Study Protocol with Statistical Analysis Plan and Informed Consent Form

Title	Details
Date	6 May 2024
NCT Number	NCT05163964
Study title	Chronotype, Chrononutrition and Glucose Tolerance Among Prediabetic Individuals: Chrono-DM™
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Study Population	Prediabetic individuals in Community Clinic Melaka
Study Design	Study Type: Prospective Longitudinal Study Sampling Method: Convenient sampling method
General Objective	To determine the associations of chronotype and chrononutrition with glucose tolerance among prediabetic individuals in Malaysia.
Specific Objectives	1) To investigate the effect of chrononutrition on glycemia measures among prediabetic individuals. 2) To investigate the associations of lifestyle factors with glycemia measures among prediabetic individuals. 3) To investigate the associations of anthropometric measurements with glycemia measures among prediabetic individuals.
Study outcomes	The main outcomes of this study are 1) The change of baseline glycated hemoglobin (HbA1c) among prediabetes adults at 6 months. 2) The change of baseline fasting plasma glucose (FPG) among prediabetes adults at 6 months. 3) The change of baseline 2-hour post-load plasma glucose among prediabetes adults at 6 months. 4) The change of baseline continuous glucose monitoring (CGM) profile among prediabetes adults at 6 months. Secondary outcome of this study are 1) The change of eating misalignment among prediabetes adults at 6 months. 2) The change of chronotype among prediabetes adults at 6 months.
Sample Size	120 prediabetic individuals
Study Duration	1 March 2022 until 31 December 2023
Name and Address of Sponsor	Fundamental Research Grant Scheme (FRGS), Ministry of Higher Education/ UMalaya (FRGS/1/2021/SKK06/TARUC/02/1)

Ethics Approval	Tunku Abdul Rahman University Management and Technology Ethics Committee (TAR UC/EC/2021/02-3) Medical Research and Ethics Committee (RSCH ID-21-00114-IVI)

Executive Summary:

Early meal timing affects the circadian rhythmicity and influenced the postprandial blood glucose level in response to meal in diabetes patients. In the other words, diabetes patients with earlier meal timing had a stable glycemic control compared with those with late meal timing. This is a new concept to switch the meal timing earlier than later in diabetes self-monitoring management. However, there is insufficient evidence from Southeast Asia to determine the optimal-meal-timing and its association with glycemic measures in prediabetes individuals. Hence, this study carried out to determine the associations of chronotype and chrononutrition with glucose tolerance among prediabetic individuals in Malaysia.

1. INTRODUCTION AND JUSTIFICATION OF THE STUDY

Diabetes Mellitus is the chronic non-communication diseases which elevated blood glucose level and human body unable to utilize it effectively, hence cause rise of complications to microvascular and macrovascular damage (IPH, NIH and MOH, 2019). There are about 463 million of patients aged 20 until 79 years old diagnosed diabetes in worldwide (IDF, 2019). It showed an increasing trend adults living with diabetes from 108 million in year 1980 until 422 million in year 2014 (IDF, 2019). International Diabetes Federation, (2019) estimated diabetes will affected in 578 million of adults globally in year 2030 and it reach up to 700 million of worldwide adults in year 2045. 373.90 million western pacific people had impair blood glucose commonly among adults aged 20 years old until 79 years old and it is the highest in global(IDF, 2019). 8% of western pacific adults had impaired glucose tolerance and 55.8% of them are undiagnosed diabetes (IDF, 2019).

Malaysia is one of the countries in Western Pacific region and have Peninsular Malaysia and East Malaysia which separated by South China Sea (Hussein *et al.*, 2015). In the latest Malaysia National Health and Morbidity Survey, the total diabetes incidence among adults ages 18 years old and above in Malaysia is about 3.9 million (18.3%), which indicated that 1 in 5 Malaysian adults have diabetes (IPH, NIH and MOH, 2019). The highest prevalence of diabetes found in state Negeri Sembilan (33.2%), followed by Perlis (32.6%) and Pahang (25.7%) (IPH, NIH and MOH, 2019). The rise of diabetes, hypertension and cholesterol increase the risk to develop cardiovascular disease and it leading to death (IPH, NIH and MOH, 2019). There are 1.7 million Malaysian adults stay with these three major risk factors of cardiovascular disease (IPH, NIH and MOH, 2019). Besides, 1 in 4 adults in Malaysia are physically not active and 95% of adults did not consume recommended both fruits and vegetables (IPH, NIH and MOH, 2019). Sugar are the preferable substitute in self-prepared drinks (coffee, tea, chocolate and malt beverages), and 4.2% adults in

Malaysia drink carbonated drinks and non-carbonated drinks daily. Instant drink products contained sugar also another choice of adults Malaysian daily (IPH, NIH and MOH, 2019). The development of diabetes related module by Ministry of Health and Malaysia Diabetes Educators Society emphasize 7 section diabetes management (MDES, 2020), included assessment, healthy eating, physical activity, medication, self-monitoring , disease complications and behavior. Despite the numerous strategies have been implemented by Malaysian government, however, it has failed to substantially reduce the prevalent of T2DM from prediabetes in Malaysia. Perhaps, there is a missing ingredient in diabetes guidelines today.

Management of excessive caloric intake and promote on exercise are both core lifestyle modification for T2DM which have been recommended in several diabetes management guidelines (MDES, 2020; MOH *et al.*, 2020; WHO, 2020). However, other factors such as dysregulation of circadian rhythms may also contribute to disease development (Onaolapo and Onaolapo, 2018). The growth of chronobiology and evidence on disturbed circadian rhythm contributed to dysfunction of carbohydrate and macronutrients metabolism and it related to hormone melatonin (Onaolapo and Onaolapo, 2018). From a chronobiological point, glucose metabolism in humans follow a circadian rhythm through diurnal variation of glucose tolerance that typically peaks during day-light hours, when food consumption usually happens and reduces when it comes to night-dark hours when fasting usually occurs (Henry, Kaur and Quek, 2020). Unusual meal timings can cause glucose intolerance by affecting the phase relationship between the central circadian pacemaker and peripheral oscillators in cells of the liver and pancreas in rodents (Bandin et al. 2015). Similarly in humans, timed meal intake is also driven by the suprachiasmatic nucleus, play a role in synchronization of circadian rhythms in peripheral tissues, thereby affecting glucose metabolism (Henry, Kaur and Quek, 2020). The effects of diet on circadian rhythmicity clearly involves a relationship between factors such as meal timings and nutrients (chrononutrition), that can contribute to circadian perturbance and influence the manifestation of metabolic disorders such as type 2 diabetes; by extension, circadian timing may also be important in prevention of T2DM development from prediabetes. A new concept is born: perhaps it is not what you eat but when you choose to eat it.

In Malaysia, there are no specific guideline for prediabetes individual and they are managed according to the latest Clinical Practice Guideline in Malaysia (MOH *et al.*, 2020). For prevention prediabetes to further develop to diabetes, lifestyle intervention such as weight loss (5-10% of initial body weight), regular physical activity (150 min/week), with dietary strategies including reduced calories and behavior modification are recommended in Clinical Practice Guideline in Malaysia (MOH *et al.*, 2020). A high fibre diet (20-30g/day with 5 to 7 servings/day) consisting of vegetables, fruits, legumes and whole grains is encouraged too (MDES, 2020). All the prediabetic individuals will be referred to dietitian and nurse educators for 6 months routine treatment, prior to T2DM diagnosis, determined by second-round of oral glucose tolerance test (OGTT) in 6-month period (MOH *et al.*, 2020). To the puzzlement of many diabetes researchers and clinicians, some of the prediabetes cases showed tremendous improvement in their blood sugar result, yet some of the prediabetes

individuals developed full-blown of T2DM with their blood sugar result higher than the normal range; despite all of them followed the same routine treatment from healthcare clinics. Perhaps, besides the usual routine treatment from Clinical Practice Guideline (MOH *et al.*, 2020) (weight management, physical activity advices and dietary modification); prediabetic individuals who able to improve their blood sugar result in 6-month period has particular meal-timing, compared to those has uncontrolled blood sugar result in 6-month period.

Although clinical practice guideline applied in the routine treatment does not include meal-timing advices, but the importance of circadian rhythms in regulating mammalian physiological responses has been recognized (Mezitis and Bhatnagar, 2018). Mezitis and Bhatnagar, (2018) from United States of America (US) has emphasized the importance of circadian timing in people with diabetes because insulin sensitivity has been shown to exhibit circadian rhythmicity. Glycemic responses to meals are exaggerated in prediabetes and diabetes patients, more so when the meal is timed to be consumed out of sync with the metabolic pacemaker. Accordingly, Mezitis and Bhatnagar, (2018) have recognized optimal hormonal and hepatic function beginning in the early morning (4:00 a.m.) and fading in the early evening (4:00 p.m.). However, the times proposed by Mezitis and Bhatnagar, (2018) suitable for diabetes patients in US, and might not be able to apply in Malaysia diabetes management. This is because circadian timing system is affected by factors consisting of cosmic events related to the universe and earth, environmental factors (light, night and day duration, seasons) and lifestyles. To the best of our understanding, neither have there been any studies that have exploring the new concept of circadian timing in Malaysia prediabetes individuals, nor in Southeast Asia. The outcome of the present study will be able to recognize the optimal-meal-timing (chrono-nutrition) and activity/ sleeping (chrono-type) among Malaysian prediabetic individuals, in order to prevent prediabetes individuals from developing T2DM. Figure 1 shows the theoretical framework of the present study, adapted from Henry, Kaur and Quek, (2020). The theoretical framework shows that a schematic representation outlining the factors affecting the circadian clock system. Meal timing and dietary components (chrono-nutrition) play an important role in regulating circadian clocks, to enhance metabolic health and reduce the risk of type 2 diabetes. These recommendations could be considered as strategies to improve glycemic control, rather than focusing on the nutritional value of a meal alone, to optimize dietary patterns of diabetics. It is necessary to further elucidate this fascinating area of research to understand the circadian system and its implications on nutrition that may ultimately reduce the burden of type 2 diabetes.

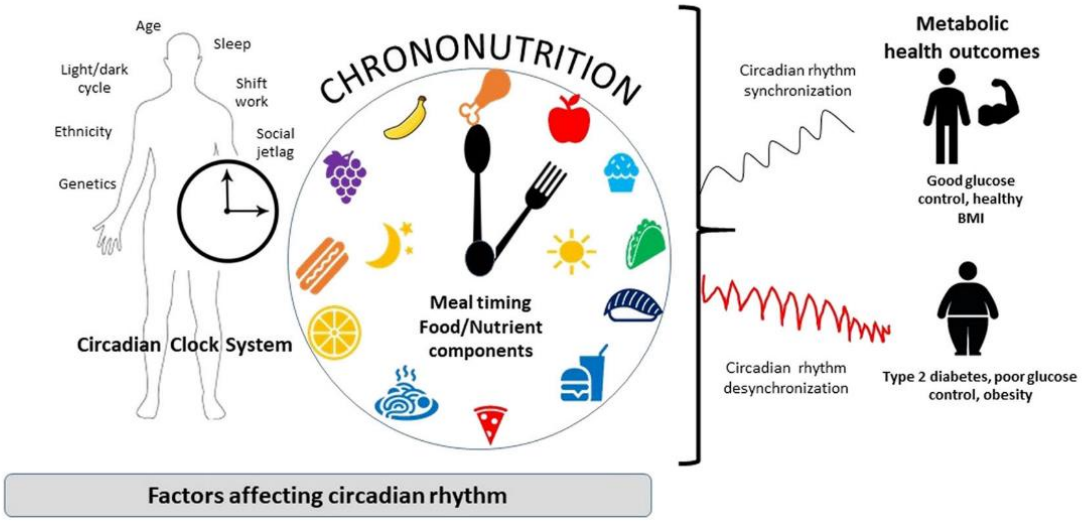


Figure 1 Theoretical framework showed factors affecting circadian rhythm.

The investigator propose that the individual is nested within their environment which includes the influences of social demands e.g. social timing resulting from demands such as school or work schedules, social activities, family obligations and routines, parenting practices, community involvement, time zone, etc., the modern lighting and climate controlled environment, as well as the effect of the earth's natural environment. Within the individual, there is an interdependence of the circadian clocks, behavior, and health. The major contribution of this model is that the circannual clock interacts with the circadian clocks to promote optimal glucose tolerance of individual's circannual influences may have health consequences. The investigator propose interactions within the individual and across levels of this model. For example, social demands influence an individual's behavior which affects alignment of the clocks either by direct entrainment of the peripheral clocks (i.e., meal timing and consistency) or by affecting exposure to the light-dark cycle via sleep timing and consistency, physical activity, and exposure to artificial light at night. It is also proposed that circadian disruption caused by the environmental factors may contribute to disruption of circannual rhythms of growth, resulting in accelerated blood sugar and contributing to the development of type 2 diabetes mellitus.

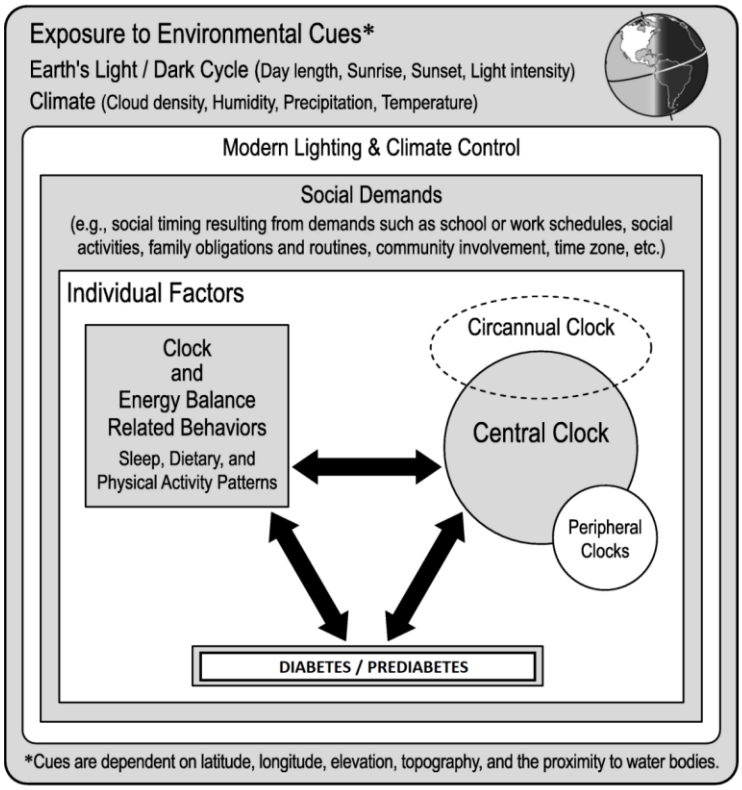


Figure 2 Conceptual framework

Identifying novel, potentially modifiable, life-style factors associated with increased blood sugar level, can lead to innovative strategies for improving health status and preventing T2DM in prediabetes individuals. The proposed study presents a novel approach as a paradigm shift in Malaysia diabetes management. The investigator anticipate that this study will not only reveal the optimal-meal timing (chrono-nutrition) for prediabetic individuals, it will also disclose the associations of chrono-nutrition with glycemic measures and anthropometric measurements in prediabetic individuals. The findings may act as guidance to government bodies to develop policies at national level. For non-governmental agencies, the outcome may be applied to plan public campaigns to improve blood sugar outcome in prediabetic and diabetic individuals. Besides, optimal-meal timing and factors associated with chrono-nutrition would be identified and management of these factors can be delivered to diabetic individuals. For example, improvement of the dietary pattern and timing coupled with physical activity to ensure good blood sugar control. Understanding the optimal-meal-timing for blood sugar control could tailor education and intervention in diabetes management. This research has potential to be converted into a larger cohort study and intervention whereby prediabetic individuals could be follow-up for longer duration to study health outcomes and blood sugar control to manage the diabetes

2. LITERATURE REVIEWS

1.0 Diabetes mellitus

The pathophysiology of type 2 diabetes mellitus (T2DM) is complex and involves insulin resistance, pancreatic β-cell dysfunction and visceral adiposity. T2DM is now a disease of major concern both globally and regionally; and is a leading cause of death in most countries (WHO 2017). In 2017, the International Diabetes Federation (IDF) estimated that 382 million people had diabetes worldwide, and by 2035, this was predicted to rise to 592 million. Almost all developing countries in the Western Pacific region and also in Southeast Asia have shown escalating diabetes rates. Eighty percent live in low- and middle income countries, and of the total, more than 60% live in Asia, with almost one-third in China (IDF 2017). An estimated 82 million adults aged 20-79 years were living with diabetes in the Southeast Asia region in 2017, representing a regional prevalence of 8.5% . About 45.8% of these diabetes case were undiagnosed (IDF 2017). In Malaysia, prevalence of diabetes has drastically increased from 6.3% (IPH 1986) to 18.3% (IPH 2019). Major increase in the prevalence of diabetes have occurred in developing countries due to rapid and ongoing socioeconomic transition and will likely lead to further rises (Nanditha et al. 2016).

1.1 Prediabetes

Prediabetes is defined as a condition in which people have higher than normal blood glucose levels but not high enough for a diagnosis of diabetes. Prediabetes prevalence is higher than that of diabetes in China and many of the Western Pacific region countries, this is true of many countries in Asia (IDF 2017). South Asian population also have a high prevalence of prediabetes and a more rapid progression to diabetes (IDF 2017). In Malaysia, National Health and Morbidity Survey (IPH 2019) found the prevalence of prediabetes was 8.9%. These prevalence were likely to be underestimates because diagnosis relied only on a single fasting blood sugar, history of physician diagnosis and information on medication (Nanditha et al. 2016). Globally, an estimated 318 million people had prediabetes in 2015, with a projected alarming increase to 482 million in 2040 (IDF 2015). Prediabetes is high-risk condition for diabetes and cardiovascular disease (Nanditha et al. 2016). Without appropriate interventions, 5.8%-18.3% of people with prediabetes develop diabetes yearly (Anothaisintawee et al. 2017). How to prevent the development of diabetes from prediabetes?

2.0 Preventing diabetes development from prediabetes

Identifying additional modifiable risk factors may lead to a research to develop novel lifestyle interventions to reduce incident diabetes from prediabetes. While there are many factors that influence the development of type 2 diabetes (T2DM), it is evident that the most influential are the behaviors commonly associated with urbanization and a modern lifestyle (Early & Stanley 2018). Randomized controlled trials from different parts of the world including United States, China, India and elsewhere have

established the proof of principle that lifestyle modification with physical activity and/ or healthy diet can delay or prevent the onset of type 2 diabetes (Anothaisintawee et al. 2017; Early & Stanley 2018). Dietary recommendations of World Health Organization (WHO) for the prevention of T2DM include limiting saturated fatty acid intake to less than 10% of total energy intake (and for high risk groups, less than 7%); and achieving adequate intake of dietary fiber (minimum daily intake of 20 grams) through consumption of wholegrain cereals, legumes, fruits and vegetables (WHO 2017). WHO strongly recommends reducing the intake of free sugars to less than 10% of total energy intake (WHO 2017), IDF fully supports these recommendations and in response published the IDF framework for Action of on Sugar (IDF 2017) Modern lifestyle are characterized by physical inactivity and long sedentary periods. Community-based interventions can reach individuals and families through campaigns, education, social marketing and encourage physical activity both inside and outside workplace (Early & Stanley 2018). IDF recommends physical activity at least between three to five days a week for a minimum of 30-45 minutes (IDF 2017). WHO has also developed recommendations on physical activity among different age groups, similar to IDF (WHO 2017), to prevent T2DM. Taking a life course perspective is essential for preventing type 2 diabetes and its complications. Early in life, when eating and physical activity habits are established and when the long-term regulation of energy balance may be programmed, there is an especially critical window to obtained a normal weight and mitigate the risk of T2DM (Zarrinpar et al. 2016). Despite recommendations from WHO and IDF have been adopted in Clinical Practice Guideline of T2DM in Malaysia (MOH 2015); to the befuddlement of many diabetes researchers and clinicians, it has failed to substantially reduce the prevalent of T2DM from prediabetes in Malaysia. Perhaps, there is a missing ingredient in diabetes guidelines today. Numerous studies suggested that the main risk factors for T2DM are excessive caloric intake and a lack of exercise; however, other factors such as disturbed sleep/wake rhythms may also contribute to disease development (Onaolapo & Onaolapo 2018). In recent years, several lines of evidence have increasingly demonstrated the role of the biological circadian clock and multiple clock genes in contributing to T2DM pathogenesis. There is a growing body of knowledge associating alterations in circadian rhythms and circadian genes with the development of T2DM (Onaolapo & Onaolapo 2018). While the implication of this advance in knowledge for the prevention and therapeutic management of diabetes mellitus is evolving, there are strong indications that circadian misalignment is relevant for the regulation of blood sugar level in chronic shift workers (Morris et al. 2017) and insulin resistance (Hashinaga et al. 2013) in rodents' model; by extension, may also be important in prevention of T2DM development from prediabetes. Human and animal studies from Western countries suggested that desynchronize between components of the circadian timing symptom and the behavioral rhythms of feeding/ fasting and activity/ sleep, or the external rhythm of light/ darkness may contribute to the pathophysiology of obesity and T2DM (Onaolapo & Onaolapo 2018). However, such relevant advices/ information are not included in pathophysiology of obesity and T2DM (Onaolapo & Onaolapo 2018). However, such relevant advices/ information are not included in any diabetes guidelines, particularly in Malaysia. Therefore, the need to drastically reduce

the global prevalence of T2DM necessitates a widening of the search for etiological factors.

3.0 Circadian timing system

To everything there is a rhythms. In most organisms, each day is organized into two phases: (1) activity and feeding and, (2) rest and fasting. The circadian system, controlled by the master circadian clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus, plays a major role in regulating daily rhythms of sleep/ wake and various metabolic outputs, such as peripheral tissue metabolism, feeding behavior and hormone secretions, generate a rhythm of approximately 24-h (hence 'circadian') (Johnson et al. 2017). As a discipline, the study of these rhythms has grown in leaps in bounds during the past quarter century. The pursuit of understanding in chronobiology has taken us from the flowering plant and the fruit fly to the mammalian nervous system and back. Within this endeavor, study of the intersection between the circadian timing system and metabolism has experienced an almost herculean pace of advancement (Perelis et al. 2016). Although interest in the topic of clock function and metabolism has percolated across the years, curiosity seems to have boomed in the early 21st century thanks to the cloning of the mammalian clock genes, the availability of various rodent genetic models, and the advances made by a number of excellent clinical sleep research labs (King et al. 1997). In 2005, a group of researchers published a brief but highly impactful paper, wherein they revealed that mice with a dominant negative mutation of the Clock locus became obese on a standard diet (Turek et al. 2005). Turek et al. (2005) suggested a new paradigm, one in which disrupting or altering the clock at the biochemical level could itself produce metabolic dysfunction. In the years that followed, it was determined that obesity in the Clock mutant mouse was likely due, in part, to a disturbance in the balance of food intake across the 24-hour day (Arble et al. 2010). That is, food intake out of phase with the bodies internal clock (eg, late night snacking) leads to considerable weight gain in otherwise healthy animals (Astiz et al. 2019). A new concept was born: perhaps it is not what you eat as much as when you choose to eat it. Although the importance of circadian rhythms in regulating mammalian physiological responses has been recognized for a long time, its impact on nutrition and metabolism is relatively new and is an area of evolving interest (Mezitis & Bhatnagar 2018).

3.1 Chrono-nutrition and blood glucose homeostasis

It has been recognized for nearly five decades that, in rodents' model, there was a diurnal rhythm in glucose tolerance. Oral glucose, intravenous glucose infusions, and identical meals all results in a significantly higher elevation of plasma glucose (i.e., lower glucose tolerance) in the evening than in the morning. Because they are nocturnal (i.e., active at night), they have lower glucose tolerance during the daytime (resting phase) than nighttime (active phase) (la Fleur 2001). There are multiple factors that could contribute to the reduced glucose tolerance, including decreased insulin sensitivity, excessive hepatic glucose production (HGP), and decreased betacell function. Study has shown that in healthy humans, both insulin sensitivity and

beta-cell responsivity to glucose are lower at dinner than at breakfast (Astiz et al. 2019). The role of HGP in the diurnal regulation of glucose homeostasis in normoglycemic humans is still not clear. While some studies showed a sleep-associated fall in HGP (Shigiyama et al. 2018) and an increased HGP at dawn (Wu et al. 2017), other studies found no diurnal rhythm in 24-hr fasting HGP (Jamshed et al. 2019) or a lower pre-meal HGP at breakfast comparing to lunch and dinner (Ferrer-Cascales et al. 2018). Different meal schedule on the test day in these studies may contribute to the different observation. However, convincing evidence has demonstrated a clear diurnal rhythm of HGP in patients with T2DM that contributes to the dawn phenomenon (hyperglycemia in the morning) often observed in the diabetic patients (Wu et al. 2017). The elevated morning HGP before breakfast could be due to a prolonged overnight fast and the resultant surge of counter regulatory hormones (e.g., cortisol, growth hormones and norepinephrine) and/or due to a circadian modulation of HGP as suggested by rodent studies (la Fleur 2001). It is well recognized that food intake, appetite, digestion and metabolism each exhibit circadian patterns (Almoosawi et al. 2016). Nutrients ingested during the active period provide substrates such as glucose, lipids and amino acids, which fuel the metabolic pathways in our cells, whereas during the resting period energy and substrates stored in our body are mobilized to sustain metabolic homeostasis (Bhadra et al. 2017). Food intake itself serves as a regulator of the circadian clock, particularly the peripheral circadian clock in tissues such as the liver and the intestine (Astiz et al. 2019). Conversely, the central circadian clock, entrained by the darklight cycle, is known to extend its effect on food absorption. More specifically, small peptides cleaved in the intestine from dietary carbohydrate have been shown to be transported in a circadian driven process (Almoosawi et al. 2016). Blood glucose homeostasis can be seen as a paradigm of the circadian control of energy metabolism. Indeed, whereas during the activity/feeding period blood glucose is mainly of dietary origin, during the resting/starvation period glucose is progressively recruited from endogenous glucose production in the liver to maintain blood glucose levels within a relatively narrow range. In this process liver glycogen content undergoes large daily fluctuations to sustain blood glucose levels, as glycogen synthesis and degradation are specifically recruited during the activity/feeding and resting/starvation periods, respectively (Karthikeyan et al. 2018). In modern societies, individuals often consume food at different time points during the day, away from home and therefore at what may be considered, from an evolutionary perspective, physiologically inappropriate times of the day (Almoosawi et al. 2016). This irregularity in eating patterns is often enforced by external pressures to conform to social schedules. Increasingly, individuals often engage in activities that are misaligned with their circadian clock system and the natural rhythm of the light-dark cycle. The resulting misalignment between the sleep-awake, fasting-feeding cycles and the light-dark cycle subsequently disrupts the natural oscillations of physiologic processes such as lipid metabolism and blood pressure, eventually manifesting itself as heightened risk of developing T2DM (St-Onge et al. 2017). Therefore, besides time-of-the-day dietary intake; prediabetes individuals may also look at the time-ofthe-day activities e.g. sleep or awake to prevent T2DM development.

3.1.1 Meal intakes timing of ingestion: morning versus dinner

Evidence suggests that meal ingestion in the morning and late evening (time of day) influence glucose metabolism in humans. Earlier studies, using mixed meals or glucose infusion, have reported circadian responses of reduced glucose tolerance and insulin sensitivity in healthy participants for the evening rather than in the morning (Almoosawi et al. 2016). An acute study which examined the effects of a late evening meal on diurnal variation of blood glucose in healthy individuals, assessed by continuous glucose monitoring (CGMS™). There was an increase in blood glucose after the late evening meal which shifted towards later at night, with peaking of blood glucose observed during sleep (Astiz et al. 2019). Late evening meals may cause postprandial hyperglycaemia with this decrease in glucose tolerance from morning towards the night. A cohort study was the first to show the relationship between late-night dinner consumption and glycaemic control in type 2 diabetics, whereby having a late dinner meal after 8 pm was independently associated with an increase in HbA1c (Sakai et al. 2018). In some acute trials, both healthy individuals (Kajiyama et al. 2018) and type 2 diabetics (Imai et al. 2017) showed significantly higher blood glucose and insulin values after night-time meals. These studies deduce that the disruption of the circadian rhythm led to the exacerbation of the physiological nocturnal decrease of glucose tolerance. Peter et al. showed that type 2 diabetic subjects who ate three identical meals had glucose excursions that were higher in the morning than in the evening (Sakai et al. 2018). There was increased glucose tolerance in response to the first and third meals of the day, irrespective of glycaemic control. There was also a change in circadian variation in insulin sensitivity in type 2 diabetics (Sakai et al. 2018). Type 2 diabetics exhibit a different daily circadian pattern from healthy individuals, with increased insulin sensitivity towards the night, and higher glucose excursions in the morning than in the evening. Methods to improve glycaemic control at dinner has been reported by some authors. Dinner that was divided into two smaller meals reduced post-meal glycaemic excursions, due to the “second-meal effect” phenomenon, by enhancing β-cell responsiveness (Karthikeyan et al. 2018) at the second dinner meal induced by the first meal (Imai et al. 2017). It was also suggested that if meal size and carbohydrate quantities were smaller, postprandial glucose can be ameliorated in both healthy and type 2 diabetics. In summary, time of day is indicative of having an influence on the postprandial glucose response to a meal. There is a defined circadian pattern for postprandial glycaemia for similar meals consumed either in the evening or morning. Glucose metabolism is not only affected by what and how much you eat alone, but also when the meal is consumed. However, more data from well-designed epidemiological studies is necessary to prove causality. Mezitis & Bhatnagar (2018) emphasize the importance of chrononutrition in people with diabetes because even insulin sensitivity has been shown to exhibit circadian rhythmicity. Glycemic responses to meals are exaggerated in these individuals, more so when the meal is timed to be consumed out of sync with the metabolic pacemaker (Almoosawi et al. 2016). Accordingly, Mezitis & Bhatnagar (2018) recognize optimal hormonal and hepatic function beginning in the early morning (4:00 a.m.) and fading in the early evening (4:00 p.m.). Review of glucose records—increasingly more abundant in the era of continuous glucose monitoring (CGM), which permits review of several months of

detailed glucose profiling and follow-up care—confirms that evening lifestyle defines morning glycaemia, thus setting the stage for the day's profile. Late-night feeding and night-shift work introduce a "jet-lag" experience, which, when sustained, virtually guarantees metabolic dysregulation.

3.2 Chrono-type and blood glucose homeostasis

Individual differences in the time at which people refer to do particular behaviors, most notably sleep, are referred to as chrono-type (Anothaisintawee et al. 2017). It is a behavioral manifestation of an individual's internal circadian clock system, which can be assessed with the use of multiple methodologies that classify individuals as having, e.g., a morning or an evening type (Gottlieb et al. 2015). Chrono-type is also used to describe personality traits associated with the preferred times-of-the-day for activities such as sleep or awake (Anothaisintawee et al. 2017). Generally, in the absence of environmental stimuli such as light, endogenous circadian clocks oscillate in a manner approximating a 24-hour cycle (Astiz et al. 2019). An individual's chrono-type is likely created by an interaction between the endogenous circadian pacemaker and its responses to light (Astiz et al. 2019) and can be modulated by factors such as age (Fischer et al. 2017) and life circumstance (e.g., needing to get to work early over years may shift preference towards earlier hours). In our modern society, night-shift work, which requires workers to eat and be active during their circadian night and sleep during their circadian day, represents and extreme form of circadian misalignment. A recent meta-analysis of 10 cohort studies (262294 participants) revealed that shift work was associated with a 40% increase in the risk of developing T2DM (Anothaisintawee et al. 2015). Recently, epidemiological and experimental studies have demonstrated that sleep quality and quantity are important determinants of whole-body metabolism. It has been suggested that impaired sleep might causally contribute to the T2DM (Sakamoto et al. 2018). Despite the clear association between short sleep and metabolic impairments, the underlying endocrine and molecular mechanisms remain only partially elucidated. Among the suggested mechanisms, the causal role of the hypothalamus-pituitary-adrenal (HPA) axis and sympathetic activation are supported by the largest body of literature. Circulating cortisol, assessed either by 24 h profiles or by single measurements of evening cortisol levels, were elevated together with markers of sympathetic activation and circulating catecholamine after total or partial sleep deprivation as well as in short sleepers (Reynolds et al. 2012). In contrast, some studies reported impairments in glucose homeostasis in sleep restricted individuals along with unchanged cortisol and catecholamine levels (Buxtone et al. 2012). The complexity of associated endocrine mechanisms can be further demonstrated by observations of elevated levels of pro-inflammatory cytokines, lower circulating testosterone, decreased thyroid stimulating hormone levels and changes in adipokines secreted from adipose tissue in sleep deprivation (Froy & Garaulet 2018). Evening types (or late chrono-types) typically have later bedtime than those who are morning types (or early chrono-types). Those with later chrono-type usually experience a mild form of circadian misalignment due to a greater degree of misalignment between social rhythms and the circadian clock, a phenomenon called "social jetlag" (Fischer et al. 2017). In an individual presenting with

an extreme evening chrono-type, the circadian phase of biological rhythms could be shifted by as much as much as 2-3 hours compared with that of an extreme morning chrono-type (Kantermann et al. 2015). This shift can result in a desynchronization between the period of biological night, as regulated by the intrinsic circadian clock system, and the environmental night governed by the light-dark cycle (Kantermann et al. 2015). Patients with T2DM, later chrono-types and evening preferences have been found to be associated with poorer glycemic control (Brady et al. 2019). A recent study revealed that evening type was associated with an increased risk of having type 2 diabetes in prediabetes individuals (Anothaisintawee et al. 2017). These data may demonstrate the contribution of circadian regulation on reversing prediabetes from developing T2DM in the future.

4.0 In summary

As shown above, evidence that provides new insight into clock function and the pathophysiology of type 2 diabetes is accumulating rapidly. Disruption of circadian rhythms, such as that due to shift work, affects not only body weight and adiposity, but glucose metabolism itself. Since the magnitude of these effects in the present increase seen in the development of type 2 diabetes is obscure, more precise mechanisms and the relative importance of these factors in the development of type 2 diabetes should be investigated. To achieve this goal, it will be necessary to establish methodologies to measure parameters of circadian systems in feeding and glucose and lipid metabolism. In addition, it is also necessary to analyse the contribution of circadian gene variations in the development of type 2 diabetes. Clinical studies related to the development of type 2 diabetes are also needed to evaluate the health-compromising behaviours that affect circadian disruption with definitions that include chrono-type or circadian typology. According to the currently available data, medical nutrition therapy for Malaysian diabetes should include meal timing as one of the strategy, to manage glycaemic control. Study should be conducted to determine the optimal-meal-timing to manage blood sugar level. Although it is very difficult to control meal timing for every individual patient with prediabetes or type 2 diabetes, a socioeconomical approach such as recommendations regarding the work environment may also be important. Although there is now insufficient evidence to evaluate the impact of these chrono-physiological disruptions on the progression or pathophysiology of diabetes precisely, future focused research on these issues can answer these questions.

Identifying novel, potentially modifiable, meal-timing, chrononutrition, life-style factors associated with glycemic measures; can lead to innovative strategies for improving health status and preventing T2DM in prediabetic individuals. The proposed study presents a novel approach as a paradigm shift in Malaysia diabetes management. The investigator anticipate that this study will not only reveal the optimal meal-feeding for Malaysia prediabetic individuals to improve their blood glucose level, it will also provide greater insight of the associations of chrononutrition, lifestyle factors and anthropometric measurements with glycemic measures. The findings will also set the directions to adopt optimal-meal-timing approach in Clinical Practice Guideline for diabetes. The investigator anticipate that our proposed study which incorporate the

circadian timing, as a novel approach, may provide the fundamental information that could be adopted by the government and policy makers. The outcome can be included in the future clinical practice guideline or to develop innovative and effective lifestyle interventions to improve the T2DM prevalent in Malaysia.

3. RESEARCH METHODOLOGY

Hypothesis:

The morning chronotype and early meal-timing are associated with low glycemic outcome among prediabetic individuals.

Research Questions:

Is the morning chronotype and early meal-timing are associated with better glucose tolerance among prediabetic individuals?

General objective:

To determine the associations of chronotype and chrononutrition with glucose tolerance among prediabetic individuals in Malaysia.

Specific objectives:

1. To examine the associations among chronotype, chrononutrition and glucose outcomes.
2. To investigate the association between lifestyle factors (physical activity level, light exposure, diet timing, sleep pattern) and chrononutrition.
3. To investigate the association between anthropometry measurements and glycemic outcomes.

Description of Methodology:

Participants will be recruited using the convenience sampling method. The proposed study will be conducted in community clinics in Malacca, Malaysia. Malacca is the capital of the coastal stage, located in southwestern Malaysia, with an estimated total population of 0.93 million in the year 2019 (Department of Survey and Mapping Malaysia, 2021). In 2019, National Health and Morbidity Survey reported that 17% of Malaccan adults had hyperglycemia and 13.7% of them had known diabetes. Malacca is 1 out of the top five states showing the highest prevalence of diabetes in Malaysia (IPH, NIH and MOH, 2019). Permission to carry out data collection has been granted by the Medical Research and Ethics Committee (MREC) (RSCH ID-21-00114-IVI). The study protocol has been reviewed and approved by the Tunku Abdul Rahman University College (TARUC) research ethics committee (TAR UC/EC/2021/02-3).

During the process of recruitment, a trained research assistant (one of the co-researcher), clinical dietitian (one of the co-researcher) or family medicine specialist

(one of the co-researcher) will inform potential prediabetic individuals research protocol verbally and written information will be provided. Newly diagnosed prediabetic individuals who are agreeable to participate will provide written informed consent. Baseline and follow up appointments will be determined based on the community clinic appointment schedules. Those who decline to participate will continue to receive their routine treatment as usual, and care provided to each prediabetic individual will not be affected by the decision to either participate or not participate in the study.

Those who meet the inclusion criteria will be invited to participate in this study. Eligible participants shall meet all the following inclusion criteria.

1) Newly diagnosed prediabetic individuals who have been first seen by clinical dietician registered under the Ministry of Health Malaysia.
2) Malaysian ≥ 18 years old.
3) Those who plan to continue prediabetes care at a community clinic in Melaka.
4) Those who can read, write and understand the Malay Language.

Participants who meet the following criteria will be excluded from continuing the study.

1) Night shift workers at least 3 times per week.
2) Known sleep disorder.
3) Pregnant Women.
4) Those on oral glucose-lowering medications, diabetes supplements, anticonvulsant medications or oral steroids currently or in the last month.
5) Those with prior explored to any information regarding time-of-eating/time-of-activity restriction.
6) Those with chronic kidney disease. They are excluded as current research lack of evidence to support the accuracy of using 24-hour continuous glucose monitoring system (CGMS) (Freestyle Libre Pro, Abbott, Germany).

Prediabetes and diabetes will be defined according to the latest Clinical Practice Guideline in Malaysia (WHO & IDF, 2006; ADA, 2020; MOH *et al.*, 2020). Diagnosis must be confirmed by measurement of venous plasma glucose (FBG and OGTT) or HbA1c level. Table 1 shows the diagnostic value for diabetes on plasma glucose. Table 2 shows the diagnostic values for prediabetes and diabetes based on OGTT. Table 3 shows the diagnostic values for prediabetes and diabetes based on HbA1c. All the tables are adapted from the Clinical Practice Guideline in Malaysia (WHO & IDF, 2006; ADA, 2020; MOH *et al.*, 2020).

Table 1 The diagnostic value for diabetes on plasma glucose

	Fasting	Random
Venous Plasma Glucose	≥ 7.0mmol/L	≥ 11.1 mmol/L

In symptomatic individual, one abnormal glucose value is diagnostic.
In asymptomatic individual, 2 abnormal glucose value are required.

Table 2 The diagnostic values for prediabetes and diabetes based on OGTT

OGTT Plasma Glucose Values (mmol/L)		
Category	0 hour	2- hour
Normal	<6.1	<7.8
IFG	6.1-6.9	
IGT	-	7.8-11.0
DM	≥ 7.0	≥ 11.1

IFG= impaired fasting glucose; IGT=impaired glucose tolerance; DM=diabetes mellitus

Table 3 The diagnostic values for prediabetes and diabetes based on HbA1c

	Normal	Pre-diabetes	Diabetes
A1c	<5.6% (38mmol/mol)	5.6-6.2% (38-44 mmol/mol	≥6.3% (45mmol/mol)

A repeated A1c should be done 4 weeks after the first positive test for asymptomatic patients.
In symptomatic individual, a single positive test is sufficient.

Location of Research:

Location of Research	**Expected number of subjects per site**
Community Clinic Batu Berendam	56
Community Clinic Bukit Rambai	55
Community Clinic Sungai Udang	55

Recruitment pamphlets that contain general information of the study will be placed in all the healthcare clinics in Malacca. During the process of recruitment, a trained research assistant, clinical dietitian (one of the co-researchers) or family medicine specialist (one of the co-researchers) will inform potential prediabetic individuals of the study both verbally and will be provided written information (Appendix A). Newly diagnosed prediabetic individuals who are agreeable to participate will be provide written informed consent (Appendix A). Study visits (recruitment and follow-up visits) of this study will be determined based on the healthcare clinic appointment schedules. Those who decline to participate continue to receive their routine treatment as usual, and care provided to each prediabetic individual is not affected nor affected by each individual's decision to either participate or not participate in the study. An overview of the study procedure is illustrated in Figure 3. All the newly diagnosed prediabetic individuals who fulfill the inclusion criteria will be invited to participate in the present study. They will be recruited when they visit the diet clinic for for the first time for their initial appointment scheduling. This would be considered the baseline visit. OGTT, HbA1c and FBS results will be traced from medical records.

Data regarding sociodemographic information and anthropometric measurements will be collected in the baseline visit. Enrolled participants will be interviewed by a trained

research assistant using the following questionnaire including (1) Munich Chronotype Questionnaires (MCTQ) (Appendix D), (2) Pittsburgh Sleep Quality Index Questionnaire (PSQI) (Appendix H), (3) Harvard Light Exposure Questionnaire (HLEQ) (Appendix G), (4) International Physical Activity Questionnaire (IPAQ) (Appendix C) and (5) Chrononutrition Profile Questionnaire (CPQ) (Appendix I). Anthropometry measurements including body fat, height, body fat percentage, visceral fat, waist circumference and hip circumference will be measured. Participants are requested to fill up 3-days dietary record (3DDR) (2 weekdays and one weekend day) and the data will be collected in the next visit. Baseline reading of fasting plasma glucose (FPG, OGTT, liver function, and HbA1c) will be retrieved from medical records in the community clinic. Routine treatment will be provided as usual. To minimize the loss of follow-up, phone conversations will be made to ensure the 3-days dietary data is completed.

At the second visit (three months from the baseline), all participants are required to wear a CGMS sensor at the back of their upper arms to measure the 24 hours glucose fluctuation over 14 consecutive days. 3DDR will be given to participants to record their consecutive 3 days dietary record and will be returned after 14 days. After 14 days, participants will return the CGMS sensor at the community clinic. Or alternative way of CGMS sensor collection by an assistant at the participant's home. All questionnaires will be interviewed by a trained research assistant again. Anthropometry measurements including body fat, height, body fat percentage, visceral fat, waist circumference and hip circumference will be measured repeatedly.

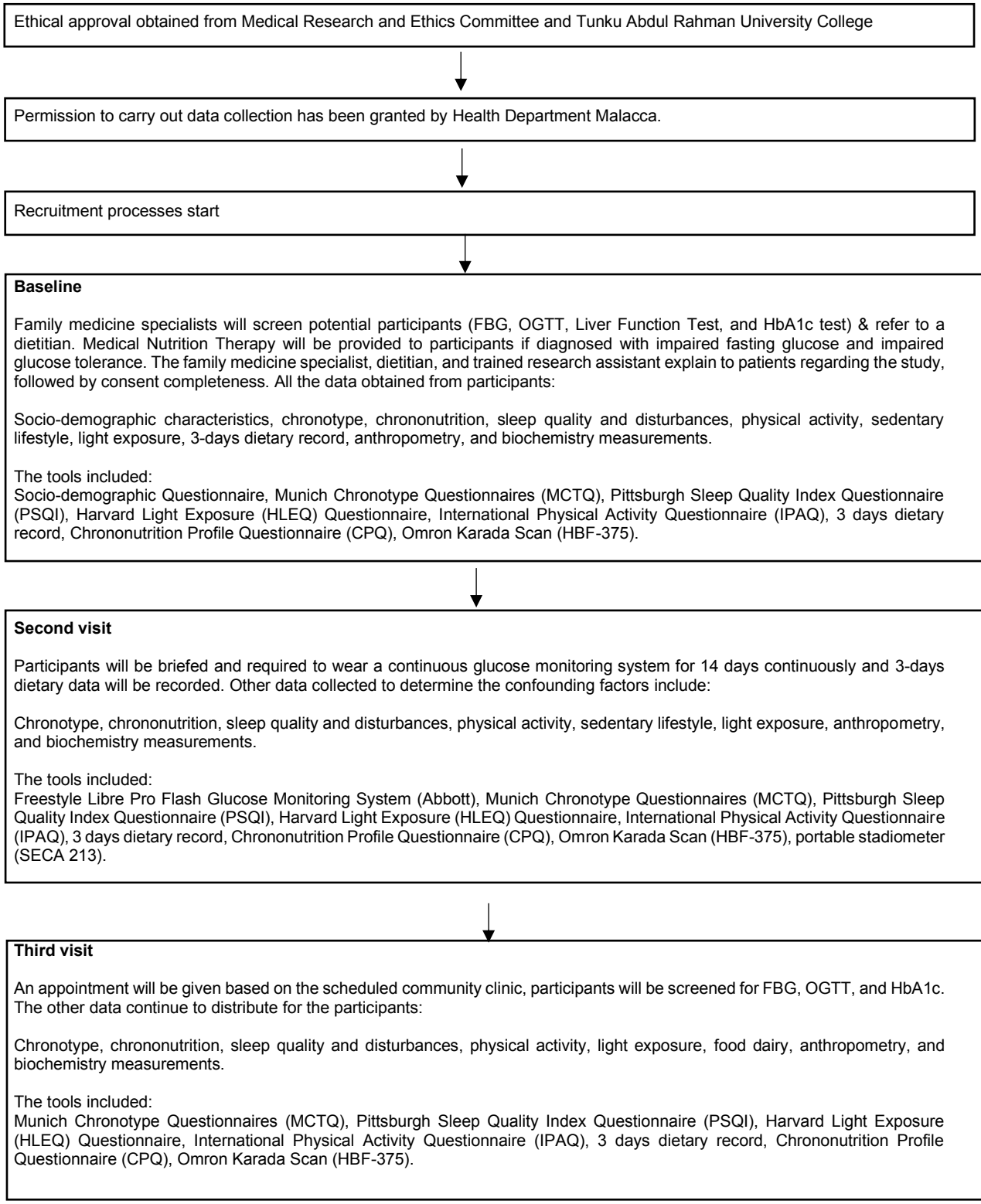
At the third visit (six months from the first visit), for FBG, OGTT and HbA1c test, all the readings will be retrieved by the research assistant from the medical record to document the glycemic outcome. The participants will be interviewed by the trained research assistant during all clinic visits using similar questionnaires. All anthropometry measurements (body fat, height, body fat percentage, visceral fat, waist circumference and hip circumference) will be conducted again. 3DDR will be given to record their 3 days dietary intake again and the record progress will be followed up through phone. The original 3DDR will be collected at the routine appointment.

Sample size Calculation:

The sample size is estimated using G*Power software version 3.1.9.4 (Erdfelder, Faul and Buchner, 1996). To determination of association between chrononutrition and glycemic control, the β-coefficient of 0.13 adopted from Sakai *et al.*, (2018), the power was set to 0.9 with 2 sided significance level and alpha error given 0.05, a total of 83 prediabetic adults expected in this study. To compensate for the non-responding and non-compliance during follow-up, a dropout rate of 50% will be considered, the total sample size required is 166 subjects in the study for 6 months.
Test Family: T Test
Statistical Test: Linear Multiple Regression Fixed Model, single regression coefficient
Tail(s): 2
Effect Size (β-coefficient): 0.13 (Sakai *et al.*, 2018)
α Error prob: 0.05
Power(1- β err prob): 0.90
Total Sample Size: 83 prediabetes individuals

50% dropout rate taken as consideration in study for 6 months follow up, total 166 prediabetes individuals shall be recruit in this study.
Dropout Rate:
$= \frac{83}{1-0.5}$ = 166 prediabetic individuals

Figure 3: Flow diagram of the study design



Participants will be undergoing OGTT, FBS and HbA1c tests after an overnight fast of 8-10 hours. This is a routine universal test for prediabetes/ diabetes diagnosis. Participants will be informed to eat their usual diet for three days prior to the test. The diagnostic value for diabetes on plasma glucose. the diagnostic values for prediabetes and diabetes based on OGTT, and the diagnostic values for prediabetes and diabetes based on HbA1c have been shown in Table 1, Table 2 and Table 3, respectively. Fasting blood sample will be taken for FBS and HbA1c tests. Subsequently, participants will be given 75-gram glucose water to drink within 5 minutes. Blood sample will be taken after 2 hours for glucose determination. Participants should rest throughout test with only plain water is allowed to drink. Figure 4 shows the procedure of OGTT. All the tests will be conducted in the healthcare clinic lab. Results will be traced from medical record.

Table 4: Summary of data collection and timeline

Data	Baseline	3 months	6 months
Sociodemographic			
Age	·		
Date of Birth	·		
Marital Status	·		
Education Level	·		
Occupation	·		
Monthly Household Income	·		
Health History	·		
Smoking Habit	·		
Supplementary Consumption	·		
Anthropometry			
Weight	·	·	·
Height	·	·	·
Waist Circumference	·	·	·
Visceral Fat	·	·	·
Body Fat	·	·	·
Step counts		·	
Questionnaires			
Munich Chronotype (MCTQ)	·	·	·
Pittsburgh Sleep Quality Index (PSQI)	·	·	·
Harvard Light Exposure (HLEQ)	·	·	·
International Physical Activity IPAQ)	·	·	·
Chrononutrition Profile Questionnaire (CPQ)	·	·	·
3 days dietary record (3DDR)	·	·	·
Glycemic Measurements			
Continuous Glucose Monitoring (CGM)		·	
Fasting Plasma Glucose (FPG)	·		·
2-hour Post-load Plasma Glucose (2hPPG)	·		·
Glycated Hemoglobin (HbA1c)	·		·

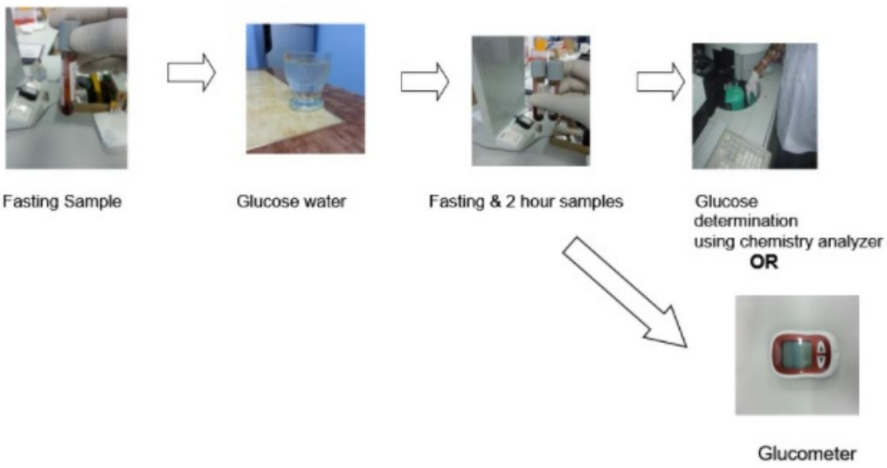


Figure 4 Procedure of OGTT 24-hour

Continuous glucose monitoring system 24-hour CGMS (Freestyle Libre Pro, Abbott, Germany) is a glucose monitoring device that measures interstitial glucose levels and its indicated for detecting trends and tracking patterns in persons with prediabetes/ diabetes. Freestyle Libre Pro is not a usual/ normal Freestyle Libre. The normal version of CGMS can be easily purchased in the market, but CGMS Pro version can only be purchased direct from Abbott Germany. For the usual/normal Freestyle Libre, reading must be read by reader every 8 hourly and participants may easily check their blood glucose, resulting the unnecessary bias, where participants might change their diet and lifestyle habit to control the blood sugar. Whereas, pro version can only be used for clinical trial purpose, cannot be commercialized out of clinical trial use. Readings from the 24-hour CGMS Pro version will not be made available to participants in real time, hence, it will prevent the bias that may arise from unmasked real-time glucose reading. Besides, blood sugar can be kept record for up to 14 days for pro version, easier for researcher to collect the data and save the cost of reader. Figure 5 shows the example of the daily glucose summary from 24-hour CGMS. 24-hour CGMS aids in understanding the person glucose profile and detecting episodes of hyperglycemia and hypoglycemia. Figure 6 shows the example of the daily pattern outcome from 24-hour CGMS.

The FreeStyle Libre Pro CGM system consists of a disposable sensor, an applicator, and a reader; that is used to start the sensor on the participant and gather the glucose data at the end of the CGMS period. The Sensor can be worn for up to 14 days, on the back of their upper arm (Figure 7). Once applied, a sensor will measure and store glucose readings every 15 minutes. The 24-hour CGMS does not require any participant action as calibration for the sensor is not needed. Readings from the 24-hour CGMS will not be made available to participants in real time, hence, it will prevent the bias that may arise from unmasked real-time glucose readings. The data from all the 24-hour CGMS (Freestyle Libre Pro, Abbott, Germany) sensors is easily accessible through the FreeStyle Libre Pro Software or The LibreView Cloud. In the present study, the 24-hour CGMS reading will be taken 14-day prior to second visit/ second-round OGTT reading. All the data from 24-hour CGMS will be analyzed to

investigate to obtain the optimal-meal-timing. The 24-hour CGMS data will be match with 3-day food diary too.

Exposure measures Meal-timing and chrononutrition

At the baseline visit, the researcher will guide the participants to fill out the 3-day food diary covering two weekdays and one weekend day (consecutive Sunday-Tuesday or Thursday-Saturday). Three-day food diary will be taken twice in the entire study. At the baseline visit, 3-day food diary will be interviewed by researcher to the participants. This food diary data will be served as baseline meal-timing data. Subsequently, participant will be given another 3-days daily dietary at second visit, 14-day prior to third visit. Participants are required to record the time, type, description and number of foods and beverages consumed throughout the day. Brand information, ingredients and cooking method will be recorded where applicable, particularly for home-cooked food. Pictures of household measuring utensils and various food portion sizes are printed in the food diary to assist participants in quantifying their food intake. For 14 day return the CGMS, researcher will be interviewed participants on the completeness of 3 days daily dietary intake.

Energy and macronutrients analysis of dietary record will be performed using Nutritionist ProTM (Axxya Systems, Stafford, TX, USA), based principally on the Nutrient Composition of Malaysian Food and the food product labels. Energy intake will be compared with estimated basal metabolic rate (BMR) to exclude participants who are under-reporting or overreporting their energy intake in their 3-day food diary. BMR is calculated using standard equations based on sex and age provided by FAO/WHO/UNU (1985) (FAO/WHO/UNU 1985). For participants in a non-dieting population, Torun et al. (1996) proposed that a ratio between energy intake and BMR of less than 1.39 and more than 2.24 be considered as under-reporting and over-reporting respectively. These cut-off points will be applied in the present trial. From the 3-day food diary data, The investigator will investigate the dietary intake as reported on the second day of recording. The second day will be selected over the first one to ensure that participants have also include night-time eating in the records. Compare to the later days, which may have been burdened by increasing exhaustion related to the record-keeping, the second day better represents the habitual dietary intake (Ahola et al. 2019). To investigate the energy and macronutrient intakes over the course of the day, five periods will be performed as follows: night (from 00:00 to 04:49), morning (from 05:00 to 09:59), midday (from 10:00 to 13:59), afternoon (from 14:00 to 16:59) and evening (from 17:00 to 23:59), adapted from Ahola et al. (2019). As these periods differed in their duration, energy intake per hour will also be calculated for each of the five periods. In addition, energy and macronutrient intakes per hour (e.g. intakes reported between 10:00 and 10:59 contributed towards the hour “10:00”). Diet quality will be derived from a 177 food items FFQ at second visit, where the Healthy Eating Index (HEI) will be calculated. This FFQ is adapted from the FFQ used by the Malaysian Adults Nutrition Survey (MANS) 2014 (IPH 2014) (Appendix C). Participants will be required to indicate frequency of foods consumed in the last 1 month. Individual portion size will be asked for each food, and pictures of the various portion size will be

provided for more accurate quantification. A validated Malaysian HEI will be applied to determine the HEI scoring (Lee et al. 2011; Goh & Norimah 2012). The instrument consists of nine components, which is make up of seven food groups and two nutrient groups, as shown in Table 5. The scoring of these components will be calculated based on the recommended serving size and nutrient intake in the Malaysian Dietary Guideline (MDG) (NCCFN 2010). The score of each food group will be calculated using the formula: (actual serving consumed based on participant's diet record /recommended serving size based on MDG) × 10. The score of each nutrient ranged from 0 to 10, which will be calculated proportionately for the in-between whole number responses, as shown in Table 6. The total score of HEI is obtained by summing up the score of each component. The composite score in percentage is calculated using the formula: (total score obtained from 9 components/maximum score of 90) × 100%. Therefore, the total score is 100%, in which less than 51% indicated poor diet, 51 to 80% indicated diet requiring improvement, and more than 80% indicated good diet (Lee et al. 2011).

Table 5: Criteria scoring for Malaysian Health Eating Index components

Components	Score range	Criteria for maximum score 0	Criteria for score 8	Criteria for maximum score 10
Food groups				
Grains and cereals	0-10	0		4-8 servings[1]
Vegetables	0-10	0		3 servings[1]
Fruits	0-10	0		2 servings[1]
Meat, poultry & Eggs	0-10	0		½ - 2 servings[1]
Fish and seafood	0-10	0		1 serving[1]
Legumes	0-10	0		½ -1 serving[1]
Milk & dairy products	0-10	0		1-3 servings[1]
Nutrients				
Total fat	0-10	≥35% energy from fat[2]		≤30% energy from fat[1]
Sodium	0-10	≥4200 mg[2]	2400 mg[1]	≤2000 mg[1]

[1]Based on the Malaysian Dietary Guidelines 2020. [2]Based on the Malaysian Adult Nutrition Survey 2014

Table 6: Malaysian Healthy Eating Index (HEI) scoring for nutrients

HEI Score	0	1	2	3	4	5	6	7	8	9	10
Energy intake from fat (%)	≥35.0	34.5	34.0	33.5	33.0	32.5	32.0	31.5	31.0	30.5	≤30.0
Sodium (mg)	≥4200	3975	3750	3525	3300	3075	2850	2625	2400	2200	≤2000

Physical activity, sedentary behaviours, sleep and light exposure questionnaire on physical activity, sedentary behavior, sleep and light exposure are also administered at the first visit. Participants are interviewed using the modified International Physical Activity Questionnaire-Short Form (IPAQ-SF)(Appendix C) to self-report their physical activity in the last 7 days (IPAQ 2005). The modified questionnaire evaluates the vigorous physical activity, the moderate physical activity and the walking time. Loy et

al. (2019) removed question asking about the sitting time from the original IPAQ-SF and included it in the questionnaire used to assess sedentary behavior. The data will be computed in metabolic equivalents (MET-min/ week) scores. Questionnaire on sedentary behavior which is modified from the Adult Sedentary Behavior Questionnaire is performed (Chu et al. 2018). The questionnaire evaluates time spent sedentary in the last 7 days, including sitting time at work, sitting/ lying down time to watch television, to use electronic devices at mealtime, while driving or reading. Participants also self-administer the Pittsburgh Sleep Quality Index questionnaire to assess their sleep habits in the last month (Buysse et al. 1989)(Appendix H), and the Harvest Light Exposure Assessment questionnaire (Appendix G) to assess their main light sources exposure in hourly basis on a typical weekday and weekend day (Bajaj et al. 2011).

Anthropometric measurements (Appendix J) will be measured at the first, second and third visit. Body weight, body fat percentage and visceral fat of each participant will be measured twice using a body fat analyzer (HBF-375, Karada) and recorded to the nearest 0.1 kg, 0.1 % and 1 unit, respectively. Participants will be weighted with their minimal cloths on, barefoot, without belt and emptied pockets. They will be asked to stand still with the body weight equally distributed on both feet. Participants have to be in the Frankfurt plane, where the body is upright, upper limbs are at the body's sides with the palms facing forward, feet flat and directed forward, directly facing the researcher assistant. The height of each participant will be measured twice using a SECA portable stadiometer Model 217 (SECA GmbH & Co., Hamburg, Germany) attached to a smooth wall and recorded to the nearest 0.1 cm. Participants will be asked to stand erect, in their bare feet, with their heels, buttocks, head and shoulder blades in a vertical line against the wall. Height will be measured in the upright position against a vertical scare and with the head positioned so that the top of the external auditory meatus is in the level with the inferior margin of the bone orbit. Waist and hip circumferences of the respondents will be measured twice, according to the standardized protocol by the WHO (2008). Waist circumference will be measured at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest. Hip circumference will be measured around the widest portion of the buttocks, with the tape parallel to the floor. For both measurements, the participants should stand with feet close together, arms at the side and body weight evenly distributed, and should wear little clothing. Both waist and hip circumferences will be measured to the nearest 0.1 cm by using a Lufkin tape model W606PM (Apex Tool Group, Sparks, MD, USA). The reported measurements are the average values from both readings.

Study Outcomes

The main outcome of the study is the glycemic outcome including FPG (mmol/L), 2hPPG (mmol/L), HbA1c (%), and glucose variability from CGMS. The secondary outcomes are chronotype from the validated questionnaire MCTQ, and chrononutrition from the validated questionnaire CPQ, 3 days dietary record, anthropometry measurements, physical activity level from the validated questionnaire IPAQ, sleep

pattern from the validated questionnaires PSIQ and light exposure from the validated questionnaire HLEQ.

Statistical Analysis

All data will be performed statistical analysis using the SPSS software version 20 (SPSS Inc., Chicago, IL, USA). The socio-demographic will be presented as mean, standard deviations, median, interquartile range, frequency, and percentage. Data cleaning will be performed to exclude the outlier data before the normality is checked using Kolmogorov-Smirnov Test. The investigator considered $p<0.05$ to be statistically significant. Chronotype, sleep quality, and light exposure will be analyzed as categorical variables whereas dietary intake, chrononutrition, anthropometry, glycemic measure, and physical activity will be analyzed as continuous variables.

The investigator will perform a multivariable generalized linear model to examine the association between night meal pattern (last night mealtime either dinner or supper with calories intake, frequency of night snacking, and the total number of the night snacking) and glycemic outcome (HbA1c, FPG, 2hPPG, CGM profile) by adjusting for the potential covariates. Specifically, nighttime will be characterized by the time range from 1900 and 0659 hours based on Malaysia solar time. The investigator will explore the late-night meal timing in 2000, 2100, and 2200 to examine its association with glycemic outcomes. The potential confounders include sociodemographic characteristics, physical activity, sleep pattern, sedentary behavior, diet quality, and light exposure will be controlled. ANCOVA test will be performed to understand the association between chrononutrition (breakfast skipping, meal frequency, eating window, sleep timing, chronotype, and fasting pattern) with glycemic variables, controlling for potential covariates (sociodemographic characteristics, light exposure, diet quality, physical activity and sleep pattern).

To investigate the association of physical activity (MET score and steps count), sedentary behaviors (MET score), sleep pattern (sleep duration), diet quality (distribution of total energy, carbohydrate, protein, and fat), and light exposure (timing responded to a different type of light) with glycemic outcome, the multivariable generalized linear model will be conducted. Meanwhile, anthropometric measures (body mass index, body fat, and waist circumference) will be categorized as either normal or overweight and obesity group, followed by using logistic regression to examine the association between anthropometry status and glycemic outcome (non-diabetes or diabetes). In addition, the glycemic outcome will be categorized into diabetes incidence (diabetes and non-diabetes) to determine the participants who had been diagnosed with diabetes at 6 months. A logistic regression test will be used to examine the association between early and late-night mealtime and diabetes incidence.

Quality control

To ensure that the database accurately reflects the data reported in the questionnaires, a pre-test will be carried out. The research assistant trained to conduct the study procedures included interviewing administrative questionnaires, CGMS device, 3 days dietary record, and pedometer. Monthly meetings are held with the principal

investigator to review study procedures and progression. A monthly report will be documented after the meeting.

Data monitoring and management

Participants are anonymized and assigned with a specific ID at study entry. To ensure accuracy and completeness of data entry, data are checked by identifying if there is any outlier or missing value. The data checking process is performed in the first, third and sixth month of the data collection, such that the experience gained can be used to train the research assistant for improvement. Paper documents are kept in a locked cabinet and electronic data are stored on password-protected computers which can only be accessed by research team members. All records will be kept for at least 5 years after completing the study. After 5 years, all papers will be shredded using an office shredder. Extra care should be taken with sensitive or confidential information and a secure paper destruction service bin used. Digital data will be destroyed by deleting or overwriting information and destroying the physical media, such as CD rom.

4. PROPOSED RESEARCH SCHEDULE

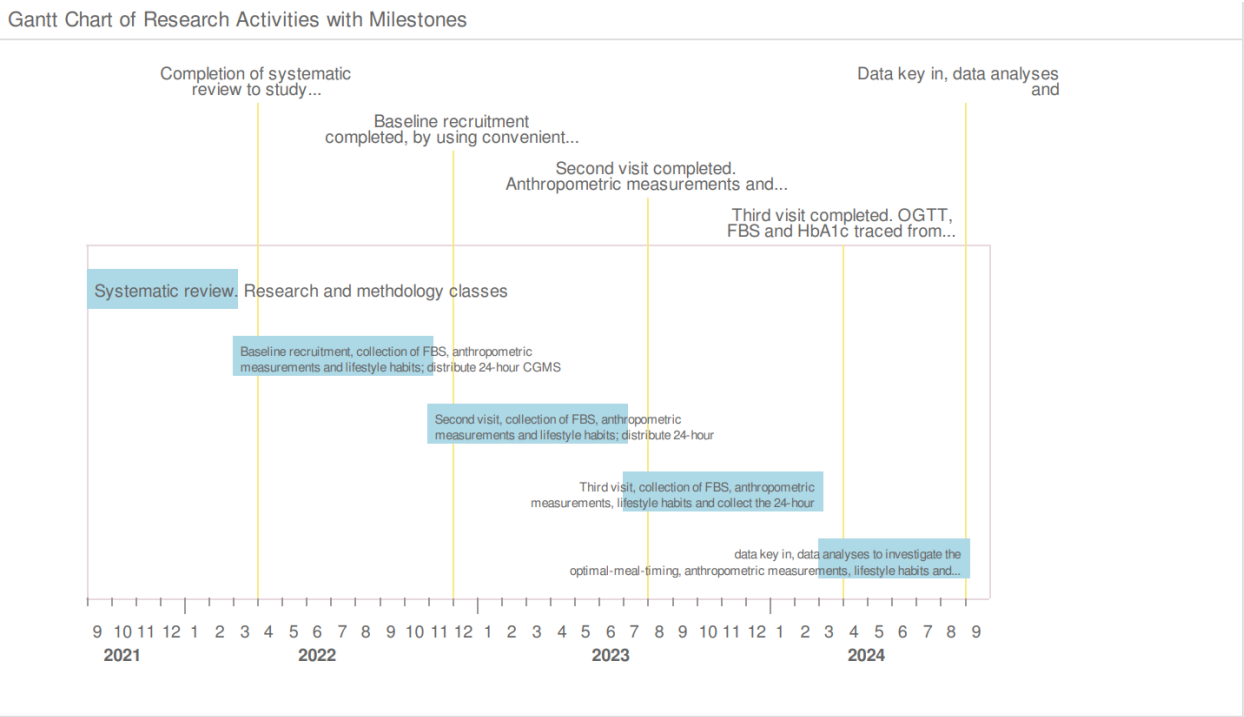
Research Activities

Activities	Start Date	End Date
Systematic review. Research and methodology classes	07/09/2021	06/03/2022
Baseline recruitment, collection of FBS, anthropometric measurements and lifestyle habits; distribute 24- hour CGMS	07/03/2022	06/11/2022
Second visit, collection of FBS, anthropometric measurements and lifestyle habits; distribute 24-hour CGMS	07/11/2022	06/07/2023
Third visit, collection of FBS, anthropometric measurements, lifestyle habits and collect the 24-hour CGMS	07/07/2023	06/03/2024
data key in, data analyses to investigate the optimal-meal-timing, anthropometric measurements, lifestyle habits and the associations between these variables; publication	07/03/2024	06/09/2024

Milestones

Activities	Date	Cumulative Project Completion Percentage (%)
Completion of systematic review to study optimal-meal-timing, chrononutrition, lifestyle factors and	31/03/2022	10

anthropometric measurements in prediabetes individuals		
Baseline recruitment completed, by using convenient sampling method. OGTT, FBS and HbA1c traced from medical record. Anthropometric measurements and lifestyle habit information collected. 3- day food diary and CGMS distributed.	30/11/2022	35
Second visit completed. Anthropometric measurements and lifestyle habit information collected. 3-day food diary and CGMS distributed.	31/07/2023	60
Third visit completed. OGTT, FBS and HbA1c traced from medical record. Anthropometric measurements and lifestyle habit information collected. 3-day food diary and CGMS colleted	31/03/2024	85
Data key in, data analyses and publication	31/08/2024	100



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APPENDIX A: INFORMATION SHEET AND CONSENT FORM

Research Title
Chronotype, Chrononutrition and Glucose Tolerance Among Prediabetic Individuals: Chrono-DM™.

Introduction
There is a growing body of knowledge associating alterations in biological clock with the development of T2DM. Perhaps, it is not what you eat but when you choose to eat it. Although the importance of biology clock in diabetes prevention had been recognized; however, such relevant information are not included in any diabetes guidelines, particularly in Malaysia.

Objective
This study aimed to determine the associations of chronotype and chrononutrition with glucose tolerance among prediabetic individuals in Malaysia.

What should I do?
This study that targets to recruit 166 prediabetes individuals who have received conventional medical nutrition therapy, from all the healthcare clinics in Malacca. Participants will be followed-up up to 6-month times:

Table 1 The overall study flow

Timeline	Services	Measurements
Baseline (first diagnosed prediabetes)	Routine Appointment in Community Service:	Fasting Plasma Glucose, Oral Glucose Tolerance Test, Liver function test, HbA1c test, Medical Officer consultation and dietitian consultation.
	Research Protocol:	1) Information data: Social-demographic Questionnaire 2) Anthropometry Measurement: Weight, Height, Body Fat Percentage, Visceral Fat, Waist Circumference 3) Pedometer Counting 4) Munich Chronotype Questionnaire (MCTQ) 5) Pittsburgh Sleep Quality Index (PSQI) 6) Harvard Light Exposure Assessment Questionnaire(H-LEA) 7) 3 days Daily Diet Record (2 weekdays and one weekend day; consecutive Sunday to Tuesday or Thursday to Saturday, within CGMS wearing period) 8) International Physical Activity Questionnaire (IPAQ)
Second visit (at 3rd month)	Routine Appointment in Community Service:	Fasting Plasma Glucose, Oral Glucose Tolerance Test, HbA1c test, Dietitian consultation
	Research Protocol:	1) Anthropometry Measurement: Weight, Height, Body Fat Percentage, Visceral Fat, Waist Circumference 2) Pedometer Counting 3) Munich Chronotype Questionnaire (MCTQ) 4) Pittsburgh Sleep Quality Index (PSQI) 5) Harvard Light Exposure Assessment Questionnaire(H-LEA) 6) 3 days Daily Diet Record (2 weekdays and one weekend day; consecutive Sunday to Tuesday or Thursday to Saturday, within CGMS wearing period) 7) International Physical Activity Questionnaire (IPAQ) 8) Chrononutrition Profile – Questionnaire (CPQ) 9) Continuous Glucose Monitoring Service (CGMS)
Third visit (at 6th month)	Routine Appointment in Community Service:	Fasting Plasma Glucose, Oral Glucose Tolerance Test, HbA1c test, Medical Officer consultation and Dietitian consultation.
	Research Protocol:	1) Anthropometry Measurement: Weight, Height, Body Fat Percentage, Visceral Fat, Waist Circumference 2) Pedometer Counting 3) Munich Chronotype Questionnaire (MCTQ)

		4) Pittsburgh Sleep Quality Index (PSQI) 5) Harvard Light Exposure Assessment Questionnaire(H-LEA) 6) 3 days Daily Diet Record (2 weekdays and one weekend day; consecutive Sunday to Tuesday or Thursday to Saturday, within CGMS wearing period) 7) International Physical Activity Questionnaire (IPAQ) 8) Chrononutrition Profile – Questionnaire (CPQ)

The interview will be like a conversation, and you can decide to stop the interview at any time. There are no right or wrong answers. The investigator are interested in learning from your personal experiences.

You will not know the reading in real time because the CGMS pro system is designed research observation and the reading will not made available to participants in real time. FDA Summary of Safety and Effectiveness Data (PMA P150021) indicated that Freestyle Libre CGMS pro version may inaccurately indicate hypoglycemia at a greater rate than other approved CGMs. The results of the clinical study conducted for this device showed that 40 percent of the time when the device indicated that user sensor glucose values were at or below 60 mg/dL(3.3 mmol/L), user glucose values were actually in the range of 81- 160 mg/dL (4.5-8.9 mmol/L). A glucometer shall be performed if you are suspect you have abnormal glucose level anytime. You will get your personal glucose result according to routine appointment with medical officer upon your request. Your personal CGMS and all measurement results will be given after 6 months measurements through email upon your request.

Benefits of Research
There are no direct benefits to you for participating in this study. However, your input will provide the vital information regarding to meal timing and glucose control in pre-diabetes patients.

Confidentiality
Your answer and information will be kept confidential by the investigators and will not made public unless disclosure is required by law. All information will be kept confidentially in Tunku Abdul Rahman University College for 3 years and then safely disposed of after 3 years. You will inform the study finding through email if there are any new publications using your information.

Risk to participants
CGMS pro system is continuous glucose monitoring system approved by US Food Drug and Administration. The investigator used this system fully to observe glucose variability personality but not the purpose to diagnose hyperglycemia or hypoglycemia. The FreeStyle Libre Pro System must be removed prior to Magnetic Resonance Imaging (MRI), Computed Tomography (CT) scan, or high-frequency electrical heat (diathermy) treatment. Potential device-related, non-serious events related to continuous glucose monitor use include: local infection, skin irritation or redness, skin inflammation, pain or discomfort, bleeding, bruising, skin edema, skin rash, itching, scarring or skin discoloration, allergic reactions to the sensor needle or adhesives, sensor or needle fracture during insertion, wear or removal. Sensor breakage with fragments retained under the skin is a potential procedure-related complication. However, based on post-market experience with this and similar devices, and the results observed in the clinical study, this event is rare and its severity does not raise major concerns.

The investigator believe participation in this study is of minimal risk. However, if you ever feel uncomfortable during the study, you are free to stop at any time. As with any study that collects information about you, there is possible risk of loss of confidentiality. However, as described below, The investigator have taken several measures to help prevent this.

Voluntary Participation
You are free to decline to participate, to end participation at any time for any reason, or to refuse to answer any individual question without penalty. Your decision whether to participate or not will not affect your relationship with this university and the community clinical services it may provide to you.

Publication
Permission will be obtained from the Director General of Health, Malaysia prior to publication. The publication will send to subject upon on their requirement.

Payment and allowance
You will not be charged any conduction fees to participate in this research study. You will not be paid any allowance in this research study. If the outcome of this research study involved in any product launch in the market in the future, you has no right to claim copyright and intellectual property from the profit of the product.

Enquiry
If you has any question regarding to this research study, please contact

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Principal investigators at the site and Name of PhD Student	Chong Guey Yong Department of Bioscience Faculty of Applied Sciences (FOAS) Tunku Abdul Rahman University Management and Technology (TARUMT) chonggy-wr21@student.tarc.edu.my
If you have any questions about your rights as a participant in this study, please contact	The Secretary, Medical Research & Ethics Committee, Ministry of Health Malaysia, at telephone number 03-3362 8407/8205/8888.

Letter of consent for research study

Give consent, Subject ID: _____

I,_____, ID card Number_____,

- Has read the Participant Information Sheet and understand regarding to the title of the research by listening to explanation from the researcher.
- Has given time for consideration and answered all question from the questionnaires.
- Understand that I can withdraw anytime during the research without giving any reason.
- Understand that all information given will stay anonymous.

I, hereby agree to take part in this research project and will follow all the research procedure and give all the related information if necessary.

_____	_____
(Signature)	(Date)
_____	_____
Witness by,	Researcher
_____	_____
(Signature)	(Signature)
_____	_____
(I/C number)	(I/C number)
_____	_____
(Date)	(Date)

APPENDIX B: SOCIO-DEMOGRAPHY

Date of interview: [ ][ ] / [ ][ ][ ] / [ ][ ][ ][ ]
d d m m m y y y y

Section 1.0 Socio-demography

1.1 What is the **highest level of education** that you have completed?

0: None *(skip to Q1.3)*

[ ]

1: Primary (eg. PSLE)

2: Secondary (eg. GCE 'O' / 'N' level)

3: Junior College / Centralised Institute (GCE 'A' Level / International Baccalaureate)

4: Institute of Technical Education [ITE/NTC (NITEC, higher NITEC or equivalent)]

5: Diploma/ Advanced diploma / Polytechnic

6: Trade or work qualification not from university

7: University Bachelor's Degree / Postgrad Diploma / Professional Degree

8: Postgraduate (Master Degree / Doctorate)

1.2 How many **years of education** have you received? *(Including home schooling but excluding pre-school, kindergarten or years spent repeating individual grades)*

[ ][ ] years [ ][ ] months

1.3 What is your current job status?

0: Not employed *(skip to Q1.5)*

[ ]

1: Self-employed

2: Part-time employed

3: Full-time employed

1.4 During the past 1 month, did you require to work **overtime** (until after 7pm)?

0: No

[ ]

1: Less than once a week

2: Once or twice a week

3: Three or more times a week

1.5 What was your weight before prediabetes diagnosis ?

[ ][ ][ ] . [ ]

1.6 What is your ethnic group?

0: Chinese

1: Malay

2: Indian

3: Other, please specify: _____

Section 2.0 Lifestyle

2.1 Have you smoked in the past?

0: Not at all for entire life

1: Yes, but stopped smoking before conceiving for this pregnancy

2: Yes, but stopped smoking since conceived for this pregnancy

3: Yes, but stopped smoking 1 month ago

4: Yes, during the past 1 month and/ or currently smoking

2.2 During the **past 1 month**, did anyone **smoke in indoor areas** where you work and/ or stay?*(Excluding area which are not fully enclosed. Should answer 'yes' if smelling the smoke).*

0: No

1: Yes

2.3 How often did you have a drink containing **alcohol** during the **past 3 months before conceiving** for this pregnancy?*(Including traditional wine, e.g. Youmeishu, DOM Benedictine)*

0: never or less than once per month

1: 1-3 times per month

2: 1-2 times per week

3: 3-4 times per week

4: 5-6 times per week

5: 1 time or more per day

2.4 Have you consumed any drink containing **alcohol since your current pregnancy**?*(Including traditional wine, e.g. Youmeishu, DOM Benedictine)*

0: No

1: Yes, but stopped drinking 1 month ago

2: Yes, current and during the past 1 month

2.5 Did you take any pills, tonics or tablets to **supplement** your diet (e.g. vitamin, minerals, iron tablets, folic acid, fish oils etc.)?*(can be more than 1 options, except if selecting 'Never')*

0: Never or without supplement for at least 3 months ago before conceiving for this pregnancy

	1: Yes, since 3 months before conceivingfor this pregnancy
	2: Yes, since conceivedfor this pregnancy
	3: Yes, current and during the past 1 month

2.6 **During the past 1 month**, how often did you **skip** your breakfast, lunch and dinner?

	Breakfast	Lunch	Dinner
0: never 1: 1-3 times per month 2: 1-2 times per week 3: 3-4 times per week 4: 5-6 times per week 5: Every day			

2.7 **During the past 1 month**, how often did you **delay** your breakfast, lunch and dinner for more than 2 hours from your regular meal timings?

	Breakfast	Lunch	Dinner
0: never 1: 1-3 times per month 2: 1-2 times per week 3: 3-4 times per week 4: 5-6 times per week 5: Every day			

2.8 **During the past 1 month**, how often did you have **snack(s) taken after 7pm**? *(Snack is defined as a portion of food often smaller than a regular meal, including beverage such as milk. Snacking frequency is defined with a time interval between episodes of at least 15 min)*

	0: never
	1: 1-3 times per month
	2: 1-2 times per week
	3: 3-4 times per week
	4: 5-6 times per week
	5: 1 time per night
	6: 2 or more times per night

APPENDIX C **International Physical Activity Questionniare**

Project Name: Meal Timing and Glucose Tolerance

Date of interview: ☐☐ / ☐☐☐ / ☐☐☐☐

d d m m m y y y y

3.0 Physical activity

*I am going to ask you about the time you spent being physically active. Think about only those physical activities that you did for **at least 10 minutes** at a time over the past week.*

During the last 7 days, how many days have you done the following kind of physical activities?

3.1 **Vigorous physical activities** which take hard physical effort and make you breathe much harder than normal.*(E.g. heavy lifting,aerobics, fast swimming or fast bicycling)*

☐ days in last 7 days ***(****If answers '0', skip to Q 3.2)*

3.1.1 On average about how much time did you usually spend on those activities?

☐☐ hrs ☐☐ mins per day (or) ☐☐ hrs ☐☐ mins per week

3.1.2 During which period(s) of the day did you usually perform those activities? *(Can be more than 1 options)*

☐ 1: Early morning (5am – 6:59am)
☐ 2: Morning (7am – 11:59am)
☐ 3: Afternoon (12pm – 4:59pm)
☐ 4: Evening (5pm – 6:59pm)
☐ 5: Night (7pm – 11:59pm)
☐ 6: Late-night (12am – 4:59am)

3.2 **Moderate physical activities** which take moderate physical effort and make you breathe somewhat harder than normal.*(E.g. carrying light loads, sweeping, scrubbing floors, washing windows, dancing, or bicycling/swimming at a regular pace. Do not include walking)*

☐ days in last 7 days ***(****If answers '0', skip to Q 3.3)*

3.2.1 On average about how much time did you usually spend on those activities?

☐☐ hrs ☐☐ mins per day (or) ☐☐ hrs ☐☐ mins per week

3.2.2 During which period(s) of the day did you usually perform those activities? *(Can be more than 1 options)*

☐ 1: Early morning (5am – 6:59am)
☐ 2: Morning (7am – 11:59am)
☐ 3: Afternoon (12pm – 4:59pm)
☐ 4: Evening (5pm – 6:59pm)
☐ 5: Night (7pm – 11:59pm)
☐ 6: Late-night (12am – 4:59am)

3.3 How many days did you **walk** for **at least 10 minutes** at a time? *(Includes at work and at home, walking from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure)*

☐

☐ days in last 7 days

(If answers '0', skip to Q 4.1)

3.3.1 On average about how much time did you usually spend on walking?

☐☐ hrs ☐☐ mins per day (or) ☐☐ hrs ☐☐ mins per week

3.3.2 During which period(s) of the day did you usually spend on walking? (Can be more than 1 options, select as appropriate)

☐ 1: Early morning (5am – 6:59am)
☐ 2: Morning (7am – 11:59am)
☐ 3: Afternoon (12pm – 4:59pm)
☐ 4: Evening (5pm – 6:59pm)
☐ 5: Night (7pm – 11:59pm)
☐ 6: Late-night (12am – 4:59am)

Section 4.0 Sedentary behaviour

*The following questions are about time spent **sitting or lying down while being awake**, **at least 10 minutes** at a time **over the past week**. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television.*

4.1 *[For those who previously answered Yes to work]*
During the last week, on an average **working day**,how much time did you spend **sitting at work**? ☐☐ hrs ☐☐ mins per day

4.1.1 During the last week, on an average **working day**, how much time did you spend on **electronic devices such as social media, computer, tablet, mobile phone at work**? ☐☐ hrs ☐☐ mins per day

4.2 *[For those who previously answered Yes to work]*
Excluding any time at work, during the last week, on average how much time did you spend……

[For those who previously answered No to work]
During the last week, on average how much time did you spend……

4.2.1 sitting or lying down and **watching television**? ☐☐ hrs ☐☐ mins per day (or) ☐☐ hrs ☐☐ mins per week

4.2.2sitting or lying down and viewing **electronic devices** other than television in your leisure time (e.g watching online video, internet surfing, social media, electronic games on computer, tablet or mobile phone etc.)? Do not include time spent on computer at work. ☐☐ hrs ☐☐ mins per day (or) ☐☐ hrs ☐☐ mins per week

4.2.3sitting or lying down at **mealtimes, driving or reading** or other time sitting [excluding TV /electronic device time] ☐☐ hrs ☐☐ mins per day (or) ☐☐ hrs ☐☐ mins per week

Section 5.0 Electronic media use before bedtime

During the last 7 days, on average how much time did you spend on the below electronic devices **before going to sleep in bed at night** (after 7pm)?

	Electronic devices	How many days in past week (0-7)	Duration per day or per week *(If the durations of using the device are inconsistent every night, sum up the duration spent for each night to obtain total time spent per week)*	Used in bedroom 0: No ; 1: Yes
5.1	**Television**	☐	☐☐ hrs ☐☐ mins per day	
5.2	**Computer:** surfing, online video, playing, reading	☐	☐☐ hrs ☐☐ mins per day	☐
5.3	**Computer:** movie, TV series	☐	☐☐ hrs ☐☐ mins per day	☐
5.4	**Tablet/ mobile phone:** surfing, online video, playing, reading, texting	☐	☐☐ hrs ☐☐ mins per day	☐
5.5	**Tablet/ mobile phone:** movie, TV series	☐	☐☐ hrs ☐☐ mins per day	☐
5.6	**Tablet/ mobile phone:** talking only	☐	☐☐ hrs ☐☐ mins per day	☐
5.7	**Audio player** (music, radio)	☐	☐☐ hrs ☐☐ mins per day	☐
5.8	**Gaming console**	☐	☐☐ hrs ☐☐ mins per day	☐
5.9	**Partner's media use**	☐	☐☐ hrs ☐☐ mins per day	☐

Reference: See Ling Loy, Yin Bun Cheung, Mary Chong, Falk Müller-Riemenschneider, Ngee Lek, YS Lee, Kok Hian Tan, Bernard Chern, Fabian Yap, Jerry Chan. 2019. Maternal night-eating pattern and glucose tolerance during pregnancy: study protocol for a longitudinal study. BMJ Open. 9:e030036. doi:10.1136/bmjopen-2019-030036.

APPENDIX D

Munich Chronotype Questionnaire

Project Name:

Meal Timing and Glucose Tolerance

Date of interview: ☐☐ / ☐☐☐ / ☐☐☐☐

d d m m m y y y y

Munich Chronotype Questionnaire (MCTQ)

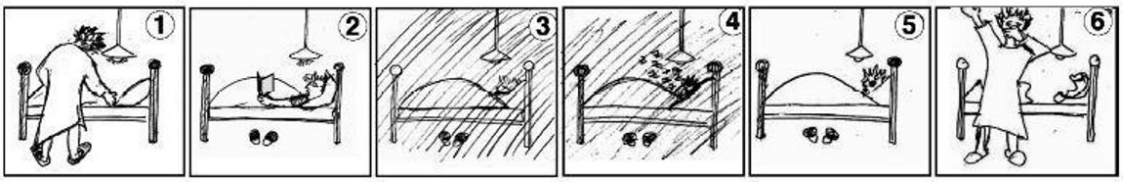
This questionnaire requires you to report your sleep patterns over the past 4 weeks. Working day and non -working day sleep patterns were asked separately. Please answer this question according to your perceptions of your weekly habits, workday sleep patterns and non -working days.

I have regular working hours (this includes housewives):

☐ Yes, I work ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ Day/week

☐ No

If your answer is 'Yes, 7 days' or 'No', please consider whether your bedtime may differ between normal weekdays and weekends and fill out the MCTQ accordingly.



Based on the following picture, answer the question below (Please answer on a 24 -hour scale, for example; 23:00):

Working Days	
Picture 1	I entered the bedroom at: _____
Picture 2	Some people stay awake even after lying in bed.
Picture 3	I'm ready to go to bed at (turn off the lights and go to sleep):_____
Picture 4	How many minutes do I need to sleep: _____
Picture 5	I woke up in the morning at: _____
Picture 6	I got out of bed after a few minutes: _____
I use an alarm clock on weekdays	☐ Yes ☐ No
If YES, I usually wake up BEFORE the alarm clock rings:	☐ Yes ☐ No
Weekend	
Picture 1	I entered the bedroom at: _____
Picture 2	Some people stay awake even after lying in bed.

Picture 3	I'm ready to go to bed at (turn off the lights and go to sleep):_____
Picture 4	How many minutes do I need to sleep: _____
Picture 5	I woke up in the morning at: _____
Picture 6	I got out of bed after a few minutes: _____
My waking hours were due to the use of an alarm clock (Figure 5):	☐ Yes ☐ No
There are certain reasons I can't determine my bedtime independently on holidays:	☐ Yes ☐ No
If 'Yes', among the factors are	
☐ Child/husband/pet	
☐ Hobby	
☐ Solat/morning prayer/meditation	
☐ Others (please state): _____	
Will I go back to sleep after doing the activity, on holidays?	
☐ Yes ☐ No	
If 'Yes' to the previous question, what time do you go to bed and wake up?	
Time to sleep again:_____	
Time to wake up again: _____	

Reference:

Ms. Fatin Hanani Mazri, Zahara Abdul Manaf, Suzana Shahar, Arimi Fitri Mat Ludin, Norwahidah Abdul Karim, Andrea Yu-Lin Ban & Rose Azzlinda Osman (2021): Modified Munich chronotype questionnaire for application to short-interval split sleep of non-shift workers, Chronobiology International, DOI: 10.1080/07420528.2021.1887209

APPENDIX E

Three Days Dietary Record

Project Name: Meal Timing and Glucose Tolerance

Date of interview: | | | / | | | | / | | | | |

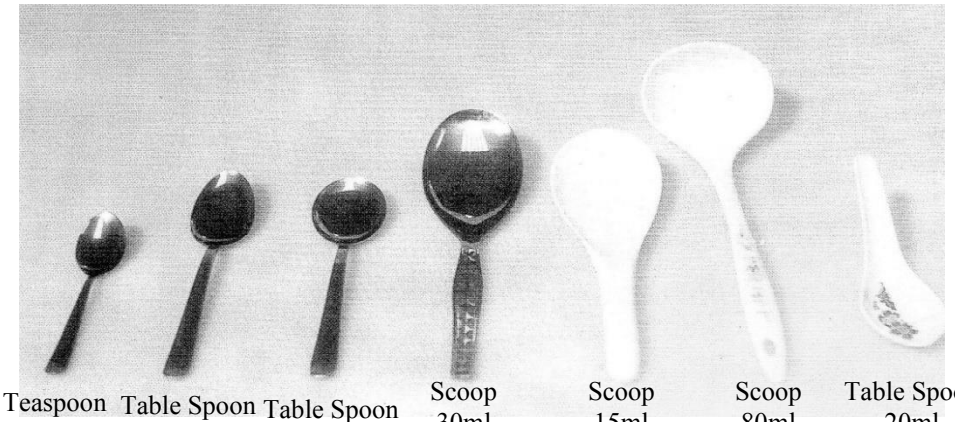
d d m m m y y y y

3 days dietary record guidelines:

1. Please fill in this food intake log book as completely as possible for 2 weekdays and 1 weekend.
2. Subjects were not allowed to change food habits while food intake records were being conducted.
3. For food records, please indicate the time, type and quantity/ size of all food and beverages taken immediately after meal time.
4. If you have any further enquiry, please contact:
   Livy Chong Guey Yong 017-7780620 chonggy-wr21@student.tarc.edu.my

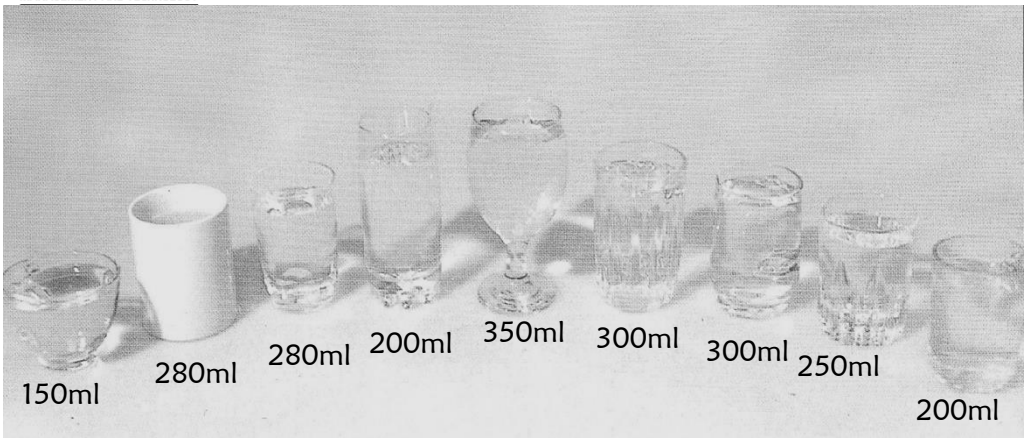
Household Measurements

A) Spoons



B) Plate and Bowls

C) Cups, mugs and glassess



Date			
Monday / Tuesday / Wednesday / Thursday / Friday / Saturday/ Sunday			
Time	Food & Beverage	Quantity	Note
Breakfast (: am)			
Morning Tea (: am)			
Lunch (: pm)			
Afternoon Tea (: pm)			
Dinner (: pm)			
Supper (: pm)			
Date			
Monday / Tuesday / Wednesday / Thursday / Friday / Saturday/ Sunday			
Time	Food & Beverage	Quantity	Note
Breakfast (: am)			
Morning Tea (: am)			
Lunch (: pm)			
Afternoon Tea (: pm)			
Dinner (: pm)			
Supper (: pm)			

APPENDIX G

Havard Light Exposure Questionnaire

Project Name: Meal Timing and Glucose Tolerance

Date of interview: | | | / | | | | / | | | | |

d d m m m y y y y

Date			
Monday / Tuesday / Wednesday / Thursday / Friday / Saturday/ Sunday			
Time	Food & Beverage	Quantity	Note
Breakfast (: am)			
Morning Tea (: am)			
Lunch (: pm)			
Afternoon Tea (: pm)			
Dinner (: pm)			
Supper (: pm)			

Harvard Light Exposure Assessment (H-LEA) Questionnaire

During a typical day, describe your exposure to the below specified light sources. Please fill in applies, for each single day of your 7 days trial and circle the hours at which you had a meal, like indicated in the example below.

H=Halogen Lamp F=Fluorescent Lamp
N=Natural Light (Indoors) S=Sunlight, Natural Light (Outdoors)
O=Other Artificial Light Sources D=Darkness

Date:			
	Night Shift Workhours From	To	
	Day Shift Workhours From	To	
	Off Work		

Time	**Exposure to Specified light sources**
1am	
2am	
3am	
4am	
5am	
6am	
7am	
8am	
9am	
10am	
11am	
Noon	
1pm	
2pm	
3pm	
4pm	
5pm	
6pm	
7pm	
8pm	
9pm	
10pm	
11pm	
12pm	

Reference:

Archna Bajaj, Bernard Rosner, Steven W. Lockley, and Eva S. Schernhamme. 2011. Validation of a Light Questionnaire with Real-life Photopic Illuminance Measurements: the Harvard Light Exposure Assessment Questionnaire. Cancer Epidemiology Biomarkers Prevention; 20(7); 1341–9.

APPENDIX H

Pittsburgh Sleep Quality Index Questionnaire

Project Name:

Meal Timing and Glucose Tolerance

Date of interview:

		/				/				
d	d		m	m	m		y	y	y	y

Pittsburgh Sleep Quality Index

Instructions: The following questions relate to your workday sleep habits over the past month only. You need to state the most accurate answer for most of the days and nights of the past month. **Please answer all questions.**

1. In the past month, what time did you usually go to bed at night on weekdays?
_____ a.m / p.m

2. In the past month, how many minutes did it usually take you to fall asleep each night on a weekday?_____ minutes

3. In the past month, what time do you usually get up in the morning on weekdays?_____a.m

4. In the past month, how many hours did you get actual sleep at night on weekdays? (This may be different from the number of hours you lie in bed).
_____ hours

Instructions: The following questions relate to your sleep habits on rest days during the past month only. You need to state the most accurate answer for most of the days and nights of the past month. **Please answer all questions.**

5. In the past month, what time do you usually sleep at night on rest days?
_____ a.m / p.m

6. In the past month, how many minutes did it usually take you to fall asleep each night on a day off?_____ minutes

7. In the past month, what time do you usually get up in the morning on a day off?_____a.m

8. In the past month, how many hours did you get actual sleep at night on a day off? (This may be different from the number of hours you lie in bed)._____ hours

Instructions: The following questions relate to your normal sleep habits over the past month only. You need to state the most accurate answer for most of the days and nights of the past month. **Please answer all questions.**

9. In the past month, how often did you have trouble sleeping because you .... (Please tick √ on the appropriate answer)

a. Can't sleep in 30 minutes
☐ No for last month
☐ Less than once a week
☐ Once or twice a week
☐ Three or more times a week

b. Wake up in the middle of the night or early in the morning
☐ No for last month
☐ Less than once a week
☐ Once or twice a week
☐ Three or more times a week

c. Need to get up to use the bathroom
☐ No for last month
☐ Less than once a week
☐ Once or twice a week
☐ Three or more times a week

d. Cannot breathe comfortably
☐ No for last month
☐ Less than once a week
☐ Once or twice a week
☐ Three or more times a week

e. Coughing or snoring loudly
☐ No for last month
☐ Less than once a week
☐ Once or twice a week
☐ Three or more times a week

f. Feeling too cold
☐ No for last month
☐ Less than once a week
☐ Once or twice a week
☐ Three or more times a week

g. Feeling too hot
☐ No for last month
☐ Less than once a week
☐ Once or twice a week
☐ Three or more times a week

h. Having bad dream
- ☐ No for last month
- ☐ Less than once a week
- ☐ Once or twice a week
- ☐ Three or more times a week

i. Having pain
- ☐ No for last month
- ☐ Less than once a week
- ☐ Once or twice a week
- ☐ Three or more times a week

j. Other reasons, please explain:

_____

_____

10. In the past month, how often have you taken medication to help you sleep (on the doctor's instructions or bought yourself at the pharmacy)?
- ☐ No for last month
- ☐ Less than once a week
- ☐ Once or twice a week
- ☐ Three or more times a week

11. In the past month, how often have you had trouble staying awake while driving, eating food, or engaging in social activities?
- ☐ No for last month
- ☐ Less than once a week
- ☐ Once or twice a week
- ☐ Three or more times a week

12. In the past month, how much trouble did you have to maintain enough enthusiasm in getting things done?
- ☐ No Problem
- ☐ A little problem
- ☐ Somewhat problematic
- ☐ Big trouble

13. In the past month, how have you assessed the quality of your sleep overall?
- ☐ Very good
- ☐ Quite good
- ☐ A little bad
- ☐ Very bad

14. Do you have a sleeping partner or roommate?
- ☐ No sleeping partner or roommate → **(You can stop answering)**
- ☐ Sleeping partner or roommate in another room

☐ Couple in the same room but not in the same bed
☐ Couple sleeping in the same bed

15. If you have a sleeping partner or roommate, ask him or her how often in the last month you have:

a. Loud snoring
   ☐ Not at all last month
   ☐ Less than once a week
   ☐ Once or twice a week
   ☐ Three or more times a week

b. Long pauses between breaths during sleep
   ☐ Not at all last month
   ☐ Less than once a week
   ☐ Once or twice a week
   ☐ Three or more times a week

c. Legs moving or jerking when you are sleeping
   ☐ Not at all last month
   ☐ Less than once a week
   ☐ Once or twice a week
   ☐ Three or more times a week

d. Episodes of disorientation or confusion during sleep
   ☐ Not at all last month
   ☐ Less than once a week
   ☐ Once or twice a week
   ☐ Three or more times a week

e. Other anxieties when you are sleeping, please explain: _____

Reference: Buysse, DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ: The Pittsburgh Sleep Quality Index (PSQI): A new instrument for psychiatric research and practice. Psychiatry Research 28:193-213, 1989

APPENDIX I

Chrononutrition Profile Questionniare

Project Name:

Meal Timing and Glucose Tolerance

Date of interview: | | | / | | | | / | | | | |

d d m m m y y y y

Chrononutrition Profile – Questionnaire (CP-Q)

Directions: The following questions are designed to assess the general timing of your eating. Please choose the one response that best fits your typical behavior and preferences. The term “eating event” refers to any time you eat something that contains calories. For example, this could be a meal, a snack, or a drink.

If you were entirely free to plan your day,

A1. What time would you prefer to wake up?
Please indicate A.M. or P.M. as part of your response.
_____ A.M./P.M.

A2. How soon after waking up would you prefer to have your first eating event of the day?
_____ hours _____ minutes

A3. How soon before bed would you prefer to stop eating?
_____ hours _____ minutes

A4. What time would you prefer to fall asleep? Please indicate A.M. or P.M. as part of your response. _____ A.M./P.M.

B1. On average, in a typical week (a 7-day period), B1. How often do you eat breakfast?
_____ 0 days _____ 1 day _____ 2 days_____ 3 days _____ 4 days _____ 5 days
_____ 6 days _____ 7 days

B2. What is your largest meal of the day?
_____ Breakfast _____ Lunch _____ Dinner/Supper
_____ Other meal (Please describe: _____)

B3. How often do you eat a snack after your last meal of the day?
_____ 0 days _____ 1 day _____ 2 days _____ 3 days _____ 4 days _____ 5 days _____ 6 days _____ 7 days

B4. How often do you wake up in the night to eat?
_____ 0 days _____ 1 day _____ 2 days _____ 3 days _____ 4 days _____ 5 days _____ 6 days _____ 7 day

On average, on a typical workday or school day,

C1. What time do you wake up?
Please indicate A.M./P.M. as part of your response.
_____ A.M./P.M.

C2. What time is your first eating event of the day?
Please indicate A.M./P.M. as part of your response.
_____ A.M./P.M.

C3. What time do you eat lunch?
Please indicate A.M./P.M. as part of your response.
Select “I do not eat lunch” if you do not typically eat lunch.
_____ A.M./P.M. _____ I do not eat lunch.

C4. What time is your last eating event before bed?
Please indicate A.M./P.M. as part of your response.
_____ A.M./P.M.

C5. What time do you fall asleep?
Please indicate A.M./P.M. as part of your response.
_____ A.M./P.M.

On average, on a typical weekend day or free day,

D1. What time do you wake up?
Please indicate A.M./P.M. as part of your response.
_____ A.M./P.M.

D2. What time is your first eating event of the day?
Please indicate A.M./P.M. as part of your response.
_____ A.M./P.M.

D3. What time do you eat lunch? Please indicate A.M./P.M. as part of your response.
Select “I do not eat lunch” if you do not typically eat lunch.
_____ A.M./P.M.
_____ I do not eat lunch.

D4. What time is your last eating event of the day before bed?
Please indicate A.M./P.M. as part of your response.
_____ A.M./P.M.

D5. What time do you fall asleep?
Please indicate A.M./P.M. as part of your response.
_____ A.M./P.M.

Reference:
Allison C. Veronda, Kelly C. Allison, Ross D. Crosby, and Leah A. Irish. 2019. Development, validation and reliability of Chrononutrition Profile Questionnaire. Chronobiology International. DOI: 10.1080/07420528.2019.1692349

Anthropometry

APPENDIX J **Anthropometry Measurement and Biochemistry Parameters**

Project Name: Meal Timing and Glucose Tolerance

Date of interview: | | | / | | | | / | | | | |

d d m m m y y y y

Measurement	Reading 1	Reading 2	Reading 3	Average
ANTHROPOMETRY				
Height (cm)				
Weight (kg)				
Body Mass Index (kg/m^2)				
Waist Circumference (cm)				
Hip Circumference (cm)				
Body Composition				
Body Fat percentage (%)				
Visceral Fat				
Subcutaneous Fat (Whole Body)				
Subcutaneous Fat (Trunk)				
Subcutaneous Fat (Arm)				
Subcutaneous Fat (Leg)				
Skeletal Muscle (Leg)				
Skeletal Muscle (Arm)				
Skeletal Muscle (Trunk)				
Skeletal Muscle (Whole Body)				
Resting Metabolism				
Body Age				
Medical Record				
Date				
HbA1C (%)				
Fasting Plasma Glucose (mmol/L)				
OGTT Plasma Glucose Value(mmol/L) 0 hour				
OGTT Plasma Glucose Value(mmol/L) 2 hours				
Blood Pressure (mm/Hg)				
Alanine Transaminase ALT (IU/L)				