INTERNATIONAL JOURNAL OF ACADEMIC RESEARCH IN BUSINESS & SOCIAL SCIENCES

Vol 14, Issue 1, (2024) E-ISSN: 2222-6990

Aqua Exercises and *Adipoq* Gene Polymorphism: Impacts on The Metabolic and Obesity-Related Traits Among Obese Women

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To Link this Article: http://dx.doi.org/10.6007/IJARBSS/v14-i1/19435 DOI:10.6007/IJARBSS/v14-i1/19435

Published Date: 07 January 2024

Abstract

Introduction: Obesity is one of the major contributors to diseases and mortality. Aqua-based exercise, also known as water-based exercise has been a safer and appropriate approach for obese people. However, less has been studied on the impact of gene polymorphism on the effects of aqua exercises in obese women. Purpose: Thus, this study aimed to examine the role of the Adiponectin (*ADIPOQ*) gene polymorphism on metabolic and obesity-related traits in response to aqua exercises among obese women. Methods: Sixty previously sedentary obese women (BMI >30 kg/m²), aged 20-59 years old were involved. All participants were subjected to aqua exercise (AE) programs over 12 weeks, 3 times per week, 60 minutes per session, with the intensity of 50-75% of the maximum heart rate. High-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride, glucose, abdominal circumference (AC), blood pressure, and body fat mass (BFM) were measured at baseline (week-0) and post-exercise intervention (week-13). The participants were genotyped for *ADIPOQ*-rs266729, *ADIPOQ*-rs17300539, and *ADIPOQ*-rs16861194. Results: The genotypes were correlated to the changes of selected metabolic and obesity-related traits except for *ADIPOQ*-rs17300539. AE demonstrated significant changes (p<.05) for most of the parameters except for LDL and blood pressure (p>.05). It was found that the participants with *ADIPOQ*-rs266729 CC genotype demonstrated superior reduction in LDL compared to GC genotype (p=.03) and *ADIPOQ*-rs16861194 AA genotype demonstrated superior reduction in AC compared with AG genotype

(p=.04) following interventions. Conclusions: 12 weeks of aqua exercise improved all health parameters in obese women, and *ADIPOQ* gene polymorphism is associated with the metabolic responses following aqua exercises. *ADIPOQ*-rs266729 G and *ADIPOQ*-rs16861194 G variants may be considered disadvantageous factors in the context of training-induced effects on metabolic and obesity-related traits.
Keywords: Aqua Exercise, Adipoq Gene Polymorphism, Metabolic, Obesity

Introduction

The prevalence of obesity has reached epidemic proportions globally, including in Malaysia (Bernama, 2020). An update on the latest reports revealed that Malaysia has the highest rate of obesity and overweight among Asian countries (Bernama, 2020; Institute for Public Health, 2020). It is also revealed that overweight and obesity levels were particularly high among women at 54.7% (Institute for Public Health, 2020). Lifestyle modifications such as high-fat diet habits and physical inactivity have triggered a sharp increase in the phenomena of obesity (Chan et al., 2017; Corbi et al., 2019). A recent study reported that people of all ages ingested more fast food and soft drinks and relied more on digital media devices to perform numerous daily tasks, which presumably reduced daily activities (Radzi et al., 2019).

Obesity refers to excessive storage of energy in the form of fat in a person. Hyperplasia and hypertrophy of fat cells result in an increase in the secretion of pathogenetic products such as free fatty acids and peptides from the fat cells to the extent they exert an adverse effect on health and shorten life expectancy (Bhale, Patil & Mahat, 2014; Bray 2004; Letchuman et al., 2010). This is due to the strong association of obesity in developing clustering of metabolic abnormalities including an increase in blood glucose, triglycerides, blood pressure, abdominal circumference, and a decrease in high-density lipoprotein cholesterol (HDL-C), all of which are related to weight gain thus, increases the risk of various non-communicable diseases and mortality (Bhale et al., 2014; Çakmakci, Uras & Tozun, 2010; Ginsberg et al., 2010; Han & Lean, 2016; Thomas et al., 2013; Yang & Barouch, 2007).

To combat health problems related to obesity, one should maintain a calorie deficit by increasing their PAL (Macera, Hootman & Sniezek, 2003). Aqua-based exercise has proven to be effective and safer conditioning for obese people due to the uniqueness of water properties. Based on the researcher's observation, Zumba® Fitness is preferred because it can be tailored to fit a wide range of population's age and culture. It is also entertaining with accompanying music and less restrictive (Nieri & Hughes, 2016). Aqua Zumba® Fitness is one of the programs under Zumba® Fitness.

A genome-wide association study (GWAS) revealed that the *ADIPOQ* gene had been demonstrated to be a susceptibility locus for obesity, and other associated health problems. In the context of genetic conditioning for a predisposition to obesity, changes in parameters associated with weight loss are modulated by genetic and environmental factors (lifestyle). To be specific, genetic variants (polymorphisms) of the Adiponectin (*ADIPOQ*) gene and exercise training contribute to the changes in metabolic and obesity-related traits, including changes in body composition, lipid parameters, glucose levels, and blood pressure (Comuzzie et al., 2001; Enns, Taylor & Zahradka, 2011; Leońska-Duniec et al., 2019; Yatagai et al., 2003). The information on the association of *ADIPOQ* gene polymorphisms with changes in metabolic and obesity-related traits in response to aqua-based exercise in obese women is warranted since studies covering genetic conditioning are lacking and reported conflicting results (Leońska-Duniec et al., 2019).

This study aims to examine the effectiveness of Aqua Zumba® Fitness in reducing metabolic and obesity-related traits and also attempts to determine the association of *ADIPOQ* gene polymorphisms with changes in metabolic and obesity-related traits in response to aqua exercise training in obese women.

Methodology

This study employed a pretest-posttest approach. The effects of aqua Zumba® were measured on metabolic and obesity-related traits comprising lipids profile, glucose, abdominal circumference, resting blood pressure, and body fat mass at baseline (week 0) and following 12 weeks of interventions (week 13). The effects of *ADIPOQ* gene variants (rs266729, rs17300539, and rs16861194) on changes in metabolic and obesity-related traits in response to aqua exercise training were also measured.

Participants

A total of 60 women were involved in this study. The characteristics of participants involved were; aged 20 to 59 years old based on year of birth, BMI, and height ranging from 30 to 40 kg/m2 and 1.5 to 1.7 meters, categorized as sedentary by participating in physical exercises less than 20 minutes a week and not be involved in any structured physical exercise in the previous six months. They are free from any metabolic or cardiovascular disease, musculoskeletal injury, physical disability, medication, or drugs or supplements known to affect metabolism. Participants with a history of chronic diseases (any type of cancer, any type of heart disease, stroke) and or musculoskeletal injuries less than one year and or currently having medical conditions including any type of heart disease, dyslipidemia (TG: ≥1.8, LDL-C: ≥4.1, and HDL-C: <1.0 mmol/L) hypertension stage 2 (<140/90 mmHG), hypotension (<90/60 mmHg), diabetes (fasting plasma glucose >7.0 mmol/L), abnormal menstruation (absence of menstrual cycles for 6 consecutive months), and hydrophobia were excluded.

Outcome Measures

A desk mercury sphygmomanometer (Accoson Dekamet, United Kingdom) was used to measure participants' resting systolic and diastolic blood pressures. The participants were instructed to sit comfortably on a chair with a backrest for at least five minutes before measurement. The examiner listened to note the first Korotkoff sound to determine the systolic blood pressure and the last Korotkoff sound to determine the diastolic blood pressure.

The participants were required to fast for 10 hours before the blood test. Venous blood (6 milliliters) samples were drawn from the participants via an antecubital vein. Blood was collected into two vacutainer tubes: a yellow top with K2EDTA (lipid profiles) and another with a grey top with potassium oxalate and sodium fluoride (fasting blood glucose). Fasting serum lipids and serum glucose were then assayed enzymatically by using the VISTA kit analyzer (SIEMENS, USA). The Dimension Vista software operating system automatically performed the processes involved in sampling the blood specimens, reagent delivery, mixing procedures, and chemical processing. Serum glucose was measured using the hexokinase (HK) method, while triglyceride was calculated based on an enzymatic procedure. The Abell-Kendall method was used to measure high-density lipoprotein (HDL) and the low-density lipoprotein (LDL) was calculated using Friedwald's formula.

An ergonomic circumference measuring tape (Seca 201, Germany) was used to measure abdominal circumference. The measurement was taken approximately at the midpoint between the lower margin of the palpable rib and the top of the iliac crest. The examiner placed the tape around the body comfortably but not too tight. The participant was asked to breathe normally and the measurement was taken at the end of normal expiration at an accuracy of 0.1 cm. The measurement was taken twice and the average value was recorded.

Lastly, the InBody720 analyzer (Biodex Co. Ltd, Seoul, Korea) was used to measure the participants' body fat mass. Direct segmental Multi-frequency Bioelectrical Impedance 70 Analysis Method (DSM-BIA) and Tetrapolar 8-Point Tactile Electrodes measurement technique were used for this machine together with Lookin'Body Software. Before the testing, the participants were asked to fast for eight hours the night before. During the weighing procedure, the participants were asked to wear light clothes and remove their socks and all accessories before stepping on the machine.

A total of 3 mL of blood was collected from each participant for genotyping purposes. All the samples were stored at -80 ºC until further use. DNA was extracted using E.Z.N.A. Tissue DNA Kit (Omega Bio-Tek, USA). The amount of DNA extracted was quantified using a NanoDrop 2000 Spectrophometer (Thermo Scientific, Wilmington, USA), which was set at a wavelength of 260 nm, while the purity of nucleic acid was estimated using the ratio of the readings at 260 nm and 280 nm. The triplicate of the readings was conducted for all the samples. The mean value for each was recorded. The integrity of the genomic DNA was confirmed by electrophoresis. Five µ L of the samples were analyzed on 0.5% agarose gel and 1 X TBE at 50 volts for approximately 2 hours or until the loading dye front was 1 cm from the bottom of the gel. The gel was visualized on an ultraviolet (UV) transilluminator. The PCR assays for identifying *ADIPOQ* variants (rs266729, rs17300539, and rs16861194), which were developed by the researchers at the Integrative Pharmacogenomics Institute (iPROMISE) Universiti Teknologi MARA (UiTM), were used (Figure 3.24). The primers for specific amplification of the SNPs were amplicons containing PCR products. The list of primers used in this study is shown in Table 3.7. The genotyping method of rs266729, rs17300539, and rs16861194 was conducted via direct sequencing (Sanger Sequencing, Life Technologies). The rs266729, rs17300539, and rs16861194 genotypes of the participants were analyzed via Sequence ScannerTM Software 2 (Life Technologies). The sample for this study was genotyped in batches, and each batch of PCR genotyping was quality-controlled using positive and negative control to detect false positive or negative results.

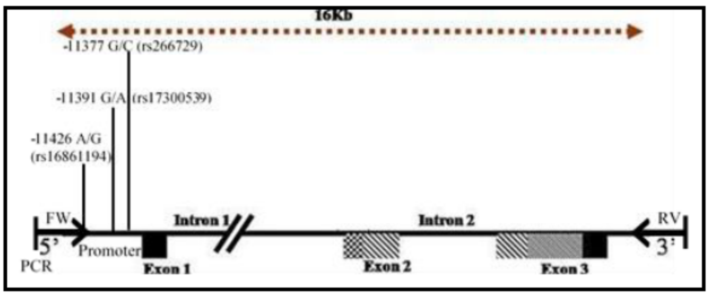

Figure 3.24 Amplification of Amplicon ADIPOQ

Table 3.7
Primers for Amplification of Amplicon *ADIPOQ*

ADIPOQ	FW	5' CTGTGTGGACTGTGGAGATG 3'
	RV	5' ACCCACTTAGGTGTTCCTA 3'

Exercise Program

All participants participated in a twelve-week aqua exercises program, 3 sessions per week, and 60 minutes per session. The aqua training sessions were performed on three different days in a week with at least one day of rest between sessions. The participants were subjected to perform aqua Zumba® with a water level fixed at chest or near chest level with an average water temperature of 24˚ Celsius. The exercise program consisted of stretching and warm-up exercises (10 minutes), endurance conditioning (40–45 minutes), and cool-down or muscular relaxation exercises (5–10 minutes) for aqua Zumba® (Table 3.10). In the warm-up session, dynamic stretching was performed. Various Latin dance movements in different rhythms and movement directions were performed from moderate to fast tempo or low to high repetitions. The exercise training intensity was gradually increased from 50% in the first week to 75% in the final week (Figure 3.26). Participants were instructed to maintain intensity during the training session, based on indicated ranges of heart rate. Maximum heart rate (HRmax) was used as an indicator of the prescribed intensity. The participants' training heart rate was monitored every 10 minutes during the exercise sessions. Missed workouts were made up by attending an additional session in the same or subsequent week.

Table 3.10
Aqua Zumba® Fitness Exercise Routines

Training week/ Intensity (Repetition: Set: Tempo)	**Step routine**
First and second week: 50– 55% Max HR (8: 4: slow – 3 to 4 seconds per execution)	Merengue: side steps, front/back steps, turns, Beto shuffle. Muscles involved: Arms, Shoulders, Chest and Back, Front and back thigh, Glutes, Calf (Upper and Lower body)
Third and fourth week: 55 – 60% Max HR (10: 4: moderate slow – 2 to 3 seconds per execution)	Cumbia: front/back, step-drag or "Sleepy leg", Machete. Muscles involved: Hips, Front and Back thigh, Glutes, Calf (Lower body)
Fifth until eighth week: 60 – 65% Max HR (8: 5: moderate slow – 2 to 3 seconds per execution)	Salsa: step out/return together, two-step. Muscles involved: Abdominals, Front and Back thigh, Glutes (Abs and Lower body)
Ninth and tenth week: 65 – 70% Max HR (10: 5: moderate to fast – 1 to 3 seconds per execution)	Reggaeton: Stomp, Running man. Muscles involved: Abdominals, Front and back thigh, Glutes, Calf, Arms, Shoulders, Chest, and Back (Total body)
Eleventh and twelfth week: 70–75% Max HR (10: 5: fast – 1 to 2 seconds per execution)	Core exercise: Hip swing, Hula-hoop, Worm, Carioca, standing pike crunch, cross knee lift, jumping jack toe touch. Muscles involved: Abdominals

Source: Adopted from Perez and Adami (2011)

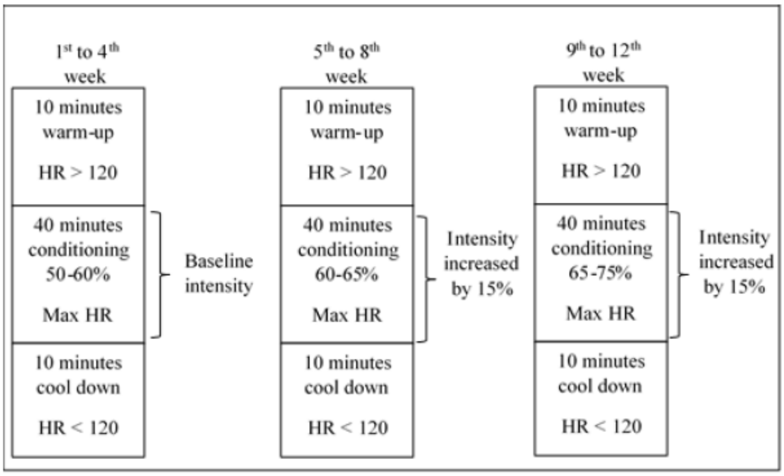

Figure 3.26 Progression Scheme of Aqua-based Exercises Training Groups

Data Analysis

The changes between pre-post on lipids profile, glucose, abdominal circumference, resting blood pressure, and body fat mass were determined using a Paired sample t-test. An Independent sample t-test was used to compare the changes in metabolic and obesity parameters between genotypes of the *ADIPOQ* gene following the aqua intervention. The expected and allele frequencies were calculated using the Hardy-Weinberg equilibrium formula below:

$$p2+2pq+q^2=1$$
$$where, p+q= 1$$

All values were reported in mean (*M*) and standard deviation (*SD*). Before the data analysis, statistical assumptions were tested and the significance level was set at .05. The data analysis of data was carried out with the aid of the IBM Statistical Package for Social Science (SPSS) version 21.

Results

Participant Characteristics

Table 1 shows physical characteristics among groups at baseline.

Table 1
Baseline physical characteristics of participants.

Variables	n=60 Mean±SD
Age (years old)	42.38±.77
Height (cm)	157.31±4.12
Weight (kg)	83.34±9.59
Body Mass Index (kg/m^2)	33.62±3.26
Body Fat Percentage	46.28±2.77

Genotype and Allele Frequencies

In the investigation of rs266729 polymorphism in 60 obese women, 2 genotypes were discovered. The heterozygous group contributed the highest proportional percentage with 53.33% (*N*=32) followed by the homozygous wild-type group with a percentage of 46.67%

(*N*=28) (Table 2). From rs266729 genotyping findings, it was found that 88 wild-type alleles dominated the total of 120 alleles contributing the allelic frequencies of 73.33% while the rest of the 32 alleles were detected to be variants alleles (26.67%) (Table 3).

Table 2
Genotype Frequencies of rs266729 among Obese Women

Percentage of genotype frequency (95% CI)				
Genotype	Observed Frequency		Observed Frequency	
	Sample (N)	Frequency (95% CI)	Sample (N)	Frequency (95% CI)
CC	28	46.67	32.27	53.78
CG	32	53.33	23.47	39.12
GG	0	0	4.27	7.12

Table 3
Allele Frequencies of rs266729 among Obese Women

Allele	Allele Frequency (Total N = 120)	Percentage of Confidence Interval (95%)
		Frequency
C	88	73.33
G	32	26.67

As for rs17300539 polymorphism, only the homozygous wild-type group was detected (100%) in all 60 obese women (Table 4). No variant allele was discovered with all 120 of the total 120 alleles observed being wild type with a percentage of allelic frequency of 100% (Table 5). Therefore, rs17300539 would not be used for further analysis.

Table 4
Genotype Frequencies of rs17300539 among Obese Women

Percentage of genotype frequency (95% CI)				
Genotype	Observed Frequency		Observed Frequency	
	Sample (N)	Frequency (95% CI)	Sample (N)	Frequency (95% CI)
GG	60	100	60	100
AG	0	0	0	0
AA	0	0	0	0

Table 5
Allele Frequencies of rs16861194 among Obese Women

Allele	Allele Frequency (Total N = 120)	Percentage of Confidence Interval (95%)
		Frequency
G	120	100
A	00	00

Two genotypes of rs16861194 polymorphism were detected. AA, homozygous wild type allele of rs16861194 was found to be the most frequently occurring genotype contributing to the percentage proportion of 78.33% (*N*=78.33), while other genotypes, heterozygous, gave a percentage proportion of 21.67% (*N*=13). Based on these genotype frequencies 107 from a total of 120 alleles observed were wild type and the remaining 13 alleles were variants with the percentage of allelic frequency of 89.17% and 10.83% respectively.

Table 6
Genotype Frequencies of rs16861194 among Obese Women

Percentage of genotype frequency (95% CI)				
Genotype	Observed Frequency		Observed Frequency	
	Sample (N)	Frequency (95% CI)	Sample (N)	Frequency (95% CI)
AA	47	78.33	47.71	79.52
AG	13	21.67	11.59	19.32
GG	0	0	0.70	1.17

Table 7
Allele Frequencies of rs16861194 among Obese Women

Allele	Allele Frequency (Total N = 120)	Percentage of Confidence Interval (95%)
		Frequency
A	107	89.17
G	13	10.83

Effects of ADIPOQ Gene Polymorphisms on Parameters in Response to Exercise

There was no significant difference in the changes in obesity-related trait parameters except for LDL between CC and GC genotypes (Table 8). Hence, it was concluded that participants with the CC genotype demonstrated a significantly greater LDL reduction than participants with the GC genotype. In contrast, although there was no significant difference between CC and GC genotypes in *ADIPOQ* rs266729, greater decrement in most of the parameters including glucose, abdominal circumference, systolic blood pressure, and body fat mass were observed in participants with CC genotype.

Table 8
ADIPOQ rs266729 Genotypes and Responses to Aqua Exercise on Metabolic and Obesity-Related Traits

Parameter	CC (n = 21)	GC (n = 19)	Independent sample t-test P – value
	Mean gain ± SD	Mean gain ± SD	
HDL (mmol/L)	.15±.15	.25±.18	.17
LDL (mmol/L)	-.74±.45	-.29±.44	.00
Triglyceride (mmol/L)	-.48±.31	-.54±.30	.50
Glucose (mmol/L)	-.57±.26	-.42±.32	.12
Abdominal circumference (cm)	-8.42 ± 3.02	-7.22 ± 2.86	.21
Systolic blood pressure (mm/Hg)	-6.76 ± 3.83	-3.76 ± 9.64	.14
Diastolic blood pressure (mm/Hg)	-5.29 ± 3.35	-7.63 ± 5.22	.10
Body Fat Mass (kg)	-4.43±1.91	-3.37±1.82	.08

There was no significant difference in the changes of all selected obesity-related traits parameters except for abdominal circumference in participants with AA and AG genotypes following 12 weeks of aqua exercise (Table 9). Hence, it can be concluded that participants with AA genotypes demonstrated a significantly greater reduction in abdominal circumference compared to participants with AG genotypes. Although there was no significant difference in participants with AA and AG genotypes of *ADIPOQ* rs16861194, greater improvement in most of the parameters including HDL, LDL, triglyceride, glucose, diastolic blood pressure, and body fat mass, were demonstrated in participants with AA genotype.

Table 9
ADIPOQ rs16861194 Genotypes and Responses to Aqua Exercise on Metabolic and Obesity-Related Traits

Parameter	AA (n = 21)	AG (n = 19)	Independent sample t-test P - value
	Mean gain ± SD	Mean gain ± SD	
HDL (mmol/L)	. 20±.25	.17±.14	.75
LDL (mmol/L)	-.53±.64	-.49±.62	.82
Triglyceride (mmol/L)	-.54±.31	-.43±.29	.40
Glucose (mmol/L)	-.57±.27	-.41±.16	.12
Abdominal circumference (cm)	-8.33 ± 2.86	-5.99 ± 2.49	.04
Systolic blood pressure (mm/Hg)	-4.81 ± 7.97	-6.00 ± 4.14	.69
Diastolic blood pressure (mm/Hg)	-6.63 ± 4.66	-5.50 ± 3.55	.53
Body Fat Mass (kg)	-4.10±1.93	-3.23±1.85	.25

Discussions

Much evidence from previous studies reported that aerobic exercise produced significant positive changes in adiponectin levels (Esposito et al., 2003; Lee, Kang & Shin, 2013). These results might be attributed to *ADIPOQ* variants (Woo & Kang, 2004). The present study investigates the difference in the influence of -11377 G>C (rs266729), -11391 G>A (rs17300539), and - 11426 A>G (rs16861194) polymorphisms in the *ADIPOQ* gene, which are well known to be associated with serum levels of adiponectin, and as a result metabolic and obesity-related trait, on effect of 12-week aqua exercise intervention in obese women. However, no further discussion on rs17300539 was included since all participants are carriers of the GG genotype.

Even though only LDL-cholesterol obtained a significant difference between rs266729 CC and GC genotypes following aqua exercise intervention, it is important to note the changes in other metabolic and obesity traits between these genotypes. Thus, our study indicates that the rs266729 CC genotype may enhance the positive effect resulting from aqua exercise training on metabolic and obesity-related traits (FM, AC, SBP) especially in LDL-cholesterol compared to the rs266729 GC genotype. It is difficult to conclude rs266729 because current findings are inconsistent with those of past studies. From the results obtained, we suggest that the G variant of rs266729 may be considered a disadvantageous factor in the context of training-induced effects on obesity traits. Thus, continued research is necessary. Comparing AA with AG genotypes, our study observed the carriers of the AA genotype (rs16861194) demonstrated greater improvement in all metabolic and obesity-related traits except for SBP. However, only AC obtained a significant difference between rs16861194 AA and AG genotypes after 12 weeks of intervention. We found that the rs16861194 AA genotype may enhance the positive effect on most of the metabolic and obesity parameters resulting from aqua exercise training, especially in reducing abdominal circumference.

To our knowledge, a limited number of published works have been found on gene-lifestyle interactions for SNP rs16861194 (A>G) in the *ADIPOQ* gene for metabolic and obesity-related improvement. However, based on the studies conducted on Chinese, Swedish, and French Caucasian populations, individuals with GG genotypes showed higher plasma glucose levels and body mass compared to individuals with AA genotypes (Chen et al., 2008; Gibson & Froguel, 2004; Gu et al., 2004). These findings supported that the G variant of this SNP is associated with a higher degree of susceptibility to obesity and metabolic complications. Therefore, carriers with GG genotypes may be considered a disadvantageous factor in training-induced effects on obesity traits among obese Malaysian women. However, the current studies still represent only the first steps towards a better understanding of the association of SNP rs16861194 that influences obesity-related traits and *ADIPOQ* gene-lifestyle interactions. Thus, future research is desirable.

Conclusion

The present study found that SNP 266729 and SNP 16861194 in the ADIPOQ gene influenced aqua exercise training's effects on metabolic and obesity-related traits. Results revealed the effect of genotype, which suggested that the C allele of rs266729 and A allele rs16861194 may enhance the training-induced favorable effect on metabolic and obesity traits.

This research significantly contributes to the existing knowledge on the interplay between genetics and lifestyle interventions in managing obesity. By exploring the specific impact of ADIPOQ gene polymorphisms on metabolic and obesity-related traits in response to aqua exercises, this study provides a nuanced understanding of individualized approaches in obesity management. It highlights the importance of considering genetic factors when designing exercise programs for obese individuals, especially women. This research not only adds to the theoretical understanding of gene-lifestyle interactions in obesity but also has practical implications in developing more effective, tailored exercise interventions. In a broader context, these findings can inform public health strategies and clinical practices aimed at combating obesity, a prevalent issue with significant health implications. By offering insights into the genetic basis of exercise efficacy, this study plays a crucial role in advancing personalized medicine and contributes to a more comprehensive approach to obesity management.

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