A STUDY OF *PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR GAMMA (PPARγ)* GENE VARIANT IN RELATION TO PHYSICAL ACTIVITY AND FAT INTAKE AMONG PRIMARY SCHOOL MALAY CHILDREN IN KOTA BHARU, KELANTAN

by:

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ABSTRACT

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Introduction: The missense mutation of Proline to Alanine at codon 12 *(Pro12Ala)* of *Peroxisome Proliferator Activated Receptor Gamma (PPARγ)* is one of the most critical genetic factors predisposing to positive energy balance that may lead to development of obesity.

Objectives: 1)To determine the association between *Pro12Ala* variant in the *PPARγ* gene and body mass indexs (BMI) status of Malay children, 2) to determine the differences of physical activity between subjects with and without *Pro12Ala*

variant in the *PPARγ* gene and 3) to determine the differences of fat intake between subjects with and without *Pro12Ala* variant in the *PPARγ* gene.

Methodology: One hundred and nineteen subjects (119) ages 9-11 years old from primary school in Kota Bharu, Kelantan were involved in this study. Anthropometric measurements: weight, height, percentage body fat, waist hip ratio and BMI were conducted. In order to determine the physical activity, activity counts of the subjects were recorded every 10 sec for 5 days (3 days of weekdays and 2 days of weekends) using accelerometer (Actigraph GT3X+). As for fat intake, a food diary was distributed to the subjects and subjects filled the diary for 2 days of weekdays and 2 days of weekends. Whereas, for genotyping, a 2ml of blood sample was collected from each subject through venipuncture. Genomic DNA was extracted from leucocyte of the blood. Afterward, High Resolution Melting (HRM) analysis was performed to identify the genetic variation of *Pro12Ala* in the *PPARγ* gene.

Results: From 119 subjects, 39.5% (n=47) were overweight, while normal weight subject was 60.5% (n=72). A statistical test of Pearson Chi square was performed, result showed that there was a significant association (p= 0.03) between *Pro12Ala* variant in the *PPARγ* gene in normal weight and overweight group, with allelic frequency among overweight children wildtype (*CC)* and heterozygous (*CG)* were 0.83 and 0.17 respectively and in normal weight group the allelic frequency of *Pro12* and *Ala12* were 0.92 and 0.08 respectively. However, there was no significant difference in activity counts between subject with and without *Pro12Ala* variant.

Thus, it is also reported that, there was no significant difference in fat intake of mutational and non mutational group of *Pro12Ala* variant.

Conclusion: There was an association between *Pro12Ala* variant in the *PPARγ* gene and BMI group of the subjects. However, there were no significant differences in physical activity and fat intake between the subject of mutation and non mutational group.

Dr Surini binti Yusoff : Supervisor

Dr Mohd Nidzam bin Jawis: Co-Supervisor

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ABSTRAK

KAJIAN TENTANG VARIASI GEN *PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR GAMMA (PPARγ)* DAN HUBUNGKAITNYA DENGAN AKTIVITI FIZIKAL DAN PENGAMBILAN LEMAK DI KALANGAN KANAK-KANAK MELAYU DI SEKOLAH RENDAH SEKITAR KOTA BHARU, KELANTAN.

Mutasi pertukaran antara Proline kepada Alanin di kodon 12 gen *Peroxisome Proliferator Activated Receptor Gamma (Pro12Ala PPARγ)* adalah salah satu daripada faktor genetik utama yang menyumbang kepada tenaga yang berlebihan dan mengakibatkan obesiti. Oleh itu, antara objektif kajian ini adalah untuk menentukan hubungkait antara variasi *Pro12Ala* pada gen *PPARγ* dan indeks jisim badan dalam kalangan kanak-kanak Melayu, menentukan perbezaan aktiviti fizikal antara subjek yang mempunyai mutasi gen dan tidak mempunyai mutasi *Pro12Ala* pada gen *PPARγ* dan menentukan perbezaan pengambilan lemak antara subjek yang mempunyai mutasi gen dan tidak mempunyai mutasi *Pro12Ala* pada gen *PPARγ.* Seramai seratus sembilan belas (119) subjek di sekolah rendah sekitar Kota Bharu, Kelantan yang berumur 9-11 tahun telah menyertai kajian ini. Ukuran antropometrik seperti berat, ketinggian, peratusan lemak dalam badan, nisbah pinggang dan pinggul serta indeks jisim badan telah diambil. Manakala analisa aktiviti fizikal dibuat berdasarkan kiraan aktiviti subjek yang direkod setiap 10 saat selama 5 hari (3 hari persekolahan dan 2 hari minggu) dengan menggunakan akselerometer (Actigraph GT3X+). Bagi pengambilan lemak, diari makanan telah diedar kepada para subjek untuk diisi selama 4 hari (2 hari persekolahan dan 2 hari minggu). Manakala, untuk analisa genetik, darah sebanyak 2ml telah diambil

daripada setiap subjek melalui prosedur tusukan di saluran darah venus. Genomik DNA telah diekstrak daripada sel darah putih. Kemudian teknik analisa *High Resolution Melting* (HRM) telah dijalankan untuk mengenalpasti variasi *Pro12Ala.* Keputusan menunjukkan, dalam kalangan 119 orang subjek, 39.5% (n=47) mempunyai berat badan berlebihan, manakala 60.5% (n=72) mempunyai berat badan normal. Ujian statistik Pearson Chi square menunjukkan terdapat hubungkait antara mutasi *Pro12Ala (P*= 0.03) dengan status berat badan normal dan berat badan berlebihan dengan frekuensi alel CC dan CG adalah 0.83 dan 0.17 dalam kumpulan subjek berat badan berlebihan dan 0.92 dan 0.08 dalam kumpulan subjek berat badan normal. Namun, tiada perbezaan yang signifikan dalam kiraan aktiviti dan pengambilan lemak antara subjek yang mempunyai mutasi dan tiada mutasi *Pro12Ala* di kalangan kanak-kanak Melayu Kota Bharu, Kelantan. Kesimpulannya, kajian menunjukkan terdapat hubungkait antara status indeks jisim badan dan kehadiran mutasi *Pro12Ala* pada gen *PPARγ.* Namun begitu tiada perbezaan terhadap aktiviti fizikal dan pengambilan lemak dikalangan subjek yang mempunyai mutasi dan tiada mutasi *Pro12Ala.*

ABSTRACT

A STUDY OF *PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR GAMMA (PPARγ)* GENE VARIANT IN RELATION TO PHYSICAL ACTIVITY AND FAT INTAKE AMONG PRIMARY SCHOOL MALAY CHILDREN IN KOTA BHARU, KELANTAN

The missense mutation of Proline to Alanine at codon 12 *(Pro12Ala)* of *Peroxisome Proliferator Activated Receptor Gamma (PPARγ)* is one of the most critical genetic factors predisposing to positive energy balance that may lead to development of obesity. Hence, the objectives of this study are to determine the association between *Pro12Ala* variant in the *PPARγ* gene and body mass indexs (BMI) status of Malay children, to determine the differences of physical activity between subjects with and without *Pro12Ala* variant in the *PPARγ* gene and to determine the differences of fat intake between subjects with and without *Pro12Ala* variant in the *PPARγ* gene. One hundred and nineteen subjects (119) ages 9-11 years old from primary school in Kota Bharu, Kelantan were involved in this study. Anthropometric measurements: weight, height, percentage body fat, waist hip ratio and BMI were conducted. In order to determine the physical activity, activity counts of the subjects were recorded every 10 sec for 5 days (3 days of weekdays and 2 days of weekends) using accelerometer (Actigraph GT3X+). As for fat intake, a food diary was distributed to the subjects and subjects filled the diary for 2 days of weekdays and 2 days of weekends. Whereas, for genotyping, a 2ml of blood sample was collected from each subject through venipuncture. Genomic DNA was extracted from leucocyte of the blood. Afterward, High Resolution Melting (HRM) analysis was performed to identify the genetic variation of *Pro12Ala* in the *PPARγ* gene. From 119 subjects, 39.5%

(n=47) were overweight, while normal weight subject was 60.5% (n=72). A statistical test of Pearson Chi square was performed, result showed that there was a significant association (*P*= 0.03) between *Pro12Ala* variant in the *PPARγ* gene in normal weight and overweight group, with allelic frequency among overweight children wildtype (*CC)* and heterozygous (*CG)* were 0.83 and 0.17 respectively and in normal weight group the allelic frequency of *Pro12* and *Ala12* were 0.92 and 0.08 respectively. However, there was no significant difference in activity counts between subject with and without *Pro12Ala* variant. Thus, it is also reported that, there was no significant difference in fat intake of mutational and non mutational group of *Pro12Ala* variant. As a conclusion**,** there was an association between *Pro12Ala PPARγ* and BMI group of the subjects. However, there were no significant differences in physical activity and fat intake between the subject of mutation and non mutational group.

CHAPTER 1

INTRODUCTION

Overweight is one of the most health related problems affecting children in developed countries. The Third Malaysian National Health and Morbidity Survey showed that the prevalence of overweight children was 5.4% (NHMS III, 2006). This number is increases each year and the overweight and obesity trends are also observed worldwide and has been viewed as a ‘global epidemic of obesity.

Generally, overweight is a multi factorial syndrome influenced by both environmental and genetic factors (Barbieri *et al*., 2005, Duran-Gonzalez *et al*., 2011). Environmental factors such as reduced physical activity, intake of energy-dense food, cultural and socioeconomic factors significantly contribute to the development of obesity (Duran-Gonzalez *et al*., 2011). A study stated that genetic factors account for 40-90% of the body mass index (BMI) variations among populations (Duran-Gonzalez *et al*., 2011).

There is a growing evidence suggesting that *Peroxisome Proliferator Activated Receptor Gamma (PPARγ)* may be one of the most critical genetic factors predisposing to positive energy balance and, ultimately obesity (Mela, 2005). Located in chromosome three and specifically expressed in adipose tissue, *PPARγ* is induced in adipocytes differentiation (Yen *et al*., 1997).

It induces the fibroblasts to differentiate into adipocytes hence leading to adipocyte differentiation and thus fat cell accumulation (Cecil *et al*., 2006). The more active the *PPARγ*, the higher the BMI and the risk for insulin resistance.

Beside genetic factor, lack of physical activity and excess caloric consumptions are some of the reasons for the development of obesity (Andersen, 2003). For instance, a cohort study in the USA revealed a higher increase in BMI for preadolescents who reported higher caloric intakes and less physical activity (Kiess *et al*., 2004). Indeed, a study reported that overweight children had significantly lower physical activity especially during weekend compared to normal weight boys (Lin *et al*., 2012). Moreover, engaging in sedentary behaviours such as television viewing is also one of primary factors of the current worldwide obesity epidemic.

Evidence also suggests that diet during childhood may have an important implication for the development of obesity and chronic diseases in later life. It has been reported that obese children consumed significantly larger amounts of total calories, protein and fat compared to the normal weight children (Soo *et al*., 2011). The causes of increased energy intakes include larger portion sizes, eating in restaurants and away from home, eating late at night, instant availability of energy dense food and fast food and frequent snacking (Kiess *et al*., 2004).

Variety of health consequences can occur due to pediatric obesity. The consequences and co morbidities of childhood obesity are not only suffered during childhood but also affect their adulthood. The consequences of obesity that commonly affect children and adolescent are increased growth then stunting, increased in fat free mass, early menarche, hyperlipidemia, increased heart rate and cardiac output, abnormal glucose metabolism and others (Schonfeld-Warden and Warden, 1997). These physical health problem then lead to lowered physical fitness consequently affecting their psycho and social wellbeing and lowering their quality of life (Lin *et al*., 2012). For example, obese children are frequently the target of discriminization and stigmatization. As a result, they always suffer significant psychosocial consequences such as being shamed, marginalized and rejected (Eisenmann, 2006).

As mentioned before, genetic influence is one of the major contributing factors in people with severe and early onset of obesity. However, there are also some individuals who develop obesity while others do not although they have obesity-related genes. The contribution of obesity-related genes is influenced by an interaction with environmental factors predisposing them to obesity, such as overeating and a sedentary lifestyle (Kiess *et al*., 2004). Because of the rising prevalence and health consequences of pediatric obesity, the assessment of factors that lead to overweight and obesity at an earlier stage of life is essential. Therefore, the present study was proposed to investigate the genetic factor of *PPARγ* gene variant which is one of the strong candidate gene influencing physical activity participation and dietary intake among Malay children

1.1 OBJECTIVES

The objectives of this study are:

1. to determine the association between *PPARγ* gene variant and BMI status of normal weight and overweight Malay children

2. to determine the differences of physical activity between subjects with and without *PPARγ* gene mutation

3. to determine the differences of fat intake between subjects with and without *PPARγ* gene mutation

1.2 SIGNIFICANCE OF THE STUDY

Findings of this study have the potential to enhance the epidemiologic data of *PPARγ* gene variant among overweight Malay children. Besides, this study also hoped to trigger further studies in larger cohorts with longitudinal data in order to understand the impact of *Pro12Ala* in *PPARγ* gene on growth and development of obesity among Malay children in Malaysia. Thus, this research finding might also provides a route for furthering our understanding of obesity susceptibility and its development, with potential to develop integrated and targeted strategies for behavioural such as diet, exercise and also pharmacological intervention.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of pediatric obesity

Obesity is a major risk factor for several common and important diseases such as type II diabetes, cardiovascular diseases, certain cancers and many more. It also inflicts large direct and indirect costs that drain healthcare and social resources (Skelton *et al*., 2009).

Obesity is defined as a condition of abnormal or excessive fat accumulation in adipose tissue, to the extent that health may be impaired (WHO,1998). Most epidemiological studies used BMI threshold to define obesity. Originally, a BMI of 25.0-29.9kg/m^2 represented pre-obesity, with BMI >30kg/m^2 defining obesity. The term overweight is commonly used to refer to a BMI in the range 25-29.9kg/m^2 (Gareth and Fruhbeck, 2009).

However, the interpretation of recommended BMI cut off point for determining overweight and obesity in Asian population is still debated. WHO consultants concluded that Asian generally have a higher percentage of body fat than the Caucasian of the same age, sex and BMI (WHO 2004). Thus, current WHO cut off-off points do not provide an adequate basis for taking action on risk related to overweight and obesity in most Asians.

Overweight is one of the problem affecting children in developed countries. The prevalence of pediatric obesity in Malaysia is increasing. A survey done by Ministry of Health Malaysia in 2001/2002 and 2007/2008 reported that there is an increasing in prevalence of overweight and obesity in children age 6 to 12 years in Peninsular Malaysia. For the overweight children, the percentage increases from 11% in 2001/2002 to 12.8% in 2007/2008. As for the obese children, the percentages increase from 9.7% in 2001/2002 to 13.7% in 2007/2008 (Ministry of Health Malaysia, 2008).

Therefore, assessment of obesity in children is important to prevent the progression of the condition and its related co morbidities into adulthood (Mela, 2005). The static BMI thresholds that are used by adults aged 18 years or older (overweight= BMI 25-29.9kg/m^2 ;obese= BMI ≥ 30kg/m^2) cannot be applied to children population to define overweight and obesity as the continuing changes in body weight and build throughout the children development. Instead, the child's BMI is compared with the distribution of BMI in a reference population of the same age and sex (Williams and Fruhbeck, 2009).

Thus, Cole *et al*. (2000) had proposed cut off points which are less arbitary and more internationally based than current alternative to provide internationally comparable prevalence rates of overweight and obesity in children with ages ranging from 6-18 years **(Table 2.1).** The data obtained on body mass index for children were derived from six large nationally representative cross sectional surveys from Brazil, Great Britain, Hong Kong, Netherlands, Singapore and the United States which each survey had 10 000 subjects and quality control measures was done to minimise measurement error (Cole *et al*., 2000).

Table 2.1: International cut off points for body mass index for overweight and obesity by sex between 2 and 18 years (Cole *et al.,* 2000)

	Body mass index 25 kg/m²		Body mass index 30 kg/m²	
Age (years)	Males	Females	Males	Females
2	18.41	18.02	20.09	19.81
2.5	18.13	17.76	19.80	19.55
3	17.89	17.56	19.57	19.36
3.5	17.69	17.40	19.39	19.23
4	17.55	17.28	19.29	19.15
4.5	17.47	17.19	19.26	19.12
5	17.42	17.15	19.30	19.17
5.5	17.45	17.20	19.47	19.34
6	17.55	17.34	19.78	19.65
6.5	17.71	17.53	20.23	20.08
7	17.92	17.75	20.63	20.51
7.5	18.16	18.03	21.09	21.01
8	18.44	18.35	21.60	21.57
8.5	18.76	18.69	22.17	22.18
9	19.10	19.07	22.77	22.81
9.5	19.46	19.45	23.39	23.46
10	19.84	19.86	24.00	24.11
10.5	20.20	20.29	24.57	24.77
11	20.55	20.74	25.10	25.42
11.5	20.89	21.20	25.58	26.05
12	21.22	21.68	26.02	26.67
12.5	21.56	22.14	26.43	27.24
13	21.91	22.58	26.84	27.76
13.5	22.27	22.98	27.25	28.20
14	22.62	23.34	27.63	28.57
14.5	22.96	23.66	27.98	28.87
15	23.29	23.94	28.30	29.11
15.5	23.60	24.17	28.60	29.29
16	23.90	24.37	28.88	29.43
16.5	24.19	24.54	29.14	29.56
17	24.46	24.70	29.41	29.69
17.5	24.73	24.85	29.70	29.84
18	25	25	30	30

2.2 Factors that contribute to childhood obesity

The etiology of obesity is multifactorial. It results from excessive weight gain from a long term imbalance between energy intake and energy expenditure (Skelton *et al*., 2009).

2.2.1 Dietary risk factors for excess adiposity in young people

Dietary habits during childhood are associated with increased adiposity in adoloscent. A survey done in United States of America showed that mean energy intake changes from 1970s- 1994 especially among adoloscent females. The mean percentage of energy from total and saturated fat decreased, but remained above recommendations, with overall means of 33.5% of energy from fat and 12.2% of energy from saturated fat (Troiano *et al*., 2000).

It is also reported that, beverages contributed 20-24% of energy across all ages and soft drinks provided 8% of energy in adolescents. Except for adolescent girls, beverage energy especially soft drink contributions were generally higher among overweight than non overweight youths (Troiano *et al*., 2000).

In Kota Bharu Malaysia, a research dietary assessment among overweight and obese Chinese children showed that protein, fat and total calorie intake were significantly higher among the overweight group (Soo *et al*., 2011).

2.2.2 Genetic as a contributing factors to paediatric obesity

The factors that contribute to development of childhood obesity are derived from environmental and genetic factors. Evidence from recent studies suggests that genetic factors account for 40-90% of the body mass index (BMI) variations among population (Duran-Gonzalez *et al*., 2011).There are many candidate genes that could play an important role in the development of obesity. One of the most studied genes associated with obesity is the peroxisome proliferater activated receptor gamma (PPARγ).

2.2.2 (a) *Peroxisome Proliferator Activated Receptor Gamma* missense mutation of *Pro12Ala*

Peroxisome proliferator activated receptor gamma, is a nuclear receptor that regulates adipocyte differentiation and possibly lipid metabolism and insulin sensitivity. The PPARγ gene is located on the short (p) arm of chromosome 3 at position 25 **(figure 2.1)**. More precisely the *PPARγ* gene is located from base pair 12,329,348 to base pair 12,475,854 on chromosome 3. It is one of candidate gene for several human disorders including obesity and type II diabetes mellitus. The *PPARγ* gene have two isoforms, *PPARγ1* and *PPARγ2*. In this study the *PPARγ2* was selected. The human *PPARγ2* has 28 additional amino acids and expressed exclusively in adipose tissue. A missense mutation was identified in these gene. Located at codon 12 at 34 nucleotide position of *PPARγ2* a missense mutation $CCA^{Pro} \rightarrow GCA^{Ala}$ was detected (Yen *et al*., 1997). The coding region of *Pro12Ala* is illustrated in (**figure 2.2).**

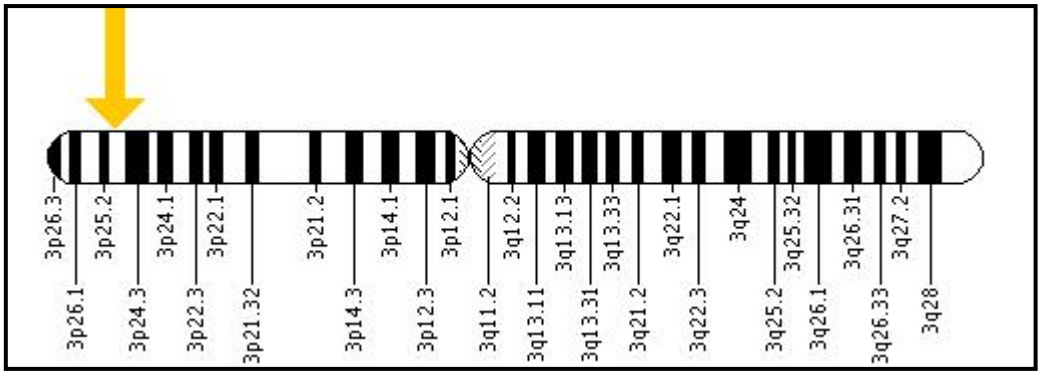

Figure 2.1: Location of *PPARγ* located on the short (p) arm of chromosome 3 at position 25

ACTCATGGGTGTATTCACAAATTCTGTTACTTCAAGTCTTTTTCT
TTTAACGGATTGATCTTTTGCTAGATAGAGACAAAATATCAGTGT
GAATTACAGCAAACCCCTATTCCATGCTGTT

ATG GGT GAA ACT CTG GGA GAT TCT CCT ATT GAC
1 2 3 4 5 6 7 8 9 10 11
GCA
CCA GAA AGC GAT TCC TTC ACT GAT ACA CTG TCT
12
GCA AAC ATA TCA CAA G

gtaaagttccttccagatacggctattggggacgtgggggcattt
atgtaagggtaaaattgctcttgtagtttgtcttccaggttgtgt
ttgttttaatactatcatgtgtacactccagtattttaatgctta
gctcgttgctatcgcgttcatttaaaaacatgttcagaaccttaa

Figure 2.2: Partial sequence of the exon in human *PPARγ*. Exonic sequence is shown in uppercase and intronic sequence in lowercase. Missense mutation of *Pro12Ala* is located at codon 12 which is encoded for Proline (CCA) which C→G substitution (GCA) Alanine at nucleotide 34 is present (Yen *et al*., 1997).

Peroxisome Proliferator Activated Receptor Gamma is the key regulator on energy balance, with polymorphisms on the *PPARγ* gene is linked to obesity and effects on body composition. Growing evidence suggest that *PPARγ* may be one of the most critical genetic factors predisposing to positive energy balance and ultimately obesity (Cecil *et al*., 2006). It is also reported that the nuclear fatty acid receptor *PPARγ* represents a potential direct link between adiposity, response to food, control of appetite and energy balance (Barbieri *et al*., 2005).

A meta analysis study examining the *Pro12 Ala* variant in the *PPARγ* gene among 19136 subjects found a positive association with BMI. In this study, the frequency of G allele, similar to other Caucasian populations was higher in obese subjects than in controls, suggesting that this variant was associated with obesity (Shawky and Sadik, 2011).

In another study among 642 northern Indians population, it was found found that there were significant association *Pro12Ala* variant in the *PPARγ* gene with morbid obesity. The researchers also found that there was a strong association of variant genotype with higher level of insulin and lower serum adiponectin concentrations (Prakash *et al*.,2012).

These results suggest that the mutant allele frequencies in *PPARγ2* gene vary among ethnic background. Allele frequencies among Caucasians are generally higher than other ethnics. Studies among Caucasians in different countries indicated that *Pro12Ala* variant in the *PPARγ* gene was greater than 10%. In contrast, Asian people have less prevalence. In native Javanese of Indonesia indicated lower incidence of that mutation which is 1.0% for diabetic and 1.7% for non diabetic subjects (Danawati *et al*., 2005).

The mechanism of the development of obesity through this gene can be explained by the ability of *PPARγ* gene to induce fibroblasts to differentiate into adipocytes thus leading to fat cell accumulation (Hwang *et al*., 2006). The more active *PPARγ* (activating mutations), the higher the BMI and the higher risk for insulin resistance (Chua *et al*., 2004).

2.2.2 (b) *Peroxisome Proliferator Activated Receptor Gamma(PPARγ)* and dietary intake

The PPARγ gene also may act as a fatty acid sensor. It has the affinity of fatty acids for the receptor varying according to their chain and degree of desaturation. It also had been mentioned that the role of *PPARγ* as a nutrient sensor regulating adipogenesis and insulin sensitivity. Several studies have shown that *Pro12Ala* variants interact with dietary nutrients to modulate body weight (Luan *et al*., 2001, Barbieri *et al*., 2005. Cecil *et al*., 2006).

The effect of *Pro12Ala* variant in the *PPARγ* gene can be altered by the character of the diet, particularly the ratio of dietary polyunsaturated fat to staturated fat (P: S ratio) (Luan *et al*., 2001). The result showed that when P:S ratio is low, the mean BMI in G carriers is greater than in *CC* homozygous, whereas when the P:S ratio is high, the G carriers is leaner than in CC homozygous (Luan *et al*., 2001).

The association between *Pro12Ala* variant and BMI according to dietary fat intake was investigated among 2141 women (1637 CC, 469 CG, 35 GG) .The BMI of *PPARγ* G variant allele carriers was higher than BMI of non-carriers (26.7 versus 25.4 kg/m2 for variant allele carriers and non carrier, respectively, P= 0.005) (Memisoglu *et al*., 2003).

Besides, a positive trend between increasing intake of total fat and BMI was observed in Pro/Pro homozygotes but not in *PPARγ 12Ala* variant-allele carriers. They also found that intake of saturated fat was directly associated with increased BMI among individuals of both genotype classes whereas intake of monounsaturated fat was inversely associated with BMI in G allele carriers. Therefore, *Pro12Ala* variant modified the association between total dietary fat intake and risk of obesity (Memisoglu *et al*., 2003).

Hence, result obtained from Quebec Family Study showed that total fat and saturated fat intakes are positively correlated with BMI, waist circumference, ratio of total cholesterol to HDL-C and fasting glucose levels in CC homozygotes but not in carriers of the G allele. They also found that in CC homozygotes, increasing fat intake was associated with a larger waist circumference. However, among subjects carrying the G allele, there was no major difference in waist circumference whether the fat intake was low or high (Robitaille *et al*., 2003).

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2.2.3 Physical activity among children

Physical activity is defined as bodily movement that is produced by the contraction of skeletal muscle and that substantially increases energy expenditure (Bouchard and Katzmarzyk, 2010). The epidemic of excess weight is driven by widespread energy imbalance favouring storage of the energy surplus not expanded.

Children and adolescents should spend at least 1 hour of moderate to vigorous physical activity per day, while strengthening exercise for their bones and muscles at least 3 times per week (Landry and Driscoll, 2011). The physical activities should be suitable for their age, fun and variety, different from their typical active daily living **(Table 2.2)**.

Some of the beneficial effects of engaging in moderate to vigorous physical activity (MVPA) during youth are improves muscular strength, cardio respiratory fitness and body composition and therefore decreases cardiovascular risk factors (Reilly and McDowell, 2003; Tao *et al*., 2007; Banks *et al*.; O.Dwyer *et al*. 2011) .

Table 2.2: Example of moderate and vigorous intensity aerobic physical activity and bone strengthening exercise activities for children and adolescent (Landry and Driscoll, 2011).

Type of Physical Activity	Age Group: Children	Age Group: Adolescents and Adults
Moderate-intensity aerobic	Active recreation, such as hiking, skateboarding, and rollerblading Bicycle riding Brisk walking	Active recreation, such as canoeing, hiking, skateboarding, and rollerblading Brisk walking Bicycle riding (stationary or road bike) Housework and yard work, such as sweeping or pushing a lawn mower Games that require catching and throwing, such as baseball and softball
Vigorous-intensity aerobic	Active games involving running and chasing, such as tag Bicycle riding Jumping rope Martial arts, such as karate Running Sports such as soccer, ice or field hockey, basketball, swimming, and tennis Cross-country skiing	Active games involving running and chasing, such as flag football Bicycle riding Jumping rope Martial arts, such as karate Running Sports such as soccer, ice or field hockey, basketball, swimming, and tennis Vigorous dancing Cross-country skiing
Muscle-strengthening	Games such as tug-of-war Modified push-ups (with knees on the floor) Resistance exercises using body weight or resistance bands Rope or tree climbing Sit-ups (curl-ups or crunches) Swinging on playground equipment/bars	Games such as tug-of-war Push-ups and pull-ups Resistance exercises with exercise bands, weight machines, hand-held weights Climbing wall Sit-ups (curl-ups or crunches)
Bone-strengthening	Games such as hopscotch Hopping, skipping, jumping Jumping rope Running Sports such as gymnastics, basketball, volleyball, and tennis	Hopping, skipping, and jumping Jumping rope Running Sports such as gymnastics, basketball, volleyball, and tennis

Note: Some activities, such as bicycling, can be moderate or vigorous intensity, depending on the level of effort.
From the U.S. Department of Health and Human Services [2].

Numerous studies have concluded that most children and adolescents in the developed countries fail to meet current public health guidelines for physical activity. O.Dwyer *et al*., (2011) stated that there was no significant difference in MVPA between overweight and normal weight children in 50 preschool children in England (P= 0.06). Physical activity was quantified using uni-axial (GT1M) accelerometer every 5 s for 7 consecutive days. Overweight boys exhibited significant lower scores than non-overweight boys for time spent in moderate intensity activity (P=0.02). However, both groups did not met physical activity recommended of 1 hour of MVPA per day.

A Dutch study, stated that activity counts measured with tri-axial accelerometers (Tracmor-4) for overweight children were significantly lowered than lean children (overweight: 46.1±6.9 vs. lean: 54.4±11.2 kCounts/day, p=0.02), even though they exerted the same movements per activity. It is also reported that, daily physical activities were inversely related to percentage body fat (r^2 =0.29, p=0.01), structured activities were not (Vogels *et al*., 2007).

Stone *et al*., (2009) observed in their study that, among 54 boys aged 8-10 years, overweight children exhibited fewer and shorter bouts compared to normal weight boys. It is also reported that less activity was accumulated during the weekends compared during the weekdays. This study used Actigraph GT1M as a method to assess the physical activity.

Whereas, a cross sectional study of 1292 children aged 9-10 years old from 4 distinct regions in Europe was conducted to examine the association between physical activity and body fatness. Physical activity was measured by accelerometer MTI. The result shows that, children who accumulated <1 hour of moderate physical activity per day were significantly fatter than were those who accumulated >2 hour per day (Ekelund *et al*., 2004).

2.3 Accelerometer as a method to measure physical activity

The accurate measurements of free living physical activity (counts) is important for research studies in which physical activity is one of the outcome measure of interest. In recent years accelerometers had been widely used to characterize physical activity in children.

Accelerometer is an objective measurement device which evaluates both physical activity quantity and quality. It is designed with large memory storage so that several days or weeks of activity can be assessed in small sampling intervals such as seconds and minutes. The device is relatively small and light weight making it unobtrusive and practical to use for extended measurement periods. The small size makes the children attracted to wear them (Fredson *et al*., 2005).

Besides, accelerometer also is a motion sensor that provides detailed information of the intensity and duration of physical activity on a minute-by-minute basis. It have good validity compared with gold standard methods and feasible to use in large scale studies as it provides information about the total amount of physical activity and its subcomponents (Ekelund *et al*., 2004).

Accelerometer provides variety of measurements on physical activity. Some of the measurements on physical activity commonly used by researchers were daily activity counts (counts/min/day) which is an indicator of the total volume of physical activity and time (min/day) spent at different physical activity intensity categories (Ekelund *et al*., 2004; Stone *et al*., 2009; O.Dwyer *et al*., 2011). It also able to

provide information on energy expenditure and able to quantify the amount of time spent in light (Crouter *et al*., 2006).

As for children,there are cutoffs for different intensity levels in children. Each cutoffs vary between studies. The cutoffs divides the intensity levels of physical activity in children into sedentary, moderate activity and vigorous physical activity. Sedentary activity defnied as activity counts/minute as less <500, light activity from 500-1999, moderate activity from 2000-2999 and vigorous activity as >3000 counts/minute.

There are various types of accelerometers. Previously, the uniaxial Actigraph is the most widely used for assessing physical activity. Actigraph GT1M is one of them. The GT1M is biaxial with an antero-posterior (AP) vector in addition to vertical (V) vector. However, in January 2009, a new model of the GT3X+ was issued.

The Actigraph GT3X-plus (GT3X+) **(Figure 2.3)** is a type of triaxial accelerometer that able to measure the amount and frequency of human activity. It provides physical activity measurement such as activity counts and vector magnitude, energy expenditure, steps taken, activity intensity levels, subject position and more. The GT3X+ accurately and consistently measures and records time varying accelerations ranging in magnitude from -6g’s to +6g’s.It is also stated that triaxial accelerometers are better than uniaxial accelerometers for predicting energy expenditure (Hanggi *et al*., 2013). .

Figure 2.3 : Actigraph GT3X+

Besides, the GT3X+ includes an inclinometer that able to detect a person posture, assuming the accelerometer is worn on the hip. The inclinometer detects sitting, standing, lying and off (non wear) (Hanggi *et al*., 2013). This is important especially in this research to check the subject sedentary activities and their relationship with health. The GT3X+ also waterproof. These qualities prevent the need to remove the monitor and participants find the monitor more acceptable for assessment of habitual activity (Rowlands and Stiles, 2011)

2.3.1 Outcome measures of the accelerometer GT3X+

Accelerometers able to collect detailed time stamped activity count data that can be distilled to time spent at various intensities of movement (Barreira *et al*., 2013). Outputs from most accelerometers are in counts. In the process of obtaining counts, the voltage signal from the accelerometer is first digitized by an analog to digital converter. Differing analytical approaches can then be applied. Commonly, the signal is rectified and integrated over a user defined epoch of between 1 second and

60 second. The summation of activity counts over epochs lead to smoothing of data (Rowlands and Stiles, 2011).

From the data of activity counts, the accelerometer able to predict energy expenditure by using formulated equation. From that, intensity threshold was able to determine. A regression equation to estimate metabolic equivalent MET was made, which than able to generate the cut points defining sedentary, light, moderate and vigorous activity using accelerometer counts (Robertson *et al*., 2010). Activity counts were translated into MET. Given is the equation derived by Freedson **(Figure 2.4)** which was used in this research.

Freedson equation:

$METs = 2.757 + (0.0015 \times counts.min^{-1}) - (0.08957 \times age\ [yr]) - (0.000038 \times counts.min^{-1} \times age\ [yr])$.

Figure 2.4 : Freedson equation of Metabolic Equivalent, MET for children

The analysis software for GT3X+ is ActiLife version 6.1. There are several sets of cut points available to users. These cut points were derived as part of past published research aimed at quantifying activity levels using ActiGraph products. All cut point sets are scaled to 60s epochs. Even if the cut point set was originally defined for sub-60s epoch files, the cut points were scaled in ActiLife (CPM = Counts Per Minute). The chosen cut points in this study was by Freedson 2005 **(Table 2.3)**.

Table 2.3: The proposed cut points define sedentary, light, moderate, vigorous and very vigorous for children. These cut points are based off of the MET formula in **Figure 2.4** with assumed MET threshold of 3, 6, and 9 METs which produce cut point boundaries of 500,4000 and 7600 CPM respectively.

Sedentary	0-149 CPM
Light	150-499 CPM
Moderate	500-3999 CPM
Vigorous	4000-7599 CPM
Very vigorous	7600 - ∞ CPM

Ref: https://help.theactigraph.com/entries/21452826-What-s-the-difference-among-the-Cut-Points-available-in-ActiLife-

Besides activity counts, step counts is also one of the outcome measure that can be collected from the accelerometer. Previously, pedometer was used to measure the step counts. Compared this two methods, pedometers are more likely to be used in a variety of clinical and public health application. This is because they are cheaper, reasonably reliable, valid, simple to use and the output is easy to understand compared to accelerometer.

Accelerometers can collect detailed timed-stamped activity count data in addition to step counts and widely used in research setting. Research had been done to compare steps/day detected by pedometer YAMAX SW-200 and accelerometer GT3X. Result shows that, the YAMAX and GT3X detected 8,025 ±

3,967 and 7131 ± 3066 steps/day respectively and the outputs were highly correlated (r=0.87) (Barreira *et al*., 2013).

A review shows that there are over 40 studies that determined physical activity studies based on free-living step values (Tudor-Locke *et al*., 2009). As for determination of physical activity among children using step counts, studies from central London in primary school children had been made. It is stated that, children attained significantly higher mean steps/day during weekdays 13,827 than weekends 10,334 ($p<0.001$), and boys attained significantly higher mean steps/day 12,263 than girls 11,748 ($p<0.05$) (Duncan *et al*., 2007).