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MODULATION OF GASTROINTESTINAL TRANSIT BY CIRCADIAN RHYTHMS AND LIFESTYLE

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Modulation of gastrointestinal transit by circadian rhythms and lifestyle

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Publications

1. Maida, A., Zota, A., Sjøberg, K. A., Schumacher, J., Sijmonsma, T. P., Pfenninger, A., **Christensen, M. M.**, Gantert, T., Fuhrmeister, J., Rothermel, U., Schmoll, D., Heikenwälder, M., Iovanna, J. L., Stemmer, K., Kiens, B., Herzig, S., & Rose, A. J. *A liver stress-endocrine nexus promotes metabolic integrity during dietary protein dilution.* The Journal of Clinical Investigation, 2016; 126(9):3263-3278.

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List of papers

This thesis is based on the following papers:

I) Jensen, M. M., Wegeberg, A.-M. L., Jensen, S. L., Sørensen, P. S., Wigh, I. M. N., Zaugg, V. S., Faerch, K., Quist, J. S., & Brock, C. *The day-night pattern of colonic contractility is not impaired in type 1 diabetes and distal symmetric polyneuropathy*. Chronobiology International, 2021;38(6):801-806.

II) Jensen, M. M., Pedersen, H., Clemmensen, K. K. B., Wegeberg, A.-M. L., Jensen, N. W., Quist, J. S., Færch, K., & Brock, C. *Human Gastrointestinal Transit and Hormonal Response to Different Meal Types: A Randomized Crossover Study.* The Journal of Nutrition, 2022, 131(2):604S-615S

III) Jensen, M. M., Pedersen, H., Clemmensen, K. K. B., Ekblond, T. S., Ried-Larsen, M., Faerch, K., Brock, C., Quist, J. S., Jensen, M. M., Pedersen, H., Clemmensen, K. K. B., Ekblond, T. S., Ried-Larsen, M., Faerch, K., Brock, C., & Quist, J. S. *Associations between physical activity and gastrointestinal transit times in people with normal weight, overweight, and obesity*. The Journal of Nutrition, published online 12 June 2023

IV) Quist, J. S., Jensen M. M., Ekblond T. S., Pedersen H. P., Clemmensen K. K. B, Blond M. B., Brock C., Færch K. *Effects of three-months 10-hour time-restricted eating on gastrointestinal transit and hormones in individuals at high risk of type 2 diabetes – the RESET randomized controlled trial.* (In preparation)

Abbreviations

BMI	Body mass index
CCK	Cholecystokinin
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
HbA1c	Haemoglobin, A1c
iAUC	Incremental area under the curve
ICJ	Ileocecal junction
IQR	Interquartile range
MVPA	Moderate to vigorous physical activity
PYY	Peptide tyrosine tyrosine
WMC	Wireless motility capsule

English summary

The gastrointestinal tract serves the vital function of transporting nutrients and water to the designated sites of mixing, digestion, absorption, secretion, and excretion. These processes rely on the coordinated movement of food particles through the gastrointestinal tract. Gastrointestinal transit is regulated by a complex interplay of neurohormonal and mechanical factors and is essential in regulating postabsorptive metabolism, appetite, and body weight. Gastrointestinal dysfunction can cause painful and debilitating symptoms and is common in metabolic disorders like diabetes and obesity. Circadian and diurnal rhythms extensively regulate gastrointestinal motility, and alterations to enteric circadian clock genes are manifest in metabolic disorders like obesity and diabetes. Time-restricted eating has been proposed as a dietary regimen for preventing adverse effects of circadian misalignment, including gastrointestinal dysfunction. Similarly, gastrointestinal transit is affected by physical activity, which may aid in relieving constipation. Many aspects of gastrointestinal transit, how it is involved in diabetes and obesity, and how it is modulated by circadian rhythms, diet, and physical activity remain to be elucidated. Different methods exist for evaluating gastrointestinal motility, but the wireless motility capsule (WMC) technique is the only approach for the pan-enteric assessment of both motility and regional transit times. The overall aim of the present thesis was to apply the WMC to investigate how gastrointestinal transit is affected by circadian rhythms and lifestyle, i.e., meal timing and physical activity, in various populations ranging from healthy individuals with normal weight to those with diabetes, overweight, and obesity.

Paper I investigated the day/night variation in colonic motility and contractility in individuals with type 1 diabetes, with and without distal symmetric polyneuropathy and healthy controls. Furthermore, we assessed whether the increased colonic motility and contractility in the morning compared to at night is blunted in the two cohorts with type 1 diabetes. We concluded that overall, the day/night pattern of colonic motility was not disrupted in the two cohorts with type 1 diabetes. However, the motility index in the evening was slightly higher in individuals with type 1 diabetes and distal symmetric polyneuropathy but without significant physiological or clinical relevance. It is worth noting that the increased motility observed from night

to morning was not affected by type 1 diabetes, with or without distal symmetric polyneuropathy.

In **paper II**, we compared the response in gastrointestinal transit times and postprandial plasma metabolites and gastrointestinal hormone concentrations after ingestion of a SmartBar compared with a standardised mixed meal. The larger volume of the standardised mixed meal only impacted gastric emptying time, which was prolonged compared to the SmartBar, while transit times through the small intestine and colon were similar for both meals. The delayed gastric emptying time observed with the standardised mixed meal increases the risk of capsule retention in individuals with abnormally prolonged gastric emptying.

Paper III explored associations between habitual physical activity and gastrointestinal transit times. Regular high light intensity physical activity was associated with accelerated colonic transit and, thereby, whole gut transit, which may help prevent constipation. No associations were observed between physical activity and small intestinal transit time or gastric emptying.

In **paper IV**, we investigated the effect of a three-month 10-hour time-restricted eating intervention on gastrointestinal hormones and transit measured using the WMC technique in individuals at high risk of type 2 diabetes. Three months of time-restricted eating did not alter pan-enteric regional gastrointestinal transit.

The knowledge obtained in the thesis contributes to elucidating the complex regulation of gastrointestinal motility and transit and how it is affected by intrinsic (diabetes, obesity, and circadian rhythms) and extrinsic factors (physical activity and time-restricted eating). Furthermore, we have provided new insights into the possible application of the WMC in metabolic research.

Dansk resumé

Mavetarmkanalens primære funktion er at transportere næringsstoffer og vand til de specifikke områder, hvor næringsstofferne blandes, fordøjes, absorberes og udskilles. Processen afhænger af velkoordinerede bevægelser af fødepartikler gennem mavetarmkanalen. Mavetarmtransit reguleres af komplekse samspil mellem neurohormonelle og mekaniske faktorer og er afgørende for reguleringen af det postabsorptive stofskifte samt appetit og kropsvægt. Mavetarmdysfunktion kan forårsage smertefulde og invaliderende symptomer, som er almindeligt forekomne ved stofskifterelaterede lidelser som diabetes og svær overvægt. Kroppens døgnrytme regulerer mavetarmmotiliteten, og ændringer i 'CLOCK'-gener, der styrer døgnrytmen i mavetarmkanalen, er observeret i metaboliske lidelser som diabetes og svær overvægt. Tidsbegrænset spisning er blevet foreslået som et anvendeligt kostregime til forebyggelse af negative virkninger af døgnrytmeforstyrrelser, herunder mave-tarmdysfunktion. Ligeledes påvirkes mavetarmtransit af fysisk aktivitet, hvilket kan hjælpe med at lindre forstoppelse. Mange aspekter af mavetarmtransit, dets involvering i diabetes og svær overvægt samt hvordan det påvirkes af døgnrytmer, kost og fysisk aktivitet, er stadig ikke fuldt klarlagt. Der findes forskellige metoder til måling af mavetarmmotilitet, men den trådløse motilitetskapsel (WMC) er den eneste metode til at måle både motilitet og transittid gennem hele mavetarmkanalen. Det overordnede formål med denne afhandling var at anvende WMC til at undersøge, hvordan mavetarmtransit påvirkes af døgnrytmer og livsstil, specifikt timing af måltider og fysisk aktivitet, i forskellige populationer, fra raske personer med normal vægt til personer med diabetes, overvægt og svær overvægt.

I **artikel I** undersøgte vi dag-/natvariationen i tyktarmens motilitet og kontraktilitet hos personer med type 1-diabetes, med og uden nervebetændelse samt raske kontrolpersoner. Desuden undersøgte vi, om den øgede motilitet og kontraktilitet, som er observeret i tyktarmen om morgenen i forhold til om natten er reduceret i de to type 1-diabetes-kohorter. Vi konkluderede, at det overordnede dag-/nattemønster af motilitet i tyktarmen ikke var forstyrret i de to type 1-diabetes-kohorter, selvom motilitetsindekset om aftenen var lidt højere hos personer med type 1-diabetes og nervebetændelse, men uden signifikant fysiologisk eller klinisk betydning. Det er værd at bemærke, at den

øgede motilitet, der blev observeret fra nat til morgen, ikke blev påvirket af type 1-diabetes, med eller uden nervebetændelse.

I **artikel II** sammenlignede vi responset i mavetarmtransittider og postprandiale plasmakoncentrationer af udvalgte metabolitter og mavetarmhormoner efter indtagelse af en SmartBar sammenlignet med et standardiseret morgenmåltid. Det standardiserede morgenmåltids større volumen påvirkede kun mave-tømningstiden, som var forlænget i forhold til SmartBar, mens transittider gennem tyndtarmen og tyktarmen var ens for begge måltider. Forsinkelsen i mavetømningstiden observeret med det standardiserede morgenmåltid øger risikoen for retention af kapslen hos personer med langsom mave-tømning.

I **artikel III** undersøgte vi sammenhængen mellem regelmæssig fysisk aktivitet og mavetarmtransittider. Regelmæssig fysisk aktivitet med høj let-intensitet accelererede tyktarmens transittid og dermed transittiden gennem hele mavetarmkanalen, hvilket kan hjælpe med at forhindre forstoppelse. Der blev ikke observeret nogen sammenhæng mellem de øvrige fysisk aktivitetsintensiteter og transittid i tyndtarmen eller mave-tømning.

I **artikel IV** undersøgte vi effekten af en tre-måneders intervention med 10 timers daglig tidsbegrænset spisning på mavetarmhormoner og -transit målt ved brug af WMC-teknikken hos personer med høj risiko for type 2-diabetes. Tre måneders tidsbegrænset spisning ændrede ikke den regionale mavetarmtransit i nogen dele af mavetarmkanalen.

Den opnåede viden i afhandlingen bidrager med ny viden om den komplekse regulering mavetarmmotilitet og transit og hvordan det påvirkes af indre faktorer (diabetes, fedme og døgnrytmer) og ydre faktorer (fysisk aktivitet og tidsbegrænset spisning). Derudover har vi bidraget med nye indsigter i den mulige anvendelse af WMC i metabolisk forskning.

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Through the ups and downs, forever by my side

/Marie

Table of contents

1. Background

The primary function of gastrointestinal motility is transporting nutrients and water through the gastrointestinal tract to the designated sites of mixing, digestion, absorption, secretion, and excretion. Motility is often described in terms of regional gastrointestinal transit times or as contractile activity of the smooth muscles lining the gastrointestinal wall. This process is achieved through tightly regulated aboral peristaltic movements of the food particles and is regulated by an intricate interplay between neurohormonal and mechanical factors[1]. In addition, gastrointestinal motility is essential in modulating postabsorptive metabolism, appetite regulation, and body weight homeostasis. Gastrointestinal dysfunction can be a source of significant discomfort to the individual with painful and debilitating symptoms. Additionally, gastrointestinal dysmotility is common in metabolic disorders like obesity and diabetes. However, the gastrointestinal tract is a complex organ system that is challenging to examine. As a result, many aspects of gastrointestinal motility and how it can be modulated remain to be elucidated. In this thesis, we have applied the wireless motility capsule (WMC) to investigate how gastrointestinal transit is affected by circadian rhythms and lifestyle, i.e., meal timing and physical activity, in various populations ranging from healthy individuals with normal weight to those with diabetes, overweight, and obesity.

1.1 Gastrointestinal transit and motility

The stomach

In the fed state, the proximal part of the stomach, the fundus, serves as a reservoir and relaxes when solids and liquids enter, a process called accommodation. Next, the contraction of the fundus drives food into the body of the stomach, where the food is mixed before it enters the distal antrum. Here, the food is ground in trituration[2], which appears to be rate-limiting for gastric emptying time[3]. After grinding the food into particles of <2 mm in diameter, the pyloric sphincter tonus decreases to allow the chyme to pass to the duodenum. When nutrients reach the ileum, a negative feedback loop known as “the ileal brake” is initiated. The ileal brake decelerates gastric emptying and thereby controls the delivery of chyme to the small intestine[4]. The motility pattern of the stomach differs between the proximal part with tonic contractions and the distal part with slow wave contractions and

peristaltic movements[4]. In the fasted state, when all liquid and digestible solids have been emptied from the stomach, non-digestible solids are pushed distally by the migrating motor complex, which is a pattern of myoelectrical activity acting as a "housekeeper" cleaning the gastrointestinal tract after a meal[5]. The migrating motor complex consists of four phases with a total duration of 90-120 minutes: 1) phase I is the most prolonged phase and is characterised by minimal or no contractile activity; 2) Phase II is the preparation phase, where irregular, weak contractions are initiated; 3) phase III is brief, but consists of forceful, coordinated slow-wave contractions clearing residual food particles; and 4) phase IV is a transition period leading to phase I. Most contractions during the migrating motor complex originate from the stomach and continue through the small intestine, but some activity is initiated in the duodenum[5].

The small intestine

The small intestine is the primary region of absorption. Therefore, small intestinal motility facilitates nutrient absorption by mixing and moving the contents to optimise contact with the absorptive surfaces and prevent stagnation. Additionally, it aids in the removal of unabsorbed material. Motility of the small intestine occurs in two contraction patterns, 1) peristaltic contractions, where anterograde contractions above and relaxation below a food bolus drive the bolus distally through the intestine, and 2) segmentation contractions with minimal anterograde movement, which aids mixing and optimises contact time with the intraluminal wall to enhance nutrient absorption and secretion from mucosal and endocrine cells[1].

The colon

The primary function of the colon is to absorb water, vitamins, and electrolytes[1]. The motility properties of the colon can be divided into two categories: the proximal colon stores and mixes stool, while the distal colon packs and expels it. The colon is idle 90% of the time, and approximately 20% of all movement is retrograde[6]. The process of colonic transit includes both segmentation, which involves non-propagating motor patterns and less frequent mass movements, characterised by propagating motor patterns[1]. In the colon, there are two types of contractile activities: 1) low-amplitude propagated contractions and 2) high-amplitude propagated contractions[7]. Low-amplitude propagated contractions can either be anterograde or retrograde and contribute to colonic tone, while high-amplitude propagated contractions trigger mass movements up to six times per day. High-amplitude propagated contractions are more frequent during the daytime after waking up

and following meals and are associated with the urge to defecate. The rectal motor complex is a braking mechanism to prevent untimely faecal flow[8]. Eating triggers the gastrocolic reflex, which modulates colonic transit. This reflex facilitates the transport of fluid and solids from the ileum to the colon. Fat is a more potent stimulant of the gastrocolic reflex than proteins or carbohydrates, especially when at least 300 kcal are ingested[1].

1.1.1 Regulation of gastrointestinal motility and transit

The regulation of gastrointestinal motility and transit involves a complex interaction between neurological, hormonal, and mechanical factors (Figure 1). The enteric nervous system is an independent network within the autonomic nervous system that controls gastrointestinal motor function. The enteric nervous system comprises hundreds of millions of ganglionated neurons organised in two primary plexuses, where the myenteric plexus controls smooth muscle activity[9]. Interstitial cells of Cajal within the myenteric plexus positioned between the smooth muscle layer and the longitudinal muscle layer of the gut wall serve as pacemaker cells that generate electrical slow waves which initiate smooth muscle contraction of the gastrointestinal walls[10]. The vagus nerve, a prominent component of the parasympathetic division of the autonomic nervous system, plays a critical role in regulating gastrointestinal motility[11]. As the longest cranial nerve, the vagus innervates various organs, including the gastrointestinal tract. The vagus nerve acts as a crucial communicator between the brain and the gut, exerting control over the complex processes of digestion and motility. Vagal afferents receive chemical and mechanical information about gastrointestinal distension, meal composition, and size. In turn, this results in the activation of vagal efferents regulating smooth muscle tone and gastrointestinal and pancreatic hormone secretion, constituting the gut-brain axis[11].

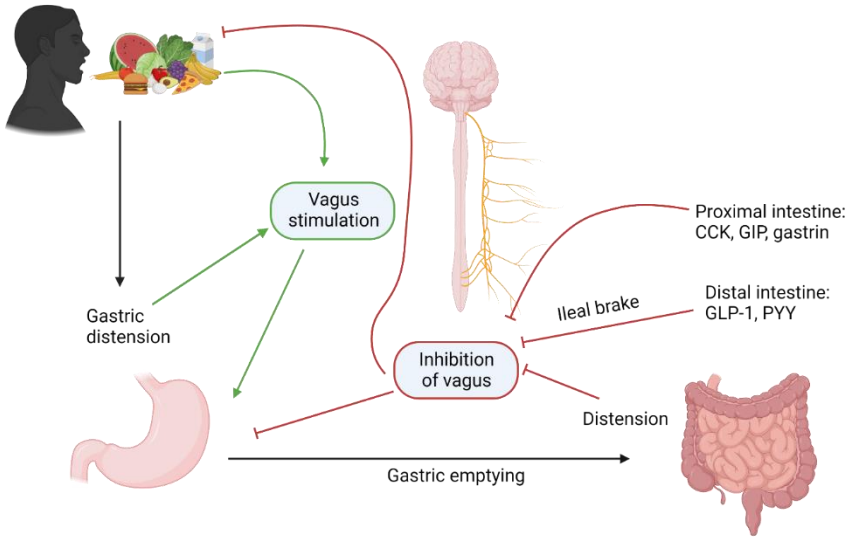

Figure 1. Neurohormonal response to food ingestion. Inspired by Camilleri, 2015 [12]

These hormones are secreted in response to a meal to aid digestion and regulate gastrointestinal motility, blood glucose, appetite, satiety (appetite to ingest a meal), and satiation (postprandial fullness) (Table 1). Even before food ingestion, motor and secretory activities are stimulated in the cephalic phase, contributing to more than 50% of the postprandial response[12].

Cholecystokinin (CCK) is primarily known for its effects on the gallbladder and pancreas. However, it is also involved in the negative feedback loop to induce satiation and terminate food intake. This function is achieved by reducing fundic and antral tone or stimulating pyloric contraction to decelerate gastric emptying[13]. The gastric hormone, Gastrin, stimulates the secretion of gastric acid[14]. Gastrin does not directly affect gastric motility but may slow gastric emptying slightly by increasing gastric volume[15]. Unlike most other appetite-regulating hormones, Ghrelin stimulates short-term food intake through hypothalamic stimulation. Glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosine (PYY) are secreted in the distal part of the gastrointestinal tract and act as crucial mediators of satiation, activating the ileal brake regulating glucose homeostasis[16,17]. GLP-1 also inhibits small intestinal motility. GLP-1 and Glucose-dependent insulinotropic polypeptide

(GIP) are the most critical incretin hormones, which augment insulin secretion from pancreatic β-cells[16,18].

Hormone/ protein/ peptide	Origin of secretion	Stimulus	Effect on the gastrointestinal tract
CCK[13]	I-cells in the duodenum and ileal and colonic neurons	- Nutrient ingestion, especially protein, amino acids, and digested fat - Gastric acid	- Inhibits gastric acid secretion and gastric emptying - Gastric fundus relaxation - Increases small bowel motility - Induce colonic contractions
Gastrin[19]	Gastric and duodenal G-cells	- Amino acids and calcium - Neuronal stimulation	- Stimulates gastric acid secretion
Ghrelin[12]	Peptide mainly expressed in the stomach	- Increased secretion with fasting, possibly regulated by plasma glucose levels	- Stimulates appetite through vagal stimulation - Contracts gastric fundus and stimulates gastric emptying - Stimulates gastric acid secretion
GIP[18]	K-cells primarily in the duodenum and proximal jejunum	- Nutrient absorption, primarily fat and carbohydrates in the duodenum	- Incretin hormone - Works primarily outside of the GI tract. - Stimulates gastric acid secretion and lower gut motility at supraphysiological levels
GLP-1[16]	Intestinal L-cells, primarily in the distal	- Nutrient ingestion, especially glucose, fatty	- Inhibits gastric emptying and antral motility - Inhibits small intestinal motility

	ileum and colon	acids and dietary fibre - Vagal stimulation - GIP secretion	- Activates the ileal brake - Promotes satiation and satiety - Incretin hormone
PYY[17]	Intestinal L-cells primarily in the distal ileum and colon and in central neurons	- Nutrient ingestion	- Regulates food intake through central regulation - Activates the ileal brake and inhibits intestinal motility - Inhibits gastric acid secretion

Table 1. Overview of selected gastrointestinal hormones and their effects on gastrointestinal motility.

These peptides and hormones are essential in regulating gastrointestinal motility, ultimately affecting appetite and food intake. Recent advances in characterising the gut microbiome have suggested a role for gut microbiota in controlling colonic motility. However, the mechanisms are not fully understood. The enteric nerve endings are placed near absorptive epithelial cells, which put them in an optimal position for interaction with gut bacteria[20]. In mice, it has been demonstrated that gut microbiota contribute to colonic motor activity by affecting enteric neuron excitability[21]. In addition, short-chain fatty acids, produced in the microbial fermentation of dietary, act as signalling molecules activating mucosal receptors of enteroendocrine cells, stimulating the secretion of GLP-1, among others[20].

1.1.2 Assessment of gastrointestinal motility and transit

The gastrointestinal tract is a diverse organ system with structures and functions that vary between regions. Disorders affecting the motility of the gastrointestinal tract are common and present with numerous symptoms, which can originate from one or more areas of the gastrointestinal tract[22]. Several methods exist for evaluating gastrointestinal motility and transit, with the majority focused on gastric emptying. Scintigraphy is the gold standard for assessing gastric emptying and small intestinal transit in the clinic. A low-fat meal consisting of ^{99m}Tc-sulfur colloid scrambled with 120 grams of egg whites, two slices of white toast, 30 grams of strawberry jelly, and 120 mL of water is ingested, followed by consecutive imaging of the abdomen at zero,

one, two, and four hours after meal consumption. Gastric counts measured during the procedure correlate directly with the volume remaining in the ventricle at the given timepoint[2]. Several other direct and indirect approaches to the evaluation of gastrointestinal transit and motility exist, such as the [^{13}C]octanoic acid breath test[23], retention of radio-opaque markers[24], the paracetamol test[25], 3D-Transit electromagnetic tracking[26], and MRI[27]; however, they present challenges in terms of precision or resource requirements. In addition, these methods necessitate sequential measurements or scans conducted in clinical or research settings, thereby constraining free-living activities. Moreover, these techniques predominantly focus on specific gastrointestinal regions and lack a comprehensive assessment of the entire gastrointestinal tract. So far, only one approach has been developed for the pan-enteric evaluation of both gastrointestinal motility and transit times, the WMC technique.

1.1.2.1 The wireless motility capsule

The WMC is an indigestible capsule that measures and transmits intraluminal pH, temperature, and pressure from ingestion until natural passage. From these measurements, regional gastrointestinal transit times (gastric emptying time, small bowel transit time, colonic transit time, and whole gut transit time) and contractility measures can be calculated. Compared to other methods, the method has several advantages: 1) it is non-invasive, 2) ambulatory, and 3) radiation-free[28]. The US FDA has approved the WMC for measuring gastric emptying time in individuals with symptoms of gastroparesis, colonic transit time in individuals with possible slow transit or constipation, and for assessment of intraluminal pH, temperature, and pressure through the entire gastrointestinal tract[29].

The WMC is administered with a standardised granola bar (SmartBar, Medtronic) with a calorie content and macronutrient composition similar to the Egg Beater meal used for scintigraphy (260 kcal, seven energy per cent (E%) fat, 74E% carbohydrate, and 19E% protein). Some WMC studies have used the Egg Beater meal instead of the SmartBar for comparison with scintigraphy[30] or manometry[31]. Validation of the WMC shows good correlations with both scintigraphy for evaluating gastric emptying time[32] and small bowel, colonic, and whole gut transit times[33]. In addition, the WMC correlates with radio-opaque markers[34] in assessing colonic transit time. Previously, there have been published normative values for gastric emptying

time, which range from three to 3.5 hours with a 95% percentile of 5.4 hours[35]. As a result, it is recommended to fast for six hours after taking the WMC.

In summary, gastrointestinal motility and transit are influenced by a complex interplay between neurological, hormonal, and mechanical factors. Well-regulated gastrointestinal motility ensures optimal post-absorptive metabolism, appetite regulation, and maintaining healthy body weight. Conversely, metabolic disorders such as obesity and diabetes often exhibit abnormal gastrointestinal motility. The WMC offers a radiation-free, ambulatory, and non-invasive assessment of pan-enteric pH, temperature, and pressure, from which regional gastrointestinal transit times can be determined. It has been validated and correlates with the gold standard, scintigraphy.

1.2 Circadian rhythm of the gastrointestinal tract

Circadian rhythms are ~24-hour intrinsic rhythms that modulate autonomous cell processes anticipating predictable environmental changes to optimise organ function, health, and performance[36]. Intrinsic factors help maintain the circadian rhythm through self-sustained oscillation independent of external cues or 'zeitgebers'. When the rhythm is synchronised with external zeitgebers such as light, ambient temperature, and food ingestion within the 24-hour day, they are termed diurnal rhythms 38. The suprachiasmatic nucleus in the hypothalamus acts as a 'central circadian clock' that responds to light through retinal ganglion cells containing blue light–sensors[37].

Most peripheral tissues contain peripheral 'clocks genes' that communicate with the suprachiasmatic nucleus through neuronal and humoral (e.g., melatonin and corticosteroids) signalling pathways[37]. Interestingly, when food is restricted to the daytime (the inactive phase) in mice, the oscillation patterns of clock genes in many peripheral organs also shift. In contrast, the clock genes in the suprachiasmatic nucleus are unaffected, underlining the importance of food as a zeitgeber for peripheral tissues independent of the suprachiasmatic nucleus[38]. In the gastrointestinal tract, clock gene expression has been observed in the epithelium, stem cells and myenteric plexus of the enteric nervous system[39], of which the latter is involved in regulating gastrointestinal motility. Also, the expression of clock genes in gastric vagal afferents is regulated by diurnal rhythms in response to mechanical stimuli from food ingestion[40]. In mice, the mechanosensitivity of gastric mechanoreceptors is reduced in the active (dark) phase allowing larger meal

sizes to be consumed during waking hours[40]. Thus, the circadian rhythm of gastric mechanosensitivity directs food intake toward the active phase of the day.

Clock genes influence circadian gene expression primarily by regulating the promoter activity of clock-controlled genes. Clock genes participate in two connected feedback circuits that regulate the expression of specific genes. In the first loop, the clock proteins, CLOCK and BMAL1, form a heterodimer that initiates the cycle by binding to the period (Per) and cryptochrome (Cry) genes' promoter regions, activating transcription. Then, the protein products, PER and CRY, inhibit the activity of CLOCK/BMAL1. In the second loop, CLOCK/BMAL1 regulates the expression of Rev-erb and Ror classes of nuclear hormone receptors that oppose the BMAL1 promoter, resulting in a 24-hour rhythm in BMAL1 transcription[39]. These clock-controlled genes can, in turn, regulate the transcription of downstream genes and thereby indirectly affect circadian variation in gene expression in various cells[37].

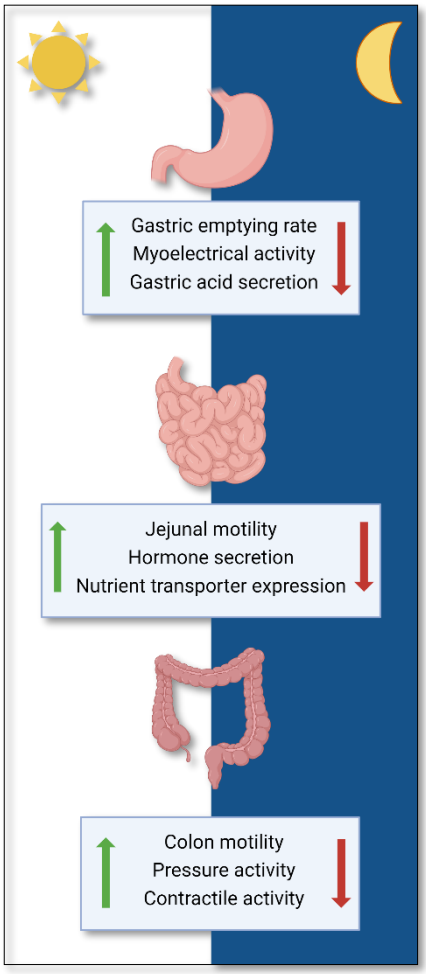

Figure 2: Simplified overview of circadian regulation of gastrointestinal motility

Circadian and diurnal rhythms extensively modulate gastrointestinal motility and transit (Figure 2). For example, gastric myoelectrical activity is reduced at night compared to daytime[41]. In support of this, Gastric emptying time has been observed to be ~50% more rapid in healthy men in the morning compared to the evening[42]. Diurnal rhythms also influence the stomach's

secretory function. For example, studies in mice have shown that Ghrelin is released in anticipation of regular mealtimes[43].

In the small intestine, decreased nocturnal motility of the jejunum has also been demonstrated in healthy individuals fasting for 24 hours[44]. Circadian rhythms also affect the secretory properties of the small intestine. For instance, the secretion of GLP-1 by small intestinal L-cells follows a circadian pattern and is sensitive to feeding[45]. GLP-1 also inhibits gastric emptying, so the secretion pattern contributes to the rhythmic variation in gastric motility. Circadian rhythms also seem to affect the expression of mucosal nutrient transporters in the small intestine and have been proposed to contribute to the anticipatory regulation of gut hormones secretion before ingestion of a meal[46].

In healthy individuals, colon motility, expressed as the sum of colonic pressure waves and periods of contractile activity, is reduced at night compared to daytime. A rapid increase in colonic pressure activity was also observed in the morning and after a meal[47]. Additionally, in healthy individuals, colonic motor activity measured by manometry increased almost two-fold from two hours before awakening to two hours after awakening. This increased pressure activity persisted throughout the waking hours of the day[48].

1.2.1 Chronodisruption

Disruption of the circadian rhythm, also known as 'chronodisruption', is widespread in developed countries due to the 24-hour availability of energy-dense food and artificial lights that manipulate the natural diurnal rhythm of eating, sleeping, and physical activity[36]. In addition, chronodisruption can be caused by, e.g., shift work, travel across time zones, or so-called social jetlag, i.e., substantial day-to-day variation in sleep- and eating patterns due to, e.g., staying up late to study or social activities.

Chronodisruption is known to induce gastrointestinal dysfunction, and common symptoms include bloating, abdominal pain, constipation, and diarrhoea[49,50]. Studies on nurses working rotating shifts have shown a connection between working night shifts and the experience of gastrointestinal dysfunction compared to nurses working daytime shifts only. Furthermore, there was a higher prevalence of functional bowel disorders such as irritable bowel disease among night shift-working nurses, and symptoms were more severe compared to nurses working daytime shifts only[50]. Similarly, it has been documented that flight crew members, especially those working on long-

distance flights, experience more dyspeptic symptoms than administrative ground-based staff[51]. These adverse effects of shift work on gastrointestinal function may be related to alterations of the clock genes caused by chronodisruption since minor polymorphisms of the clock genes CLOCK and PER3 have been linked with reduced gastric motility in the morning in women[52].

Besides gastrointestinal disorders, chronodisruption has also been linked to metabolic disorders, such as overweight and diabetes[36]. Despite gastric emptying being more rapid in the morning, the postprandial glucose response to a meal has been reported to be reduced in the morning compared to when an identical meal is ingested at lunch or dinner. The increased glucose tolerance in the morning was partly caused by increased β-cell responsiveness and insulin action[53]. In contrast, individuals with obesity exhibit attenuated, phase-delayed, or even absent circadian variation in glucose tolerance and insulin sensitivity[54]. In these individuals, glucose tolerance in the morning was even lower than the evening glucose tolerance of individuals with normal weight[55]. The same pattern has been documented in individuals with type 2 diabetes[54], indicating that chronodisruption is a distinct feature of obesity and type 2 diabetes, although causation has not been established.

1.2.3 Time-restricted eating

Time-restricted eating (time-restricted feeding in animals) is an emerging intervention designed to align eating behaviour with the body's circadian rhythm to optimise digestion, metabolism, and appetite regulation. A study of American individuals showed that calorie intake was frequent and erratically distributed throughout the day. The duration from the first to last daily caloric intake, also known as the "eating window", was 14.75 hours and paralleled time awake[56]. A time-restricted eating intervention is intended to reduce the daily eating window to four to twelve hours, typically eight to ten hours, and keep the timing of the eating window constant throughout the week. The interest in time-restricted eating was sparked by animal studies showing that mice fed an ad libitum obesogenic diet could prevent weight gain, fat deposition, and insulin resistance through time-restricted feeding[57].

Interestingly, mice fed only during the active phase gained less weight than those fed only during the inactive phase despite comparable caloric intakes in the two groups. This study indicates that time-restricted eating could induce

weight loss without calorie restriction[58]. However, this observation has not been replicated in human trials.

It has been suggested in humans that time-restricted eating promotes weight loss and health in individuals affected by chronodisruption[36]. In time-restricted eating studies, the main focus has been on body weight, glucose tolerance, and metabolism. Overall, time-restricted eating with ad libitum energy intake within the selected eating window induces a modest weight loss of one to four per cent of body weight in four to twelve weeks, usually caused by a reduction in energy intake[59].

The effect of time-restricted eating on the gastrointestinal tract is yet to be fully understood, but a few studies have been published so far, primarily in rodents. For example, in mice fed a high-fat diet, the diurnal rhythmicity of mechanosensitive gastric vagal afferents caused by food-related stimuli was attenuated. However, time-restricted feeding prevented this obesity-induced effect[60]. The study indicates that obesity-induced chronodisruption of the gastric vagal afferents may contribute to increased appetite at night and that time-restricted feeding may counter this effect. Supporting this is the observation of decreased perceived hunger at night in individuals after a 16-week time-restricted eating intervention[56].

Gut microbiota also displays circadian oscillations in both function and composition. Time-restricted feeding enhanced the gut microbiome composition in mice by improving the ratio between obesogenic and obesity-protective microflora. However, time-restricted feeding did not improve gut microbiota diversity, although it is generally believed that great diversity in microbial species protects against obesity and metabolic diseases[57].

In individuals with suspected gastroesophageal reflux disease, following a two-day time-restricted eating intervention with an eight-hour eating window improved symptoms of regurgitation and heartburn. However, it only tended to reduce gastric acid exposure[61]. This study was not randomised and lacked a control group; therefore, the results should be interpreted cautiously. However, it indicates that gastric acid secretion may be improved with time-restricted eating in individuals with gastrointestinal symptoms.

In summary, circadian and diurnal rhythms extensively modulate gastrointestinal motility and transit while affecting the secretory function and pattern of the stomach and small intestine. Peripheral tissues have 'clock genes' that communicate with the suprachiasmatic nucleus to regulate circadian gene expression. Food acts as a zeitgeber for peripheral tissues independent of the suprachiasmatic nucleus. Chronodisruption, caused by shift work and exposure to artificial light, can lead to gastrointestinal issues such as bloating and constipation. It has also been linked to metabolic disorders like obesity and diabetes, and studies suggest that alterations to clock genes may be responsible for these adverse effects. However, the exact causal relationship is still unclear. Time-restricted eating aligns eating behaviour with the circadian rhythm of the body. Studies suggest that it can promote weight loss and reinforce circadian rhythms. Time-restricted eating can improve the composition of the gut microbiome, reduce perceived hunger at night, and improve symptoms of gastroesophageal reflux disease. Still, no randomised controlled trial has previously investigated how gastrointestinal transit and motility are affected by time-restricted eating.

1.3. Obesity

Nearly 60% of adults and 30% of children in the European region are affected by overweight or obesity. Additionally, overweight and obesity have been estimated as the fourth most common risk factor for noncommunicable diseases in the European region by the World Health Organization (WHO)[62]. Individuals with overweight (body mass index (BMI) of 25-29.9kg/m^2) have a two-fold increased risk of developing cardiometabolic multimorbidity (i.e., a minimum of two from type 2 diabetes, coronary heart disease, and stroke) compared to people with normal healthy weight (BMI 18-24.9 kg/m^2). For individuals with class I obesity (BMI 30-34.9 kg/m^2) or class II/III obesity (BMI >35 kg/m^2), the risk is five and ~15 times higher than individuals with a BMI in the normal range[63].

Obesity is also associated with gastrointestinal dysfunction, and the prevalence of functional gastrointestinal disorders such as functional dyspepsia and diarrhoea is high among individuals with obesity[64]. Obesity is influenced by the amount and composition of the food ingested and the absorption of nutrients, and these processes are linked to gastrointestinal motility. Alterations in the stomach's motility can impact sensations of hunger and satiety, and alterations in small intestinal motility can affect the absorption

of nutrients[64]. The impact of obesity on gastric emptying is inconclusive, with studies showing accelerated[65–68], similar[69–72], or delayed[73] gastric emptying times in individuals with obesity compared to normal weight. Only one study by Steenackers et al.[70] has applied the WMC technique to investigate gastrointestinal transit and contractility in individuals with normal healthy weight and obesity. In that study, gastric emptying time was similar between the fasted and fed groups. However, food-induced gastric contractility was increased in individuals with obesity compared to individuals with normal weight, which contradicts the finding of similar gastric emptying time or suggests that gastric motility is ineffective in obesity.

The conflicting reports on obesity and gastric emptying may result from methodological differences in the study populations, BMI classification, group matching, test selection, and meal standardisation. Lastly, most studies have included small study populations with 11-24 participants in each group. With a day-to-day variation in gastric emptying of ~13%[74], such small study populations may be underpowered to detect potential group differences. One large study including 509 participants with normal healthy body weight, overweight, obesity class I, and obesity class II/III has been published[68]. In that study, accelerated gastric emptying ½-times assessed by scintigraphy of both solids and liquids were reported for all BMI classes above the normal range, thus tipping the scale towards a more rapid gastric emptying in obesity (Figure 3).

Most studies report overall gastric emptying time, but some evidence suggests that the initial phase of gastric emptying rate may be accelerated in obesity[75,76]. In a carefully conducted study by Verdich et al., [76] overall gastric emptying rate after three hours was similar between individuals with normal healthy weight and obesity. However, there was a trend towards an accelerated gastric emptying rate within the first 30 minutes. Furthermore, it was speculated that the more rapid initial gastric emptying in obesity is linked to a higher absorptive capacity in the proximal part of the small intestine[71]. In that study, individuals with obesity absorbed more energy in the proximal part of the small intestine despite a decreased exposure time caused by a faster small intestinal transit time. This would reduce the nutrient load sensed by the small intestine and may, in turn, decrease the activation of the ileal brake, thereby reducing the inhibition of gastric emptying. In agreement with this, the two gut hormones involved in the ileal brake, GLP-1[77], and PYY[78], exhibit blunted responses to a meal in individuals with obesity. Other proposed mechanisms

for accelerating gastric emptying in obesity are altered vagal activity and sensitivity. Vagal efferent neuron activity involved in maintaining gastric tone and secretion of insulin and glucagon is increased in obesity[79]. Additionally, animal studies have suggested blunted sensitivity of vagal afferents to enteroendocrine hormones such as CCK and leptin in obesity[80].

In the study by Steenackers et al., small bowel transit time measured with the WMC was accelerated, but only in the fasted state[80]. In addition, small intestinal contractility was higher, and pH was reduced in the fasted and fed state in individuals with obesity compared to individuals with normal weight[70]. It seems intuitive that accelerated small intestinal transit would lead to weight loss because the nutrient exposure time and absorption would be reduced. However, transit time is not the only factor in energy absorption. As mentioned above, evidence suggests that individuals with obesity have a more efficient energy harvest capacity and absorb more energy from the small intestine despite accelerated transit time[71]. In line with this, increased in vitro smooth muscle contractility was observed in resected small intestinal samples from individuals with obesity undergoing Roux-en-Y gastric bypass surgery compared to individuals with normal weight[81]. Lastly, individuals with obesity have a higher incidence of small intestinal bacterial overgrowth, which may affect contractility of the small intestine, although there was no association with orocecal transit[82].

The literature on colonic motility in obesity is sparse. However, BMI was negatively associated with colonic transit time measured with scintigraphy in a cohort of 287 individuals with lower functional gastrointestinal disorders such as irritable bowel disease, functional constipation, and functional diarrhoea and 170 healthy controls[83]. In that study, individuals with BMI >30 kg/m^2 had accelerated colonic transit compared to individuals with BMI <30 kg/m^2 when adjusted for age, sex, and subject group (functional gastrointestinal disorders vs. healthy controls). Similarly, colonic transit time tended to be faster ($P = 0.08$) in healthy individuals with BMI >30 kg/m^2 when adjusted for sex[72]. A more rapid colonic transit time in obesity may explain the higher prevalence of diarrhoea in individuals with obesity, although constipation is also more frequent in obesity[72]. However, when measured with the WMC, colonic transit time, motility index, and pressure were not affected by obesity[80].

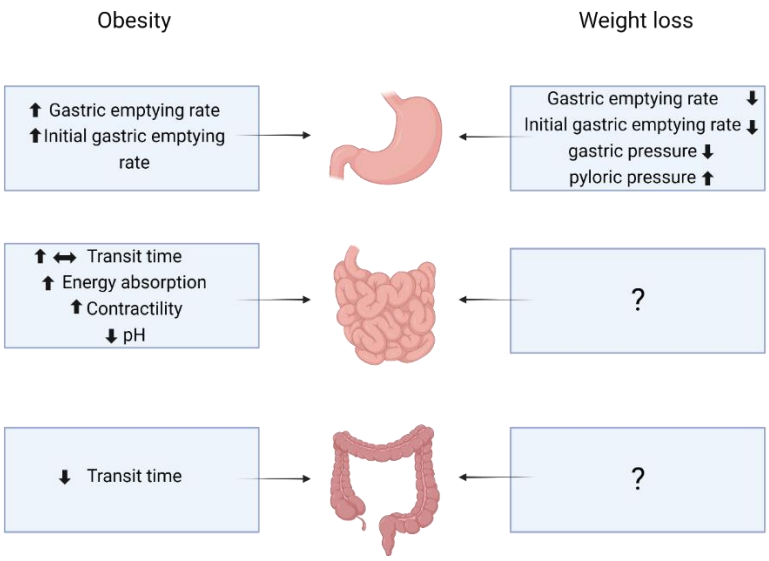

Figure 3. Effects of obesity and weight loss on gastrointestinal motility and transit

1.3.1 Weight loss

Various weight loss methods, including pharmacological, surgical, and behavioural interventions, impact the gastrointestinal tract differently. Although surgical and pharmacological interventions provide insights, the effects on gastrointestinal motility are not solely caused by weight loss *per se* but also by the direct and indirect effects of the drug or procedure applied. Therefore, the focus of this section will be on dietary interventions (Figure 3).

In line with the prevailing finding of accelerated gastric emptying in obesity, diet-induced weight loss seems to decelerate gastric emptying[66,76], although contradictory results also have been published[76]. For example, in the study by Verdich et al.[76], a substantial weight loss of ~19 kg and an eight-week weight stabilisation period resulted in a slower initial gastric emptying rate, which returned to the level of individuals with normal weight. In contrast, the overall gastric emptying rate was unaffected. It was suggested that the weight loss-induced deceleration of the initial emptying rate might delay meal termination and lead to an increased susceptibility to excessive eating and the regain of lost weight. Similarly, a four-month weight loss intervention with or without an intragastric balloon slowed gastric emptying in both groups[66]. In addition, a four-day period of caloric restriction increased the ‘sensitivity’ to an

intraduodenal infusion of a lipid emulsion in individuals with obesity, meaning that the number of antral and duodenal pressure waves was reduced. In contrast, pyloric pressure increased, indicating inhibition of gastric emptying[84]. This may explain the reduced hunger and energy intake at a buffet meal.

The effect of diet-induced weight loss on small intestinal and colonic transit times or motility has not been reported and warrants investigation.

In summary, gastric emptying time and colonic transit time seem to be accelerated in obesity, although contradictory report exists. The initial gastric emptying rate may be accelerated in obesity, possibly due to higher absorptive capacity in the small intestine. Other proposed mechanisms include altered vagal activity and blunted hormone responses. The relationship between small intestinal transit time and obesity is less clear. However, individuals with obesity absorb more energy from the small intestine despite the faster transit time. This could be a factor in the development of obesity. Dietary interventions seem to decelerate gastric emptying; however, contradictory results have also been published. Weight loss-induced deceleration of the emptying rate may lead to an increased susceptibility to excessive eating and weight regain.

1.4. Diabetes

Diabetes is a heterogeneous collection of chronic diseases collectively characterised by hyperglycaemia but with considerable variation in clinical presentation and disease progression. Five hundred thirty-seven million adults worldwide are living with diabetes[85], with negative consequences for both the individual's quality of life and public and personal healthcare expenses. The most common variants of diabetes are type 1 diabetes, which accounts for 5-10% of diabetes cases, and type 2 diabetes, which accounts for 90–95% of diabetes cases worldwide[86]. Type 1 diabetes is caused by cell-mediated autoimmune destruction of the β-cells in the pancreas and often presents early in life. Type 2 diabetes is characterised by insulin deficiency and peripheral insulin resistance, often associated with overweight or obesity[85].

Although type 1 and type 2 diabetes have different causes, they share similar complications associated with disease duration and the degree of glycaemic

control. These range from acute and potentially life-threatening conditions like severe hypoglycaemia or ketoacidosis to chronic and debilitating macro- and microvascular complications that affect various organ systems, including cardiovascular disease, retinopathy, nephropathy, and neuropathy[87]. Additionally, diabetes frequently leads to gastrointestinal disorders, which may result in considerable discomfort for the individual[88]. However, dysmotility has been reported in asymptomatic individuals, so symptom ratings are a poor indicator of gastrointestinal dysfunction[89]. As described previously, postprandial plasma glucose concentrations both regulate and are regulated by gastrointestinal transit. Therefore, the interplay between glycaemic control and gastrointestinal dysmotility plays a central role in the progression of diabetes[90].

Several studies report decelerated gastric emptying time, colonic transit time, and whole gut transit times in individuals with type 1 and type 2 diabetes using different methodologies[89,91–96]. In a study by Rouphael et al., [91] gastric emptying time and colonic transit times were delayed in most individuals with diabetes referred to WMC testing at their clinic. In total, 72% of the referred individuals had dysmotility, but there was no correlation between gastrointestinal dysmotility and either haemoglobin, A1c (HbA1c) or microvascular complications. However, gastrointestinal motility is altered within minutes in response to hypo- or hyperglycaemia[97]. Since HbA1c reflects the average glycaemic level within the past months, it may not be an optimal surrogate for glycaemic control in this context[98]. In another study, colonic and whole gut transit times were prolonged in asymptomatic individuals with type 1 diabetes and not type 2 diabetes, indicating that enteric neurons may be affected even before neuropathy symptoms arise[89]. Meanwhile, gastric emptying time and small bowel transit time were unaffected.

Gastrointestinal dysfunction may arise even before a diagnosis of type 2 diabetes is established. Gastrointestinal symptoms are more prevalent in individuals with prediabetes (defined as impaired fasting glucose and/or impaired glucose tolerance and/or HbA1c of 39–47 mmol/mol) than in healthy individuals[99]. Another study reported an accelerated gastric emptying rate in individuals with prediabetes compared to healthy controls[100]. In that study, individuals with type 2 diabetes had normal gastric emptying time. However, this may be because the glucose control of the participants was well-regulated. Additionally, they had a shorter disease duration than other studies in which

gastric emptying has been delayed. It seems counterintuitive that individuals with prediabetes have accelerated gastric emptying while individuals with diabetes exhibit prolonged gastric emptying. However, this may be explained by the BMI. The individuals with prediabetes had a BMI of ~28 kg/m^2, and as previously described, most studies have reported accelerated gastric emptying in individuals with overweight and obesity. Thus, it is possible that hyperglycemia in prediabetes has not yet caused nerve damage and accelerated gastric emptying.

1.4.1 Diabetic neuropathy

Gastrointestinal disorders in diabetes are partly caused by prolonged hyperglycaemia leading to neurotoxicity and damage to the nerves of the enteric nervous system that regulates gastrointestinal function. It has been widely accepted that gastrointestinal dysfunction in diabetes is related to neuropathy, but emerging evidence suggests that other factors, including autoimmunity, endothelial dysfunction and gut microbiota, may also contribute[101]. Neuropathy is the most common complication, affecting at least 50% of individuals with diabetes[102]. Diabetic neuropathy displays various patterns of nerve injury. Because the enteric nervous system is part of the autonomic nervous, it is common to evaluate the autonomic function and use it as a proxy for the function of the enteric nervous system. Consequently, cardiac autonomic neuropathy is the most extensively studied proxy of dysautonomia, as the vagus nerve constitutes the parasympathetic nerve in both organs. Diabetes-induced parasympathetic withdrawal is caused by a mismatch in autonomic regulation through the gut-brain axis, leading to harmful changes in the function and structure of the gastrointestinal tract[88]. Meanwhile, distal symmetric polyneuropathy is the most common type of neuropathy in diabetics and is characterised by sensory and motor disruptions in the lower limbs and hands[102]. However, studies have suggested that this type of neuropathy can be accompanied by gastrointestinal dysmotility in individuals with diabetes, indicating systemic neurodegeneration[92,103]. WMC studies of gastrointestinal function in individuals with type 1 diabetes with and without distal symmetric polyneuropathy have shown that gastric emptying time and antroduodenal transit time are prolonged in individuals with type 1 diabetes and distal symmetric polyneuropathy compared to individuals with type 1 diabetes only. The antroduodenal transit time refers to the duration of WMC passage through the pyloric sphincter. Antroduodenal transit time can be delayed in individuals with pylorospasms caused by dysfunctional and dyscoordinated gastric and duodenal motility, for instance, as a result of, e.g.,

neuropathy[92]. It appears that individuals with type 1 diabetes and distal symmetric polyneuropathy are more prone to gastric dysmotility and pylorospasms than those with type 1 diabetes alone, indicating systemic neurodegeneration.

In summary, both type 1 and type 2 diabetes are associated with complications that affect various organ systems and gastrointestinal function, which is a critical factor in glucose control and, therefore, the progression of diabetes and its complications. Research has revealed that people with diabetes have delayed pan-enteric transit times, leading to gastrointestinal symptoms. Interestingly, individuals with prediabetes experience more gastrointestinal symptoms, but their gastrointestinal transit seems to be accelerated in contrast to individuals with type 2 diabetes. In individuals with type 1 diabetes and distal symmetric polyneuropathy, the gastric function is negatively affected, causing pylorospasms, likely due to effects on the enteric nervous system.

1.5. Physical activity

When discussing physical activity, it's crucial to differentiate between the immediate effects of acute physical activity or exercise and the long-term effects of maintaining a physically active lifestyle over an extended period, from here on termed habitual physical activity. The following paragraph will describe how gastrointestinal transit is affected by both acute and habitual physical activity.

1.5.1 Acute physical activity

The effects of acute exercise on gastric emptying time have been extensively studied, primarily in healthy individuals with normal weight. Most studies were conducted to optimise nutrient and water absorption to enhance exercise performance. These studies have revealed that the impact of exercise on gastric emptying time is influenced by exercise intensity[104]. Short bouts of low to moderate-intensity exercise typically accelerate gastric emptying, while extended periods of vigorous-intensity exercise can delay gastric emptying both during and after exercise[104]. There is limited research on how acute exercise affects small intestinal and colonic transit. Studies on orocecal transit have produced conflicting results, with some showing accelerated transit during exercise[105,106], while others show no change[107]. However, these differences may result from varying exercise protocols. Acute exercise also seems to impact the orocecal transit in an intensity-dependent manner, with

decelerating transit with increasing intensity[104]. Low to moderate-intensity exercise has been found to either accelerate[108] or have no effect[107] on small intestinal transit time. However, acute vigorous intensity exercise has been shown to accelerate both small bowel and colonic transit time[109].

The mechanisms by which acute exercise affects the gastrointestinal tract include reduced blood flow, increased gastrointestinal motility, mechanical bouncing of the intestines, and neuro-endocrine alterations[110–112]. In a meta-analysis, a moderately decreased response in acylated Ghrelin and small to moderate increases in plasma PYY, GLP-1, and pancreatic polypeptide were observed in response to acute exercise[113], indicative of prolonged gastric emptying. Accordingly, these exercise protocols were predominantly of high intensity[113]. As previously described, these hormones and peptides modulate gastric and small intestinal motility in various ways. Therefore, it seems likely that they play a role in how acute exercise affects gastrointestinal motility.

1.5.2 Habitual physical activity

Only a few studies have investigated the associations between habitual physical activity and gastrointestinal motility and transit. One study has reported an inverse association between physical activity energy expenditure and gastric emptying time[114], indicating that higher physical activity levels may lead to faster gastric emptying. Another study has suggested a reversed U-shaped association, where individuals with moderate levels of habitual physical activity had the fastest gastric emptying compared to those with low or high-intensity activity levels[115]. However, the latter relied on questionnaires to assess physical activity, which can be subject to recall bias and may not accurately reflect actual activity levels.

In one study, habitual physical activity energy expenditure did not correlate with colonic transit time[116]. However, these correlations were not based on intensity but total energy expenditure. In a study by Oettlé et al.[117] one week of moderate-intensity aerobic exercise accelerated colonic and whole gut transit time in healthy individuals. In line with this, a ~2-fold slower colonic transit time has been reported in middle-aged men after two weeks of reduced physical activity[118]. However, the mechanisms driving this inverse association are not fully understood. However, the effects of habitual physical activity may be related to the mechanisms driving the effects of acute exercise.

In summary, the relationship between physical activity and gastrointestinal motility, particularly pan-enteric regional transit, requires further investigation. The available evidence suggests that acute exercise can affect gastric emptying time in an intensity-dependently manner. Still, the effects on other gastrointestinal tract regions need to be clarified. The association between habitual physical activity and gastric emptying time still needs to be fully understood. However, understanding these relationships can provide valuable insights into optimising glycemia, managing body weight, and promoting overall gastrointestinal health.

2. Rationale and aims

The overall aim of the present thesis was to investigate aspects of gastrointestinal transit and how it is modulated by circadian rhythms, diabetes, and lifestyle factors, i.e., overweight and obesity, the timing of food intake and physical activity.

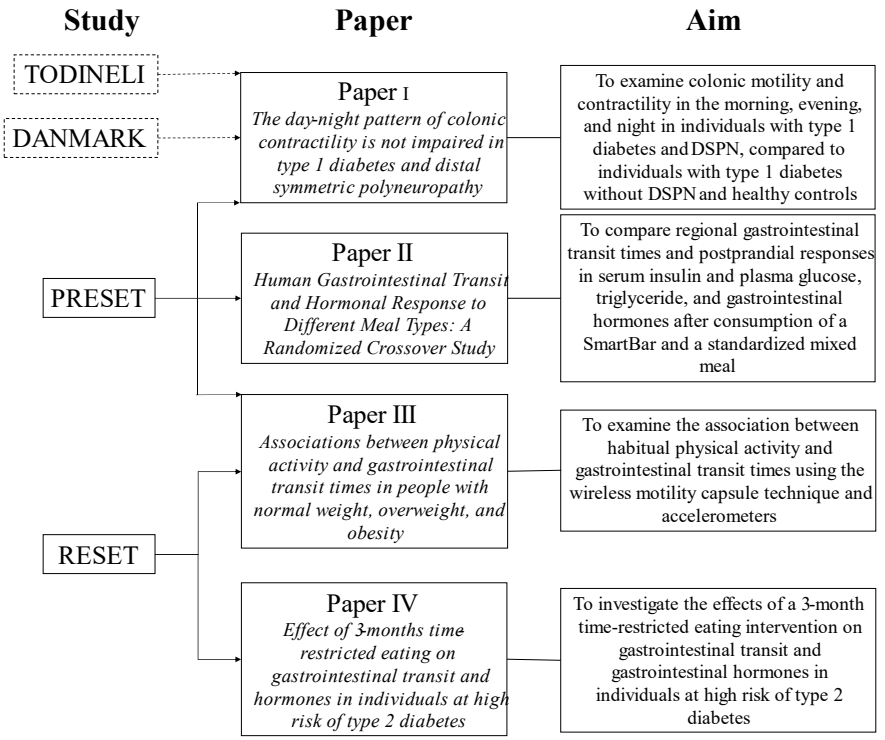

Figure 4. Overview of the studies and papers included in the thesis. Studies in solid boxes are conducted at Steno Diabetes Center Copenhagen, and studies in dashed boxes are conducted at Aalborg University Hospital. Papers I-III are published, and paper IV is in preparation. DSPN, distal symmetric polyneuropathy.

The thesis includes four papers, of which papers I-III are published in peer-reviewed journals, and paper IV is in preparation (Figure 4). The papers are based on four studies described in the methods section (page 42). The studies

were conducted at Steno Diabetes Center Copenhagen and Aalborg University Hospital.

The specific rationales, aims, and hypotheses of the four papers were as follows:

2.1 Paper I

Colonic motility follows a circadian rhythm and usually is minimal during the night and increases dramatically after waking up in the morning and after the ingestion of a meal. This day-night rhythm is partly controlled by the peripheral 'gut clock' by regulating clock genes in the myenteric plexus. Thus, the rhythmicity of colonic motility is partly controlled by the enteric nervous system. Distal symmetric polyneuropathy is the most common type of neuropathy in individuals with type 1 diabetes; up to 50% of individuals with type 1 diabetes develop the condition, and it is commonly concomitant with diabetic autonomic neuropathy, with asymptomatology reflecting the various end-organs. Common gastrointestinal symptoms include colonic dysmotility and overlapping upper and lower abdominal symptoms, of which constipation is the most predominant[9]. It is not known, however, whether disruption of the day-night rhythm of colonic motility is associated with type 1 diabetes with/without distal symmetric polyneuropathy.

Aim: We aimed to investigate whether the day-night rhythm of colonic motility and contractility was affected in individuals with type 1 diabetes with and without distal symmetric polyneuropathy compared to healthy controls. In addition, we examined whether the increase in colon motility and contractility from night to morning was dampened by type 1 diabetes with or without distal symmetric polyneuropathy.

Hypothesis: We hypothesised that individuals with type 1 diabetes and distal symmetric polyneuropathy had decreased colonic motility index and contractility and a reduction in the night-to-morning increase in motility index compared to healthy controls and individuals with type 1 diabetes without distal symmetric polyneuropathy.

2.2 Paper II

The WMC offers a time-efficient examination of regional and whole-gut transit times and motility during free-living conditions. In metabolic research,

the WMC represents a potential method to evaluate gastrointestinal transit, pH, and contractility. For instance, this would be useful when performing a mixed meal tolerance test to examine metabolic responses to a meal, such as postprandial excursions of metabolites and gastrointestinal hormones. However, the WMC is usually administered with a granola bar (SmartBar, Medtronic) which is low in calorie- and fat content (260 kcal, seven energy per cent (E%) fat, 74E% carbohydrate, and 19E% protein) compared to a typical western meal which is used for mixed meal tolerance tests in which postprandial hormone- and metabolite responses are evaluated. Such measures had never previously been reported in response to ingestion of the SmartBar. Therefore, there was a need for studies comparing gastrointestinal transit times and postprandial responses in hormones and metabolites to a SmartBar and a standardised mixed meal, respectively. Because the WMC is expelled from the stomach with the migrating motor complex, the validity of the WMC method is dependent on gastric emptying of the capsule before ingesting a subsequent meal. Since gastric emptying is prolonged with increasing caloric loads, it needed to be established whether a mixed meal with higher calorie content than the SmartBar would imperil the integrity of the test.

Aim: We aimed to compare the effect of a SmartBar and a standardised mixed meal on regional gastrointestinal transit measured with the WMC and postprandial responses in gastrointestinal hormones.

Hypothesis: We hypothesised that the standardised mixed meal would elicit prolonged regional transit times compared with the SmartBar. In addition, we hypothesised that the standardised mixed meal, which was larger in volume and had a higher fat content, would cause increased postprandial excursions in plasma gastrointestinal hormone concentrations compared with the SmartBar.

2.3 Paper III

Gastric emptying time is a rate-limiting factor in postprandial glycemia and is involved in developing type 2 diabetes[119]. Exercise has been shown to acutely accelerate gastric emptying at low intensities and decelerate gastric emptying at high intensities[104]. However, while the impact of acute exercise on gastric emptying has been studied extensively in healthy individuals, few studies have examined the relationship between habitual physical activity and gastric emptying. Concerning physical activity focus has been on the effects of gastric

emptying, partly because gastric emptying is relatively simple and inexpensive to assess. On the other hand, small intestinal transit has been proposed to influence postprandial glucose- and calorie absorption from the small intestine[120]. Thus, it is relevant to investigate how small intestinal transit can be affected and manipulated. However, the association between pan-enteric transit and habitual physical activity had not been explored.

Aim: We aimed to explore associations between habitual physical activity, i.e. time spent performing physical activity at different intensities and total physical activity and gastrointestinal transit times.

Hypothesis: As this was an explorative post hoc analysis, no hypotheses were predefined.

2.4 Paper IV

Time-restricted eating has been shown to induce body weight loss, improve insulin sensitivity and reduce blood pressure and appetite in the evening in humans[36]. In obese animal models, circadian rhythms are blunted but can be restored with time-restricted eating[57]. The circadian clock regulates gastrointestinal transit, motility[47], and hormone secretion[121], which are exacerbated in the evening compared to the morning. By aligning food intake with the circadian rhythm with time-restricted eating, it may be possible to improve gastrointestinal function. However, whether time-restricted eating affects gastrointestinal transit and motility in individuals with overweight and prediabetes or obesity has yet to be investigated. We also plan to analyse postprandial excursions of several gastrointestinal hormones in plasma and gastrointestinal symptom scores assessed by questionnaires. However, these have yet to be analysed and will not be included in this thesis.

Aim: We aimed to explore whether gastrointestinal transit times and postprandial hormone responses to a meal were affected by a three-month 10-hour time-restricted eating intervention in individuals with overweight and prediabetes or obesity. Furthermore, we wished to assess whether time-restricted eating affected gastrointestinal symptoms.

Hypothesis: As this was an explorative post hoc analysis, no hypotheses were predefined.

3. Materials and methods

The papers included in this thesis are based on data from four studies, two randomised controlled trials. (PRESET and RESET) and baseline data from a randomised trial (TODINELI) and one prospective cross-over study (DANMARK). PRESET and RESET were conducted at Steno Diabetes Center Copenhagen, Denmark, and TODINELI and DANMARK were conducted at Aalborg University Hospital, Denmark (Figure 4). The studies were conducted in accordance with the ethical standards of the regional ethics committees and with the Declaration of Helsinki.

3.1. My contributions

I was involved in the preparation, execution, data processing, and manuscript preparation of the PRESET and RESET studies. Collaborating with colleagues, I designed the studies, wrote the study protocols, applied for ethical approval, recruited and screened participants, and conducted test days. Furthermore, I co-supervised one bachelor's student and two master's students. The TODINELI and DANMARK studies were conducted by colleagues at Mech-Sense, Aalborg University Hospital. For **papers I-III**, I performed the data analyses and drafted the manuscript; for **paper IV,** I performed the WMC data analyses and data management; colleagues conducted the statistical analyses, and I contributed to drafting the manuscript.

3.2 The PRESET study

The PRESET study was a randomised, open-label, cross-over study conducted at Steno Diabetes Center Copenhagen (NCT03894670). The PRESET study also served as a pilot study for testing the protocol of the experimental test day for the RESET study. In brief, 14 participants completed two mixed meal test days which only differed by the meal ingested. The participants underwent baseline measurements, including body weight, height, Dual-energy X-ray Absorptiometry, self-assessed appetite using visual analogue scales (VAS), and blood sampling. An accelerometer was placed on the lower back to assess physical activity during a 6-day free-living period following the test day. Regional gastrointestinal transit was evaluated using a WMC (SmartPill; Medtronic, Minnesota, USA), which was swallowed immediately after ingestion of either 1) a muesli bar (SmartBar; Medtronic, Minnesota, USA) or 2) a standardised mixed breakfast meal in random order. Blood samples were

drawn before and after ingestion of the WMC, and self-assessed appetite was evaluated consecutively for four hours while the participants stayed seated or supine. Gastrointestinal contractility measures and motility indices from the SmartBar test day were included in **paper I**, data on gastrointestinal transit data, postprandial hormone concentrations and appetite scores from both test days were included in **paper II** and data on gastrointestinal transit and physical activity from the mixed meal test day was included in **paper III**.

3.3 The RESET study

The RESET study was a randomised, controlled trial conducted at Steno Diabetes Center Copenhagen (NCT03854656). The study was designed to investigate the effects of three months of time-restricted eating on body weight, metabolism, and behaviour in individuals with either 1) overweight (BMI: 25-29.9 kg/m^2) and prediabetes (HbA1c ≥39 to <48 mmol/mol) or 2) obesity (BMI: ≥30 kg/m^2) with or without prediabetes[122]. The present thesis includes a secondary analysis in a sub-sample of the study participants (**paper IV**) evaluating the effect of time-restricted eating on gastrointestinal transit. Fifty-three men and women were randomised to three months of either 1) time-restricted eating, in which participants were instructed to consume all foods and beverages (except water) within 10 hours and thereby fast for the remaining 14 hours of the day or 2) habitual living (control group). At baseline and after three months of intervention, participants underwent the same measurements as on the mixed breakfast meal test day of the PRESET study. Gastrointestinal transit data and physical activity data from the baseline visit were included in **paper III**. Gastrointestinal transit data from baseline and after three months of intervention were included in **paper IV.** In the final version of paper IV, data on postprandial hormone concentrations will also be included. Still, these analyses have not been finalised in time to be included in the thesis.

3.4 The TODINELI study

The TODINELI study was a randomised, placebo-controlled trial conducted at Mech-Sense, Department of Gastroenterology and Hepatology at Aalborg University Hospital (NCT-02138045). The study was designed to explore the neuroprotective effect of liraglutide in experimental neurophysiological pain models in individuals with type 1 diabetes and distal symmetric polyneuropathy[123]. Forty-eight individuals were examined at baseline and

after 26 weeks of treatment with either liraglutide or placebo. Regional gastrointestinal transit times and motility indices were measured with the WMC technique after ingestion of a SmartBar. Data from the baseline visit is included in **paper I**.

3.5 The DANMARK study

The DANMARK study was a prospective, cross-sectional study conducted at Mech-Sense, Department of Gastroenterology and Hepatology at Aalborg University Hospital (N-20170045). The study aimed to explore sensorimotor and autonomic complications in adults with type 1 – and type 2 diabetes to identify potential biomarkers for evaluating the progression and severity of complications related to diabetes. In this thesis, WMC-assessed gastrointestinal transit times and motility indices from 56 individuals with type 1 diabetes were included in **paper I**.

3.6 Methods

3.6.1 Regional gastrointestinal transit and motility

Regional gastrointestinal transit times and motility indices were assessed in all four studies using a WMC (Medtronic, Minnesota, USA). The WMC is an indigestible capsule containing sensors measuring pH, temperature, and pressure as it passes through the gastrointestinal tract. The capsule reports pH with a range of 1–9 and an accuracy of ±0.5 pH units, pressure with a range of 0–350 mmHg and an accuracy of ±5 mmHg and temperature with a range of 20–40ºC with an accuracy of ±1ºC[33]. Before any measurement, the WMC was activated and connected to a portable data receiver by a docking station connected to a computer with the software program MotiliGI (Medtronic, Minnesota, USA). The WMC was instantly swallowed with 200 mL water after ingesting either a standardised mixed breakfast meal or a SmartBar (Medtronic, Minnesota, USA) per the study protocol.

Following ingestion of the WMC, participants fasted for a minimum of six hours to ensure the capsule had passed the pyloric sphincter before consumption of a second meal. The WMC is often emptied from the ventricle with the phase III migrating motor complex. If a second meal is consumed before initiating the migrating motor complex, the WMC will remain within

the ventricle until the next migrating motor complex[124]. The study participants were instructed to keep the data receiver within <25 cm of their body for one week or until expulsion, indicated by a loss of signal to the capsule after a bowel movement. On the data receiver, a button was used to register daily events such as bowel movements, eating, bedtime, wake-up time, and gastrointestinal symptoms (i.e., pain in the abdominal region, bloating, nausea, vomiting). The participants also noted the time and type of the events in a personal diary.

Data from the WMC were uploaded and analysed in MotiliGI (SmartPill, Medtronic) by two researchers independently in accordance with standard practice. Gastrointestinal landmarks were identified based on the pH and temperature curves. The time of ingestion and expulsion were determined based on a rapid increase and decrease in temperature. The point of passage through the pyloric sphincter (gastric emptying) was identified as a sustained increase in pH of >3 pH units, and the point of passage across the ileocecal junction (ICJ)) was identified by a sustained decrease of >1 pH units (Figure 5). Gastric emptying time was defined as the time from ingestion until a sustained increase in pH of >3 pH units; small bowel transit time was defined as the time from gastric emptying to the ileocecal junction; colonic transit time was defined as the time from ICJ to expulsion and whole gut transit time was defined as the time from ingestion to expulsion.

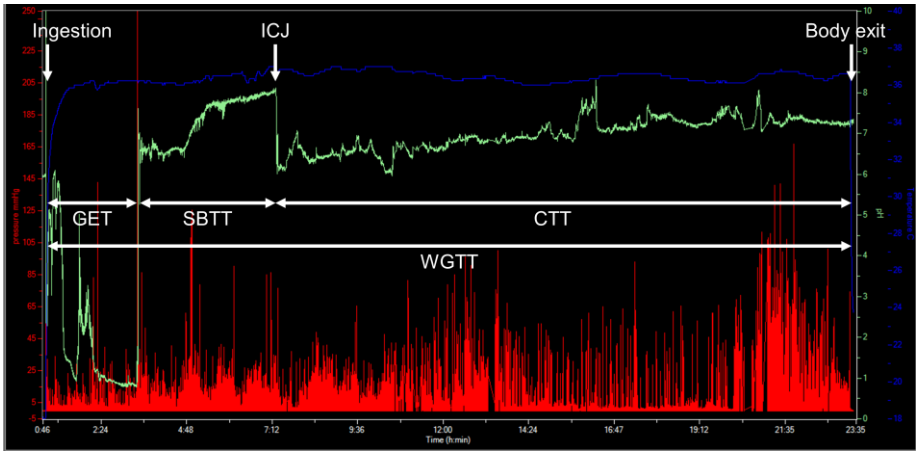

Figure 5. Representative trace from the wireless motility capsule. pH (green), temperature (blue) and pressure (red) curves measured with the wireless motility capsule. Ingestion and body exit are evaluated from the temperature curve, and regional transit times are assessed from the pH curve. ICJ, ileocecal junction; GET, gastric emptying time; SBTT, small bowel transit time; CTT, colonic transit time; WGTT, whole gut transit time.

For paper I, maximum amplitude, mean peak amplitude/contraction, mean contractions/min, and motility indices were calculated at three designated time points during the measurement period using the MotiliGI software. The motility index was calculated as the natural log of the sum of amplitudes*number of contractions + 1 and expressed in mmHg*S/min[28].

3.6.2. Blood analyses

Blood samples were collected from an antecubital vein in the PRESET study and were analysed in the central laboratory at the Steno Diabetes Center Copenhagen.

All samples were centrifuged at 3500 × g for 15 min at 4◦C to separate plasma/serum. Glucose was measured in plasma from tubes containing a citric acid buffer. Whole blood was incubated for 20–30 min at room temperature for the insulin measurements before centrifugation. Alanine aminotransferase (ALAT), aspartate transaminase (ASAT), triglyceride, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and very-low-density lipoprotein (VLDL) cholesterol were analysed in plasma from lithium-heparin–coated tubes. Plasma/serum was analysed with the Cobas 8000 system (Roche Diagnostics) on sampling day.

Total ghrelin, GIP, GLP-1, glucagon, leptin, and PYY were measured in plasma separated from blood samples collected in EDTA sample tubes with valine-pyrrolidine chilled on ice and centrifuged at 3500 × g for 15 min at 4◦C. After the study was completed, all samples were analysed simultaneously with a sandwich immunoassay using electrochemiluminescence (MSD)[125]. All analytes were analysed by the high-sensitivity U- PLEX Metabolic Group 1 (Human) Multiplex Assay Kit, which was coated with a linker-coupled antibody solution (MSD). Dipeptidyl peptidase IV Inhibitor (MSD) was added to a prepared reagent (Metabolic Assay Working Solution). Samples were diluted 1:2 in buffer, and all calibrations and samples were analysed in duplicates. Data were read on MESO QuickPlex SQ 120 (Model. No.: 1300; MSD). The data are included in **paper II** and will be included in the final version of **paper IV**.

3.6.3 Physical activity

Physical activity was assessed with accelerometers (Axivity AX3, Newcastle upon Tyne, UK) placed on the lower back in the PRESET and RESET studies. Data were analysed in the software, Actilife (version 6.13.4 ActiGraph, LLC, Pensacola, Florida) and is reported in **paper III**.

Records were validated and evaluated based on the following criteria: 1) minimum 10 hours of awake wear time per day, 2) minimum three weekdays and one weekend day (Saturday-Sunday) with valid data. The first day of the recording was excluded because the participants were physically inactive during the test day.

Total sleep duration was calculated based on self-reported bedtimes and time out of bed using the Cole-Kripke algorithm[126] and was categorised as non-wear time. Non-wear time was determined with the Choi 2011 algorithm[127] in the Actilife software. Time spent on physical activity in the intensity categories listed below was calculated in 60-sec epochs on the vertical axis in the software. Cut points for the different intensity categories were chosen based on cut points previously used in a population similar to ours[128]: Sedentary activity: 0-100 counts/min; low light activity (e.g. sitting or watching television[129]): 101-759 counts/min (e.g. light housework such as vacuuming[129]); high light activity: 760-1951 counts/min (e.g. more demanding housework such as gardening[129]); moderate and vigorous activity: ≥1952 counts/min (e.g. ascending stairs, brisk walking or running[129]).

4. Key results

4.1 Paper I

Key results:

- The motility index in individuals with type 1 diabetes and distal symmetric polyneuropathy tended to be higher in the evening compared to healthy controls (mean group difference of 1.74% (95% CI: 1.08; 2.81), $p = 0.064$) but not individuals with type 1 diabetes without distal symmetric polyneuropathy
- The motility index did not differ between groups in the morning or night.
- Max amplitude, mean peak amplitude, and mean contraction did not differ between the groups at any of the three time points.
- Overall, max amplitude, mean contraction, and motility index peaked in the morning, decreased in the evening, and reached their nadir at night. In contrast, the mean peak amplitude did not differ between the three time points.
- The increase from night to morning did not differ between the groups in any of the contractility measures.

Interpretation:

Type 1 diabetes with or without distal symmetric polyneuropathy was not associated with a disruption in the day-night pattern of colonic motility and contractility. The motility index tended to be higher in individuals with type 1 diabetes and distal symmetric polyneuropathy in the evening. However, the effect size was small and may not be physiologically or clinically relevant. Furthermore, type 1 diabetes with or without distal symmetric polyneuropathy did not blunt the increased motility observed from night to morning.

4.2 Paper II

Key results:

- The standardised mixed meal resulted in prolonged gastric emptying time in all participants with a median (IQR) difference of 98.0 (70.0; 113) min ($p < 0.001$) compared with the SmartBar
- The two meals resulted in comparable small bowel transit time [median difference (Q1, Q3) = 20.0 min (−12.0; 79.8); colonic transit time (median difference =−79.5 (−182; 666) min, p= 0.952); and whole gut transit time (median difference =−206 (−344; 284) min, p = 0.463)
- Higher plasma GIP iAUC (p = 0.003) and PYY iAUC (p = 0.017) were observed with the standardised mixed meal compared with the SmartBar, whereas the responses of plasma GLP-1, glucagon, ghrelin, or leptin did not differ between the two meals

Interpretation:

The WMC can be administered with a higher-calorie standardised mixed meal without imperilling the validity of the test. However, it is essential to note that the meal-induced decelerated gastric emptying time increases the risk of capsule retention in individuals with pathologically prolonged gastric emptying. Higher volume, calorie and fat content in a meal only seemed to affect gastric emptying time, whereas small bowel, colon and whole gut transit times were similar between the two meals. The higher plasma PYY iAUC may contribute to the prolonged gastric emptying time with the standardised mixed meal by activating the ileal brake.

4.3 Paper III

Key results:

- An inverse association was observed between time spent performing high light intensity physical activity and colonic transit time and also whole gut transit time, meaning that for every additional hour spent performing high light intensity physical activity, colonic transit time

decreased by 25.5 % (2.7; 3.10) (p = 0.028) and whole gut transit time decreased by 16.2 % (8.4; 1.84) (p = 0.028)
- No association was observed between gastrointestinal transit times and time spent on sedentary activities or physical activity at a low light intensity, MVPA or total counts per minute per day.
- There was no difference in gastrointestinal transit times between the two cohorts, i.e. individuals with normal weight (PRESET) and overweight or obesity (RESET)

Interpretation:

Engaging in regular high light intensity physical activity, such as demanding housework, seems to accelerate colonic and whole gut transit, which supports the National clinical guideline for non-surgical treatment of constipation.

4.4 Paper IV

Key results:

- Three months of time-restricted eating did not affect regional gastrointestinal transit times in individuals with overweight and prediabetes or obesity
- Time-restricted eating led to a within-group weight loss of 1.2 (0.5, 1.8) kg, but this was not different from the control group (P = 0.099)

Pending results:

- Fasting and postprandial excursions of gastrointestinal hormones
- Gastrointestinal symptom scores
- Association between gastrointestinal transit times and glucose variability measured with a continuous glucose monitor

Interpretation:

Three months of time-restricted eating did not alter pan-enteric gastrointestinal transit times in individuals with overweight and prediabetes or obesity. This may be caused by selection bias, as we excluded individuals with gastrointestinal symptoms or diseases, limiting the potential for improvement. The effects are independent of weight loss since there was no difference between the groups.

5. Discussion

The papers included in this thesis explored aspects of gastrointestinal transit and how it is modulated by circadian rhythms, diabetes, overweight, and lifestyle factors such as food intake and physical activity. A summary of the main findings is presented in Figure 6. The following discussion will put these results in the context of existing literature and critically evaluate the methods applied. The discussion will be divided into four sections: 1) circadian rhythm and diabetes, 2) overweight and obesity, 3) physical activity and 4) methodological considerations.

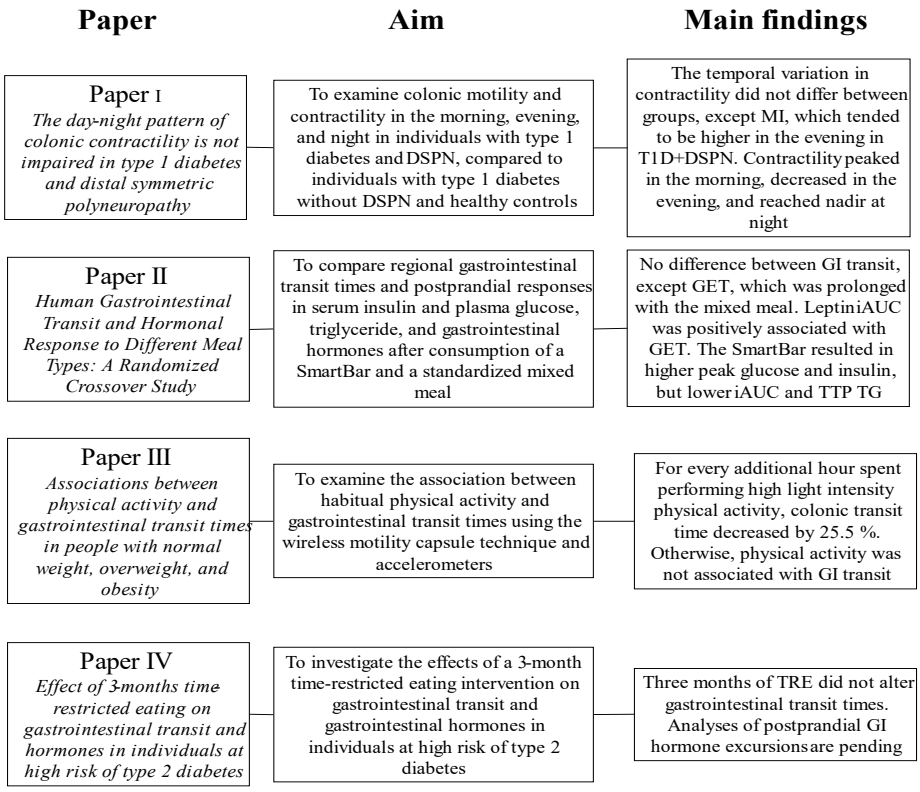

Figure 6. Simplified overview of aims and main findings. DSPN, distal symmetric polyneuropathy; GI, gastrointestinal; GET, gastric emptying time; iAUC, incremental area under the curve; MI, motility index; T1D, type 1 diabetes; TG, triglyceride; TRE, time-restricted eating

5.1 Circadian rhythm and diabetes

In **papers I and IV,** we examined aspects related to the day-night rhythm of gastrointestinal transit. In **paper I**, we observed a trend towards a minor increase ($p = 0.064$) in motility index during the evening for individuals with type 1 diabetes and distal symmetric polyneuropathy compared to healthy individuals. However, the effect size was small and hence clinically irrelevant. However, we confirmed that colonic contractility and motility display temporal variation, with peak activity in the morning and minimal activity at night. Similar observations have previously been reported in healthy individuals. In 24-hour manometry studies, colonic pressure activity was blunted at night compared to morning[47] and increased rapidly upon awakening[47,48]. In our study, we could not identify the position of the WMC at the given time points. In addition, we assume that the position of the capsule changes between each of the examined time points. As colonic contractility differs between the different colonic regions[130], the basis of comparison is unequal. However, in a study where manometric probes were placed from the cecum to the rectum of healthy individuals, the increase in motility in the morning was instant and co-occurred throughout the colon[48].

5.1.1 Diabetes

In **paper I**, we observed that individuals with type 1 diabetes, with or without distal symmetric polyneuropathy, experienced similar diurnal variations in colonic contractility as healthy individuals. This finding was novel and surprising since the cohort with type 1 diabetes and distal symmetric polyneuropathy had prolonged colonic transit, indicative of damage to the enteric neurons controlling colon smooth muscle tone. However, it appears that the nerve injury does not affect the clock genes of the myenteric plexus[39], at least not in our cohort. Streptozotocin-induced type 1 diabetes in mice causes a phase delay and decrease in the amplitude of the circadian rhythmicity of the *per1* and *per2* clock gene mRNA expression when compared to wildtype mice[131]. Mice lacking these clock genes also exhibit blunted circadian variation in the intracolonic pressure changes and smooth muscle contractile activity. This implies that these clock genes are essential in maintaining normal circadian rhythmicity of colon motility and that they may be negatively affected in type 1 diabetes. However, whether these

observations can be replicated in humans is unknown, and our data do not support it.

The type of neuropathy may partly explain the similar diurnal variation in colonic motility between the groups of individuals. Since distal symmetric polyneuropathy primarily affects peripheral nerves, this may not constitute a disturbance in the gastrointestinal circadian rhythm. Whether individuals with diabetic enteropathy experience gastrointestinal chronodisruption remains to be investigated. Furthermore, glucose control did not appear to influence the temporal variation in colonic motility. Individuals with type 1 diabetes and distal symmetric polyneuropathy were less well-regulated in terms of HbA1c (65 [60, 72] mmol/mol) than individuals with type 1 diabetes without distal symmetric polyneuropathy (58 [53, 69] mmol/mol), but exhibited similar diurnal variation in motility. In support of this, HbA1c has been demonstrated to correlate poorly with gastrointestinal dysmotility[91], possibly because it reflects long-term glucose control, whereas the postprandial plasma glucose response affects gastrointestinal motility almost instantly[97]. Thus, continuous glucose monitoring may be a more appropriate surrogate marker for glucose homeostasis concerning gastrointestinal transit. In **paper IV**, we plan to investigate this relationship further. In that study, we applied continuous glucose monitoring concomitantly with assessing gastrointestinal transit[122]. In the RESET study, we did not include individuals with diabetes, but individuals at high risk of developing type 2 diabetes with one of two risk profiles, 1) BMI ≥25 kg/m^2 with prediabetes (HbA1c of 39-47.9 mmol/mol), or 2) BMI ≥30 kg/m^2 with or without prediabetes. This enables us to investigate whether gastrointestinal transit times are associated with glucose homeostasis below the diabetic glycaemic range. Furthermore, we plan to examine whether gastrointestinal transit times differ between individuals with and without prediabetes based on HbA1c. These research questions have never been assessed before. They will provide novel insight into the link between glucose homeostasis and gastrointestinal function and contribute to elucidating possible mechanisms involved in developing type 2 diabetes.

5.1.2 Time-restricted eating

In **paper IV**, we report that three months of time-restricted eating with a daily eating window of 10 hours did not alter pan-enteric gastrointestinal transit in individuals at high risk of type 2 diabetes. We conducted the first study to investigate the influence of time-restricted eating on pan-enteric motility. However, some studies have investigated time-restricted eating-induced

effects on gastric emptying and other aspects of gastrointestinal function. Similar to our findings, Hutchison et al.[132] reported similar gastric emptying rates before and after two seven-day time-restricted eating interventions separated by two weeks. The two intervention periods differed by the timing of the eating window (early: eight AM to five PM vs late: 12 PM to nine PM). However, the lack of intervention effect may also be explained by the short duration of the intervention.

Furthermore, a recent study reported that gastric acid exposure time tended to decrease in individuals with suspected gastroesophageal reflux disease after only two days of time-restricted eating with an eight-hour eating-window[61]. This was accompanied by reduced symptoms of heartburn and regurgitation. Although the intervention period was very short and no control group was included in the studies, these results suggest that time-restricted eating improved gastrointestinal function. In our study, exclusion criteria encompassed gastrointestinal diseases and regular (weekly) gastrointestinal symptoms, and as such, the participants did not exhibit any symptoms of gastrointestinal dysmotility. Indeed, this has diminished the potential for improvement of gastrointestinal function and motility, which may explain the lack of intervention effects.

In mice, time-restricted feeding prevented the obesity-induced chrono-disruption of mechanosensitive vagal afferents in the stomach[60]. As these are known to regulate gastric motility and emptying, these results indicate that time-restricted eating may enhance to stomach's response to food and thereby improve appetite regulation. Whether this connection also applies to humans is unknown, but time-restricted eating has been demonstrated to reduce perceived hunger at night[56,132]. In **paper IV**, we did not assess appetite at different times of the day.

In the RESET study, we have yet to analyse gut microbiota and, therefore, cannot contribute further to this discussion. However, previous studies have shown that gut microbiota are involved in colonic motility by directly and indirectly affecting enteric smooth muscle contractility[20]. Furthermore, the composition and diversity of the gut microbiota are associated with obesity[133] and display circadian oscillation[59]. Therefore, previous studies on gut microbiota may provide information on the mechanisms through which time-restricted eating can affect colon motility. In a study in mice, Time-restricted feeding resulted in protection against weight gain by enhancing the ratio

between obesogenic and obesity-protective bacteria[59]. A few human studies have reported the effects of time-restricted eating on gut microbiota with conflicting results[134–137]. One study showed no difference in microbiota composition or α and β diversity[137] between the time-restricted eating group and the control group after a 12-week intervention period. Similarly, time-restricted eating did not alter the diversity or composition of gut microbiota in individuals with obesity[136]. In contrast, 25 days of eight-hour time-restricted eating enhanced microbial richness while enhancing BMAL1 and CLOCK gene expression, which is suggested to be caused by activation of sirtuin-1, which has been linked with improved microbial richness[134]. Furthermore, in what appears to be the same cohort (however, this is not stated in the papers), time-restricted eating was associated with microbial composition and relative abundance[135].

In **paper IV**, we plan to examine the effect of time-restricted eating on postprandial excursions of gastrointestinal hormones involved in regulating gastrointestinal motility, appetite, and glucose homeostasis. In the study by Hutchison et al.[132], seven days of early and late time-restricted eating did not affect gastrointestinal hormone secretion in response to a liquid meal in individuals with obesity. However, in the RESET-study, the intervention period was three months which may result in different intervention effects.

5.2 Overweight and obesity

The role of gastrointestinal motility has been studied, especially in relation to appetite regulation, which is a crucial element in maintaining a healthy body weight. This thesis reports gastrointestinal transit times of individuals with normal weight (**papers II and III**) and overweight or obese (**papers III and IV**). The data are derived from two different studies, the PRESET study, which included individuals with normal weight and the RESET study, which included individuals with overweight and obesity. Since the studies were conducted at the same research facility, mainly by the same staff and using identical study protocols, it seems reasonable to compare the two cohorts. **In paper III**, we included individuals with normal weight from the mixed meal test day of the PRESET study and individuals with overweight and prediabetes or obesity with or without prediabetes from the baseline visit of the RESET study. There was no difference in gastrointestinal transit times between study groups, indicating that gastrointestinal motility is unaffected by overweight

and obesity. However, it may also be an issue of insufficient statistical power because the PRESET study only included 14 participants.

Overall, previous studies indicate that gastric emptying is accelerated[65–68] in obesity, although conflicting results have been published as well[69–73]. It has been proposed that GLP-1 and PYY may be involved in the mechanisms by which gastric emptying is accelerated in obesity[76,78]. GLP-1 and PYY are essential hormones activating the ileal brake in response to nutrients in the ileum, and the postprandial responses of these two hormones are blunted in individuals with obesity[76,78].

It is possible that we did not find a difference between individuals with normal weight and individuals with overweight and obesity because we excluded individuals with gastrointestinal illness and symptoms in the RESET and PRESET studies. Gastrointestinal disorders are more prevalent in individuals with obesity than those with normal weight[138]. By excluding individuals with gastrointestinal symptoms, we may have eliminated the potential difference between the cohorts leading to selection bias. Methodological differences between studies may also explain it. Only one other study has applied the WMC to investigate the relationship between body weight and gastrointestinal transit times[70]. Similar to our observation, there was no difference in gastrointestinal transit times in the fed state between individuals with normal weight and obesity. Compared to a meal, the capsule's indigestible properties cause it to empty differently from the stomach. This allows for measuring the overall gastric emptying time but not the gastric emptying rate, which varies between the initial lag phase and the emptying phase[139]. It has been suggested that the initial 30 minutes of gastric emptying is accelerated in obesity[76], but this would not be evident when assessed by the WMC technique.

In the WMC study by Steenackers et al.[70], small intestinal and colonic transit were not altered in obesity in the fed state, similar to our observation in **paper III**. They did, however, observe an accelerated small intestinal transit time in individuals with obesity in the fasted state. In our study, we did not assess gastrointestinal transit times in the fasted state. Previously, colonic transit time has been suggested to be accelerated in obesity with[83] and without[72] gastrointestinal disorders, i.e., irritable bowel disease, functional constipation, and diarrhoea. These studies included 457 and 165 participants, respectively, and considering the large intra- and interindividual variation in colonic transit[35], it is plausible that our studies were underpowered to detect a possible

difference in colonic transit time between the two study populations. Furthermore, selection bias, as described earlier, may also play a role in this matter.

5.2.1 Weight loss

In **paper IV**, the individuals in the time-restricted eating group lost 1.2 (0.5, 1.8) kg body weight compared to baseline. However, there was no difference from the control group (RESET main paper, under review). This insignificant weight reduction may explain the lack of intervention effects on gastrointestinal transit. Previous studies have reported that overall gastric emptying, or the initial 30 minutes, was decelerated by weight loss. However, in these studies, participants lost more weight than in the RESET study. For instance, the initial phase of gastric emptying was decelerated after a 19 kg weight loss in obese individuals. In contrast, Quist et al.[140] reported that a reduction of 3.4kg of fat after three months did not alter gastric emptying. At the same time, 4.5 kg fat loss after six months accelerated gastric emptying in individuals with overweight and obesity. In that study, however, the weight loss was obtained through an exercise intervention, which in itself affects gastrointestinal transit independently of weight loss, as discussed below. On the other hand, three months of intervention may not be sufficient to alter gastric emptying.

5.3 Physical activity

In **paper III,** we explored the association between habitual physical activity and gastrointestinal transit in individuals with normal weight, overweight and obesity**.** Here, we reported that the more time the participants spent performing high light intensity physical activity, such as gardening or painting[129], the more rapid the colonic and whole gut transit times were. One study has previously examined the association between habitual physical activity and colonic transit. Physical activity energy expenditure was estimated from one-week accelerometry, while colonic transit time was assessed by radio-opaque markers. In that study, colonic transit time did not correlate with habitual physical activity. This may be explained by the fact that physical activity was expressed as physical activity energy expenditure and not intensity. This is equivalent to total counts per minute, as reported in **paper III**, which was not associated with colonic transit time in **paper III.**

Especially performing MVPA and minimising sedentary time have been promoted by healthcare authorities to reduce the risk of various chronic

diseases and mental health issues[141]. In line with this, increasing MVPA is recommended to prevent and treat chronic constipation in inactive individuals[142]. Colonic and whole gut transit times were accelerated after one week of moderate-intensity running and cycling for one hour per day compared to one hour of sedentary activity per day for one week. However, it should be noted that the radio-opaque markers were consumed after the initial exercise session. Thus the measurement was simultaneous with the intervention, indicating that any observed effects were likely due to the acute exercise rather than habitual physical activity. Conversely, daily time spent on MVPA did not associate with colonic transit time in our study. This may be explained by the low variation in MVPA in our cohort. Another possible explanation is that hip-worn accelerometers, such as the one applied in paper III, are not sensitive to static activities like weightlifting or bicycling[143]. Since bicycling is a very common means of transportation in Denmark, MVPA may have been underestimated.

Although we did not observe an association between gastric emptying time and habitual physical activity at any intensity, previous studies have suggested that physical activity may have an inverse relationship with gastric emptying ½-time[114,115]. However, there have been discrepancies in the findings of various studies, which could be attributed to methodological differences. For example, the use of questionnaires in one study may have led to recall bias[115]. In addition, a liquid meal was used instead of a solid mixed meal. The gastric emptying rate of a liquid meal is constant and lacks the lag phase observed after a solid meal, in which the food is broken down to smaller particle sizes. Thus, the emptying profile differs between a liquid and a solid meal. Additionally, in another study[114], the grouping of participants in dichotomised categories based on their level of physical activity may have skewed the results.

Intervention studies have also explored the link between physical activity and gastric emptying, with mixed results. Most studies have shown no effect on gastric emptying after four weeks to three months of moderate to vigorous intensity exercise[140,144,145]. However, in one study, three months of vigorous intensity exercise training did not affect gastric emptying, whereas gastric emptying was accelerated after six months[140]. Based on these findings, over three months of exercise training is necessary to affect gastric emptying. However, the specific mechanisms behind this duration-dependent relationship remain unclear. The mechanisms behind these effects may involve hormonal regulation, mechanical stimulus, and neurological factors.

In **paper III**, no exercise intervention was introduced, and the measurement of physical activity was assumed to reflect the participants' habitual physical activity level.

It is important to note that **paper III** was a cross-sectional study, and therefore causality cannot be established from our findings. Food intake and acute physical activity during the measurement may have affected gastrointestinal transit. Nevertheless, these findings can generate hypotheses for further research.

5.4 Methodological considerations

In this thesis, the primary method employed is the WMC. While this approach has numerous benefits, some weaknesses will be addressed in the following discussion.

In **paper II**, we assessed the potential of the WMC methodology for application in research areas beyond gastroenterology. By administering a standardised mixed breakfast meal with the WMC, gastric emptying time was prolonged compared to the SmartBar in all participants without exception.

The length and diameter of the WMC are 26.8 mm and 11.7 mm, respectively, and thereby exceed the maximal particle size of <2mm, which can be emptied from the stomach during the fed phase. The WMC is, therefore, most often emptied from the stomach with the phase III migrating motor complex[146]. Ingestion of a secondary meal before the capsule has passed to the small intestine can cause retention of the capsule in the stomach until the next phase III migrating motor complex is initiated. Therefore, the validity of the WMC measurement requires fasting until the capsule has passed from the stomach to the small intestine**. In paper II**, we found that the capsule was emptied from the stomach within the recommended 6-hour post-ingestion fasting period for 86% of the participants with the standardised mixed meal. The participants in the study were healthy, normal-weight individuals with no self-reported systematic gastrointestinal symptoms. Based on our findings, we conclude that the WMC is safe to consume along with a standardised breakfast meal and will not compromise the accuracy of the test in healthy individuals without suspected gastric dysmotility. However, caution is due when working with individuals with known or suspected prolonged gastric emptying, such as individuals with diabetes. In these cases, it may be necessary to implement a more extended fasting period to avoid capsule retention in the stomach resulting in invalid measurements.

When analysing data for the papers included in this thesis, we noticed a considerable intraindividual variation in repeated measurements of all transit measures and motility indices measured with the WMC. This observation is in line with reported coefficients of variation of 20-40.4% for gastric emptying time, 20-30.2% for small bowel transit time, 35.1-42.4% for colonic transit time, and 26.4-31.8% for whole gut transit time[35]. In contrast, intraindividual variation in gastric emptying is approximately 13% when measured by

scintigraphy and the [13C]octanoic acid breath[74,147]. This discrepancy may be partly explained by the ambulatory nature of the WMC test, which introduces the possibility of engaging in various activities which may affect the measurement, such as physical activity or consuming a large amount of dietary fibre or coffee. Although it is a cause of more significant intraindividual variation, the option of free-living activity during the measurement is also an advantage of the method in that the measurement reflects the gastrointestinal transit and motility of the individual in their everyday environment and daily life. This is impossible with other methods, such as scintigraphy or breath test requiring consecutive scans. Additionally, the WMC is expelled from the stomach with the migrating motor complex and thus reflects the transit time of the entire meal. In contrast, gastric emptying measured by scintigraphy and breath test is given as a rate at a given timepoint[2]. It should be noted, however, that gastrointestinal transit times measured with the WMC agree well with scintigraphy[32,33]. Detecting statistically significant differences or effects becomes challenging due to the large intraindividual variation. This issue is particularly important for **papers II and IV**, which involve repeated measurements. A study with large population size is necessary to overcome this challenge. Compared to other gastrointestinal tests, the WMC method is quite expensive, and as a result, there is a relatively high risk of underpowering a study that uses the WMC method. Further, in **paper IV**, the participants included were not recruited to have abnormal gastrointestinal transit or symptoms thereof, so the potential for improvement was relatively low. Therefore the possible intervention effect was expected to be small. Thus, the analysis may have been underpowered.

The thesis includes studies that involve both men and women, which improves the translation of the results to the general population. However, it also increases the variation in the data because previous studies have revealed that men tend to have faster gastric emptying, colonic transit time, and whole gut transit time measured with the WMC compared to women[30]. Furthermore, the menstrual cycle and menopausal status of the women may also affect the results. In response to an oral glucose tolerance test, gastric emptying, plasma glucose, insulin and GLP-1 are attenuated during the follicular phase compared with the luteal phase of the menstrual cycle[148]. Similarly, postmenopausal women's pan-enteric transit is slower in postmenopausal women compared with premenopausal women[149]. We did not consider the menstrual cycle when planning test days in either of the studies included in the thesis. Neither did we enquire about menopausal status.

A limitation of the WMC technique is that it cannot assess the direction of the peristaltic movement. This is possible with other methods, such as manometry or 3D-transit[150]. The proportion of retrograde and anterograde peristaltic waves is an indication of the efficiency in the coordination of gut motility, especially compared to measurements of contractile activity only. However, the WMC technique combines contractility measures and transit times, which allows for assumptions to be made about gut efficiency.

In **papers I and III**, data were pooled from three and two different studies. Although the administration of the WMC and the data analysis are standardised by the manufacturer, several aspects of the studies differed. In **paper, I**, the three studies included were conducted at two different research facilities by different staff members. Furthermore, it is important to note that the analyses conducted were secondary in nature. As a result, none of the studies were specifically designed or adequately powered to address the research question posed in the paper. However, the interpretation of the results has been made with these uncertainties in mind, and they have been addressed openly in the papers. Therefore, the analyses are exploratory and may be considered hypothesis-generating.

6. Conclusions

This PhD thesis aimed to explore aspects of gastrointestinal transit and how it is modulated by circadian rhythms, diabetes, and lifestyle factors, i.e., overweight and obesity, timing of food intake and physical activity. Based on the four papers included in the thesis, we conclude the following:

I: Individuals with type 1 diabetes, with or without distal symmetric polyneuropathy, do not appear to have a disrupted day/night pattern of colonic motility and contractility. While the motility index was slightly higher in those with distal symmetric polyneuropathy in the evening, the effect size was small and may not have significant physiological relevance. Additionally, type 1 diabetes with or without distal symmetric polyneuropathy did not affect the increased motility observed from night to morning. The combination of slowed colonic transit and similar or even amplified motility index in individuals with type 1 diabetes with or without distal symmetric polyneuropathy may indicate that the neuronal activity of colonic contractility and propulsion may be disturbed and inefficient.

II: It is possible to use a higher-calorie standardised mixed meal when administering the WMC without compromising its accuracy. However, it is important to remember that the meal may cause a delay in gastric emptying time, which could increase the risk of capsule retention in individuals with abnormally prolonged gastric emptying. The size, calorie content, and fat content of the meal only seem to impact gastric emptying time, while transit times through the small intestine, colon, and entire gastrointestinal tract are similar for both meals.

III: Regular high light intensity physical activity seems to accelerate colonic transit and, thereby, whole gut transit, which may help prevent constipation. No associations were observed between physical activity and small intestinal transit time or gastric emptying. However, previous studies have observed faster gastric emptying in individuals who were habitually physically active compared to inactive individuals. This discrepancy may be explained by methodological differences or by insufficient statistical power.

IV: Three months of time-restricted eating did not alter pan-enteric regional gastrointestinal transit. This may be due to the exclusion of individuals with

gastrointestinal symptoms or diseases, which limits the potential for improvement. Other possible explanations include that the study may be underpowered and that the participants did not lose weight compared to the control group.

6.1 Perspectives

In **paper II,** we explored the potential of the WMC methodology for use in metabolic research by examining the applicability of a standardised mixed meal, similar to those often applied in a mixed meal test, with the WMC. Although the study was too small to provide normative values for gastrointestinal transit times with the standardised mixed meal, our study offers an indication and insights into the advantages and pitfalls of applying the WMC technique with a standardised mixed meal. These results may contribute to widening the WMC method's application and aid in qualifying the decision of what method to apply when measuring gastrointestinal transit and motility.

In future studies, it is first of all essential to be aware of including a sufficient number of study participants to ensure adequate statistical power. Second, the selection of the study population is also imperative. Many unanswered questions remain regarding the link between obesity and gastrointestinal motility. When studying gastrointestinal transit in individuals with overweight and obesity, individuals with dysmotility or gastrointestinal symptoms should also be included to reflect the actual symptom burden of the population.

The relationship between gastrointestinal transit and glycaemia is poorly elucidated and warrants further investigation. It is interesting to investigate whether gastrointestinal dysmotility is a cause or reaction to diabetes and whether the link may be related to overall glycaemia or the daily fluctuations in plasma glucose levels. We plan to explore this matter in **paper IV,** in which we will assess whether gastrointestinal transit times differ between individuals with and without prediabetes defined by HbA1c. Furthermore, we wish to examine whether gastrointestinal transit is associated with markers of glycemic control measured by continuous glucose monitoring.

Time-restricted eating has gained much attention recently, and new studies are published frequently. However, there are still many knowledge gaps regarding

the impact on human physiology and health. Most studies have focused on body weight and metabolism, and only a few studies have investigated the effects of time-restricted eating on the gastrointestinal tract. Still, the fact that circadian rhythms extensively modulate the functions of the gastrointestinal tract and may be adversely affected by chronodisruption calls for studies on how to prevent such conditions. Therefore, time-restricted eating may have a role to play in the treatment or symptom relief of gastrointestinal disorders such as irritable bowel disease or inflammatory bowel disease.

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