AN INVESTIGATION OF PHYSICAL ACTIVITY LEVEL, SEDENTARY TIME AND BONE MINERAL DENSITY IN RELATION TO SCLEROSTIN LEVEL IN POSTMENOPAUSAL WOMEN WITH TYPE 2 DIABETES MELLITUS

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by

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ALMI	Appendicular lean mass index
AGE	Advanced glycation end-products
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BMSi	Bone mineral strength index
CI	Confidence interval
cm	centimeters
CPM	Count per minute
CVS	Cardiovascular disease
DXA	Dual-energy x-ray absorptiometry
ELISA	Enzyme-Linked immunosorbent assay
ESS Lab 2	Exercise and Sport Science Laboratory II
FBS	Fasting blood sugar
FFQ	Food frequency questionnaire
FRAX	Fracture risk assessment
FSH	Follicle-stimulating hormone
GRF	Ground reaction force
HbA1c	Hemoglobin A1c
HHQ	Health history questionnaire
HR-pQCT	High-resolution peripheral quantitative computed tomography
HPUSM	Hospital Pakar Universiti Sains Malaysia
JRF	Joint reaction force

kg	kilogram
KPP	Klinik Pakar Perubatan
LED	Light-emitting diode
LMI	Lean mass index
MLT	Medical Laboratory Technologist
MVPA	Moderate-to-vigorous physical activity
NCDs	non-communicable diseases
PA	Physical activity
RTC	Real-time clock
SD	Standard deviation
Sed	Sedentary
SPSS	Statistical Package for the Social Sciences
T1D	Type 1 diabetes mellitus
T2D	Type 2 diabetes mellitus
USB	universal serial bus
USM	Universiti Sains Malaysia
WHR	Waist-hip circumference
YT2D	Years T2D

LIST OF APPENDICES

KAJIAN TAHAP AKTIVITI FIZIKAL, MASA SEDENTARI DAN KETUMPATAN TULANG SERTA HUBUNGKAIT DENGAN PARAS SCLEROSTIN DALAM KALANGAN WANITA MENOPOS YANG MENGHIDAP DIABETES MELLITUS TAHAP 2

ABSTRAK

Individu yang menghidap diabetes tahap 2 (T2D) dikaitkan dengan peningkatan risiko kepatahan tulang pada mana-mana bahagian tulang rangka disebabkan oleh kualiti tulang yang lemah, sungguhpun mempunyai kepadatan mineral tulang yang lebih tinggi, berbanding dengan individu bukan T2D. Kajian awal membuktikan wanita menopos yang mengalami tulang rapuh dan patah tulang mempunyai tahap sclerostin yang tinggi berbanding mereka yang tidak mengalaminya, manakala aktiviti fizikal (PA) adalah berkait songsang dengan tahap sclerostin. Dalam kajian ini, tahap aktiviti fizikal seperti sedentari (Sed), sederhana cergas (MPA) dan sederhana hingga cergas, (MVPA) diukur bagi melihat perkaitan dengan tahap sclerostin wanita menopause yang menghidap T2D. Kajian rentas ini melibatkan 71 wanita menopaus yang mempunyai T2D, purata berumur 59.5±4.1 tahun dari Klinik Pakar Perubatan (KPP), HPUSM. Sejarah kesihatan peserta, tempoh menopaus dan tempoh didiagnosis T2D direkodkan di dalam borang Soal-Selidik Sejarah Kesihatan, manakala jumlah pengambilan kalsium harian (mg/hari) juga direkodkan di dalam borang Soal Selidik Kekerapan Makanan. Tahap sclerostin (pmol/L) juga diukur melalui analisa serum darah melalui protokol ‘sandwich Enzim Linked Immunosorbent Assay (ELISA). Kepekatan sclerostin kemudiannya ditentukan oleh ‘optical density’ (OD) melalui alat ‘microplate reader’ jenama Thermo Fisher Scientifiec (model Varioscan Flash). Bacaan gula berpuasa (FBS, mmol/L) dan

kawalan glycemiks (HbA1c, %) pula diperolehi melalui rekod terkini rawatan peserta di KPP, HPUSM. Ukuran antropometri seperti berat badan (kg) dan tinggi (cm) turut dilakukan bagi penentuan Indeks Jisim Tubuh (BMI, kg/m^2) dan juga pengimbasan dual-energy x-ray absorptiometry (DEXA) dibuat untuk mendapatkan maklumat tentang ketumpatan mineral tulang (BMD, kg/cm^2), T-skor (SD), peratusan lemak badan (%) dan jumlah tisu tanpa lemak, (LMI, %), indeks tisu tanpa lemak bahagian kaki dan tangan (ALMI, kg/m^2). Intensiti aktiviti fizikal pula diukur berdasarkan jumlah gerakan tranlasi per minit (CPM) dengan menggunakan accelerometer jenama Gulf Coast Data Concepts (GCDC), model X16-D dan X16-4 selama 7 hari berturut-turut. Data yang memenuhi penggunaan minima 10 jam dalam sehari, selama 4 hari dikira sah dan dianalisa dengan menggunakan pakej GGIR, perisian R. Menggunakan perisian SPSS versi 27, statistik output deskriptif, analisis korelasi dan regresi berbilang pembolehubah turut diperolehi. Secara keseluruhannya, lapan wanita (11%) telah dikenalpasti menghidap osteoporosis dengan purata T-skor pada -3.3±0.6 SD. Dapatan analisis korelasi menunjukkan, tempoh T2D, tahun (r=0.50), purata MPA, min/day (r = -0.39) dan purata MVPA, min/hari (r= -0.39) berkait secara signifikan dengan tahap sclerostin, manakala masa sedentari (339.3±83.6 min/hari), aktiviti fizikal ringan (205.2±37.1 min/hari), sederhana (32.0±17.2 min/hari) dan cergas (0.4±0.9min/hari) tidak dikaitkan dengan tahap sclerostin. Model regresi berbilang pembolehubah meramalkan 33% ($F=5.63$, $p<0.003$) hasil sklerostin dipengaruhi oleh ALMI, kg/m^2 (β= - 0.001, p= 0.990), tempoh T2D didiagnos (β= 1.070, p=0.009) dan MVPA (β= -0.292, p= 0.056). Kesimpulannya, tempoh T2D berkait rapat dengan tahap sclerostin dalam wanita T2D, disamping mengambil kira ALMI. Walaupun tidak ketara, tahap sclerostin cenderung lebih tinggi pada tahap MVPA yang rendah.

Penyiasatan yang lebih mendalam diperlukan untuk memahami hasil novel ini tentang perkaitan sclerostin dengan sedentari dan tahap PA dalam wanita menopaus T2D.

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ABSTRACT

People with type 2 diabetes (T2D) have been associated with an increased risk of fractures at any skeletal site due to the poorer quality of the bone, despite having greater bone mineral density when compared to osteoporotic and normal non-T2D people. Studies showed that postmenopausal women with fragility fractures have high sclerostin levels compared to those without fractures, while physical activities (PA) were inversely related to sclerostin levels. In this study, different levels of PA (sedentary, moderate, vigorous PA and MVPA) levels were objectively measured to assess their influence on sclerostin levels in T2D postmenopausal women. This cross-sectional study involved 71 postmenopausal T2D women, aged 59.5±4.1 years from the Diabetic Clinic, Hospital Pakar Universiti Sains Malaysia. Health history of postmenopausal and T2D duration, also other chronic diseases were obtained, followed by calcium intake (mg/day) using a validated food frequency questionnaire. Anthropometry measurements were recorded and fasting blood glucose (mmol/L), HbA1c (%) and sclerostin (pmol/L) were obtained via blood assay. Using dual-energy X-ray absorptiometry (DEXA), bone mineral density (BMD, kg/cm^2), T-score (SD), body fat (%), total lean mass (%), and appendicular lean mass index (ALMI, kg/m^2) were derived. Participants wore an accelerometer for a week during waking hours where those with a minimum of 10-h wear/day and for a minimum of four days of valid data were analysed. Accelerometer data were analysed using the GGIR package

for R-software. Using SPSS version 27, descriptive output, correlation and multivariable regression analyses and outcomes were obtained. Overall**,** eight women (11%) were newly identified as having osteoporosis with average T-scores -3.3±0.6 SD. From our correlation analysis, duration of T2D, years (r = 0.50), ALMI, kg/m^2 (r = 0.14) and average MVPA, min/day (r = -0.4) were significantly related to sclerostin levels ($p<0.05$). Sedentary (339.3±83.6 min/day), light (205.2±37.1 min/day), moderate (32.0±17.2 min/day) and vigorous PA (0.4±0.9min/day) were not correlated to sclerostin levels. The multivariable regression model predicted as much as 33% (F = 5.63, $p<0.003$) of sclerostin outcomes are influenced by the duration of T2D diagnosed (β= 1.070, p=0.009) and MVPA (β = -0.292, p = 0.056) while controlling for appendicular ALMI, kg/m^2 (β= -0.010, p= 0.990). In conclusion, the duration of diagnosed T2D influenced the sclerostin levels, which may impair bone formation while accounting for appendicular muscle. More in-depth investigations are needed to understand these results of possible MVPA influence on sclerostin in T2D postmenopausal women.

CHAPTER 1

INTRODUCTION

1.1 Introduction of type 2 diabetes mellitus (T2D)

In recent years, diabetes has emerged as a significant global public health concern with a remarkable increase in its prevalence. Worldwide, the estimation of people with diabetes is 537 million and it is projected to reach 643 million by 2030 (Magliano, 2021). In Malaysia, diabetes affected 4.4 million out of total 22.3 million adults (IDF Diabetes Atlas, 2021). The Ministry of Health Malaysia (MOH) predicts that diabetes will affect 30% of Malaysian persons aged 18 and over by 2025. Thus, prevention and intervention of complications is important to reduce morbidity and socioeconomic costs (Institute for Public Health, 2020).

Diabetes can be categorised as type 1 and type 2 (Antar *et al*., 2023). Among those types, more than 90% are type 2 (T2D), caused by a progressive loss of adequate β-cell insulin secretion frequently on the background of insulin resistance (American Diabetes Association Professional Practice, 2021). T2D is well-known to affect patients' renal, vision, nerve, and cardiovascular systems (Picke *et al*., 2019).

Individuals with sedentary lifestyles, obesity, and a lack of physical activity are more vulnerable to this incidence (Li *et al*., 2022). In addition to diabetic medication, lifestyle modifications such as increased physical activity, a nutritious diet, and weight loss (for those who are overweight or obese) are crucial for reducing the risk of T2D and enhancing general health (Syeda *et al*., 2023).

1.2 T2D in postmenopausal women

T2D can be developed at any age, but more likely to people at 35years and above (American Diabetes Association Professional Practice, 2021). Besides, T2D develops mostly in postmenopausal women, compared to premenopausal women (Li et al., 2019). During menopause, phenotypical and metabolic changes will influence body weight, adipose tissue distribution, energy consumption, insulin secretion, and sensitivity (Paschou and Papanas, 2019). Thus, menopausal women who are deficient in the hormone estrogen are more likely to develop non-communicable diseases such as T2D, osteoporosis, cancer, and coronary heart disease (MOH, 2022).

1.3 Bone health issues in aging and T2D people

Numerous physiological changes associated with aging include changes in muscle mass, bone density, and joint integrity. Osteoporosis is frequently linked to aging and characterized by reduced bone mass, increased bone fragility, and an elevated risk of fracture (Gulzada and Khurram Shahzad, 2024; Walker, 2023). Worldwide, 1-in-3 women aged 50 years and above are diagnosed with osteoporosis and may experience a fragility fracture (IOF, 2023). Osteoporosis is classified as BMD below the standard deviation (SD) of -2.5, compared to general population that is stated as a T-score, derived from dual-energy x-ray absorptiometry (DEXA) (Salari *et al*., 2021; Schini *et al*., 2024; Vilaca *et al*., 2022).

T2D is associated with various complications, including bone fragility and associated with an increased risk of fracture (Faienza *et al*., 2022; Linde *et al*., 2018). People with T2D have a 40–70% increased risk for fractures at any skeletal site, despite preserve a normal to increased bone mineral density, suggesting that other factors besides bone quantity must account for increased bone fragility (Picke *et al*.,

2019). The elevated fracture risk in people with T2D could also be attributable to a lower bone quality, which includes bone mass, bone turnover, and bone material qualities (Linde *et al*., 2018).

1.4 Bone condition of people with T2D and the circulation of sclerostin

The skeletal system is incredibly dynamic and goes through repair and remodelling (Alramah *et al*., 2024). Regular bone remodelling is required for healing of fractures and skeletal adaption to mechanical applications. An imbalance between the production of bones and resorption processes can cause disorders relating to the bones, such as osteoporosis (Alramah *et al.*, 2024). However, individual with T2D have a nearly twofold increased incidence of fractures compared to healthy people, although BMD value is normal or above normal compared to healthy individuals (Faienza *et al.*, 2022; Linde *et al*., 2018).

Previous studies reported that serum sclerostin is increased in T2D and become a risk factor to fragility fracture in people with T2D (García-Martín *et al*., 2012; Napoli *et al*., 2017). Besides, circulating levels of sclerostin have been associated with low BMD in patients with T2D (Wang *et al*., 2017). Sclerostin acts as an inhibitor of bone formation and generated by osteocytes that acts as an antagonist for the Wnt/β-catenin signaling pathway, which controls bone formation by osteoblasts (Baron and Rawadi, 2007; Van Bezooijen *et al*., 2004). Sclerotin is the biochemical marker encoded by the SOST gene which is related to bone and glucose metabolism (Janik *et al*., 2018; Marzullo *et al*., 2021; Meier *et al*., 2023). Higher sclerostin levels result in decreased bone material or, in other words, increased bone resorption (Vasiliadis *et al*., 2022).

Researchers are interested to know the effects of sclerostin and its relationship to bone development. Previous study found that sclerostin levels were associated to

vertebral fractures in postmenopausal women with T2D compared to healthy individuals, indicating that sclerostin may contribute to diabetes-related bone fragility (Black and Rosen, 2016). Recent study then showed that sclerostin level is significantly increased in individual with T2D, compared to non-T2D individuals and it showed a significant positive correlation with gender, age, fasting blood glucose, HbA1c and insulin (Alramah *et al*., 2024).

Currently, sclerostin inhibitors are being tested in clinical trials to promote bone growth in premenopausal and postmenopausal women, positive results without adverse side effects (Aditya and Rattan, 2021). Earlier, a study by Ardawi *et al*., (2012) reported that physically active premenopausal women (>120 min/week of PA, measured by accelerometer) have lower sclerostin levels, compared to less active women (<30 min/week). This indicated that it is possible that physical activity may be a natural inhibitor of sclerostin levels. However, there are no data about sclerostin levels in relation to physical activity in T2D postmenopausal women.

1.5 The significance of physical activity to sclerostin level and T2D

World Health Organization (World Health Organization. WHO, 2020) has established the significance of physical activity as a preventive tool to manage and overcome non-communicative diseases, such as heart disease, stroke, cancers, and type 2 diabetes. Regular and sufficiently engaged in PA prevents premature death and improves bone health and muscle strength (Bull *et al*., 2020).

Modernization and technology are evolve rapidly, favoring the occurrence of sedentary behavior, as recent occupational roles and lifestyle shift to be less active and more time is spent sitting (Farhana *et al*., 2022; Park *et al*., 2020). While exercise is strongly advised, WHO also noted that sedentary behaviour should also be reduced

(Siddiqi, 2021). Regular participation in sports activities and or being physically active could minimized the physiological alterations due to ageing and may contribute to healthier lives and well-being (Angba, 2022).

WHO recommended that adults and older adults should do minimum moderate-intensity aerobic PA for at least 150-300 minutes a week or 75-150 minutes of vigorous-intensity PA or a combination of both moderate and vigorous-intensity PA throughout the week for a good health outcome (World Health Organization. WHO, 2020). In addition, International Diabetes Federation (IDF) also stated that the combination of aerobic exercise and resistance exercise is the most effective way to prevent and treat T2D, as well as reducing the sedentariness (Forouzanfar *et al*., 2016).

Studies also showed a negative correlation between physical activity and increased sclerostin, but the specific influence of physical activity on the sclerostin level remains unclear (Oniszczuk *et al*., 2022). Ardawi *et al*., (2012) did a longitudinal study on active premenopausal and sedentary women (using the accelerometer to measure their PA). From the study, physically active women showed lower sclerostin levels compared to their sedentary counterparts. Then, Catalano and colleagues shared their study findings that premenopausal participants with BMI 30kg/m^2 or less who went for physical training for 8 weeks (running, cycling, set up, stretching, and mobilise upper and lower body) showed lowered sclerostin levels by 34% compared to pre-test, while no significant differences within-group change in sclerostin levels for their sedentary control group (Catalano *et al*., 2020). However, recent review studies have reported that the type, intensities, regulations and frequency of the exercise, as well as the number of body parts involved during the exercise, will influence the sclerostin level. Besides, more recent outcomes studies proved that

moderate to vigorous exercise will inhibit protein secretion, and promote higher BMD (Oniszczuk *et al*., 2022).

While various studies showed physical activity may be a natural inhibitor of sclerostin level, data on sclerostin level in relation to physical activity in postmenopausal women with T2D are still lacking. With few studies that investigates bone health in postmenopausal women who also have T2D, this study intends to provide insights to the relation of objectively measured physical activity levels and sclerostin in postmenopausal women with T2D. By examining the association between different physical activity levels measured objectively and sclerostin levels in T2D postmenopausal women, we will have the initial evidence to determine if physical activity may be a positive step towards better bone health and diabetic control in T2D postmenopausal women.

1.6 Problem statement

Diabetes mellitus is a risk factor for osteoporotic fractures. Sclerostin is an inhibitor of bone formation. However, there are no data about sclerostin levels in T2D. As both T2D and osteoporosis are prevalent conditions that severely affect quality of life and incur exorbitant medical costs, the possibility of physical activity to prevent or treat both issues of skeletal fragility and blood glucose control in T2D postmenopausal women requires immediate attention. By examining the association between different physical activity levels measured objectively and sclerostin levels in T2D postmenopausal women, we will have the initial insight to determine if physical activity may be a positive step towards better bone health and diabetic control in T2D postmenopausal women.

1.7 Objectives of study

1.7.1 General objectives

To investigate physical activity level, sedentary time and bone mineral density in relation to sclerostin level in postmenopausal women with type 2 diabetes mellitus (T2D).

1.7.2 Specific objectives

I. To identify the association of moderate-to-vigorous physical activity levels (MVPA) to sclerostin levels in post-menopausal women with T2D.
II. To identify the association of sedentary time (SED) to sclerostin levels in post-menopausal women with T2D.
III. To identify the association of MVPA with SED to sclerostin levels in post-menopausal women with T2D.
IV. To identify bone mineral density in relation to sclerostin level in postmenopausal women with T2D.

1.8 Hypothesis of the study

I. Ho_1: There are no significant association of moderate-to-vigorous physical activity levels (MVPA) to sclerostin levels in post-menopausal women with T2D.

   H_{A1}: there are significant association of moderate-to-vigorous physical activity levels (MVPA) to sclerostin levels in post-menopausal women with T2D.

II. Ho_2: There are no significant association of sedentary time (SED) to sclerostin levels in post-menopausal women with T2D.

   H_{A2}: There are significant association of sedentary time (SED) to sclerostin levels in post-menopausal women with T2D.

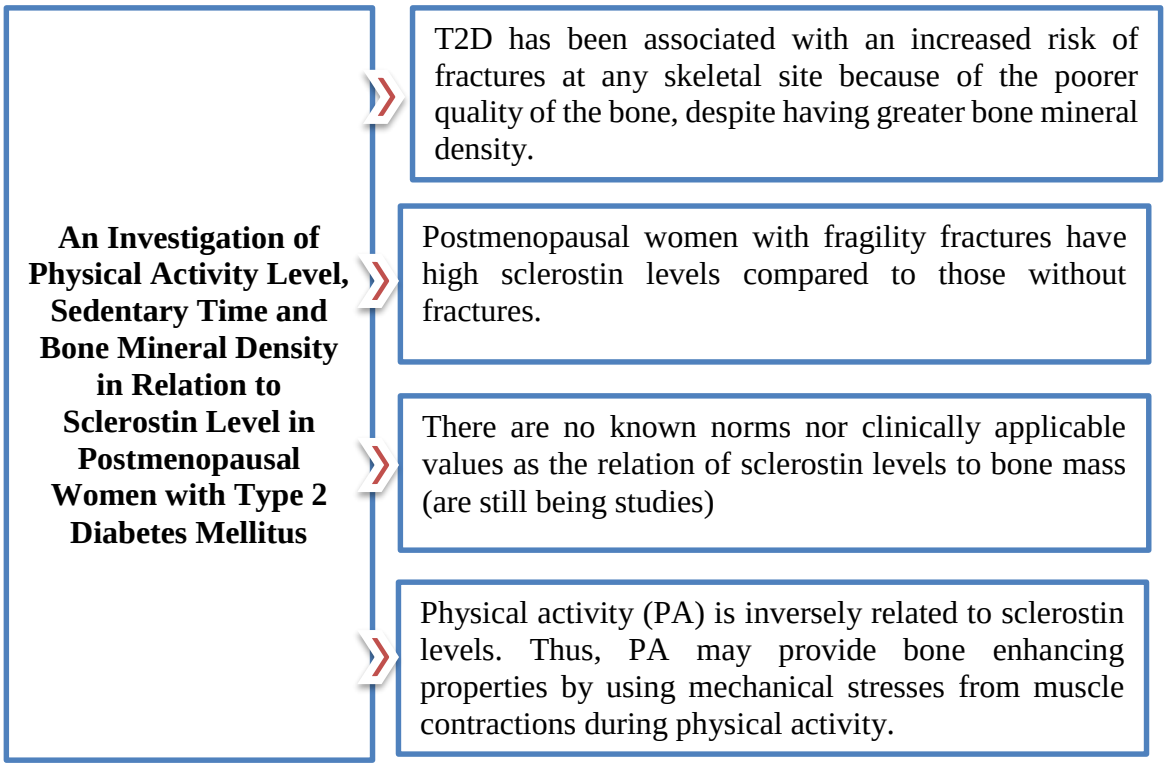
III. Ho_3: There are no significant association of MVPA with SED to sclerostin levels in post-menopausal women with T2D.

H_{A3}: There are significant association of MVPA with SED to sclerostin levels in post-menopausal women with T2D.

IV. Ho_4: There are no significant association of bone mineral density in relation to sclerostin level in postmenopausal women with T2D.

H_{A4}: There are significant association of bone mineral density in relation to sclerostin level in postmenopausal women with T2D.

1.9 Conceptual Framework



CHAPTER 2

LITERATURE REVIEW

2.1 Postmenopausal women with T2D

More than half a billion people are living with diabetes globally, and it affects every group of age, including women (IDF Diabetes Atlas, 2021). Among diabetes, T2D encounters 90-95% of the prevalence (WHO, 2022). T2D is the most common chronic disease in postmenopausal women, as the risk of having T2D is high in the postmenopausal phase and they are susceptible of having early menopause (Li *et al*., 2019). In Malaysia, the National Diabetes Registry reported that the prevalence of T2D is high among Malay women aged 50-59 years (Chandran *et al*., 2019).

Drastic hormonal changes during midlife age due to ovarian aging and the menopausal transition play a prominent factor that changes almost everything in women's lives (Sipilä *et al*., 2020). Besides, the progressive transition from perimenopause to postmenopausal will later lead to insulin resistance and increased risk of metabolic syndrome such as T2D, cardiovascular disease (CVS), and osteoporosis (Harlow *et al*., 2012, Li *et al*., 2019; The Lancet Diabetes & Endocrinology, 2022).

Menopause is the decline of endogenous oestrogen and the permanent cessation of menstruation after the end of follicular activity. A woman is considered to have reached menopause after 12 consecutive months of amenorrhoea (Cheer *et al*., 2022; Opoku *et al*., 2023). World Health Organisation (WHO) stated that menopause occurs generally at the age of 45-55 years worldwide (World Health Organisation, 2023), while the menopausal age in Malaysia is around 50 years old (MOH, 2022).

2.2 Risk factors for T2D among postmenopausal women and their body composition

People are more likely to develop diabetes as a person ages 35 and above, and the risk is increased as the person gets older (National Institutes of Health, 2022). In postmenopausal women, the risk factors associated with developing T2D are increased due to the menopausal transition phase that contributes to the increasing fat mass and dyslipidaemia and, later will result in the prevalence of overweight and obesity (The Lancet Diabetes & Endocrinology, 2022).

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a health risk. Both are characterized by the body mass index (BMI, kg/m^2) where total body weight in kg is divided by body height in meters squared (m^2). An individual is considered overweight when their BMI is over 25 kg/m^2, while obese is over 30 kg/m^2 (World Health Organization, 2023).

Besides, people spending more time sitting and living a sedentary lifestyle will increase their diabetes risks (National Institutes of Health, 2022). Women should also be physically active, reduce their BMI to not more than $25kg/m^2$, and lower their abdominal fat (waist-hip circumference, WHR) to less than 35 inches, as a larger WHR will increase the risk of diabetes and heart disease, even if they have a normal BMI (National Institutes of Health, 2022).

2.2.1 Obesity among postmenopausal women with T2D

Obesity itself has become a global pandemic and is terrifying people's lives as it could affect almost every organ system (Ruze *et al*., 2023). In almost every country, obesity is more prevalent in women than men, especially in the middle and older age groups (Opoku *et al*., 2023). A study in the US stated that over 43% of their cohort of

postmenopausal women are obese and the prevalence is also contributing to the leading risk factor of T2D (Hales *et al*., 2020).

The increasing prevalence of T2D significantly parallels to the increases in overweight and obesity, especially abdominal obesity (Hussein *et al*., 2015). For a study to define level of physical activity among T2D cohort Shazwani *et al*., (2010) reported that abdominal obesity is significantly more prevalent in diabetic participants (mean age 59 years), compared to male cohort. Meanwhile, a cross-sectional study in India showed that obesity is a highly prevalent comorbidity in diabetic women participants, where abdominal obesity was reported to be the highest type of obesity and was also associated with physical inactivity and hyperglycemia (Vasanthakumar and Kambar, 2020).

People with obesity have a BMI of more than 30kg/m^2, but a BMI higher than 35kg/m^2 is up to 20 times at risk of the prevalence (Pan and Yeh, 2008). Recent dietary trends that consume energy-dense foods, which are high in fat and free sugar, have contributed to the imbalance of calories consumed and calories expanded. These factors become the fundamental cause of overweight and obesity and its associated complications, including blindness, limb amputations, and kidney failure (World Health Organization, 2023). Besides, severe obese also contributed to muscle weakness, poor gait and mobility, and balance limitation, which led to falls and fractures (Amato, 2022).

Among postmenopausal women, the menopausal transition affected lipid metabolism, energy consumption, insulin resistance, and body fat composition and later resulted in excessive fat accumulation in adipose tissue, skeletal muscle, and abdominal area (Kim and Ko, 2023; Opoku *et al*., 2023). Postmenopausal women with obesity are also at higher risk of non-vertebral fracture compared to healthy people,

although peripheral fat tissue was once established to influence bone health positively (Marzullo *et al*., 2021).

2.2.2 Body mass index and bone mineral density

Previous epidemiological and clinical studies showed that high level of fat mass might be the risk factor for osteoporosis and fragility fracture (López-Gómez *et al*., 2022). DEXA scanner could measure bone mineral density (BMD) and it also measures lean mass index (LMI, kg/m^2) and appendicular lean mass index (ALMI, kg/m^2) of the participants. The appendicular skeletal muscle mass index (ASMI) is an important risk indicator for osteoporosis due to the anatomical proximity and metabolic connection between muscle and bone mass (da Cruz *et al*., 2021). Total body lean mass and appendicular lean mass absolute value can be normalized to the body mass index, BMI (kg/m^2), which account for allometric differences in body size, and later obtaining the LMI or the ALMI that enable the comparisons among the different subjects independently of their body size *(Minetto et al*., 2021).

Table 2.1 The cut off points proposed to discriminate between the normal and low lean mass

Variables	Men	Women	Reference
ALMI (kg/m^2)	7.26	5.45	Baumgartner *et al*., 1998
ALM (kg)	19.75	15.02	Cawthon *et al*., 2014
ALM/ BMI	0.79	0.512	Cawthon et al., 2014
ALM (kg)	20	15	Cruz-Jentoft *et al*., 2019
ALMI (kg/m^2)	7.0	5.5	Cruz-Jentoft et al., 2019
LMI (kg/m^2)	14.58	12.14	Suetta *et al*., 2019
ALMI (kg/m^2)	6.60	5.03	Suetta et al., 2019

Appendicular Lean Mass (ALM), Appendicular Lean Mass Index (ALMI), Body Mass Index (BMI), Lean Mass Index (LMI) (Minetto *et al*., 2021)

2.3 T2D, osteoporosis and other risk factor for bone fracture among postmenopausal women

T2D and osteoporosis are common metabolic diseases among the elderly *(Si et al*., 2020). Osteoporosis is a disease characterized by low mineral bone mass due to the deterioration of bone tissue micro-architectural, which makes it susceptible to bone fragility and fractures (Lin *et al*., 2021). Recent statistics on osteoporosis stated 37 million fragility fractures among individuals aged over 55 years occur every year, which is equivalent to 70 fractures per minute (Wu *et al*., 2021). Besides, the prevalence is estimated to occur about 40% of the postmenopausal time, where the weakened skeletal structure and strength gradually decrease, later predisposing to increasing of bone fragility and increases risk of fracture, commonly at the hip, vertebral and wrist areas (Abdulameer *et al*., 2018).

Meanwhile, people with T2D are also at high risk of fractures, although their bone mineral density (BMD) often normal or even elevated compared to non-T2D counterparts (Viggers *et al*., 2020). Poiana and Capatina stated people with diabetes, both T1D, and T2D significantly increase the risk of fracture at the area of vertebral, hip and all non-vertebral (Poiana and Capatina, 2017), while Picke and team added that people with T2D are at 40-70% increased risk for fracture, especially at the hip, wrists and feet (Picke *et al*., 2019).

In T2D, bone mineral density (BMD) may underestimate the risk of low-energy fractures as bone quality is reduced and insufficiently predicted by BMD, which is estimated by a dual-energy X-ray absorptiometry (DXA) scan (Baleanu *et al*., 2019). The fracture risk may be attributed to the reduction of bone quality, as well as impairments in bone material properties and increases in cortical porosity *(Khosla et*

al., 2021). The condition has led to a hypothesis that people with T2D may have abnormalities, probably in bone microarchitecture or the material composition that affects the decline in bone quality (Piccoli *et al*., 2020). The pathogenesis of increased fracture risk in T2D may also be due to low bone turnover caused by osteocyte dysfunction, resulting in increased cortical porosity, bone micro-cracks, and fracture (Linde *et al*., 2018). Besides, altered collagen structure from advanced glycation end-products (AGE) due to constant high blood glucose levels in T2D people may also be the cause that affects cortical structure and quality, and consequently will reduce bone strength (Picke *et al*., 2019)

The development of T2D will also increase micro and macrovascular complications where these people are at risk of higher propensity for falls and decreased bone quality (Napoli e*t al*., 2018; Sarodnik *et al*., 2022). Besides, longer disease duration and poor glycemic control could also negatively affect a patient's quality of life, which includes fragility fractures (Ferrari *et al*., 2018).

Various methods have been used to study the bone quality among T2D and non-T2D people. Trabecular bone score (TBS) is an example tool for predicting lower bone quality in TD2 patients as it could provide information regarding bone quality independent of bone mineral density (BMD) in T2D people. Besides, high-resolution peripheral quantitative CT (HRpQCT) is also an example of current bone imaging technology that capture volumetric BMD of peripheral sites. In addition to BMD and geometric measures, high-resolution peripheral QCT (HR-pQCT) provides quantitative access to bone microarchitecture, which affects bone quality and strength. In these two decades, this has been used to demonstrate the increased cortical porosity in diabetic postmenopausal women and helped our understanding of how T2D adversely impacts both bone metabolism and fracture risk (Rubin and Patsch, 2016).

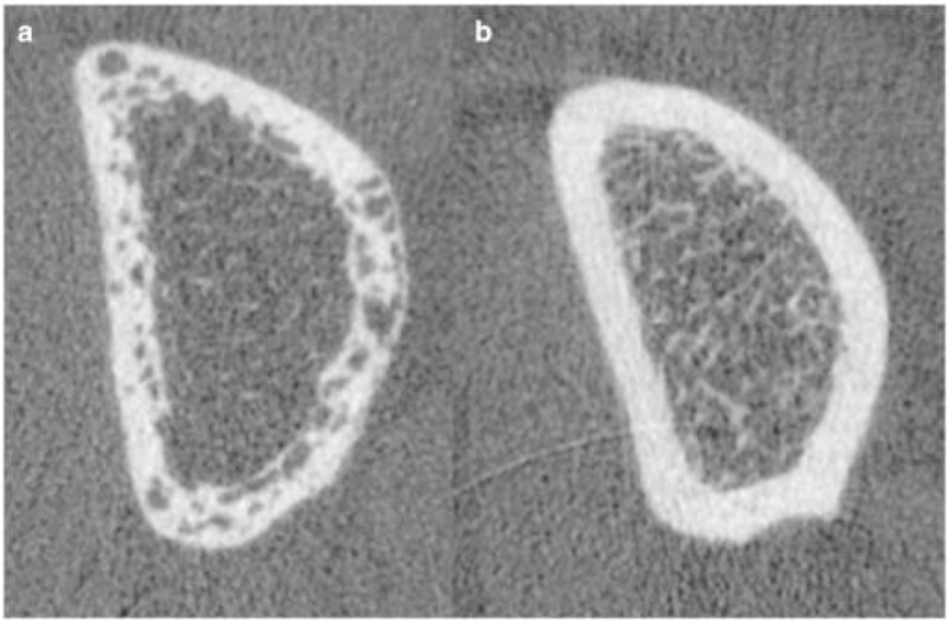


Figure 2.1 Cortical porosity in diabetic bone disease with fractures.

Figure 2.1 above is an image courtesy of Thomas M. Link, Department of Radiology and Biomedical Imaging, The University of California, San Francisco (Rubin and Patsch, 2016). The figure shows the high-resolution peripheral quantitative computed tomography (HR-pQCT) of the distal radius in type 2 diabetic women. The left side (a) showed the bone fragility of women with T2D, while on the right side (b) is without bone fragility fractures and corticol area seem denser.

Another tool to study bone strength is using Bone Mineral Strength Index (BMSi) in postmenopausal women. It had been proven that impairment in bone materials is associated with long duration of having T2D and poor glycaemic control in individuals (Farr and Khosla, 2016)

2.3.1 Daily calcium intake

Sufficient calcium intake also plays a key role in maintaining skeletal mineralization which store 99% of body calcium (Shlisky *et al*., 2022). Calcium deficiency due to inadequate intake contribute to the cause of reduced bone mass and osteoporosis (Ministry of Health Malaysia, 2017). It is recommended that daily calcium intake for Malaysian women aged 50 and above is 1200mg/day for daily intake (Ministry of Health Malaysia, 2017).

Inadequate calcium intake was also recorded among local younger group in Malaysia with mean calcium intake, 351±227 (mg/day), which attained 29% from the recommended intake (Ismail *et al*., 2020). Balk and team reported that inadequate calcium intake was recorded in many Asian countries with average dietary calcium intake less than 500 mg/day, while countries in Africa and South America was between 400 and 700 mg/day (Balk *et al*., 2017). Low- and middle-income countries (LMICs) are at greatest risk of low calcium intakes as compared to high-income countries (HICs). Northern European, for instance have national calcium intake greater than 1000 mg/day, while American ranging from 918 to 1296 mg/day for daily calcium derived from food and supplements intake. Paradoxically, osteoporotic fracture showed lower rates in LMICs, compared to HICs (Shlisky *et al*., 2022).

2.3.2 Treatment and medication

Drugs or hormone intake for treatment or medication purposes such as sex steroids/hormone-replacement therapy, glucocorticoids, warfarin, bisphosphonate, teriparatide, denosumab, strontium ranelate, tamoxifen, anticonvulsants, antacids, and other similar medication classes could affect bone metabolisms (Pitts and Kearns, 2011). In addition, medication for rheumatoid arthritis, hyper/hypothyroidism, kidney diseases stage IV & V could also affect bone metabolism (Unnanuntana *et al*., 2011).

2.4 Sclerostin as bone markers

The higher bone mass in patients with T2D does not sufficiently protect against fractures, suggesting that other factors are likely responsible for this increased fracture risk (Farr and Khosla, 2016). Our skeletal structure requires balance of bone resorption and bone formation to achieve its optimal bone health condition (Herbert *et al*., 2019). However, T2D is associated with reduced bone turnover, perhaps with an imbalance between bone resorption and bone formation (Costantini and Conte, 2019). One possible vital factor underlying the low bone turnover and contributor to fracture risk is the increased of circulating serum sclerostin (Rubin and Patsch, 2016).

Sclerostin is a bone inhibitor that involved in bone formation (Piccoli *et al*., 2020). Sclerostin is produced by osteocytes and acts as the antagonist for Wnt/β-catenin signaling pathway, the regulator of bone mass (Baron and Rawadi, 2007; Van Bezooijen *et al*., 2004). Osteocytes play a major role to control the balance of bone resorption and bone formation, due to any mechanical stimuli to the muscle (Rosen, 2019).

Since long time ago, researchers are interested to know the effects of sclerostin and its relationship to bone development. Serum sclerostin is increased in T2D and become a risk factor to fragility fracture in people with T2D (García-Martín *et al*., 2012; Napoli *et al*., 2017). Circulating levels of sclerostin have been associated with low BMD in patients with T2D (Wang *et al*., 2017). Sclerostin levels were also found to be related to vertebral fractures in T2D women independent of lumbar BMD, bone turnover and diabetic medications (Meier *et al*., (2023); Yamamoto *et al*., (2013). Among postmenopausal women with T2D are also associated to vertebral fractures as compared to healthy individuals, indicating that sclerostin may contribute to diabetes-

related bone fragility (Black and Rosen, 2016). Individual with T2D that is significantly increase in sclerostin level also showed a significant positive correlation with gender, age, fasting blood glucose, HbA1c and insulin (Alramah *et al*., 2024).

Recent study by Sylvawani *et al*., (2021) among T2D and non-T2D group of premenopausal women showed that serum levels of sclerostin were significantly higher in the T2D group than in the non-T2D group (132.1 pg/mL and 96.0 pg/mL, respectively; $p < 0.001$). However, study by Ardawi and colleagues reported that physically active premenopausal women have lower sclerostin levels, with accumulating 60-120 min/week and >120 min/week of PA (measured by accelerometry) having 21.6±6.2 pmol/L and 17.6±4.2 pmol/L sclerostin, respectively. This is significantly lower ($p<0.001$) compared to less active women (<30 min/week) with averaged sclerostin levels at 27.8±5.0 pmol/L (Ardawi *et al*., 2012). It indicated that physical activity may be a natural inhibitor of sclerostin levels. However, there are no data about sclerostin levels in relation to physical activtiy in T2D postmenopausal women.

With regards to sclerostin levels, there are no known norms nor clinically applicable values as the relation of sclerostin levels to bone mass are still being studied. In addition, Modder and colleagues noted that postmenopausal women on estrogen therapy had significantly lower sclerostin levels, 22.7±1.2 pmol/L ($p<0.001$), than postmenopausal women not on estrogen therapy 27.8±0.8 pmol/L (Mödder *et al*., 2011). Ardawi and colleagues reported that postmenopausal women with fragility fractures have high sclerostin levels, 77.7±12.3 pmol/L, compared with those without fractures, 50.1±13.1 pmol/L (20). Thus, sclerostin levels may be one other indicators of the likelihood of having a fragility fracture in healthy, postmenopausal women.

Therefore, data about sclerostin levels in relation to physical activity are needed as it might be useful as a natural inhibitor of sclerostin levels and may promote bone health in T2D postmenopausal women (Mödder *et al*., 2011). Both T2D and osteoporotic prevalent demand high cost of treatment and worsening with postmenopausal effect, duration of T2D, physically inactive and living with sedentary lifestyles. However, physical activity can affect the level of sclerostin, but depend on the level and types of the PA. (Oniszczuk *et al*., 2022)

2.5 The importance of physical activity for postmenopausal women and T2D

Over the years, WHO has highlighted the importance of physical activity (PA) and reducing sedentary behaviour as effective ways to prevent the rising prevalence of T2D (World Health Organization, 2023). Physical activity itself is defined as voluntary movements related to physical mobility that include exercise, sports, occupational and household task and activities of daily living (Caspersen *et al*., 1985). Meanwhile, exercise is the subset of physical activity that is planned, structured, and repetitive and has a final or an intermediate objective for the improvement or maintenance of physical fitness (Caspersen *et al*., 1985). Sedentary behavior involve sitting and low levels of energy expenditure such TV viewing, computer use, or sitting in an automobile typically are in the energy-expenditure range of 1.0 to 1.5 METs (multiples of the basal metabolic rate) (Tremblay *et al*., 2017)

Exercise is a recommended strategy to increase BMD and strengthen the bone, especially with resistance and impact-related exercise program (Pinheiro *et al*., 2020). During exercise, the muscles exert mechanical forces upon bone and the stimulus directs bone to develop and become stronger (Janik *et al*., 2018). This follows the mechanostat theory proposed by Harold Frost where bone adapts to loads and result in

bone formation, maintenance or resorption as a response to withstand forces or to maximize energy for movement (Frost, 2003). Viggers *et al*., (2020) also summarized that a combination of aerobic exercise such as jogging or walking plus resistance training using free weight or band is recommended as prevention and treatment for osteoporosis and T2D. Thus, physical activity may provide bone enhancing properties by using mechanical stresses from muscle contractions and impact forces for example from jumps that is conducted from targeted exercises.

Exercise is also recommended for diabetes management where muscles provide a pathway in blood glucose management by promoting glucose uptake via muscle contractions that are independent of insulin action a (Colberg *et al*., 2010). Physically active person will benefit positive health outcome and experience good quality of life (Bull *et al*., 2020). Besides, being physically active was indicated to lower the fracture risk and reduce osteoporotic prevalence in postmenopausal women (Pinheiro *et al*., 2020). Thus, physical activity is proven to be a beneficial mechanism to not only control blood glucose levels in postmenopausal women with T2D but may also bring positive benefits to bone health.

2.6 Physical Inactivity and Sedentary Behavior

The evolution of technology due to modernization and rapid development has changed the nature of work as occupational and daily tasks mostly are assisted but more efficient and less labour intense (Woessner *et al*., 2021). These have caused a decrease in physical activity, a changes in lifestyle and healthy diet where people become inactive and live sedentary lifestyles, predispose to the occurrence of overweight and obesity (IDF Diabetes Atlas, 2019; Lee *et al*., 2022).

In Malaysia, the National Health and Morbidity Survey in 2019 reported that 1 in 4 adults was physically inactive and the rate was higher compared to other Asian countries such as China, India and Hong Kong (Alias *et al*., 2022). Worldwide, 1 in 3 women was reportedly inactive (World Health Organization. WHO, 2020). In high-income countries, physically inactive and sedentariness is twofold high compared to low-income countries (World Health Organization. WHO, 2020). Due to the evolution of technology that makes occupational and daily tasks more efficient with less labour, people have become more inactive and sedentary (Woessner *et al*., 2021).

Sedentary behaviour has been highlighted as one key contributing factor to the prevalence of overweight and obesity and caused the increasing of co-morbidities including type 2 diabetes and cardiovascular disease (Chau *et al*., 2013; Panahi and Tremblay, 2018; Petersen *et al*., 2014).

Sedentary behaviour can be an independent predictor for metabolic disease risks (Panahi and Tremblay, 2018). Despite achieving the physical activity recommendation levels, large amount of sedentariness or prolonged sitting behaviour is associated to adverse health outcomes such as increased risks of obesity, metabolic syndrome, cardiovascular diseases type 2 diabetes, colon cancer and musculoskeletal disorders (Chau *et al*., 2013; Farah Farhana et al., 2022). Besides, Park and colleagues presented that risk of getting T2D was significantly increased with increased daily sedentary time (Y. M. Park et al., 2021).

Sedentary behaviour is defined as engaging in sociocultural activities that reduce human energy expenditure by 1.5 metabolic equivalents (METs) above resting (Thivel *et al*., 2018). Meanwhile, for movement observation using accelerometer, people are considered to be sedentary when their count per minutes (cpm) is less than 99 cpm (Troiano *et al*., 2008). Activities such as sleeping, sitting for a long time,

commuting in the workplace and at home, and lying down is always are always associated to sedentary behaviour (Thivel *et al*., 2018).

In the modern era, being sedentary means making use of technology and engaging in more screen-based activities. People spend the majority of their screen time on computer-related tasks, watching TV, or using social media (Nakshine *et al*., 2022). Besides, times spent during driving or travel for working has also contributed for sedentary activity (Mackay *et al*., 2019)

2.7 The association of sedentary time, moderate, vigorous and moderate to vigorous physical activity (MVPA) with sclerostin level

WHO had stated that adult age 19-64 years, as well as people living with chronic condition are suggested to do brisk walking, cycling on ground level, gentle swimming and a few other moderate physical activities for 150-300 minutes per week to obtain good health outcome (World Health Organization. WHO, 2020). Studies proved that moderate to high levels of physical activity and cardiorespiratory fitness are associated with substantially lower morbidity and mortality in people with diabetes.

Previous studies proved that sclerostin levels are inversely proportional to the increasing time of moderate activity. Janik *et al*., (2018) did a study where a cohort of 50-75 years women with osteopenia showed their sclerostin was significantly lower after involved for scheduled cycling training for 12 weeks. Then, Armamento-Villareal and team also did a study among the cohort of older adult aged ≥65 years and obese (BMI ≥30 kg/m^2). The participants were randomly assigned to control, diet, exercise and combined diet-exercise group for one year. The result showed sclerostin level was increased in diet group but become lower in diet with exercise group. Their

study also found that increasing sclerostin levels with weight loss was prevented by exercise as it partly mediates the negative effects of weight loss on bone metabolism and the osteoprotective effect of exercise training (Armamento-Villareal *et al*., 2012).

Meanwhile, studies also showed significantly and positive association of vigorous PA with the sclerostin level. Kouvelioti and team for instance, they did a study on potential exercise-induced changes in sclerostin and in bone turnover markers in young and active women. The participants were with mean age of 22.5±2.7 years and they performed high intensity of interval running and cycling. The result showed a significant increase of sclerostin level after five minutes doing the two types of high intensities exercise. Interestingly, the sclerostin level return to baseline values after one-hour they finished the exercise. The study also showed that the result is not accompanied by a response in either bone formation or resorption markers (Kouvelioti *et al*., 2018). Besides, Sliwicka and team reported that among the healthy men, it also showed a 1.3 fold of sclerostin increase after 72hours they finished the marathon compared to baseline values (Śliwicka *et al*., 2021).

Anna and team summarised that MVPA is an appropriate PA that beneficial for bone health, as it could inhibit the secretion of sclerostin and induced the bone mineral density. Proper exercise will have an osteogenic effect (a process of creating new bone tissue), however, it must be based on the type, intensity, regularity and frequency of exercise and the number of body parts involved (Oniszczuk *et al*., 2022). Ardawi had proven that through a moderate exercise (30-120 minutes per week of walking), sclerostin level was decreased, but higher in bone formation markers (Ardawi *et al.,* 2012*).* According to WHO, adults and people with T2D should perform a combination of moderate and vigorous activity (MVPA) for minimum of 150

min/week to achieve minimal good health outcome (World Health Organisation WHO, 2010).

2.8 The physical activity assessment of adults with type 2 diabetes

For those with type 2 diabetes, programmed exercise was effectively proven in enhance the glycaemic control, reducing insulin dependent, anti-hyperglycaemic agents and insulin, and producing modest but sustained weight loss. Traditional self-report PA questionnaires has become a challenge due to imprecise, recall bias and lack of standardization (Celis-Morales *et al*., 2012). Luckily, the invention of the accelerometer since decades ago has shown a reliance and convincing method in objectively measure the body's acceleration in at least one of three orthogonal planes (anteroposterior, mediolateral, and vertical). The acceleration then will be converted to activity counts and reported as counts per minute and later to categorize PA or exercise into intensity levels (light, moderate, vigorous) (Chen and Bassett, 2005). There are many types and brands of accelerometer in the market today, but the most reliable and commonly used for research and intervention is G3TX of Actigraph (Pensacola, FL, USA) (Aadland and Ylvisåker, 2015). Another option is GCDC, an affordable model of accelerometer, long lasting battery and capable for y-axis record as well as measure vigorous activity (http://www.gcdataconcepts.com/GCDC_X16 - and 1D_User_Manual.pdf., 2016)

Accelerometer can measure the body's acceleration via tri-orthogonal plane (anteroposterior (z-axis), mediolateral (x-axis), and vertical (y-axis)) and later convert it into activity counts, counts per minute (CPM). Few years back, most of the device can only measure the body's acceleration via two planes only. CPM categorize

exercise into a few intensity levels, such as light, moderate and vigorous and even sedentary.

Troiano *et al*., (2008) mentioned that accelerometer-based cut points were used to classify the PA intensity of middle-aged to older adults with type 2 diabetes as sedentary, light, moderate, vigorous, and very vigorous. For light PA, the CPM is set at 100 to 2019, moderate was set at 2020 to 5998, while vigorous PA was set for count for over 5999 CPM (Moldovan *et al*., 2022). Examples for light PA including casual walking, doing household chores or activities of daily living. Brisk walking and bicycling are considered as moderate and vigorous PA includes sports activities produces large increases in breathing or heart rate, such as jogging, aerobic dance or bicycling uphill (Dupré *et al*., 2023). Adults and people with T2D should perform a combination of moderate and vigorous activity (MVPA) for minimum of 150 min/week to achieve minimal good health outcome (World Health Organisation WHO, 2010).