values (Gorber et al. 2007, Maukonen et al. 2018). Therefore, the validity of self-reported height, weight, and WC against measured values have been examined in the DILGOM 2014 data; the self-reported values have been found to correlate strongly with the measured values (for height, intraclass correlation coefficient 0.96 in men and 0.97 in women; for weight, intraclass correlation coefficient 0.99 in men and women; for WC, intraclass correlation agreement 0·96 in men and 0.95 in women) (Kanerva et al. 2018). However, regarding the WC, the difference between self-reported and measured WC increased towards the larger WCs. Analyses were conducted with and without self-reported WC values in substudy III to take this finding into account, but this did not affect the findings.

4.3.4 GENETICS

Venous blood samples were taken by trained study nurses at the FINRISK study sites. The samples were stored in minus 70°C. DNA extraction was conducted at the THL. Genotyping was done at the Sanger Institute, Broad Institute, or Institute for Molecular Medicine Finland in five batches using the following Illumina GWAS arrays: HumanCoreExome, Omniexpress and 610K. Four batches were substudies of FINRISK 2007 (including COROGENE controls (610K), PREDICTCVD cases and controls (Omniexpress), and one batch with HumanCoreExome, whereas the fifth batch included participants from FINRISK 2012 (HumanCoreExome). The same standard quality control methods and standard imputation procedures were centrally applied for the data from each batch; this was followed by a joint quality control (minor allele frequency [MAF] ≥0.05 [5%], Hardy-Weinberg equilibrium [HWE] $P>1x10^{-7}$, imputed information score [INFO] >0.7 and missing proportion <0.02 [2%]) to harmonize the data content. Imputation of all GWAS data was done against a Finnish population-specific, whole-genome sequence backbone, the SISu (Sequencing Initiative Suomi) reference database version 3 (www.sisuproject.fi). The genotyping and imputation have been performed as described in Locke et al. (2019). Furthermore, closely related individuals were excluded (n=125) from the final dataset (PLINK pi_hat>0.20).

The following 20 key clock genes (see Figure 2, on page 18) were included in the clock gene analyses: *ARNTL, ARNTL2, BHLHE40, BHLHE41, CLOCK, CRY1, CRY2, CSNK1E, CSNK1D, NFIL3 NPAS2, NR1D1, NR1D2, PER1,*

PER2, PER3, RORA, RORB, RORC, TIMELESS (Hayes et al. 2005, Takahashi 2017, Sato et al. 2018, Kurien et al. 2019, Patke et al. 2020). A total of 4022 clock gene SNPs passed the quality control out of 8668 SNPs and were included in the study. Among these SNPs, altogether 66 SNPs were previously associated with chronotype (Katzenberg et al. 1998, Carpen et al. 2005, 2006, Matsuo et al. 2007, Lee et al. 2011, Etain et al. 2014, Kripke et al. 2014, Parsons et al. 2014, Dmitrzak-Weglarz et al. 2016, Song et al. 2016, Jankowski and Dmitrzak-Weglarz 2017, Jones et al. 2019).

For the GWAS analysis, 5,842,835 SNPs passed the quality control out of 12,954,971 SNPs and were included in the GWAS. Of these, 7741 SNPs were found to be among the top 10,000 chronotype-associated SNPs in the previous GWAS meta-analysis of chronotype based on the UK Biobank and the 23andMe data (Jones et al. 2019).

The GRS was based on 313 lead SNPs from the previous GWAS of chronotype (Jones et al. 2019). The GRS was created by summing the total number of minor alleles that were weighted by their corresponding regression coefficients for risk of being an evening type for each participant. These regression coefficients were based on the previous GWAS study; however, the direction of the regression coefficients for the analyses was reversed because the coefficients were originally reported for risk of being a morning type (Jones et al. 2019). The individual associations of these 313 SNPs with chronotype were also analyzed.

4.3.5 SOSIOECONOMIC AND LIFESTYLE FACTORS

The information of the participants' background variables was obtained from self-administered questionnaires.

Education was assessed from the reported total numbers of school years, which were categorized into thirds (low, middle and high) according to birth cohort. Birth cohort was taken into account to adjust for the extension of the basic education system and the increase of average school years over the last decades.

Participants' smoking status was derived from questions on smoking history, and current smoking habits were assessed with a four-level scale (never a smoker, quit ≤6 months ago, quit ≥6 months ago, and a current smoker).

The average leisure-time physical activity over the previous 12 months was assessed with four categories: inactive (light activities such as reading and watching television); moderately active (walking, gardening or other activities ≥4 hours/week); active (running, swimming or other physically demanding activities ≥3 hours/week); or very active (competition or other heavy sports several times per week). Answers were then categorized into three categories: inactive, moderately active and active (combining active and very active categories).

Participants were asked average hours of sleep a night. Subjective sleep sufficiency was assessed with four categories: always sufficient, often sufficient, seldom or never sufficient, or cannot say. Participants were also asked if they experienced insomnia often, sometimes, or never.

General health status was determined by asking participants to rate their current health and physical fitness status according to these categories: very good, fairly good, satisfactory, fairly poor, or very poor.

Evening or night shift work was based on a question on the participants' usual working schedules that included the following options: regular working hours; regularly working on two or three shifts; evening or night shift; irregular working hours; not working currently; or part-time job/part-time pension.

4.4 STATISTICAL ANALYSES

The main statistical analyses used in the thesis were linear regression, logistic regression and analysis of co-variance (ANCOVA). Analyses were performed with SPSS statistical computing software (IBM SPSS Statistics), version 22.0 (II) and version 24.0 (III), R statistical computing software, version 2.13.1 (I) and version 3.5.1 (IV and additional analyses for I) (R Core Team, 2018), and PLINK, versions 1.9 and 2.0 (IV) (Purcell et al., 2007).

The analyses were generally conducted together for men and women because no gender interactions occurred (except for I and III regarding anthropometrics findings). The normality of the variables was tested with QQ-plots and histograms when needed. Logarithmic transformations were applied if normality assumptions were not met.

Confounding variables were initially selected based on the previous literature. The final selection was made based on linear regression analysis using the method proposed by Rothman (Rothman 1986). Two models were used based on this analysis. The first model (model 1) was adjusted for age and sex. The second model (model 2) was further adjusted for education, smoking, leisure-time physical activity and sleep duration (I) or experienced sufficiency of sleep time (II and III). Regarding substudy I, the associations between chronotype and the BSDS and baseline anthropometrics were adjusted for age (model 1). Otherwise, the findings are mainly reported according to model 2, because the findings between the models did not differ remarkably in general. Substudy III was further adjusted (model 2) for baseline weight/BMI/WC when changes in these anthropometrics were examined and for total energy intake when energy intake timing was examined as the main explanatory variable. Substudy IV on the genetic basis of chronotype was adjusted for age, sex, genotyping batch and the first five principal components to account for population structure, as there can be a systematic difference in allele frequencies between a population's subpopulations due to different ancestry.

4.4.1 ASSOCIATIONS BETWEEN CHRONOTYPE AND DIETARY HABITS (I, II)

In substudy I the association between chronotype and overall diet quality was examined by determining *P*-value for trend with linear regression analysis (ME score was used as a continuous variable).
In substudy II associations between chronotype and energy/macronutrient intake timing were examined, in addition to linear regressions analysis, by determining the differences between morning and evening types (chronotype as categorical variable) with ANCOVA followed by a Bonferroni corrected post-hoc test.

Furthermore, to unify the findings, additional analyses for the thesis were conducted regarding substudy I with ANCOVA to determine the differences between morning and evening types. Chronotype categorization was conducted as in the original paper by dividing the ME score into thirds to get more even groups by numbers to be able to stratify them by the BSDS fifths. Further analyses were also conducted with model 2.

4.4.2 ASSOCIATION BETWEEN CHRONOTYPE AND OBESITY (I, III)

Chronotype associations with baseline BMI, WC and BF% (I) and with changes in weight, BMI and WC were examined over the seven-year follow-up period (III). In substudy I these associations were examined with linear (for continuous anthropometric variables) and logistic regression (for categorized anthropometric variables) analysis to determine *P*-value for trend (the ME score was used as a continuous variable). In substudy III these associations were examined by determining the differences between morning and evening types (chronotype as categorical variable) with ANCOVA (for continuous anthropometric measure variables) followed by a Bonferroni-corrected post-hoc test and with logistic regression (for categorized anthropometric measure variables).

Again, additional analyses for the thesis were conducted to unify the findings. Regarding substudy I, additional analyses were conducted to determine the differences between morning and evening types with ANCOVA. Chronotype categorization was conducted as in the original paper by dividing the ME score into thirds to get more even groups by numbers to be able to stratify them by the BSDS fifths. The longitudinal analysis of anthropometrics (III) included weight as an outcome variable; thus, weight was also further included as an outcome variable in the baseline anthropometrics analysis (I). Furthermore, the analyses were also conducted with model 2. Additional analyses with linear and logistic regression were conducted for substudy III to determine the *P*-value for trend.

4.4.3 INTERRELATIONSHIPS BETWEEN DIETARY HABITS, OBESITY AND CHRONOTYPE (I, III)

In substudy I, the Baron and Kenny test for mediation was used (Baron & Kenny, 1986), which included four-step multiple linear regression analyses to determine whether overall diet quality mediates an association between chronotype and obesity (Figure 4).

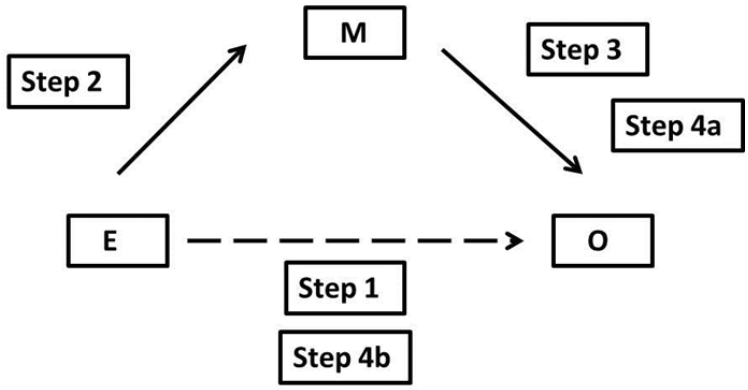

Figure 4 Baron and Kenny (1986) four-step test for mediation. In the first step (step 1), explanatory variable (chronotype) should be associated with the outcome variable (BMI, WC and BF%). In the second step (step 2), explanatory variable (chronotype) should be associated with the mediator (BSDS). In the third step (step 3), mediator (BSDS) should be associated with the outcome variable (BMI, BF% and WC). In the fourth step (step 4), a) mediator (BSDS) should be associated with the outcome variable (BMI, BF% and WC) when controlled for the explanatory variable (chronotype) and, b) the association between explanatory variable (chronotype) and outcome variable (BMI, BF% and WC) (step 1) should be greatly reduced, if not non-significant when controlled for the mediator (BSDS). E=explanatory variable, M=mediator, O=outcome.

Furthermore, in substudy I the likelihood ratio test was used to determine whether chronotype modified the association between overall diet quality and anthropometric measures followed by chronotype stratified linear regression analysis in case of statistically significant interactions emerged.

In Substudy III the overall association between morning/evening energy intake and anthropometric measures with logistic regression (energy intake timing variables as categorical variables) and with linear regression (energy intake timing variables as continuous variables) were first examined. Then, an interaction term between chronotype and energy intake evening/morning (as continuous variable) was added to the models to determine whether chronotype modified the association between morning/evening energy intake and anthropometric measures. Statistically significant interactions were further analyzed in a chronotype-stratified analysis.

4.4.4 ASSOCIATIONS BETWEEN CHRONOTYPE AND GENETICS (IV)

All the genetic-association analyses of chronotype were conducted with linear (continuous chronotype) and with logistic (binary chronotype) regression analysis using additive models, which is the most common practice for GWASs (Hill et al. 2008, Bush and Moore 2012). Furthermore, the Benjamini-Hochberg (BH) and Benjamini-Yekutieli (BY) false discovery rate methods were used to correct the *P*-values for multiple testing (except for the full GWAS analysis of chronotype) with *P*-values < 0.05 considered significant. As for the full GWAS results, *P*-values < 5×10^{-8} were considered genome-wide significant and suggestive for *P*-values $<1\times10^{-6}$. Furthermore, regarding the clock gene analysis, significantly associated SNPs (based on BH and BY corrected *P*-values) were further linkage disequilibrium (LD) clumped (using clump in PLINK, with threshold $P<0.05$, $r^2>0.5$, range: 250kb) to reveal independent associations signals. Regarding the GRS, the proportion of chronotype variance the GRS explained was estimated with adjusted partial R^2 (continuous sMEQ) and partial pseudo R^2 (Nagelkerke) (binary chronotype), using R-package 'rsq'.

5 RESULTS

5.1 SOCIO-ECONOMIC AND LIFESTYLE ASSOCIATIONS OF CHRONOTYPE

In the DILGOM 2007, 53% of the population were morning, 39% intermediate and 8% evening types (Table 7). Evening types were almost 10 years younger, more often women and more likely had higher education than morning types ($P<0.001$). They were also more likely smokers and more often physically inactive ($P<0.05$). Evening types also reported experiencing insufficient sleep and insomnia more often than morning types ($P<0.05$). Furthermore, evening types rated their health and physical fitness as good ($P<0.001$) less often. These associations also emerged when testing trends for ME score ($P<0.01$).

Table 7. *Percentages (%) or mean values (SE) of socio-economic and lifestyle factors by chronotypes in the DILGOM 2007 (n=4421).*

	Chronotype[1]				
	Morning	**Intermediate**	**Evening**		
	***n*=2343 (53%)**	***n*=1712 (39%)**	***n*=366 (8%)**	*P*[2,3]	*P-trend*[2,4]
ME score, range	19-27	13-18	5-12		
Age, years	54 (0.3)	50 (0.3)	46 (0.7)	<0.001	<0.001
Female, %	51	58	62	<0.001	<0.001
High education[5] (%)	31	41	45	<0.001	<0.001
Current smoker (%)	15	18	26	<0.001	0.002
Physically inactive[6] (%)	14	21	30	<0.001	<0.001
Sleep h/night	7.3 (0.02)	7.4 (0.02)	7.2 (0.5)	0.095	0.75
Sleep ≥10 h/night, %	1.2	1.8	4.7	<0.001	<0.001
Sleep ≤5 h/night, %	3.7	2.9	8.0	<0.001	0.001
Experienced insufficient sleep[7], %	7.5	16	34	<0.001	<0.001
Insomnia[8], %	52	61	65	<0.001	<0.001
Good self-rated health[9], %	64	61	50	<0.001	<0.001
Good self-rated physical fitness[9], %	54	46	36	<0.001	<0.001
Evening or night shift work[10], %	0.8	1.6	6.0	0.001	0.003

ME score, morningness-eveningness score; SE, standard error

[1] Chronotype was categorized according to the pre-defined cut-off values (Merikanto et al. 2012).

[2] Model 1: adjusted for age and sex (adjusting variables not included in the model when examined as response variable).

[3] *P*-value for the difference between morning and evening types (ME score was used as a categorical variable) was determined with a multiple comparisons post-hoc test with Bonferroni correction for continuous variables and with logistic regression for categorical variables

[4] *P*-value for trend was determined with linear regression (continuous variables) or logistic regression (categorical variables)

[5] Thirds of self-reported total school years according to birth cohort to adjust for the extension of the basic education system and the increase of average school years over time.

[6] Self-reported leisure-time physical inactivity.

[7] Percentage of participants experiencing insufficient sleep often or always.

[8] Percentage of participants reported having insomnia sometimes or often.

[9] Percentage of participants rating their health/physical fitness as very good or fairly good.

[10] n=2884 (morning types n=1434, intermediate types n=1182, evening types n=268).

5.2 CHRONOTYPE AND DIETARY HABITS (I, II)

Overall diet quality (I)

Overall, lower ME scores (eveningness) were associated with lower BSDS in women ($P<0.001$, model 1) and in men ($P=0.04$, model 1) (Table 8). Of the specific BSDS components, lower ME scores (eveningness) were associated with lower consumption of cereals and fish (men) and with higher intakes of alcohol and total fat (E%) (women) ($P<0.05$, model 1). Furthermore, a lower percentage of evening-typed men were in the highest fifth (13% vs. 23%) of the BSDS, whereas it was the other way around in the lowest fifth of the BSDS (evening 28% vs. morning 19%). In women, a lower percentage of evening types were similarly in the highest fifth of the BSDS (11% vs. 19%), whereas the percentages were 27% vs. 17% in the lowest fifth, respectively.

In additional analyses conducted for the doctoral thesis with the further adjusted model (model 2), the positive associations between ME score and the BSDS score remained significant (women $P=0.011$, men $P=0.016$) (data not shown). Regarding the specific BSDS components, cereal consumption (women $P<0.001$, men $P=0.03$) and alcohol intake (only in women $P=0.007$) remained significant, but the other associations were attenuated ($P>0.05$), whereas an association emerged between lower ME scores (eveningness) and lower consumption of fruits and berries (women $P=0.03$, men $P=0.047$) and red and processed meat (only in men $P=0.035$). Furthermore, when chronotype was categorized into thirds, the total scores of the BSDS did not differ between morning and evening types in either sex ($P>0.05$, model 2) (data not shown). Of the specific BSDS components, evening types had a lower consumption of cereals (women $P<0.001$, men $P=0.008$, model 2) and a higher consumption of alcohol (only in women $P=0.02$, model 2).

Table 8. *Associations between chronotype and the Baltic Sea Diet Score (BSDS) (mean values with their standard errors (SE)).*

	Chronotype[1]			
	Morning	**Intermediate**	**Evening**	
	Mean (SE)	**Mean (SE)**	**Mean (SE)**	*P*-trend,[2,3]
Women	*n*=816	n=923	n=669	
BSDS, range	1–25	1–25	1–25	
BSDS, mean	13.3 (0.1)	12.9 (0.1)	12.6 (0.1)	<0.001
Energy intake (kJ/d)	9489 (103)	9433 (64)	9389 (105)	0.54
BSDS components				
Fruits and berries (g/d)	158 (4)	152 (3)	146 (4)	0.076
Vegetables (g/d)	325 (6)	321 (4)	319 (7)	0.55
Cereals (g/d)	85 (1)	79 (1)	74 (1)	<0.001
Low-fat milk (g/d)	254 (8)	246 (5)	239 (8)	0.23
Fish (g/d)	40 (1)	39 (1)	38 (1)	0.33
Red and processed meat (g/d)	112 (2)	110 (1)	108 (2)	0.25
Total fat (E%)	30	31	31	0.0018
Fat ratio	1.5 (0.03)	1.5 (0.02)	1.5 (0.04)	0.6
Alcohol (g/d)	3.6 (0.2)	4.4 (0.13)	5.1 (0.2)	<0.001
Men	n=839	n=606	n=568	
BSDS, range	2–25	2–25	2–25	
BSDS, mean	13.0 (0.1)	12.8 (0.1)	12.5 (0.2)	0.04
Energy intake (kJ/d)	11597 (130)	11676 (90)	11776 (159)	0.43
BSDS components				
Fruits and berries (g/d)	113 (4)	110 (3)	107 (5)	0.42
Vegetables (g/d)	262 (6)	255 (4)	245 (7)	0.08
Cereals (g/d)	89 (2)	84 (1)	78 (2)	<0.001
Low-fat milk (g/d)	337 (11)	342 (8)	349 (13)	0.53
Fish (g/d)	55 (2)	53 (1)	49 (2)	0.044
Red and processed meat (g/d)	176 (3)	173 (2)	169 (3)	0.12
Total fat (E%)	32	32	32	0.67
Fat ratio	1.5 (0.04)	1.5 (0.03)	1.5 (0.04)	0.7
Alcohol (g/d)	10.6 (0.5)	11.8 (0.4)	13.3 (0.6)	0.003

E%; percentage of energy intake

[1] Chronotype was categorized based on study-specific thirds.

[2] Model 1: adjusted for age and energy (except for total fat (E%) and fat ratio) .

[3] *P*-value for trend was determined with linear regression analysis.

Energy and macronutrient intake timing (II)

The total daily energy intake did not differ between chronotypes (morning type; 7808 kJ, SE 170 vs. evening type; 7881 kJ, SE 210, P=1.00), but differences were found in the timing of energy intake between morning and evening types. Most of the evening (80%) and nearly all of the morning types (99%) had some energy intake (>0 kJ) in the morning hours (by 10:00 a.m.). Energy intake in the morning hours among evening types was 350 kJ lower than that of morning types (P<0.001) (Table 9). In the evening hours (after 8:00 p.m.), 94% of evening and 81% of morning types had some energy intake, and the energy intake among evening types was, on average, 430 kJ higher than that of morning types (P=0.001). These associations were also found when trends for ME score were tested (P<0.001).

The total daily macronutrient intakes did not differ between morning and evening types (except for protein, of which the evening types had a lower intake (16.4E%, SE 0.3, vs. 17.3E%, SE 0.3, P=0.017), whereas the timing of macronutrient intakes differed. Evening types had higher intakes of sucrose in the morning (difference between evening and morning types +0.6 E% units, P<0.01) and in the evening (difference between evening and morning types +1.1 E% units, P<0.01) (Table 9). Furthermore, evening types had lower intakes of all the other macronutrients in the morning hours than morning types (P<0.01). In the evening hours, the evening types had 5.0 E% units more fat and 1.5 E% units more saturated fat (P<0.05) than morning types (Table 6). When trends were tested for the ME score, in addition to these findings, lower scores (eveningness) were associated with higher carbohydrate, protein and alcohol intakes in the evening hours (P<0.05).

At the weekend, these findings in the timing of energy intake were more pronounced, because the evening types had, on average, 380 kJ lower energy intake in the morning hours, whereas in the evening hours they had, on average, 590 kJ more energy than the morning types (P<0.01) (data not shown). The findings were similarly more pronounced in macronutrients, particularly regarding evening hour intakes. For example, the evening types had 3.1 E% units more sucrose (P=0.025), 8.7 E% units more fat (P=0.001) and 3.5 E% units more saturated fat (P=0.003) than the morning types. These associations were also found when trends for the ME score were tested (P<0.001).

Table 9. *Means with their standard errors (SE) of energy and macronutrient intakes in morning and evening hours by chronotypes (n=1854)*[1]

	Chronotype[2]				
	Morning	**Intermediate**	**Evening**		
	***n*=904**	***n*=726**	***n*=224**	*P*[3]	*P*-trend[4]
ME score, range	19–27	13–18	6–12		
By 10:00 a.m.[5]					
Energy[6], kJ	1505 (70)	1328 (70)	1157 (87)	<0.001	<0.001
Energy, %TEI	18.7 (0.7)	16.4 (0.7)	14.3 (0.9)	<0.001	<0.001
Carbohydrate, E%	52.8 (1.3)	50.5 (1.3)	47.1 (1.6)	<0.001	<0.001
Sucrose, E%	10.7 (1.0)	10.9 (1.0)	11.3 (1.2)	<0.001	<0.001
Fiber, E%	2.8 (0.1)	2.6 (0.1)	2.4 (0.2)	0.008	<0.001
Fat, E%	23.8 (1.0)	23.3 (1.0)	19.6 (1.2)	<0.001	0.002
Saturated fatty acid, E%	9.0 (0.5)	9.0 (0.5)	7.3 (0.6)	0.002	0.018
Protein, E%	16.8 (0.6)	16.1 (0.6)	13.6 (0.7)	<0.001	<0.001
Alcohol[7], g	0.13 (0.1)	0.003 (0.1)	0.49 (0.1)	0.69	0.47
After 8:00 p.m.[8]					
Energy[6], kJ	1153 (76)	1371 (76)	1581 (94)	<0.001	<0.001
Energy, %TEI	14.0 (0.9)	16.7 (0.9)	20.0 (1.1)	<0.001	<0.001
Carbohydrate, E%	48.8 (2.0)	51.3 (2.0)	51.2 (2.4)	0.84	0.008
Sucrose, E%	12.5 (1.2)	13.4 (1.2)	13.6 (1.5)	0.002	<0.001
Fiber, E%	2.9 (0.2)	2.8 (0.2)	2.8 (0.2)	1.00	0.58
Fat, E%	21.5 (1.2)	23.4 (1.2)	26.1 (1.5)	0.002	<0.001
Saturated fatty acid, E%	8.8 (0.6)	9.7 (0.6)	10.3 (0.7)	0.033	<0.001
Protein, E%	12.4 (0.8)	13.1 (0.8)	13.4 (0.9)	0.67	0.041
Alcohol, g	1.8 (0.7)	1.9 (0.7)	4.0 (0.9)	0.088	0.018

%TEI; percentage of total energy intake, E%; percentage of energy intake, ME score; morningness-eveningness score.

[1] Including all days of the week (except for Fridays).

[2] Chronotype was categorized according to the pre-defined cut-off values (Merikanto et al. 2012).

[3] *P*-value for the difference between morning and evening types was determined using multiple comparisons post-hoc test with Bonferroni correction. Adjusted for age, sex, education, leisure-time physical activity, smoking and experienced sufficiency of sleep.

[4] *P*-value for trend (ME score was used as a continuous variable) was determined with linear regression. Adjusted for age, sex, education, leisure-time physical activity, smoking and experienced sufficiency of sleep.

[5] Intakes between 3:00 a.m.- 9:59 a.m..

[6] Alcohol was not included in energy intake.

[7] In all, 13 participants had alcohol intake > 0 g by 10:00 a.m.. Adjusted for age and sex because of the high amount of zero intakes.

[8] Intakes between 8:00 p.m.-2:59 a.m..

5.3 CHRONOTYPE AND OBESITY (I, III)

Chronotype was not associated with baseline anthropometrics (BMI, WC, BF%) in either sex (P>0.05, model 1) (Table 10).
A lower ME score (eveningness) was associated with lower BMI in men (P=0.040) in additional analyses conducted for the thesis with model 2 (data not shown). Associations between chronotype and baseline weight were also examined, but no associations emerged in either sex (P<0.05, model 2). No associations were found, either, when differences between morning and evening types (chronotype categorized into thirds) were examined in either sex (P>0.05, model 2).

Table 10. *Mean values (SE) or percentages (%) of baseline anthropometrics by chronotypes (n=4421).*

	Chronotype[1]			Model 1[2,3]
	Morning	**Intermediate**	**Evening**	*P*-trend
Women	n=923	n=816	n=669	
ME-score, range	22-27	17-21	5-16	
BMI				
BMI, kg/m²	26.7 (0.17)	26.7 (0.11)	26.6 (0.17)	0.84
BMI ≥ 30.0, %	21.8	22.7	22.2	0.94
WC				
WC, cm	86.0 (0.42)	86.5 (0.27)	86.9 (0.43)	0.19
BF%				
BF, %	35.2 (0.23)	35.2 (0.15)	35.3 (0.24)	0.92
Men	n=839	n=606	n=568	
ME-score, range	22-27	18-21	6-17	
BMI				
BMI, kg/m²	27.2 (0.13)	27.1 (0.09)	26.9 (0.16)	0.19
BMI ≥ 30.0, %	21.4	16.5	17.9	0.27
WC				
WC, cm	96.1 (0.37)	96.3 (0.26)	96.7 (0.46)	0.68
BF%				
BF, %	24.8 (0.2)	24.7 (0.14)	24.6 (0.25)	0.36

ME score; morningness-eveningness score, SE; standard error; WC; waist circumference
[1] Chronotype was categorized based on study specific thirds.
[2] Model 1: adjusted for age.
[3] *P*-value for trend (ME score was used as a continuous variable) was determined with linear regression for continuous anthropometrics and with logistic regression for categorical anthropometrics.

Substudy III examined changes in the anthropometric measures (weight, BMI and WC) over the seven-year follow-up period. A sex-stratified analysis showed that weight gain was higher among evening-typed women during the follow-up period than among morning-typed women; evening-typed women gained 2.3 kg, on average, while morning-typed women gained 0.3 kg on average (P=0.019) (Table 11). No such associations emerged in men (P>0.05). Furthermore, because depression links to chronotype and obesity (Zhao et al. 2009, Merikanto et al. 2013a, 2015), those diagnosed with depression (n=130) were excluded from the analyses. After the exclusion, weight gain among evening-typed women was 1.5 kg, on average, while it was 0.5 kg among morning-typed women, but the difference between morning and evening types was no longer statistically significant (P>0.05).

In additional analyses conducted for the thesis, trends for the ME score were tested. Lower ME scores (eveningness) were associated with higher increases in weight (P-trend =0.003, model 2) and BMI (P-trend= 0.003, model 2), which remained significant after excluding those with depression (weight change, P-trend=0.016, BMI change P-trend=0.012, model 2).

Table 11. *Mean (SE) changes in weight and BMI over 7-year follow-up period (*n*=1097).*

	Chronotype[1]			
	Morning	**Intermediate**	**Evening**	$P^{2,3}$
Women	n=293	n=254	n=72	
Weight change, kg	0.31 (0.32)	1.04 (0.35)	2.32 (0.65)	0.019
BMI change, kg/m²	-0.09 (0.13)	0.21 (0.13)	0.72 (0.25)	0.024
Men	n=259	n=179	n=40	
Weight change, kg	1.03 (0.38)	0.53 (0.45)	-0.25 (0.96)	1.00
BMI change, kg/m²	0.17 (0.12)	-0.03 (0.15)	-0.17 (0.32)	1.00
Participants diagnosed with depression were excluded (*n*=995)				
Women	n=270	n=221	n=59	
Weight change, kg	0.46 (0.32)	1.09 (0.35)	1.48 (0.67)	0.51
BMI change, kg/m²	-0.04 (0.12)	0.24 (0.14)	0.45 (0.26)	0.48
Men	n=245	n=165	n=35	
Weight change, kg	0.88 (0.37)	0.34 (0.45)	-0.03 (0.99)	1.00
BMI change, kg/m²	0.12 (0.12)	-0.10 (0.15)	-0.09 (0.33)	1.00

ME score; morningness-eveningness score, SE;standard error, WC; waist circumference

[1]Chronotype was categorized according to the pre-defined cut-off values (Merikanto et al. 2012)

[2] Adjusted for age, education, smoking, leisure-time physical activity, experienced sufficiency of sleep time, baseline BMI/weight depending on which of these were examined. Weight change was further adjusted for baseline height.

[3] P value for the difference between morning and evening types was determined with a multiple comparisons post-hoc test with Bonferroni correction for continuous variables and with logistic regression for categorical variables

5.4 INTERRELATIONSHIPS BETWEEN CHRONOTYPE, DIETARY HABITS AND OBESITY (I,III)

Chronotype, BSDS and obesity (mediation test, I)

For men, the requirements of the first two steps of the four-step mediation test were met as in step 1, a statistically significant positive association between chronotype and BMI emerged (but not for the other anthropometric measures), and a positive association between chronotype and the BSDS was found (P<0.05, model 2) (Figure 1) in step 2. However, the requirements for steps 3 and 4 were not met because no association between BSDS and anthropometric measures was found in step 3. The associations between the BSDS and anthropometric measures were unaffected in step 4a when chronotype was added to the model. Furthermore, the association between chronotype and BMI remained significant in step 4b when the BSDS was added to the model. For women, only the requirements of step 2 were met because a positive association between chronotype and the BSDS was found (P<0.05) (data not shown). Together, these findings indicated that the BSDS did not mediate the association between chronotype and obesity because the requirements for the steps were not sufficiently met in either sex.

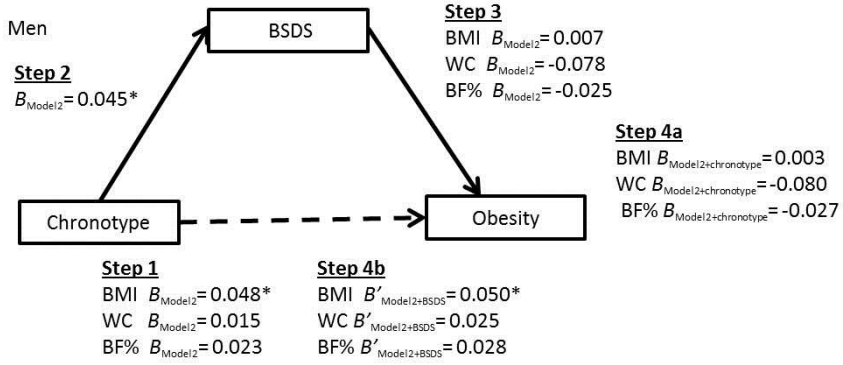

Figure 5 The Baltic Sea diet score (BSDS) mediated pathway between chronotype and anthropometric measures for men. *P<0.05, model 2 was adjusted for age, education, smoking, leisure-time physical activity and sleep duration.

Chronotype, BSDS and obesity (effect modification, I)

The interaction analysis with the likelihood ratio test to determine whether chronotype modified the association between the BSDS and obesity indicated an interaction between chronotype and the BSDS on WC (*P*=0.046) and BF% (*P*=0.036) but only for men (Table 12). However, in the chronotype-stratified analysis, the directions of associations between the BSDS and WC were parallel in all chronotypes, whereas for BF% the association seemed positive in evening types and inverse for the other chronotypes, but none of these associations were statistically significant (model 2) (*P*>0.05). For women, no significant interaction was found between chronotype and BSDS on anthropometric measures (data not shown).

Table 12. ***Mean*** *BMI, WC and BF% according to the Baltic Sea Diet Score by chronotypes in men.*

	Baltic Sea Diet Score fifths[1]				
	1 (low) Mean (95% CI)	3 Mean (95% CI)	5 (high) Mean (95% CI)	*P*-trend[2]	*P*-int.[3,4]
BMI (kg/m2)					0.10
Morning	27.9 (26,6-27.6)	27.2 (26.9-27.4)	27.2 (26.7-27.7)	0.76	
Intermediate	26.7 (26.1-27.2)	26.6 (26.3-26.9)	26.5 (25.9-27.5)	0.69	
Evening	26.4 (25.8-26.9)	26.5 (26.1-26.8)	26.6 (25.8-27.4)	0.68	
WC (cm)					0.046
Morning	96.7 (95.2- 98.1)	96.5 (95.8- 97.3)	96.4 (95.0- 97.8)	0.84	
Intermediate	95.2 (93.7- 96.7)	94.6 (93.8- 95.5)	94.1 (92.4- 95.7)	0.41	
Evening	95.3 (93.7- 97.0)	95.0 (94.0- 96.0)	94.4 (92.2- 96.6)	0.57	
BF%					0.036
Morning	25.8 (24.3- 25.9)	25.3 (24.9- 25.7)	25.5 (24.7- 26.3)	0.53	
Intermediate	24.9 (24.6- 25.8)	24.2 (23.7- 24.7)	23.5 (22.5- 24.5)	0.073	
Evening	23.9 (23.0- 24.8)	23.9 (23.3- 24.5)	24.0 (22.7- 25.2)	0.90	

CI; confidence interval, BF%; body fat percentage, BSDS; the Baltic Sea Diet Score, WC; waist circumference.

Data are presented as means and 95% confidence intervals.

[1] BSDS fifths for men: 1st, 2–9 points; 2nd, 10–12 points; 3rd, 13–14 points; 4th, 15–16 points; 5th,17–25 points.

[2] *P*-value for trend between the BSDS and anthropometric measures and between the BSDS and anthropometric measures by chronotypes was determined with linear regression. Significance testing was at *P*<0.05.

[3] *P* value for interaction was determined with likelihood ratio test. Significance testing was at *P*< 0.05.

[4] Adjusted for age, energy intake, education, physical activity, sleep duration and smoking.

Chronotype, energy intake timing and anthropometric measures (III)

In the morning, participants in the second-highest fourth of morning energy intake were less likely to have increases (5%) in anthropometrics (weight [OR 0.48, 95% CI 0.31, 0,73], BMI [OR 0.57, 95% CI 0.37, 0.89], WC [OR 0.66, 95% CI 0.45, 0.97]) than those in the highest fourth of morning energy intake over the follow-up period.

In the evening, those participants in the highest fourth of evening energy intake were almost two-times more likely with obesity at baseline (OR 1.95, 95% CI 1.18, 3.22) and at follow-up (OR 1.97, 95% CI 1.21, 3.21) than those in the lowest fourth (*P*-trend<0.05, model 2).

A significant interaction emerged between chronotype and morning energy intake on increases in weight (≥5%) and BMI (≥5%) when effect modification by chronotype was tested (Table 13). Among morning types, higher morning energy intake seemed to increase the risk of gaining weight and having increases in BMI, whereas for evening and intermediate types these tendencies were in the opposite direction, although none of these associations were significant. As for evening energy intake, no significant interactions emerged between chronotype and evening energy intake on any of the anthropometric measures (data not shown).

Table 13. *Association between morning energy intake (continuous variable) and weight gain and BMI increase by chronotypes* (n=*1097).*

	Morning energy intake (%TEI)									
	Morning (*n*=552)			**Intermediate (*n*=433)**			**Evening (*n*=112)**			
	OR	**95% CI**		**OR**	**95% CI**		**OR**	**95% CI**		*P*-int.[1,2]
Weight gain ≥ 5%	1.02	1.00	1.04	0.98	0.96	1.01	0.96	0.91	1.01	0.025
BMI increase ≥ 5%	1.02	1.00	1.04	0.97	0.95	1.00	0.97	0.92	1.02	0.012

%TEI; percentage of total energy intake, CI; confidence interval, OR; odds ratio

[1] Model 2 adjusted for age, sex, education, smoking, leisure-time physical activity, experienced sufficiency of a sleep time, baseline energy intake and with baseline BMI/weight depending on which of these was examined. Weight change was further adjusted for baseline height.

[2] Interaction was tested by adding an interaction term between chronotype and %TEI in the morning (as a continuous variable) to the model.

5.5 GENETIC ASSOCIATIONS OF CHRONOTYPE (IV)

Clock gene analysis

Seven independent associations signals were found within three clock genes (*CRY1*, *NFIL3*, *NR1D2* aka *Rev-erbβ*) with continuous ME score and single-item chronotype when *P*-values were corrected with the BH method ($P<0.05$), whereas no associations were found by the binary ME score. Within *CRY1*, three independent association signals with evening chronotype emerged. Two of the signals (lead SNPs rs8192440, with 39 correlated SNPs and rs77706154) emerged with both a continuous ME score and a single-item chronotype. An SNP (rs1017168A) that has previously been associated with evening type was among the correlated SNPs associated with rs8192440 (Jones et al., 2019). The third of the signals within *CRY1* emerged solely with a continuous ME score (lead SNP rs3741891, with 43 correlated SNPs). Within *NFIL3*, three independent signals associated with the morning chronotype emerged ($P<0.05$). One signal was found with both a continuous ME score (lead SNP rs2482702, with 5 correlated SNPs) and a single-item chronotype (lead SNP rs9409419, with 6 correlated SNPs), whereas one signal emerged solely with a continuous ME score (lead SNP rs2440590, with four correlated SNPs) and another one solely with a single-item chronotype (lead SNP rs2440592, with four correlated SNPs). One of the *NFIL3* associated SNPs (rs2482705A) has previously been associated with the morning type (Kripke et al., 2014). Within *NR1D2*, one independent association signal with evening chronotype emerged with both the continuous ME score and the single-item chronotype (lead SNP rs4131403, with 21 correlated SNPs).

One independent association signal within *NR1D2* (*Rev-erbβ*) (lead SNP rs4131403, with 22 correlated SNPs) remained significantly associated with a continuous ME score and a single-item chronotype with the BY method ($P<0.05$).

GWAS of chronotype

No genome-wide significant associations were found in the GWAS of chronotype; however, a few suggestive ($P<1\times10^{-5}$) associations emerged, among which one intergenic SNP (rs79036472) was found in continuous and binary ME scores. The previously genome-wide associated top 7741 SNPs of the recent meta-analysis based on the UK Biobank and the 23andMe data (Jones et al. 2019) were not replicated in the present study, either, but the directions of the SNPs' effects were mostly in the same direction. Regarding the continuous ME score, 80.1% (6202 of 7741) of the SNPs had the same direction of effect (binomial test $P<2.2\times10^{-16}$) for the binary ME score, 82.8% (6409 of 7741) of the SNPs had the same direction (binomial test $P<2.2\times10^{-16}$), and for the single-item chronotype, 87.4% (6766 of 7741) of the SNPs had the same direction of effect (binomial test $P< 2.2\times10^{-16}$) as the meta-analysis.

Genetic risk score for chronotype

The GRS of 313 SNPs (Jones et al. 2019) predicted chronotype in the present study population because a higher GRS was associated with the evening type in both the continuous and binary ME score and the single-item chronotype (P<0.001) (Table 14). Furthermore, the GRS explained for ~1.4% (continuous ME score, R^2=0.01387), ~1.3% (binary ME score, Nagelkerke's pseudo R^2=0.01312), and ~1.9% (single-item chronotype, Nagelkerke's pseudo R^2=0.01893) of chronotype variation. No associations were found when the individual SNPs of the GRS were examined (P>0.05).

Table 14. *Linear or logistic regression analysis of a genetic risk score (GRS) association with chronotype based on 313 chronotype associated SNPs (Jones et al., 2019).*

	continuous ME score			**binary ME score**			**single-item chronotype**		
	B	SE	*P*[1]	OR	95% CI	*P*[1]	OR	95%CI	*P*[1]
GRS	-0.49	0.05	<0.001	1.24	1.18,1.31	<0.001	1.3	1.24,1.37	<0.001

Decreasing beta and increasing odds ratios refer to evening type.
CI; confidence interval, OR; odds ratio, ME; Morningness-Eveningness
[1] Adjusted for age, sex, five principal components and genotyping batch.

6 DISCUSSION

Chronotype trait is determined half by genetics and half by environmental factors (Koskenvuo et al. 2007). The associations of chronotype with dietary habits, obesity and genetics are recognized but relatively understudied in population-based samples. This thesis aimed to explore dietary habits (overall diet quality, energy and macronutrient intake timing), obesity/weight change and genetic associations of chronotype using population-based samples. Findings from the thesis suggested that, compared to morning types, evening types had a lower overall diet quality and postponed timing of energy and macronutrient intakes. However, despite their unhealthier dietary habits, evening types were not significantly more prone to obesity or weight change than morning types. No evidence was found, either, that chronotype would modify the association between the BSDS and obesity or that the BSDS would mediate the association between chronotype and obesity, whereas a higher energy intake in the evening hours was associated with a higher obesity risk independent of chronotype. Furthermore, a novel association between chronotype and one of the key clock genes *NR1D2* was found when the genetic basis of chronotype was studied. Instead, the GWAS of chronotype did not yield any genome-wide significant associations, but the GRS based on 313 SNPs that have previously been associated with chronotype successfully predicted chronotype in the current study population.

6.1 DIETARY HABITS (I,II)

Overall diet quality

Those with a higher tendency towards eveningness had a lower adherence to the healthy diet assessed with the BSDS in both sexes. The proportion of evening types was also higher in the lowest fifth of the BSDS, whereas in the highest fifth it was lower compared to morning types (difference in proportions between morning and evening types in both cases was approximately 10%). However, the mean difference between the total scores of the BSDS in morning and evening types was non-significant in both sexes.

The adherence to the BSDS by chronotypes has not been examined before, but the findings are in line with three very recent studies that have found an association between evening type and lower adherence to the healthy diet assessed with the Mediterranean Diet score in healthy Italian adults (n=416) (De Amicis et al. 2020), middle-aged Italians (n=172) (Muscogiuri et al. 2020) and in Spanish university students (n=457) (Rodríguez-Muñoz et al. 2020). However, a small-scale study on pregnant Brazilians (n=100) found

no association between chronotype and the revised version of Brazilian Healthy Eating Index (Gontijo et al. 2019).

Of the individual BSDS components, evening types had a lower consumption of cereals and a higher alcohol intake (women) than morning types. A Japanese study of female dietetics students (n=3304) (Sato-Mito et al. 2011a), a Finnish population-based study including DILGOM 2007 participants (n=4493) (Kanerva et al. 2012) and a Spanish study of university students have reported lower cereal intake among evening types (n=457) (Rodríguez-Muñoz et al. 2020), while a study on pregnant Brazilians (n=100) found opposite results (Gontijo et al. 2019), whereas some studies report no association between chronotype and cereal intake (Sato-Mito et al. 2011b, Mota et al. 2016, Silva et al. 2016, Muñoz et al. 2017). However, it should be noted that the definition of cereals in the present study included Nordic cereals (rye, oat and barley), whereas in the other studies the definition was wider and also included other grains. Higher alcohol intake among evening types has been reported earlier by numerous studies (Sato-Mito et al. 2011a, Kanerva et al. 2012, Suh et al. 2017). A recent review that has gathered the current evidence on dietary habit differences between chronotypes (the review also included substudies I-III of the thesis) also listed higher alcohol intake among evening types as one of the foods and nutrients with strongest evidence of chronotype associations (Mazri et al. 2020). Other foods and nutrients listed in the review included lower vegetable consumption and higher intakes of sweet foods, sugared beverages and caffeine among evening types (Mazri et al. 2020). Of these foods/nutrients, vegetables were also a component of the BSDS in addition to alcohol, and the direction of the association was similar although not statistically significant in the present study. Thus far, only one population-based, larger-scale study has been published on the associations between single nutrient and food intakes and chronotype (Kanerva et al. 2012), which could affect the findings and the strengths of the associations. Most of the previous studies have also not evaluated sex differences between chronotypes on food and nutrient intakes. Furthermore, the use of different methods for assessing dietary intake and differences in confounding factors may also have affected the findings.

In conclusion, despite the slight discrepancies in the findings, it can be concluded that the overall evidence, together with the present findings, suggest that the evening types have unhealthier food and nutrient intakes and poorer overall diet quality compared to morning types.

Timing of energy and macronutrient intakes

The present study's findings indicated that total daily energy intake between chronotypes did not differ, but differences were found in distribution of energy and macronutrient intakes throughout the day. In the morning (till 10:00 a.m.), evening types had lower intakes of energy and all the other macronutrients except for sucrose than did morning types, whereas in the

evening (after 8:00 p.m.) they had higher intakes of energy, sucrose, fat and saturated fat. These findings were even more pronounced at the weekend.

These findings are in line with the literature. Previous studies have shown that evening types have a higher energy intake after 8:00 p.m. than morning types (Baron et al. 2011, Lucassen et al. 2013) (see Table 3 on page 35). Of macronutrients, a small-scale US study (n=52) found that evening types had higher percentages of their total daily carbohydrate, protein and fat intake after 8:00 p.m. (Baron et al. 2013), whereas another US study on short-sleeping participants with obesity (n=119) found no differences between chronotypes in total macronutrient intakes (carbohydrates, protein and fat) after or before 8:00 p.m. (Lucassen et al. 2013). Morning distribution of energy and macronutrient intakes by chronotypes has not been evaluated before, although evening types more often report skipping breakfast (e.g., Nakade et al. 2009, Sato-Mito et al. 2011a, Meule et al. 2012, Teixeira et al. 2018).

The later energy intake timing of evening types may simply be a reflection of their preferences to time the daily activities later; however, the differences between chronotypes were smaller in the present study compared to the previous studies (Baron et al. 2011, 2013, Lucassen et al. 2013) (see Table 3 on page 35). This may indicate that evening types may have a slightly earlier phase in the present population-based study, whereas the study populations may have been more selected, including participants with more flexible timetables in the previous studies (Baron et al. 2011, 2013, Lucassen et al. 2013).

Later timing of energy intake among evening types may also be reflected in their lower overall diet quality. Few studies have indicated that eating breakfast associates with higher carbohydrate intake, whereas individuals are more likely to eat fatty foods, particularly foods that include saturated fat, in the evening hours (Khare and Inman 2006, Myhre et al. 2015, Gibney et al. 2018, Beaulieu et al. 2020). A laboratory study of 44 healthy young adults examined the impact of meal timing and chronotype on appetite and food reward (Beaulieu et al. 2020). They found that liking and wanting high-fat foods were lower in the morning (8-10 a.m.) than in the early evening (4-6 p.m.) and that evening type was associated with a greater desire for high-fat food compared to morning type. It has also been suggested that it may be easier to make healthier food choices in the morning than in the evening because self-control tends to diminish as the day wears on (Boland et al. 2013). It could be speculated that this may particularly apply for evening types, because they are also more likely to live against their biological rhythms (Wittmann et al. 2006), and they more often report insufficient sleep (found also in the present study, see Table 7 on page 56), which can make them feel more tired during the day, thus leading to unhealthier food choices.

Food intake is the primary entrainer of the peripheral clocks, so dietary habits, including timing of dietary intake and composition of diet, may both

have a potential to modulate the phase of circadian clocks and even to disrupt the synchronization between central and peripheral clocks, which may lead to circadian misalignment (Asher and Sassone-Corsi 2015, Ribas-Latre and Eckel-Mahan 2016, Oike 2017, Reinke and Asher 2019). Most of the evidence thus far regarding a phase shift in peripheral clocks caused by feeding timing or composition of food is mainly from rodent models (Damiola et al. 2000, Stokkan et al. 2001, Ribas-Latre and Eckel-Mahan 2016, Oike 2017), although some evidence in humans also exists. For example, a laboratory study of 10 healthy men demonstrated the effect of a five-hour delay in meals (breakfast, lunch and dinner) on markers of the master clock and multiple peripheral circadian rhythms (Wehrens et al. 2017). They found a delay in glucose rhythms (6 hours) and in adipose tissue PER2 expression (~1 hour). No changes in the levels of central clock markers were found that indicated the entrainment of peripheral clocks but not the master clock. Additionally, another laboratory study also of 10 healthy men demonstrated the effects of a morning versus evening carbohydrate-rich meal on the circadian phase of core body temperature, heart rate, and salivary melatonin rhythms (Kräuchi et al. 2002). They reported an advanced circadian phase with a carbohydrate-rich meal in the morning in body temperature (59 min) and heart rate (43 min) compared to a carbohydrate-rich meal in the evening. No effect on dim light melatonin onset was found. The role of dietary composition and the timing of energy intake in the phase alignment of chronotypes, however, is yet to be determined.

In conclusion, the present findings, together with the overall evidence on differences in energy intake timing of chronotypes, suggest that the evening types have postponed energy intake compared to morning types. Chronotype differences in timing of energy intake could also provide one explanation for the overall unhealthier dietary habits of evening types compared to morning types.

6.2 OBESITY (I,III)

In contrast to the hypothesis, the findings suggested that men with a higher tendency towards eveningness more likely had a lower baseline BMI than morning-typed men, whereas no baseline associations between chronotype and obesity emerged in women. Evening-typed women were more likely to gain weight and have increases in their BMI than morning-typed women during the seven-year follow-up period. However, after the exclusion (n=130) of those with depression, the difference between morning and evening types attenuated to non-significant, although a positive association between a higher tendency towards eveningness and weight gain remained. Thus, it is likely that depression influenced the findings, at least to some extent, but also the lower sample size and lower statistical power to detect differences after excluding those with depression.

Cross-sectional studies on the associations between chronotype and obesity have generally yielded inconsistent results (see Table 4 on page 41). Some studies have reported higher BMI/obesity risk or unhealthier body composition among evening types compared to morning types (Yu et al. 2015, Celis-Morales et al. 2017, Sun et al. 2020), while some studies have reported no associations (Johnsen et al. 2013, Knutson et al. 2017). Furthermore, the longitudinal evidence on these associations is scarce and is based thus far on small-scale studies with selected study populations (Culnan et al. 2013, Ruiz-Lozano et al. 2016). Evening-typed US college freshmen (n=54) were more prone to increases in BMI during an eight-week follow-up (Culnan et al. 2013), whereas bariatric surgery patients with evening preference types were less likely to lose more weight after the surgery than morning types during a six- year follow-up in a Spanish study (n=252) (Ruiz-Lozano et al. 2016).

Furthermore, the present study's findings indicated interaction between sex and chronotype on anthropometric measures. Some of the previous studies have also addressed sex differences. Among the cross-sectional studies, the present study's finding are in line with a Korean study of a middle-aged population in which evening-typed women had higher WC, fat mass and visceral mass, but no associations were found in men (Yu et al. 2015). However, studies from Norway (Johnsen et al. 2013) and the USA (Knutson et al. 2017) found no sex-stratified differences between chronotypes and anthropometrics, while three studies did not report sex interactions but adjusted their analyses for sex (Celis-Morales et al. 2017, Anothaisintawee et al. 2018, Sun et al. 2020). Regarding the two longitudinal studies, the weight gain was more pronounced among male than female college freshmen in a US study (Culnan et al. 2013), while the Spanish study on obese participants did not evaluate sex differences (Ruiz-Lozano et al. 2016). Regarding the US study of college freshmen, it was based on self-reported data on anthropometrics, which may have affected the findings, particularly among women, together with the very short follow-up period (Culnan et al. 2013).

There may be several reasons behind these overall inconsistent findings. First, the different study populations used may affect the findings; some of the studies were conducted on certain age groups (e.g., college students, middle aged participants) (Culnan et al. 2013, Yu et al. 2015, Sun et al. 2020), on different ethnic subgroups (Knutson et al. 2017, Sun et al. 2020) or on health-compromised participants (Ruiz-Lozano et al. 2016, Anothaisintawee et al. 2018). Second, different chronotype assessment methods used and differences in confounding factors may affect the findings. Moreover, none of the previous studies have taken depression into account when studying these associations. All these perspectives also make it hard to compare these studies.

In conclusion, findings from the present study indicated that a higher tendency towards eveningness in men associated with lower BMI. Evening-

typed women were more likely to gain weight than morning-typed women, but these associations were at least partly explained by depression. Based on the whole evidence, it is impossible to draw conclusions on these associations due to the finding's inconsistencies.

6.3 CHRONOTYPE, DIETARY HABITS AND OBESITY (I,III)

Chronotype, healthy diet and obesity (I)

No evidence that adherence to the healthy diet would mediate the association between chronotype and anthropometric measures was found in the present study. Furthermore, no evidence was found that chronotype would modify the association between the healthy diet and obesity in the present study, either. This is at least partly explained by the fact that no strong associations were found between chronotype and obesity or between the BSDS and anthropometric measures.

This is in line with a recent study of Italian adults (n=416) in which a healthy diet, assessed with the Mediterranean Diet score, did not explain the association between chronotype and WC (De Amicis et al. 2020). They found that evening types had a lower adherence to the Mediterranean Diet and that morning type was inversely associated with WC, but the association between chronotype and WC was independent of the adherence to the Mediterranean Diet.

In conclusion, it is impossible to make definite conclusions based on these studies. It seems, however, that evening types are not significantly more prone to obesity in the present study population despite a lower overall diet quality. This could partly be due to the fact that there were no differences in total daily energy intake between chronotypes. However, it should be noted that evening types also reported lower physical activity, which most likely affects the energy requirements. It is also well known that people are more likely to gain weight with age (Pajunen et al. 2012). Therefore, another likely explanation could be related to the fact that the evening types were, on average, almost a decade younger than the morning types, and the possibility of some remaining residual confounding despite adjusting the analysis for age cannot be completely ruled out.

Chronotype, timing of energy intake and obesity (III)

Chronotype played no role in the association between morning or evening energy intake and obesity in the present study. Evening energy intake was associated instead with obesity independent of chronotype; participants in the highest fourth of evening energy intake were almost two-times more

likely with obesity at the baseline and at follow-up than those in the lowest fourth despite chronotype.

Similar findings regarding evening energy intake have been reported in a small-scale study of American adults (n=52) in which a higher evening energy intake was associated with a higher BMI independent of sleep timing (Baron et al. 2011) (see Table 3 on page 35). Another US study of middle-to-older-aged participants (n=872) found that higher evening energy intake within two hours before bedtime was associated with a higher risk of being overweight or obese but, unlike in the present study, the association was even stronger among evening types (Xiao et al. 2019). Furthermore, the same study also reported that morning types particularly benefited from higher energy intake in the morning within two hours after waking up in terms of obesity risk. A study of college-aged US participants (n=110) also indicated that eating closer to the biological night is associated with higher body fatness (assessed with DLMO), while they found no differences in clock hour of energy intake in terms of body fatness (McHill et al. 2017). A Spanish study of university staff (n=171) suggested that those chronotypes eating more in accordance with intrinsic circadian rhythm were less likely with obesity (Muñoz et al. 2017). Finally, a Thai study (n=210) suggested that later breakfast timing by evening types could mediate the association between chronotype and BMI on participants with type 2 diabetes (Nimitphong et al. 2018).

In conclusion, the majority of these studies suggested that a later eating time or eating closer to the biological night seem to play a role in obesity, although the role of chronotype in this association seems unclear. Furthermore, the role of chronotype in the association between morning energy intake and obesity remains unclear and requires further study. Overall, different study designs and definitions of timing of energy intake/meal make comparing these studies difficult and may account for the inconsistencies between studies. Some of the previous studies suggested that instead of examining clock hours of eating, it might be useful to analyze time of eating by measuring the time of food intake relative to the sleep and wake-up times, which could better capture the actual circadian timing of eating. The sleep and wake-up times were unavailable in the current study.

6.4 GENETIC ASSOCIATIONS OF CHRONOTYPE (IV)

Clock gene analysis

Findings of the present study indicated a novel association between chronotype and the *NR1D2* gene, because an independent association signal within *NR1D2* (lead SNP rs4131403) with chronotype emerged. *NR1D2* is part of an additional feedback loop in circadian clock machinery encoding a repressor that controls *ARNTL* transcription with retinoic acid orphan receptors (RORs) as opposing activators (Liu et al. 2008, Takahashi 2017)

(see Figure 2 on page 18). SNPs within this gene have not been associated with chronotype earlier, but a difference in timing of expression of this gene has been found between extreme morning types and extreme evening types based on hair follicle cells from fourteen individuals with extreme morning or evening chronotype (Ferrante et al. 2015). Furthermore, NR1D2 has been shown to have a role in carbohydrate and lipid metabolism in the liver and skeletal muscle (Ramakrishnan et al. 2005, Wang et al. 2007). Mouse studies have demonstrated that administration of synthetic NR1D2 ligands or NR1D2 agonist may result in increased energy expenditure, reduced fat mass and improved dyslipidemia and hyperglycemia (Solt et al. 2012).

Although no strong associations were found between chronotype and obesity in the present study, these findings are supported by several epidemiological studies that have reported an association between chronotype and metabolic diseases (Merikanto et al. 2013b, Celis-Morales et al. 2017, Knutson and von Schantz 2018). Thus, the genetic background of chronotype may have a potential to at least partly explain the adverse health and health behavior related to evening types.

GWAS of chronotype

The current study represents the first GWAS of chronotype in which chronotype has been assessed with a validated chronotype questionnaire in addition to the simple self-evaluation question. However, no genome-wide significant associations were found in the present study. This is probably due to the small sample size in terms of conducting a GWAS, which limits the power to detect genome-wide associations. The previous GWASs of chronotype, which have identified 351 independent chronotype- associated loci, have all included large sample sizes based on two large cohorts, the 23andMe (Hu et al. 2016, n=89,283) and the UK Biobank (Lane et al. 2016; n=100,420, Jones et al. 2016; n=128,266) or a meta-analysis of these two cohorts (Jones et al. 2019; n=697,828).

No replication of the top 7742 genome-wide significant SNPs of the previous GWAS meta-analysis of chronotype (Jones et al. 2019) was found, either. However, the directions of effects of the SNPs were mostly in the same direction, showing a good consistency between our results and the GWAS of Jones et al. (2019).

More GWASs of chronotype in large sample sizes and different populations are needed to make definite conclusions. More GWASs in which chronotype assessment should be based on validated questionnaires are also warranted.

GRS of chronotype

The GRS was associated with chronotype in the present study population and accounted for ~1.3% (binary ME score), ~1.4% (continuous ME score) and ~1.9% (single-item chronotype) of chronotype variation. The estimate for chronotype heritability was 50% and that for additive genetic effect was 12%

(Koskenvuo et al. 2007) in the Older Finnish Twin Cohort Study data (n=8753). It has been generally noticed that the variance in the phenotype of interest explained by findings from GWASs tend to be much lower than what is found by using classic twin and family studies on assessing heritability (Mayhew and Meyre 2017). This gap is called "missing heritability," and there are many suggested reasons behind it, such as epigenetics, gene-by-gene interactions (epistasis), and small-size effect variants. It has also been suggested that the classic heritability studies could overestimate heritability.

Another study have also developed and tested a GRS for chronotype based on independent GWAS findings (Vera et al. 2018). This study included Spanish overweight and obese participants (n=1693), and the GRS for chronotype was based on 15 previously chronotype-associated SNPs. The GRS also successfully predicted chronotype in their study, although they did not assess the variance that the GRS explained. It can be concluded that, together, this evidence suggests that GRS based on GWAS studies may be a useful tool for capturing the genetic component of chronotype in different populations. Developing a GRS based on the previous findings of GWASs may also be a more powerful tool to reveal chronotype associations in smaller sample sizes that may lack the power to detect individual genome-wide significant associations.

6.5 MAIN STRENGTHS AND LIMITATIONS OF THE STUDY

6.5.1 STUDY SAMPLES AND DESIGNS

This thesis was based on large population-based studies: FINRISK 2007 (and related substudies DILGOM 2007/2014 and FINDIET 2007) and FINRISK 2012 with acceptable participation rates, which can be considered a strength of the study. However, the possibility that health-conscious people tend to be those who are more likely to participate in health surveys may have affected the results to some extent (Jousilahti et al. 2005, Tolonen et al. 2010). Furthermore, some selection may have occurred regarding the DILGOM follow-up study, because those who were lost during the follow-up were more likely evening (29%) than morning types (21%). They also tended to be younger, more likely with less education, more physically inactive (particularly evening types), more often smokers and with higher anthropometric measurements (particularly evening types) than those who attended. Thus, it is possible that the differences between morning and evening types may be even more distinctive in reality than was found in the thesis.

Furthermore, all the substudies were observational (cross-sectional [I, II, IV] and longitudinal [III]) and cannot reveal causality. However, the

longitudinal design of substudy III provided an opportunity to assess changes in the outcome measures over time. It should be noted, however, that the changes in outcome measures (anthropometric measurements) were predicted with baseline predictors (chronotype, energy intake timing), which does not take into account possible changes in predictors during the follow-up period and whether it has any bearing on the findings. However, because chronotype was also assessed in the DILGOM follow-up, it provided a possibility to study whether participants reported a change in chronotype during the follow-up period. Thus, the main findings of substudy III were also analyzed without participants whose chronotype had changed from morning to evening type or vice versa during the follow-up period (n=9), which did not alter the findings.

6.5.2 MEASURES

Chronotype was assessed with a shortened ME questionnaire that included six items from the original 19-item MEQ (Horne and Östberg 1976, Hätönen et al. 2008). The original MEQ has been validated against circadian variation in body temperature (Horne and Östberg 1976, Bailey and Heitkemper 2001) and DLMO (Kantermann et al. 2015). The shortened version accounted for 83% of the total variance of the original MEQ with Cronbach's α of 0.80 indicating a good internal consistency (Hätönen et al. 2008). Two different cut-off value strategies were used to categorize chronotype: categorizing the ME sum score into study-specific thirds (I) and using pre-defined cut-off values that reflected the scaling of the original MEQ sum score (Merikanto et al. 2012) (II and III). This could potentially affect the results and their comparison. However, it has been found when comparing these two cut-off value strategies that categorizing into thirds yields more even group sizes, which means that some of those categorized as intermediate types by the pre-defined method were categorized as evening types when the ME score was divided into thirds (Maukonen 2015). Furthermore, regarding differences in energy and macronutrient intakes, a slight attenuation of mean differences in energy and macronutrient intake values when using thirds was detected. However, because the group size of evening types was also larger when using thirds, the statistical power to detect differences was also higher. Nevertheless, all analyses (I and II, when applicable) have been conducted in the thesis with both categorization methods, and they generally yielded similar results. It should also be noted that chronotype trait is a continuum with extreme types in the opposite ends of the continuum; therefore, drawing the lines or making cut-off values to a continuous trait is not straightforward in any case. For this reason, all analyses have also been conducted with the ME score used as a continuous variable. Moreover, the possibility of reporting biases related to self-reported data affecting the total sum score and categorizing of chronotype cannot be ruled out.

A limitation concerning all dietary assessment methods in general is that individuals tend to overestimate the consumption of healthy foods and underestimate the consumption of unhealthy foods. Women and people with obesity are particularly more likely to misreport their food intake (Paalanen et al. 2006). Evening types were more often women in the present study. Thus, it could be that evening types, particularly evening-typed women, may be more prone to underreport their food intake than morning types, affecting the dietary intake differences between morning and evening types. Thus, it could be that the chronotype differences in dietary intake may be even more distinctive in reality.

The FFQ is a widely used and accepted method in nutritional epidemiology studies (Willett 2013). The FFQ has also been found to be a valid tool in assessing the overall diet quality with dietary scores (Benítez-Arciniega et al. 2011, van Lee et al. 2013). Furthermore, validation studies of FFQ have shown that the FFQ used in substudy I has a good ability to rank participants according to their food and nutrient consumption, which is the FFQ's main purpose (Männistö et al. 1996, Paalanen et al. 2006, Kaartinen et al. 2012). The FFQ also seems to be a rather valid tool for assessing absolute intakes. Regarding nutrients, the FFQ seems to slightly underestimate nutrient intakes in general compared to food records, with alcohol being the most underestimated nutrient (Männistö et al. 1996, Paalanen et al. 2006). For example, in the first validation study of the FFQ used in the thesis among the Kuopio Breast Cancer Study participants (the FFQ was validated against two 7-day food records and the participants completed the same FFQ twice three months apart), the underestimation of alcohol was -22% (FFQ1) and -33% (FFQ2) compared to the absolute mean values derived from the food records (Männistö et al. 1996). Furthermore, the FFQs tended to under- or overestimate the intakes of food groups slightly more than nutrient intakes. The greatest overestimation among food groups concerning the BSDS components was for vegetables (FFQ1 +30%, FFQ2 +35%). To control for the underreporting of energy intake in substudy I, participants with low energy intake in relation to the estimated basic metabolic rates were excluded from the analysis in additional analyses (Goldberg et al. 1991). This did not generally alter the findings. Moreover, all analyses have been adjusted for energy intake.

Furthermore, although the selection and scoring of the BSDS components is based on the careful consideration of scientific evidence on the healthy Nordic diet, it is still arbitrary. However, in terms of nutrient intakes, the BSDS has been shown to be a valid tool for assessing a healthy diet; higher adherence to the BSDS has been associated with, e.g., higher intakes of carbohydrates (E%), fiber and lower intakes of SFA (E%) and alcohol (E%) (Kanerva et al. 2014b).

The 48-hour dietary recall (II and III) covered two consecutive days, and there was likely some correlation between the days. Furthermore, the 48-hour dietary recall may not be an accurate estimate of long-term energy and

macronutrient intakes or habitual timing of energy or macronutrient intakes. It should also be noted that with regard to combining the 48-hour dietary recalls and the 3-day food diaries for weekdays and weekend analysis (II), the possibility of methodological differences affecting the results cannot be completely ruled out, although both methods generally yielded similar results when energy and macronutrient intakes were compared (Maukonen 2015) (see page 47 for further details).

Furthermore, anthropometric measurements (I) were based on measured values that were assessed according to standardized international protocols that can be considered a strength (Tolonen et al. 2008), whereas in substudy III measured and self-reported values were used. The validity of self-reported anthropometric measures has been examined in the DILGOM 2014 data because self-reported anthropometric measures are particularly subject to reporting biases (Gorber et al. 2007, Maukonen et al. 2018). The self-reported values have reached a high agreement with measured values (Kanerva et al. 2018) (see page 49 for further details).

In substudy IV genotyping was conducted in five batches with three genotyping platforms (FINRISK 2007; Illumina 610K (COROGENE controls), Illumina Omniexpress (PREDICTCVD cases and controls), Illumina HumanCoreExome and FINRISK 2012; Illumina HumanCoreExome), which could potentially affect the findings. However, the same standard quality control methods and standard imputation procedures were centrally applied for the data from each batch. This was followed by a joint quality control to harmonize the data content and the analyses were further adjusted for the different batches used.

The genome of the Finnish population differs from other populations because of a relatively small number of founder individuals and strong genetic isolation over centuries; therefore, some variants may be more enriched in the Finnish population and, at the same time, some variants may be more rare here than elsewhere. This could either increase or decrease the probability of finding associations compared to genetically more heterogenic populations. This may also potentially affect the comparison of the studies. The GWAS replication analysis (although not statistically significant) in the current study, however, showed a good consistency with the previous GWAS of chronotype on participants with European ancestry (Jones et al. 2019). Furthermore, the function of the gene and the role of a single variant are considered to be similar across the different populations.

All the studies allowed an extensive control for confounding factors (e.g., smoking, education, and other sleep factors). However, some residual confounding may still remain despite this.

6.6 IMPLICATIONS FOR FURTHER RESEARCH

Different strategies to tackle the complex obesity epidemic are needed, and in recent years an increasing interest has been addressed towards health implications related to sleep and circadian rhythms. Sleep behavior plays an ever more important role alongside healthy diet and physical activity in weight management and the individual's overall wellbeing. Chronotype links directly to sleep behavior and, consequently, the number of studies examining the role of chronotype in obesity and dietary habits has also increased during recent years, and even from the start of this thesis project. However, many of these studies have still been rather small scale and included selected study populations, which hamper the generalizability of the results and may even partly account for the inconsistent findings. Therefore, more larger-scale, population-based studies and longitudinal studies on these associations are still needed.

In particular, the association between chronotype and obesity and the role of chronotype in the association between food intake timing and obesity remain inconclusive and require further study. Future studies should also examine the role of energy intake timing relating not only to obesity but also to a wider scale with overall metabolic health (e.g., type 2 diabetes) and take chronotype into account when studying these associations. An important point for future studies on energy intake timing is also to seek unification in the methodological aspects related to the timing of food intake. That would make comparison of the studies easier and further unify the findings.

Another interesting area that requires further study is the role of social jetlag in chronotype associations. Evening types are prone to social jetlag (Wittmann et al. 2006), and social jetlag has been associated with adverse metabolic health (Koopman et al. 2017, Islam et al. 2018), so it would be important to examine whether social jetlag could mediate or modify the associations between chronotype, dietary habits and obesity.

This thesis also examined the genetic basis of chronotype. Findings suggested novel associations between chronotype and a clock gene that has previously been associated with carbohydrate and lipid metabolism. Furthermore, GRS based on a large GWAS of chronotype was associated with chronotype in the current study population. Future studies should explore the potential of these findings in capturing chronotype in various study populations. Furthermore, the degree to which genetics and environment could explain the adverse health behaviors and health of evening types should be further explored.

This study's results provided novel, population-based information on the associations between chronotype, dietary habits and obesity and on the genetic basis of the chronotype. Despite some inconsistencies in the findings, this thesis suggested that evening types had an unhealthier overall diet quality and a postponed energy intake, which is line with the current

evidence. Furthermore, findings from the thesis added to the evidence related to the overall unhealthier behaviors associated repeatedly with evening type, such as smoking and physical inactivity. Thus, this points to the need to assess the potential beneficial health effects in identifying those with later circadian preference in health promotion work. Awareness of one´s chronotype could also further advance or encourage adapting a healthier lifestyle. Furthermore, there is a trend in society towards a greater flexibility in work schedules, and the results of this study could further support this trend. Greater flexibility in work schedules could especially promote the health of those whose working schedules are against their biological rhythms (e.g., evening types, shift workers) and improve their work efficiency.

7 CONCLUSIONS

The aim of this thesis was to study the associations between chronotype, dietary habits (overall diet quality, energy and macronutrient intake timing) and obesity (weight, BMI, WC, BF%) and the interrelationships between these factors. This thesis also aimed to clarify the genetic basis of chronotype. The following main conclusions can be drawn from the thesis:

1. Evening type was associated with lower adherence to the healthy diet (assessed with the BSDS), with lower energy and macronutrient intake (except for sucrose) in the morning (till 10:00 a.m.) and with higher energy, sucrose, fat and saturated fat intake in the evening (after 8:00 p.m.) compared to morning types. Differences between morning and evening types in energy and macronutrient intake timing were even more pronounced at the weekend. No differences, however, emerged between chronotypes in total daily energy intake.

2. A higher tendency towards eveningness in men was associated with lower BMI at the baseline but not in women. Evening chronotype was associated with higher increases in weight and BMI only in women during a seven-year follow-up period, but these associations were at least partly explained by a higher prevalence of depression among evening types.

3. Associations between chronotype and obesity were not mediated by the healthy diet (assessed with the BSDS), nor did chronotype modify the association between the BSDS and obesity. Furthermore, a higher evening energy intake was associated with a higher obesity risk. This association was not modified by chronotype, either. Thus, it seemed that despite the unhealthier dietary habits (lower adherence to the BSDS, later energy intake timing) of evening chronotypes, chronotype played no role in the association between healthy diet and obesity nor in the association between energy intake timing and obesity.

4. Chronotype associated with the *NR1D2* clock gene that has previously been demonstrated to have a role in carbohydrate and lipid metabolism. Furthermore, a GRS developed based on 313 SNPs that have previously been associated with chronotype was able to capture the genetic aspect of chronotype in the current study population. However, the GWAS of chronotype did not yield any genome-wide associations.

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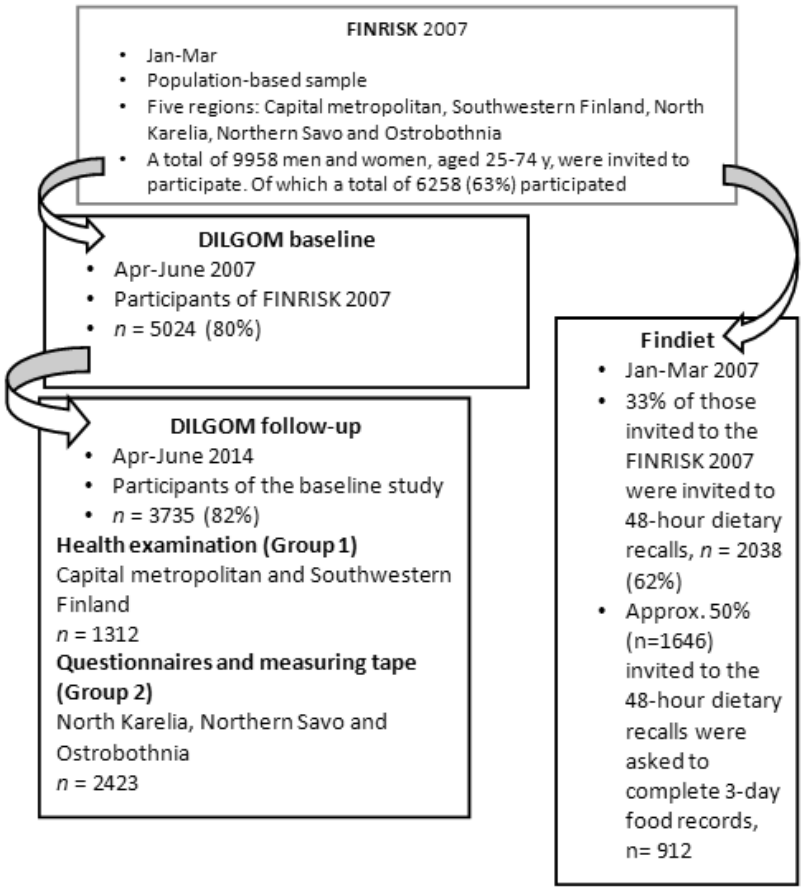
APPENDICES

Appendix I. Flowchart of the substudies of FINRISK 2007



Appendix II. A shortened six-item version of Horne and Östberg's (1976) Morningness-Eveningness Questionnaire.

Item 4

"Assuming adequate environmental conditions, how easy do you find getting up in the morning?"

1="not easy at all"

2="not very easy"

3="quite easy"

4="very easy"

Item 7

"During the first half-hour after woken up in the morning, how tired do you feel?"

1="very tired"

2= "quite tired"

3= "quite rested"

4="very rested"

Item 9

"You have decided to engage some physical exercise. A friend suggests that you do this one hour twice a week and the best time for your friend is between 7.0- 8.0 AM. Bearing in mind nothing else but your "feeling best" rhythm, how do you think you would perform?"

1= "Would be in good form"

2= "Would be in moderate form"

3= "Would find it quite difficult"

4= "Would find it very difficult"

Item 15

"You have to do two hours of hard physical work. Considering only your own "feeling best" rhythm, which of these following times would you choose?"

1="8:00-10:00"

2="11:00-13:00"

3="15:00-17:00"

4="19:00-21:00"

Item 17

"Suppose you can choose your own work hours. Assume that you worked a five hour day. Which five consecutive hours would you select?"
"1-2", "2-3", "3-4", "4-5", "5-6", "6-7", "7-8", "8-9", "9-10", "10-11", "11-12", "12-13", "13-14", "14-15", "15-16", "16-17", "17-18", "18-19", "19-20", "20-21", "21-22", "22-23", "23-24", "24-01"

Item 19

"There are so called morning people and evening people, which are you?"

1="definitely a morning type"

2="rather more a morning than an evening type"

3= "rather more an evening than a morning type"

4="definitely an evening type"