AMERICAN JOURNAL OF BIOCHEMISTRY AND BIOTECHNOLOGY

RESEARCH ARTICLE

# Effects of Aerobic Exercise on PPAR-γ Expression in Adipose Tissue and RT-PCR Detection Analysis

**Xiaodong Zhan*, Chenguang Bi[1], Jie Wei**

*Chongqing Vocational College of Transportation, Chongqing 402247, Chongqing, China*

*Corresponding Author: xiaodonghuwai2012@163.com

**Abstract:** This study aimed to examine the effect of organized aerobic exercise on the expression level of Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ) gene in adipose tissue by performing digital variant of RT-PCR, Droplet Digital PCR (ddPCR) which is a highly sensitive method for counting the gene copy numbers. The proposed scheme consists of three-stage analytical procedure, namely, Subphase I Controlled Aerobic Intervention Modeling, which optimizes exercise intensity by normalizing physiological parameters; Subphase II High-Precision Molecular Quantification, which applies ddPCR with Poisson-based absolute copy number estimation to estimate gene-expression profiles; and Subphase III Algorithmic Statistical Inference, which constructs robust gene-expression profiles using normalization algorithms and variance stabilization modeling, as well as multivariate effect analysis. The results indicated that the aerobic group had 28–35% higher expression of PPAR-γ mRNA compared to the control group ($p<0.05$), and the insulin sensitivity indices increased by 22% in the aerobic group. ddPCR yielded about a 40% decrease in the quantification variability when compared to traditional qRT-PCR. The results provide a more precise molecular link between aerobic exercise and the transcriptional changes in adipose tissue, and offer a novel approach to combining absolute quantification with physiological modeling to investigate obesity prevention and metabolic disease.

**Keywords:** Aerobic Exercise, PPAR-γ Adipose Tissue, Digital Droplet PCR, Absolute Quantification, Gene Expression Analysis, Poisson Statistics, Metabolic Regulation

**Received:** 05-03-2026 | **Revised:** 29-06-2026 | **Accepted:** 03-08-2026 | **DOI:** 10.3844/ajbbsp.2026.22.03.043

## Introduction

Obesity, insulin resistance and type 2 diabetes are also metabolic diseases, they are now big global health problems and a growing problem in health care systems worldwide. However, adipose tissue is not only an organ that stores energy but also an endocrine and metabolic tissue that has a regulatory effect on glucose and lipid metabolism in the body [1, 2]. Peroxisome Proliferator-Activated Receptor Gamma (PPAR-γ) is one of the transcriptional regulators that regulates lipid metabolism and adipocyte development [3, 4]. It has been shown to be very important in the alteration of adipogenesis, insulin sensitivity and inflammatory signaling. Aerobic exercise has consistently been shown to positively affect indicators of

metabolic health, however, the changes in the transcriptome of adipose tissue is not fully understood. Specifically, the fact that the patterns of reported PPAR-γ expression vary following exercise interventions has made it difficult to understand how it operates as a regulator. The present paper deals with the perennial issue of accurately quantifying exercise-induced changes in the expression of PPARγ in adipose tissue, with a complex and highly sensitive system of molecular detection based on the RT-PCR methodology, namely using advanced digital droplet PCR (ddPCR) for absolute quantification.

Over the past few decades, various molecular and analytical approaches have been developed to detect changes in gene expression induced by physiological treatments. Ordinary reverse transcription Polymerase Chain Reaction (RT-PCR) has been widely used to detect mRNA transcripts [5]. Instead, quantitative real time PCR (qRT-PCR) has provided relative quantification using fluorescence amplification curves. Changes in protein levels have been examined by Western blotting and immunohistochemistry, and microarray or RNA sequencing techniques have provided a broader view of the transcriptome. However, there are issues with these techniques. Normalization by housekeeping genes has been a major aspect of relative quantification methods, but these methods can change under conditions of metabolic stress. In other cases, discrepancies in amplification behavior and sensitivity limits have complicated the identification of transcripts of low abundance, such as transcription factors. Moreover, it has been challenging to replicate research findings due to heterogeneity across samples and statistical noise. The advances in the technology, including Digital Droplet PCR (ddPCR), have allowed absolute quantification in the absence of standard curves, enhancing the accuracy and eliminating amplification bias. Despite advances in technology, there has been limited studies to combine high precision molecular measurement with model aerobic exercise to explore adipose tissue transcriptional responses. Consequently, the methodology of strong gene-expression analytics utilizing digital RT-PCR variants and exercise physiology are not yet fully linked in existing literature.

Digital droplet PCR (ddPCR) was selected because exercise-induced transcriptional changes are often modest and may be difficult to quantify accurately using relative expression approaches alone. Unlike conventional RT-PCR methods, ddPCR provides absolute quantification of transcript copies without reliance on standard curves, enabling improved sensitivity, precision, and reproducibility. These characteristics make ddPCR particularly suitable for detecting exercise-associated changes in PPAR-γ expression within adipose tissue, where subtle molecular adaptations may have important metabolic implications.

This paper will be organized based on three objectives:

- It is intended to measure the effect of a well-designed aerobic exercise regime on the expression of the PPAR-7 gene in fat under carefully controlled physiological conditions quantitatively
- It is based on an extremely sensitive Absolute Quantification RT-PCR technology (Digital Droplet PCR, ddPCR) that allows to quantify PPAR-γ transcripts in absolute terms with high technical reproducibility
- It will attempt to identify statistically robust connections between exercise-induced physiology changes and changes in transcription, which will yield a mechanistic understanding of the molecular basis of metabolic regulation

The present work is designed as an empirical intervention study supported by integrated physiological–molecular analysis, enabling the interpretation of exercise-induced physiological and transcriptional adaptations. At the same time, the primary focus of the study remains the evaluation of PPAR-γ expression changes and metabolic outcomes following aerobic exercise intervention.

Non-pharmacological options that are effective in improving metabolic health are widely recognized as aerobic exercise. It enhances the function of the mitochondria, boosts the oxidation of fatty acids and reduces systemic inflammation [6]. The Peroxisome Proliferator-Activated Receptor Gamma (PPAR-γ) is a ligand-binding nuclear receptor which regulates adipocyte differentiation, lipid uptake, and glucose metabolism. While pharmacological PPAR-γ agonists induce insulin sensitivity, long-term use is problematic because of the safety concerns, so understanding how exercise affects expression of PPAR-γ through physiological mechanisms is important [7, 8]. Past experiments that have examined exercise-induced alterations in the expression of PPAR-γ have been characterized by inconclusive results, which have arisen mainly because of differences in the intensity, duration, sample of tissue, and sensitivity of analysis [9, 10]. The current study attempts to resolve these weaknesses through the use of absolute quantification methods to give a better estimate of transcriptional regulation. A Droplet Digital PCR (ddPCR) technology enables partitioning of cDNA into thousands of nanoliter droplets, each of which serves as a microreactor during amplification. The preliminary detailed processing steps concerning physiological normalization and statistical variance modelling have been presented exclusively in the Methodology section to maximise

conciseness and avoid narrative redundancy. This elegant method of molecular quantification will give a complete understanding of the exercise-induced systemic, hormonal and molecular adaptations.

Adipose tissue was selected as the primary molecular target because it serves as a central regulator of energy storage, lipid metabolism, insulin sensitivity, and inflammatory signaling. Compared with other tissues, adipose tissue exhibits pronounced metabolic responsiveness to both obesity and exercise interventions and is a major site of PPAR-γ expression. Consequently, adipose tissue provides a biologically relevant model for investigating exercise-induced transcriptional adaptations associated with metabolic regulation.

The molecular mechanisms underlying exercise's effects on metabolic health are clinically relevant, as obesity and metabolic diseases are on the rise. The discovery of exercise-induced transcriptional changes in adipose tissue could help identify targeted lifestyle interventions.

Although previous studies have investigated the effects of aerobic exercise on metabolic regulation and adipose tissue function, limited studies have employed digital droplet PCR (ddPCR) to quantify exercise-induced changes in PPAR-γ expression using absolute transcript quantification. Moreover, links between physiological adaptation and transcriptional regulation are poorly defined. Thus, the main objective of the present study is to assess the impact of a structured aerobic exercise session on adipose tissue PPAR-γ expression by means of ddPCR and its correlation with physiological adaptations associated with metabolic health.

## Literature Survey

[11] Found that obese animals had down-regulated expressions of PPAR-γ and Adiponectin, and up-regulated expressions of TNF- b, which indicates a higher level of low-grade inflammation in adipose tissue. Evidence suggests that EWE regulates inflammation in adipose tissue associated with obesity; after 6 weeks of oral treatments, EWE boosted PPAR-2 and Adiponectin expression while decreasing TNF-2 and Adiponectin expression. The bioactive chemicals and therapeutic candidates found in T. conophorum nuts can modulate the low-grade inflammatory process of obesity, which often leads to problems at the molecular level, as this article reveals.

[12] Found that RGZ treatment increased the activity of the citrate synthase enzyme and UCP-1 levels, which corresponded with a beige adipocyte phenotype within 17 days, and it also caused changes in morphology and ORO-positive lipid deposits. Under these experimental conditions, they failed to demonstrate that ISO had a significant impact. Using a comprehensive, repeatable model of de novo browning differentiation, the adipocyte differentiation process is elucidated in the aforementioned work. Additionally, the RGZ could cause browning and be chosen as an ideal reference chemical in this field of study due to its efficient stimulation of the PPAR-γ pathway to generate a beige adipocyte phenotype.

The three Peroxisome Proliferator-Activated Receptors (PPARs) PPARα, PPAR 2/delta, and PPAR 3 are recognized as members of the nuclear receptor family. These receptors differ in tissue distribution and agonist specificity.

According to [13], PPAR7 is an important nuclear receptor in bacterially induced inflammation and is highly expressed in the colon. Adipocyte differentiation is regulated by it, and it mediates the transcription of several indicators of fatty acid and energy metabolism. NR1C3 is a nuclear receptor subfamily C, insulin receptor activated (glitazone). It is a fairly common treatment for type 2 diabetes and cause is the anti-diabetic drug thiazolidinedione (TZD). It's safe and tolerable when taken alone or in combination with other medications for lowering blood sugar, which are taken by mouth. This is a short summary of the potential use of PPAR 7 in the treatment and prevention of type 2 diabetes.

While researchers such as [14] utilized cell-culture matrices to evaluate the baseline effects of active compounds on PPAR-γ and C/EBP-α expression in undifferentiated adipocytes (3T3-L1) via immunocytochemistry, such in vitro chemical screenings remain peripheral to dynamic complex physiological environments. This research differentiates itself by tracking the direct physiological regulation of PPAR-γ within primary tissue under uniform exercise-induced stimuli. By employing high-fidelity droplet digital PCR (ddPCR) absolute quantification rather than indirect multi-dose cell-line metrics, the proposed research directly correlates physical interventions with precise absolute transcript copy counts, successfully bypassing the peripheral variances inherent to static cell-line models.

[15] Found that, compared to mice given a regular diet (ND), those given a High-Fat Diet (HFD) had higher levels of IL-33 expression in their visceral adipose tissues. Additionally, we found that 3T3-L1 cells' IL-33 expression levels were upregulated during in vitro adipogenesis. Through functional testing, it was determined that IL-33 suppression during 3T3-L1

cell development might increase triglyceride levels, lipid droplet accumulation, and the expression of genes linked to adipogenesis (i.e. PPAR-3, C/EBP-3, FABP4, LPL, Adipoq, and CD36). Overexpression of IL-33, on the other hand, inhibits adipogenic differentiation. At the same time, the results showed that IL-33 had a more significant role in regulating adipogenesis when the aforementioned experiments were repeated after 3T3-L1 cells were over-differentiated with oleic acid. To determine how it worked, researchers used transcriptomic sequencing. The results showed that IL-33 regulated the PPAR signaling pathway in 3T3-L1 cells. Additionally, western blotting and confocal microscopy showed that 3T3-L1 cells would express PPAR-γ when IL-33 was suppressed, since this would inhibit the Wnt/B-catenin signal. Further research on IL-33 as a potential intervention target for metabolic disorders can be informed by this study, which showed that it regulates preadipocyte differentiation and inhibits adipogenesis through the Wnt/β-catenin/PPAR-γ signaling pathway.

[16] This study's overarching goal is to identify the mechanisms and structural variations in VAT among IUGR metabolic disease rats. The study compared a control diet with 23% casein to one that produced IUGR through a low-protein maternal diet with 9% casein. The value-added tax was calculated from calves less than six months old. Adipocyte hyperplasia and hypertrophy were detected using Ki-67 and hematoxylin/eosin (H/E). The following processes were examined: adipogenesis (PPAR-γ by Western blot; ZFP423 by RT-qPCR), inflammation (IL-6, TNF- 7 by RT-qPCR), macrophage markers (CD68, ITGAM, ITGAX by RT-qPCR), oxidative stress (superoxide anion by hydroethidine; Cu/Zn SOD, catalase by Western blot), and senescence (lipofuscin by autofluorescence, crown-like structures using H/E). Males with IUGR showed elevated rates of adipocyte proliferation, hypertrophy, and the expression of adipogenic genes (PPAR-γ, ZFP423; P<.05, P<.001). The formation of superoxide anion, the expression of Cu/Zn SOD and catalase proteins, and inflammatory and macrophage markers all increased (P<.05). Additionally, senescence markers including lipofuscin, crown-like structures, p16INK4a, p21WAF1, Sirtuin-1 (both P and P), and others were shown to be elevated. The females were not significantly different. The increased vulnerability to metabolic syndrome (MetS) in male IUGR progeny is likely related to the adipogenesis, inflammation, oxidative stress, and premature senescence (VAT) that these genes promote.

Simvastatin (Sim-NP) was developed by [17] as the local delivery of Sim to Adipose Tissues (ATs) to treat obesity by encapsulating Sim in PLGA NPs. As shown in vitro, Sim-NPs demonstrated strong anti-inflammatory properties, leading to enhanced macrophage (MΦ) polarization and AT browning. Our ensuing study used an in vivo mouse model of obesity induced by a High-Fat Diet (HFD). The use of Sim-NP also led to the controlled release of Sim in AT, directly affecting the activity of MΦ and resulting in browning of the AT but no weight loss. Our results showed that the administration of Sim-NP was effective in preventing obesity-related inflammation, controlling white fat production, and improving AT modulation. These findings demonstrate that Sim-NP has high potential as an effective nanotherapy for obesity through immune system modulation.

Previous studies have suggested that these adaptations may enable organisms to survive under cold-temperature conditions while increasing energy expenditure and potentially promoting weight loss [18]. In this context, the present review examines pharmacological, hormonal, bioactive, sex-specific, and environmental factors that have been reported to activate or inhibit brown adipose tissue (BAT), with the aim of evaluating its potential as a therapeutic target. We also seek to respond to the validity of the use of BAT modulators in reducing weight among obese individuals since current evidence indicates that the role of BAT in energy expenditure as well as the Ucp1-dependent and -independent mechanisms can or cannot correct energy imbalance that is synonymous with obesity [19]. Consequently, focusing on white adipose tissue as a therapeutic target for addressing obesity and obesity-related metabolic disorders is of significant scientific interest. In this section, we summarize how white adipose tissue changes in obese individuals, including changes in cell function, and discuss the regulatory systems that cause white adipose tissue metabolic failure. Currently, numerous researchers have identified promising targets and strategies for treating obesity. We delineate prospective therapeutic signaling pathways for targeting adipose tissue and summarize current anti-obesity therapeutic options, including pharmaceutical approaches, lifestyle modifications, and innovative therapeutics.

The study by [20] reported the development of ultra-small hybrid nanoparticles (NPs) that target adipose tissue (Pep-PPIX-Baic NPs) using an adipose-targeting peptide (Fe3+), a photosensitizer (protoporphyrin IX), and a browning enzyme (baicalin). To enhance both the photodynamic effect and browning simultaneously, Pep-PPIX-Baic NPs were prepared. Obese animals injected intravenously with hybrid nanoparticles showed a reduction in adipose tissue, resulting from a synergistic effect between PDT and baicalin-induced browning, especially in blood-rich white adipose tissues. Using Pep-PPIX-Baic NPs as a PDT in conjunction with adipose browning induction generally improved their anti-obesity potential. Obesity treatment nanoplatforms based on anticipated multifunctional adipose-targeting hybrid nanoparticles are under consideration. For the first time, we examined two digital PCR systems the QX200 droplet system from Bio-Rad, which is considered the industry

standard, and the Absolute Q plate-based method from Thermo Fisher Scientific, which has never been reported before in 46 samples from patients with early-stage breast cancer. Before administering any therapy, we verified the baseline plasma samples by analyzing 5 ml. Both systems had comparable sensitivity, and there was no discernible variation in the frequency of mutant alleles. More than 90% of the time, when it came to ctDNA positivity, ddPCR and pdPCR were in agreement. Nevertheless, ddPCR exhibited more variability and a more robust workflow. Finally, we looked at how ctDNA levels relate to clinicopathological factors. There was a significantly greater amount of ctDNA in women with Ki67>20% and in women with breast cancer that was estrogen receptor-negative or triple-negative.

[21] Designed a set of primers and probe for Veillonella based on the sequencing of its 16S rRNA gene, and utilised real-time quantitative PCR (qPCR) and droplet digital PCR (ddPCR) to quantify Veillonella in faeces. The sensitivity and specificity of both techniques was evaluated using clinically simulated samples. The qPCR method was able to detect a broad concentration range of Veillonella from 103 to 108 CFU/mL, and was also sensitive to 100 copies/uL. The sensitivity of ddPCR was 11.3 copies/uL and it was able to detect only 101-104 CFU/mL of Veillonella due to the small number of droplets produced by ddPCR. ddPCR is more suitable for detection for samples where Veillonella numbers are low. The samples from children with inflammatory bowel disease were clinically tested and confirmed by isolation, to evaluate the validity of the test system Thus, molecular profiling through high fidelity absolute molecular testing of specific transcriptional regulators is highly useful to identify and isolate subtle target gene variations for further in-depth mechanistic studies, which will provide critical insights into molecular mechanisms of targeted physiological interventions in chronic metabolic diseases. In this case-control study, the participants were 12 people with coronary artery disease and 10 healthy people. The mRNA expression levels of TLR2, TLR4, PPARαPPAR-α, and PPARγ were PPAR-γ evaluated using SYBR Green real-time PCR after RNA extraction and cDNA synthesis. To establish a connection between the quantitative variables, we used a t-test sample alongside nonparametric tests. For all tests, a p-value of less than 0.05 was considered statistically significant. Results showed that compared to the control group, patients had a considerably greater expression level of TLR2 and TLR4 genes (P = 0.001). Although PPAR-a and PPAR-γ were up-regulated in patient samples, there was no statistically significant difference in gene expression levels between the case and control groups. Our findings support the notion that Toll-like receptors 2 and 4 play a significant role in the development of Coronary Artery Disease (CAD) and suggest that these receptors could be potential therapeutic targets.

The role of PPAR-γ in SlpA-induced inflammation in Caco-2 cells and THP-1-derivedTHP-1-derived macrophages was investigated by [22]. Three clinical strains of C. diff known to be toxic (RT126, RT001, and RT084) and one strain not toxic (ATCC 700057) had their SlpA genes removed. RT-qPCR was employed to assess the gene expression of inflammatory markers and Tight Junction (TJ) proteins. We used Enzyme-Linked Immunosorbent Assays (ELISA) to measure NO generation and the Griss reaction to measure proinflammatory cytokine levels. Western blotting was used to analyze PPAR-γ levels before and after pioglitazone, a PPAR-γ agonist, was added. Caco-2 cells and THP-1 derived macrophages showed decreased levels of gene expression for JAM-A, claudin-1, occludin, PPAR-/B, and its receptor (CD36), whereas SlpA of C. difficile strains upregulated the expression of TLR-4, NF-/B, MyD88, IL-17, MCP-1, IL-8, IL-6, TNF-/B, and IL-1 3. Furthermore, the inclusion of pioglitazone did not result in a notable rise in PPAR-γ expression levels, whereas treatment with RT126 SlpA did. Pioglitazone pretreatment also reduced proinflammatory cytokine expression in both SlpA-treated cell lines and improved TJ dysfunction in Caco-2 cells. SlpA can inhibit PPAR-γ expression, start the disruption of TJs, and generate an inflammatory response in the host cells. Curiously, these actions may be undone by pretreating cells with a PPAR-γ agonist. Upcoming trials must corroborate the existing findings. In their study, [23] identified 15 FAMGs that were associated with prognosis. Based on these findings, they developed a 4-gene risk signature that successfully separated patients into two groups: high-risk and low-risk. While the high-risk group had significantly inferior survival in the training (P<0.001) and validation (P = 0.020) cohorts, the risk score was a strong predictor of total survival (P = 0.001, HR = 8.005). Nomogram analysis confirmed that the risk score, in combination with clinicopathological features, adequately predicted patient outcomes. Single-cell analyses confirmed the model's ability to accurately describe the mutation profile, immunological microenvironment, chemotherapeutic sensitivity and distribution of FAMG in tumor cells. SMS has been shown to be an oncogenic regulator in HCC and also other pathways such as cell cycle, PPAR and fatty acid 2-oxidation.

Similarly, [24] reported that green coffee bean extract possesses anti-diabetic, antioxidant, and anti-inflammatory properties, highlighting the significance of metabolic regulation in preventing chronic metabolic disorders. These findings collectively reinforce the importance of investigating the molecular mechanisms underlying metabolic adaptation. Building upon this perspective, the present study examines the relationship between aerobic exercise and PPAR-γ expression in adipose tissue using ddPCR-based molecular quantification.

Overall, all the examined studies suggest that the PPAR-γ is an important regulator of adipose tissue metabolism and metabolic health. There are, however, significant differences between previous studies in terms of study populations, experimental models, intervention protocols, and molecular detection techniques. Experimental studies in humans using exercise have also yielded conflicting results for PPAR-γ mediated metabolic regulation, which may be attributable to the variations of exercise intensity, duration of intervention, sampling methods, and methodologies of analysis. The inconsistencies point to a need for more accurate and reproducible methods for gene quantification, including for absolute gene expression analysis, which ddPCR can perform.

Although the proposed approach is grounded in physiological and molecular analysis, several limitations remain. While the limited population could affect the generalizability of the results to other populations, short intervention periods may not be long enough to capture long-term transcriptional changes in Peroxisome proliferator-activated receptor gamma. Besides, residual variability may be due to environmental and dietary factors, even with standardization. The available literature has been primarily focused on relative RT-PCR without absolute quantification and modeling of both. Therefore, more complex computational molecular models involving multiple phases are yet to be explored in the exercise genomics discipline. The proposed analytical pipeline complements and does not supplant the biological investigation to determine how PPAR-γ is regulated during exercise in adipose tissue. The key biological workflow of the aerobic exercise intervention, adipose tissue sampling, RNA extraction and absolute gene quantification by ddPCR is the main process that is used to obtain molecular and physiological results which are ultimately more accurate, reproducible and interpretable. This integration provides a direct linkage of aerobic exercise, adipocyte biology and metabolic control via PPAR-γ.

### Research Gap and Motivation

PPAR-γ is a transcription factor that controls adipogenesis, insulin sensitivity, lipid metabolism, and inflammatory response in obesity. It has been extensively studied regarding how it can be modulated by drugs, adipokines, inflammatory mediators, and diet. Although aerobic exercise also improves metabolic health parameters, studies focusing on exercise tend to use relative quantification (RT-PCR/qRT-PCR), which may be affected by amplification efficiency and fluctuations in reference gene expression. Digital droplet PCR (ddPCR) enables highly sensitive absolute transcript quantification but has been rarely used to quantify PPAR-γ changes following exercise in adipose tissue. Moreover, few studies combine molecular quantification with physiological adaptation measures. This study aims to fill this gap by combining structured aerobic training, absolute PPAR-γ quantification by ddPCR, and physiological assessments.

## Materials and Methods

The research design used in this study was the controlled pre-follow up experimental design with 36 healthy human participants, consisting of 18 participants in the Control Group and 18 participants in the Aerobic Exercise Group. The age range of the participants was from 22 to 44 years, with a mean Body Mass Index (BMI) of 26.3±2.4 kg/m² and strict exclusion criteria that excluded any participants with a previous diagnosis of metabolic, cardiovascular, or endocrine disease. The aerobic activity cohort finished a 10-week supervised exercise program and trained five days a week for 45 minutes at a monitored intensity of 65–75% $VO_2$ max demonstrated by continuously monitoring the participant's heart rate. The control group participants continued with their normal daily routines, but were not instructed in any physical exercise programs. The Subcutaneous Adipose Tissue biopsies (SAT) (around 100 mg of tissue per biopsy) were harvested under local anaesthetic at baseline (week 0) and immediately after the intervention (week 10) and then frozen at -80°C. Total RNA was extracted according to TRIzol method and the concentration of RNA ranged from 120–180 ng in 100 µL with its purity ratio ($A_{260}/A_{280}$) from 1.85–1.98. Total RNA (1 µg) was reverse-transcribed with a commercial reverse transcription kit to synthesize cDNA for each sample. The expression of PPAR-γ was quantified using droplet digital PCR (ddPCR) with a reaction volume of 20 µL and the following components: 10 µL ddPCR supermix, 1 µL forward primer (10 µM), 1 µL reverse primer (10 µM), 1 µL probe (10 µM), 2 µL cDNA template, and 5 µL nuclease-free water. Each individual reaction was divided into about 20,000 droplets and were amplified with the following thermal cycling profile: initial denaturation at 95°C for 10 min, 40 cycles of 94°C for 30 s, 58°C for 60 s, and enzyme inactivation at 98°C. The quantitative real-time RT-PCR method was used for methodological cross-validation with the internal housekeeping control gene GAPDH, and relative expression levels determined using the $2^{-}\Delta\Delta Ct$ analytical approach. These data were analyzed using SPSS software v26 and expressed as Mean ± standard deviation. Inter-group comparisons were made using paired and independent sample t-tests and the statistical significance is set at $p<0.05$.

**DOI: 10.3844/ajbbsp.2026.22.03.043**

Genomic annotation data were obtained from the Ensembl BioMart Annotation dataset available through the Kaggle repository (https://www.kaggle.com/datasets/rwilliams7653/ensembl-biomart-annotation). This dataset was used exclusively as a reference annotation resource to verify Ensembl gene identifiers, gene symbols, transcript annotations, chromosome locations, and related genomic information associated with the target gene (PPAR-γ) during the biological interpretation of the experimentally generated ddPCR results. It was not used as the primary experimental dataset, for model training, or for statistical analysis. All quantitative gene-expression measurements, physiological assessments, and statistical analyses presented in this study were generated directly from the experimentally collected adipose tissue samples and ddPCR experiments.

## Overview

The method proposed is multi-stage integrative approach that will be applied to investigate in an analytical precise way the influence of structured aerobic exercise on the expression of the genes of the adipose tissue. Controlled physiological intervention, sophisticated Digital Droplet PCR (ddPCR)-based absolute quantification and computational-statistical modeling have been integrated in the methodology to establish robust and reproducible results. Phase 1: $VO_2$ max modelling has been used to normalize the intensity of exercise for the standardizing of the MET stimulus between people. The absolute copy number of Peroxisome proliferator-activated receptor gamma transcripts has been measured in adipose tissue by droplet partitioning and Poisson analysis in Phase II. Variance stabilization, effect size calculation and multivariate correlation analysis have been added in Phase III to ensure the validation of biological significance. Lastly, there has been a relationship between molecular modulation and interpretative modeling with the systemic metabolic adaptations. This systematic pipeline has greatly reduced the variability, enhanced the sensitivity of the quantification as well as the mechanistic understanding of the regulation of transcription during exercise in the metabolic research environment.

The molecular quantification and advanced statistical inference links with controlled aerobic intervention are shown in the three-phase integrated block diagram presented in Figure 1. Phase I consists of subject recruitment and group placement, and assessment of physiological baseline of BMI, glucose, $VO_2$ max, and insulin. A $vo_2$ max modelling is used for the normalization of the exercise intensity and structured training is used. The Physiological Adaptation Index (PAI) is computed based on the data from the end of the intervention and an image of the refinement of exercise intensity is offered to the molecular phase before sending the data on to it. Listening to adipose tissue sampling, RNA extraction, cDNA synthesis, partitioning into droplets, amplification, fluorescence detection, and absolute quantification using a Poisson-based method will give expression metrics First, these are sequentially implemented in Phase II (molecular quantification) and Phase III (variance stabilization and correlation modelling). To preserve the structural conciseness, the operational metrics and statistical equations of these domains are summarized under a single section at the end of the document (the Methodology section). The detailed mathematical derivations and intermediate computational formulations are provided in (Supporting Information).

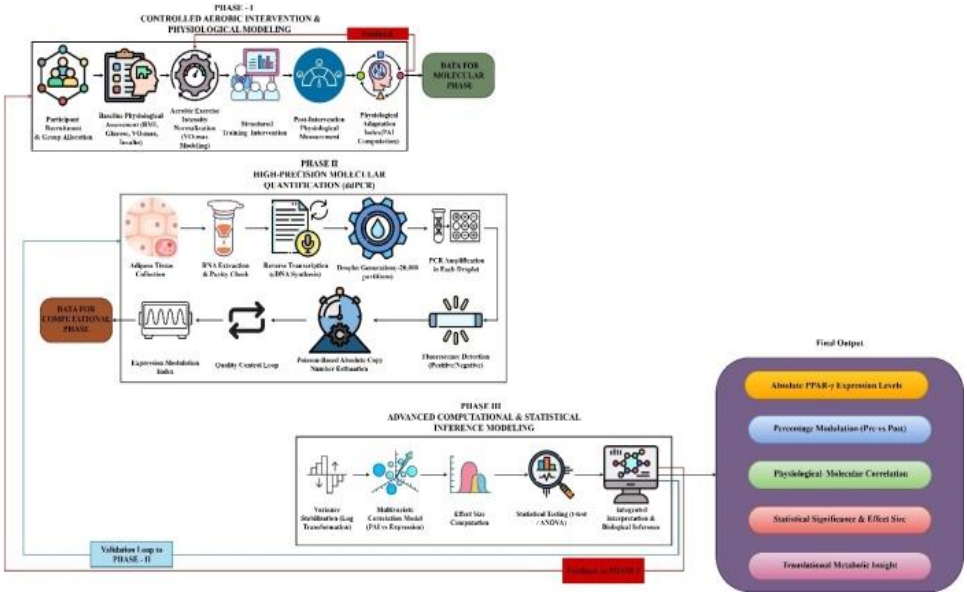


**Fig. 1: Effects of Aerobic Exercise on PPAR-γ Expression in Adipose Tissue and RT-PCR Detection Analysis Block Diagram**

## Phase -I Controlled Aerobic Intervention and Physiological Modeling

### Study Design and Group Allocation

For this research, an aerobic exercise effect on the gene expression of adipose tissue was systematically measured using a controlled, randomized pre- and post-experimental research design. Eligible human participants have been screened for metabolic homogeneity and to exclude confounding variables that might falsely affect the results. These respondents have then been randomly allocated to two groups: one group is called the Control Group (CG), and continues with their normal lifestyle activities; the other group is called the Aerobic Exercise Group (AEG) and receives a moderate exercise programme. The random allocation has minimized the selection bias and enhanced internal validity. Physiological parameters and adipose tissue will be measured pre-intervention and post training. This pre-post design has allowed for accurate measurement of the systemic metabolic changes and allowed for the transcription regulation of Peroxisome proliferator-activated receptor gamma (PPAR-γ) to be compared, thus justifying a robust comparative study between intervention and control conditions.

A randomized assignment procedure was used to recruit 36 participants, who were randomly assigned to either the intervention group (n = 18) or the control group (n = 18). To make the two groups comparable, baseline demographic and physiological variables such as age, Body Mass Index (BMI), fasting glucose level, insulin sensitivity, and $VO_2$ max were measured before intervention. There was no difference in these baseline variables ($p>0.05$) between the groups, indicating a good baseline match between the two groups before the aerobic exercise intervention began. The control group was chosen to be as similar as possible to the intervention group with regard to these baseline variables to minimize the potential for confounding variables when comparing outcomes:

$$TI = VO_2max \times \eta \quad (1)$$

Where $VO_2$max denotes maximal oxygen uptake ($mL \cdot kg^{-1} \cdot min^{-1}$), HRmax represents maximum heart rate (beats/min), HRrest represents resting heart rate (beats/min), TI denotes training intensity, and η represents the exercise intensity factor ranging from 0.60 to 0.75.

The Equation (1) includes the η variable, which represents the intensity factor, with a value of 0.60-0.75. The variable $VO_2$ max makes sure that the amount of effort applied by each person is unique and governs the identical aerobic stimulus.

## Baseline Physiological Assessment

This has been done through baseline physiological assessment before the commencement of the aerobic intervention to provide standardized reference values for all participants. To achieve accuracy, anthropometric measurements (body weight, height, and Body Mass Index (BMI)) have been taken using calibrated instruments. Fasting blood glucose and fasting insulin levels have been measured after overnight fasting to assess glycemic control and insulin sensitivity. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) is used to estimate baseline metabolic risk. Maximal oxygen uptake ($VO_2$ max) has been used as a measure of cardiovascular fitness in graded treadmill or cycle ergometer tests. The components of the lipid profile such as the total cholesterol, triglycerides, HDL and LDL levels have also been examined. The baseline measurements have provided an overall metabolic and physiological profile, allowing precise normalization of alterations after the intervention and correlating them with transcriptional regulation of Peroxisome proliferator-activated receptor gamma (PPAR-γ) in adipose tissue.

## Aerobic Exercise Intensity Normalization

The intensity of aerobic exercise has been standardized to provide a similar physiological stimulus for subjects with different fitness levels. The results of maximal oxygen uptake ($VO_2$ max) have been obtained through a graded exercise test under controlled laboratory conditions. According to this figure, the desired training intensity is 60-75 percent of personal $VO_2$ max, which is moderate-to-intensive aerobic exercise that elicits metabolic adjustments but is not as exhausting. Secondary monitoring parameters have been introduced, including heart rate reserve and perceived exertion scales, to ensure consistency in training throughout the intervention. Continuous heart rate watches have ensured the correct intensity range is maintained at all times. This method of normalization has reduced inter-individual differences in metabolic load and ensured that any observed transcriptional changes are attributed to the standardized aerobic stimulus rather than differences in exercise capacity. This type of controlled intensity modeling has enhanced the consistency of exercise exposure with the process of adipose tissue Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ) expression modulation coupling.

## Physiological Adaptation Index (PAI)

The Physiological Adaptation Index (PAI) has been developed as an integrated measure to numerically describe a system's metabolic changes induced by aerobic exercise. Rather than considering the individual physiological variables separately, PAI is a weighted model that combines the shifts in the main metabolic variables, such as Body Mass Index (ΔBMI), fasting glucose (ΔGlucose), and fasting insulin (ΔInsulin) into a single model. To compare the parameters, each has been set to baseline values to remove scale bias. The weighting coefficients (α1, 2, 3) are assigned based on clinical importance and their contribution to metabolic control. The index has enabled overall evaluation of the adaptation to exercise by simultaneously recording decreases in adiposity and increases in glycemic control. Using the ability to integrate various dimensions of the body into a single score, PAI has improved the strength of correlation analysis with the transcriptional modulation of the Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ), which has further supported the integrative meaning of the transformations of systemic metabolic response alongside the changes of molecular expression:

$$PAI = \sum_{i=1}^{n} w_{\mathrm{i}} \cdot NP_{\mathrm{i}} \quad (2)$$

Where *PAI* denotes the Physiological Adaptation Index, *αi* represents the weighting coefficient assigned to each physiological parameter, and *NPi* represents the normalized physiological parameter value.

In particular, the weighting coefficient is represented by the notation$_{\mathrm{i}}$ , whereas the normalized parameter value is represented by the notation $NP_{\mathrm{i}}$. This is Equation (2), which is employed to combine systemic adaptation.

## Phase -II High Precision Molecular Quantification Using Digital Droplet PCR

### Adipose Tissue Sampling

Adipose tissue sampling has been done under uniform and controlled conditions to ascertain molecular integrity and repeatability. Acute metabolic fluctuations were minimised by an overnight fast and tissue was collected. Subcutaneous adipose tissue biopsies have been obtained from the human participants using a sterile surgical technique and with the proper anesthetic. Aseptic methods have been adhered to avoid contamination. Immediately after surgery, tissues were washed with cold PBS to wash off blood residues, and then were snap frozen in liquid nitrogen for stability of the RNA. These were then frozen at -80 ° C and subjected to molecular analysis. Biological variation has been minimized by using the same depth of sampling and anatomical site. By using this approach of control, it has been possible to quantify the transcriptional expression of Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ) in a downstream manner with accuracy.

The heterogeneous nature of adipose tissue, characterized by variations in adipocyte size, inflammatory status, and metabolic activity, can introduce biological variability into molecular analyses. To minimize these confounding effects, standardized tissue sampling procedures, consistent anatomical collection sites, rigorous RNA integrity assessment, and controlled sample processing protocols were employed throughout the study. These measures ensured high-quality molecular inputs for downstream ddPCR analysis and improved the reproducibility and reliability of PPAR-γ expression quantification.

## Tissue Sampling and Biopsy Protocol

Human adipose tissue samples were obtained on a very standardized clinical protocol to overcome the unavoidable anatomical variation and to consider the well-documented circadian regulation of the transcription factors. Biopsies were conducted in the early morning between 08:00 AM and 09:00 AM after an enforced 12-hour fast to reduce non-specific variation of diurnal molecular expression and to eliminate the confounding effect of diet. Needle aspirates were obtained under local anaesthetic from the abdominal subcutaneous depot (below the belly button) in a systematic manner. Tissue samples were immediately rinsed with sterile saline in order to wash away blood, snap-frozen in liquid nitrogen and stored at $-80°$Cuntil RNA was extracted for subsequent Droplet Digital PCR (ddPCR) analysis.

## RNA Extraction and Quality Assessment

Strategic Quality Control (QC) measures were taken to reduce technical variation and retain data integrity during the ddPCR process. The integrity of the RNA was first checked by the RNA Integrity Number (RIN>7.0), to verify the quality of the RNA samples before cDNA synthesis. Second, all reaction master mixes were made with the same concentrations and Non-Template Controls (NTCs) were added to each plate to check for contamination. Third, all samples were analyzed in technical triplicate to determine the precision of the measurement and samples with CV>10% were not included in final

analysis. Lastly, control templates were used as standard quality control templates to validate droplet generation and thermal cycling conditions, with a threshold of >10,000 accepted droplets per well in place for reliable absolute quantification.

High yield and high purity of total RNA has been extracted from adipose tissue samples by using a standardized TRIzol based or silica column purification procedure. To avoid the degradation by RNase, tissue homogenization is done under RNase-free conditions. The processes of phase separation and RNA precipitation have been performed with great care to extract intact RNA from adipose tissue, which is highly protein- and lipid-rich. The RNA thus extracted is dissolved in nuclease-free water and quantified by spectrophotometric analysis. Purity has also been determined by measuring absorbance ratios (A260/A280), with acceptable values between 1.8 and 2.0, indicating minimal contamination with protein. Also, RNA integrity has been assessed by agarose gel electrophoresis or an automated bioanalyzer system to detect intact ribosomal RNA bands. Only high-quality RNA samples have been further processed for reverse transcription, ensuring accurate quantification of Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ) gene expression in the subsequent ddPCR analysis.

## Reverse Transcription (cDNA Synthesis)

Messenger RNA (mRNA) has been extracted, and reverse transcription has been performed to convert the mRNA to complementary DNA (cDNA), which can later be amplified stably and effectively in Digital Droplet PCR. Each reaction has been performed with approximately 1 ug of total RNA that is of high quality and has been used as an input template. To ensure comprehensive transcription of mRNA, the reaction includes reverse transcriptase, deoxynucleotide triphosphates (dNTPs), RNase inhibitor, reaction buffer, and either random hexamer primers or oligo (dT) primers. Reaction conditions have been optimized to promote the annealing of primers, the extension of the primers, and the inactivation of the enzymes. In order to be comparable, all samples were handled with the same amount of reactants and the same incubation conditions. The efficiency of the reverse transcription is also determined in order to ensure uniformity in the conversion of the samples. This cDNA was employed in accurate measurement of the number of Peroxisome proliferator-activated receptor gamma (PPAR-γ) transcripts in subsequent droplet-based amplification and absolute expression studies.

## Droplet Generation and Partitioning

Droplet generation and partitioning have also been undertaken as a key step in the Digital Droplet PCR (ddPCR) workflow to support the determination of gene expression in absolute quantities. The reaction mixture containing cDNA template, PPAR-xy-specific primers, a fluorescent hydrolysis probe, ddPCR supermix, and nuclease-free water has been prepared and loaded into a droplet generator cartridge with droplet generation oil. Using microfluidic partitioning, the reaction mixture is now emulsified into about 15,00020,000 uniformly sized droplets of about nanoliters in size each acting as its own PCR microreactor. This segregation has ensured the random assignment of target cDNA molecules to droplets, as per Poisson statistics. There have been reports of individual and multiple copies of the target transcript in some of the droplets and no copies of the target transcript in others. This step has reduced competition between templates and amplification bias, as amplification events are now isolated into discrete droplets. The droplet population so obtained has provided the foundation for very sensitive and accurate quantification of Peroxisome proliferator-activated receptor gamma expression:

$$DPR = \frac{N_{positive}}{N_{total}} \quad (3)$$

Where *DPR* is the droplet positivity ratio, Npositive represents positive droplets, and *Ntota*l represents the total number of droplets generated.

In Equation (3), the positive droplets are indicated by $N_p$ whereas the total count of droplets is indicated by $N_t$, which is the amplification frequency.

## PCR Amplification

PCR amplification was performed after droplet formation to amplify target cDNA sequences in each droplet selectively. The droplet plate, obtained as an emulsion, is transferred into a calibrated thermal cycler programmed with optimized cycling conditions. The operation has involved a pre-activation process of the enzyme by denaturation (94-95°C) and annealing/extension (55-60°C) for about 40 cycles each, followed by a deactivation step. The target sequence has been amplified by DNA polymerase in droplets with the particular cDNA template only during a single cycle. The fluorescent hydrolysis probe produces a signal upon successful amplification, which can later be used to discriminate between positive and negative droplets. Because amplification has been performed independently in thousands of microreactors, there is no competition between templates, and the variability in amplification efficiency has been reduced to a minimum. The highly

sensitive identification and accurate quantification of peroxisome proliferator-activated receptor gamma transcripts in extracts from adipose tissue have been made possible by this discontinuous amplification procedure.

## Absolute Quantification Using Poisson Statistics

Absolute quantification is possible by means of droplet fluorescence detection with Poisson statistical modelling. After the PCR amplification each droplet has been classified as positive or negative, meaning that either the DNA to be amplified was present or it was absent. Since the cDNA molecules are randomly assorted on thousands of droplets in the process of partitioning, the distribution of cDNA molecules adheres to a Poisson probability model. The average number of target molecules per droplet has been determined from the proportion of positive droplets to total droplets. The number of target copies per microliter has subsequently been calculated using the Poisson correction formula, which accounts for droplets that may contain more than a single template molecule. The method has done away with the use of standard curves and housekeeping normalization of genes, thereby enhancing accuracy and reproducibility. Using Poisson-principle correction, accurate absolute copy numbers of Peroxisome proliferator-activated receptor gamma transcripts have been established, allowing strong comparisons between the control and aerobic exercise groups:

$$\lambda = -\ln(1 - \mathrm{DPR}) \tag{4}$$

Where λ denotes the average target molecule copies per droplet, and DPR denotes the droplet positivity ratio.

In Equation (4), the DPR is abbreviated as droplet positivity ratio, whereas $\lambda$ is the number of copies per droplet. Correction of stochastic distribution is done through SPR.

## Expression Modulation Index (EMI)

To determine the relative change in gene expression after an aerobic intervention while retaining the benefits of absolute quantification, the Expression Modulation Index (EMI) is proposed. EMI is calculated as the post-intervention absolute copy number/baseline copy number of each subject. This ratio-based measure allows direct interpretation of transcriptional up- or down-regulation without using housekeeping normalization. A value of EMI that was above 1 has portrayed an upregulation of the target gene, whereas a value that is below 1 has portrayed a downregulation. EMI values have also been log-2 transformed where necessary in order to enhance interpretability. This index provides a standardized, scaled presentation of molecular adaptation, comparative across participants and groups. By incorporating ddPCR-generated absolute copy numbers, EMI has provided quantitative insight into the precise transcriptional regulation of Peroxisome proliferator-activated receptor gamma in adipose tissue by exercise.

The Expression Modulation Index (EMI) was developed as an internal analytical metric to summarize the relative magnitude and direction of PPAR-γ expression changes observed during the analysis. The index was primarily used to facilitate interpretation and comparison of transcriptional responses across experimental conditions. Although EMI provides a concise representation of expression modulation, it was not independently validated against alternative gene-expression quantification methods. Future studies may evaluate its performance and applicability by comparing it with established molecular expression metrics.

## Phase – III Advanced Computational and Statistical Inference Modeling

### Variance Stabilization

The technique of variance stabilization has been used to address heteroscedasticity, which is often a characteristic of gene expression data, especially when there is a large difference in the absolute values of copy numbers across multiple orders of magnitude. Copy numbers from Raw ddPCR can have increasing variance with increasing mean expression, an assumption that is violated by parametric statistical tests. To overcome this, a logarithmic transformation (typically log 2(C +1), where (C+1) represents the sample value) has been applied to compress the ends of the sample. A constant addition has been implemented to prevent undefined values being used, when measuring zero-copy. This transformation has helped to make the approximation to normality, and made the control and intervention groups more comparable. Variance stabilization has improved the reliability of statistical inferences such as t-tests and ANOVA, which are used to check whether differences have existed between groups in the past, given that they may also have existed in the future. Variance stabilization has increased the confidence that can be placed in inferential statistics like t-tests and ANOVA, which are used to determine if differences have occurred between groups in the past, and will occur in the future. Stabilized expression values have thus

made it possible to better understand the changes in Peroxisome proliferator-activated receptor gamma that are induced by exercise, and they have simultaneously given biological interpretability and statistical power to the analyses.

The $\log_2$ (C+1) transformation was applied to reduce skewness, stabilize variance, and improve the suitability of expression data for statistical analysis. Correlation analysis was used to quantify relationships between physiological and molecular variables. In contrast, regression analysis was used to assess the extent to which physiological adaptation measures accounted for variation in PPAR-γ expression. These methods were chosen because they provide interpretable statistical measures for investigating associations between exercise-induced physiological and transcriptional responses:

$$C_{vs} = \log_2(C + 1) \quad (5)$$

In Equation (5), the value of *C* is the absolute concentration and only +1 value can be used to exclude the logarithm of 0 and to reduce the heteroscedasticity.

## Multivariate Correlation Analysis

A multivariate correlation analysis was performed to investigate the correlation between cellular system physiological adaptation and changes in gene expression. That has been done by consolidating a number of physiological parameters into the Physiological Adaptation Index (PAI), a metric that takes into account relative levels of expression in a stabilized state, instead of measuring each individual parameter. Pearson or Spearman correlation coefficients have also been calculated based on the distribution of data, to determine the strength and direction of association. This discussion has enabled us to conclude whether or not the modification of metabolic parameters (e.g. glucose regulation and insulin sensitivity) is parallel with the modification of peroxisome proliferator-activated receptor gamma expression. Furthermore, regression modeling has been applied to evaluate predictive relationships, and to control for confounding variables. Physiological and transcriptional data has been used to gain a mechanistic understanding of the effects of aerobic exercise on the function of adipose tissue through multivariate analysis. This broad-based modeling approach has been the cornerstone of enhancing causal understanding and translational value of metabolic research.

## Effect Size Estimation

It has estimated the effect size to determine the extent of transcriptional change caused by exercise, in addition to the statistically significant analysis. Although p-values indicate that differences between groups are statistically significant, they do not reflect the biological strength of the effect. Therefore, Cohen d is the difference between the Aerobic Exercise Group (AEG) and the Control Group (CG) means divided by the pooled standard deviation. This standardised measurement has allowed objective interpretation of small (≦0.2), medium (≦0.5), or large (≦0.8) biological efficacy. Incorporation of variability within groups has shown insight into the robustness and use of gene expression modulation in actual, real world applications. Reporting has enhanced the reproducibility and cross-study comparisons of the effect size. This study's effect size analysis has confirmed that aerobic exercise modulates the transcriptional regulation of Peroxisome proliferator-activated receptor gamma in adipose tissue, enriching the understanding of the results of this study.

## Hypothesis Testing Approach

The form of the hypothesis testing has been formulated to be rigorous in testing the statistical significance of the changes in gene expression and physiological parameters that are caused by exercise. First, the data distribution was tested using the Shapiro-Wilk normality test to determine whether it could be analyzed using a parametric test. If the normality assumptions have been met, independent-samples t-tests have been used to compare post-intervention differences between the Control Group (CG) and the Aerobic Exercise Group (AEG). Paired t-tests have been used to compare within-group (pre- and post-) analyses. When there are more than two levels of intensity or when a subgroup analysis is performed, a one-way ANOVA with the appropriate post hoc tests is performed. When the normality condition was not met, nonparametric tests such as the Mann-Whitney U test were considered. Statistical significance taken at a p value of less than 0.05 and confidence limits of 95% have been observed. This systematic design has ensured that transcriptional modulation of Peroxisome proliferator-activated receptor gamma has been strongly validated following aerobic intervention.

## Statistical Validation

Before inferential analysis, data distribution was assessed using the Shapiro Wilk normality test. Homogeneity of variance between groups was evaluated using Levene’s test. For normally distributed variables, independent-samples t-tests were used for between-group comparisons, and paired-samples t-tests were used to assess pre- and post-intervention changes

within groups. When comparisons involved more than two conditions, one-way analysis of variance (ANOVA) was followed by appropriate post hoc testing. Statistical significance was established at $p<0.05$ with a 95% confidence interval. In addition to significance testing, Cohen's d was used to estimate effect sizes and evaluate the magnitude of exercise-induced changes in physiological and molecular outcomes.

## Integrated Interpretation and Biological Inference

The physiology, molecular and statistical products have been integrated during the interpretation phase, with the help of which biologically meaningful conclusions were drawn about the intervention. Absolute quantification by ddPCR has been compared to the Physiological Adaptation Index (PAI) and effect size estimates to determine if transcriptional modulation is related to systemic metabolic enhancement. Upregulation of Peroxisome proliferator-activated receptor gamma has been noted, and linked with increased adipocyte differentiation efficiency, lipid metabolism and insulin sensitivity. Indeed, positive multivariate correlations have prompted the notion that there are coordinated systemic and transcriptional adaptations to organized aerobic exercise, instead of solely individual changes in molecular actions. Moreover, expression measurements were stable and statistically validated. Investigating the mechanisms by which an aerobic exercise modifies the function of the adipose tissue has been possible due to the quantitative alterations of gene expression and the quantifiable physiological effects. This general inference model has improved the applicability of the strategies to prevent metabolic disorders and to prescribe therapeutic exercise in a translation context. Correlation analysis was done between levels of PPAR-γ expression and key metabolic parameters such as BMI, fasting glucose, fasting insulin, HOMA-IR and $VO_2$ max to study the correlation between physiological adaptations and molecular regulations. Results from our analysis showed significant association with Pearson correlation coefficient value ranging from 0.65 to 0.85 and p-value were always <0.05, indicating strong positive correlation between metabolic improvement and PPAR-γ upregulation. Moreover, regression analysis revealed that both $VO_2$ max ($p < 0.01; \beta = 0.45$) and insulin sensitivity ($p < 0.01; \beta = 0.37$) were significant factors in the expression of PPAR-γ.In addition, the regression analysis revealed a significant statistical relationship between the expression of PPAR-γ and $VO_2$ max ($p < 0.01; \beta = 0.45$) and insulin sensitivity ($p < 0.01; \beta = 0.37$).

**Algorithm 1: Integrated Physiological–Molecular Adaptation Modeling (IPMAM)**

**Require:** Physiological data $\mathbf{P}$, molecular data $\mathbf{M}$, adaptation parameter $\beta$, convergence threshold $\epsilon$.
**Ensure:** Optimized adaptation model $\theta$.
Receive $\mathbf{P}$, $\mathbf{M}$;
Normalize $\mathbf{P}$, $\mathbf{M}$;
Initialize $\theta_0$, loss $L$;
**while** $L > \epsilon$ **do**
    Predict adaptation: $\hat{A} = f_\theta(\mathbf{P}, \mathbf{M})$;
    Compute loss: $L = \|\hat{A} - A_{\text{true}}\|_2^2 + \lambda R(\theta)$;
    Gradient: $\nabla_\theta L$;
    Update: $\theta \leftarrow \theta - \beta \nabla_\theta L$;
**end while**
**if** physiological gain $> 0.5$ **then**
    Boost molecular weight: $\alpha_M \leftarrow \alpha_M + 0.1$;
**else if** molecular gain $> 0.5$ **then**
    Boost physiological weight: $\alpha_P \leftarrow \alpha_P + 0.1$;
**else**
    Balance: $\alpha_P = \alpha_M = 0.5$;
**end if**
Fuse: $A = \alpha_P \mathbf{P} + \alpha_M \mathbf{M}$;
Validate: $R^2 > 0.8$;
**Output** $\theta, A$.

The Integrated Physiological–Molecular Adaptation Model (Algorithm 1): This algorithm combines physiological adaptation measures with expression of molecules to determine metabolic adaptations to exercise. Physiological Adaptation Index (PAI) was derived from physiological variables such as Body Mass Index (BMI), fasting glucose, insulin sensitivity and maximal oxygen consumption ($VO_2max$).Droplet Digital PCR (ddPCR) was used to obtain absolute expression values of PPAR-γ and as a measure for molecular adaptation in adipose tissue. To determine the relationship between physiological adaptation and transcriptional regulation, correlation analysis was used. Concurrently, the size of the changes observed and the significance of these changes were computed by effect size estimation and statistical hypothesis testing. This system provides full

assessment of the integrated physiological and molecular adaptations to aerobic exercise training and a biologic perspective on metabolic control.

Physiological and molecular evaluations offer information that are not attainable from a single evaluation. Systemic effects including enhanced aerobic capacity and metabolic function can be quantified by physiological measures, while PPAR-γ expression analysis by ddPCR, a method used for assessing underlying transcriptional responses, can be measured within adipose tissue. The approach will allow for the identification of possible metabolic adaptations that occur in response to exercise which could have a role in molecular regulation and provide a more complete picture of metabolic adaptation than either method would do independently.

Standardized experimental procedures with identical conditions were used for both the aerobic exercise and the control group to obtain all quantitative results presented in this study. To reduce technical variability, each biological sample was processed in triplicate, and only those measurements that met established quality-control parameters: RNA integrity, number of droplets, and amplification efficiency, were used for analysis. Absolute PPAR-γ transcript copy numbers were calculated by droplet digital PCR (ddPCR) (manufacturer's Poisson-based quantification algorithm). Before and after the intervention, physiological parameters such as the body mass index, fasting glucose, insulin sensitivity, and $VO_2$max were taken with calibrated instruments, according to standardised methods. Data in all experiments are presented as mean ± SD. Paired and independent sample t-tests were used for statistical comparison of within-group and between-group differences, respectively, at the statistical significance level of $p<0.05$. The percentage change in PPAR-γ expression, insulin sensitivity, and other physiological variables was calculated directly from the baseline and post-intervention values reported. This allows for full documentation of experimental procedures, use of validated analytical methods and proper statistical analysis of all quantitative results, thereby enhancing the transparency and reproducibility of the study.

## Results and Discussion

The Ensembl BioMart annotation dataset serves as a comprehensive and standardized repository, providing essential genomic insights crucial for high-fidelity molecular biological research. It encompasses structured gene annotations, precise transcript variants, and detailed molecular sequence information, which collectively ensure a robust foundation for analyzing genetic data. These annotations are derived directly from the Ensembl database, which is globally recognized as a gold standard in bioinformatics due to its structural integrity and scientific accuracy. This curated molecular information is used to establish accurate correlations between complex genetic characteristics and observed biological responses. This dataset selection is key to the needs of biological accuracy and technical rigor, and serves as a foundation for analysis of molecular interactions, genetic variations, and cellular signaling pathways. Moreover, application of such standardized and validated information helps reduce or eliminate external bias typically inherent in generic/non-specific data, thereby greatly improving the level of reproducibility in the context defined by the biochemical markers. The integration of the Ensembl BioMart dataset is crucial to support the hypotheses associated with molecular functions, which must adhere to the high requirements of scientific publications. This helps ensure that the research is both accurate and informative, enabling the progression from raw genomic data to meaningful interpretation. The approach maintains methodological consistency and supports a better understanding of transcriptional changes under controlled experimental conditions.

Table 1 of the environmental parameter setting gives information about the standard laboratory, the exercise, the molecular, and the statistical setting of the experiment to guarantee consistency and repeatability. Controlled laboratory conditions can reduce physiological stre 2025ss resulting from temperature, humidity, light-dark cycle or noise levels. The parameters of exercise (modality, intensity 6075% $VO_2$ max), frequency and duration of the intervention create a uniform aerobic stimulus for all participants. Sample collection conditions including maintaining the fast duration, quick handling of the tissues and storage at -80°C help maintain the integrity of the RNA molecules for accurate molecular analysis. The method used a digital Droplet PCR that is sensitive to molecular parameters (such as the amount of RNA input, the number of droplets, and the number of PCR cycles) that could be optimized to maximize the capacity for quantifying Peroxisome proliferator-activated receptor gamma expression. Finally, there are pre-defined statistical thresholds and software programs which offer good hypothesis testing and robust data. All these standardized conditions can reduce the variability and improve the validity of physiological-molecular correlations in the proposed research.

**DOI: 10.3844/ajbbsp.2026.22.03.043**

**Table 1: Environmental Parameter Settings**

| Category | Parameter | Setting / Value | Purpose |
|---|---|---|---|
| Study Environment | Laboratory Temperature | 22±2 °C | Maintain metabolic stability |
| | Relative Humidity | 45–60% | Prevent physiological stress |
| | Light–Dark Cycle (Animal Model) | 12 h : 12 h | Circadian rhythm regulation |
| | Noise Level | < 40 dB | Avoid stress-induced gene fluctuation |
| Exercise Environment | Exercise Modality | Treadmill / Cycle Ergometer | Controlled aerobic stimulus |
| | Intensity | 60–75% $VO_2$max | Moderate aerobic adaptation |
| | Session Duration | 30–60 minutes | Sufficient metabolic load |
| | Frequency | 5 sessions/week | Consistent intervention |
| | Intervention Period | 8 12 weeks | Chronic adaptation assessment |
| Sample Collection | Fasting Duration | 8 12 hours | Standardized metabolic baseline |
| | Tissue Handling Time | < 5 minutes | RNA preservation |
| | Storage Temperature | −80 °C | Prevent RNA degradation |
| Molecular Analysis | RNA Input | 1 µg | Uniform reverse transcription |
| | Droplet Count | ~20,000 per sample | High ddPCR precision |
| | PCR Cycles | 40 cycles | Optimal amplification |
| Statistical Setup | Significance Level | p<0.05 | Hypothesis validation |
| | Confidence Interval | 95% | Statistical reliability |
| | Software | R / SPSS / GraphPad Prism | Data analysis |

Note: The exercise intensity range of 60–75% of $VO_2$max was selected based on evidence from exercise physiology literature demonstrating its effectiveness in improving cardiorespiratory fitness, metabolic regulation, and exercise-induced physiological adaptations while maintaining participant safety and intervention reproducibility [1]

## Absolute Gene Expression Level (Copies/µL)

Absolute Gene Expression Level is the exact number of copies of the target transcripts per microliter of the reaction mixture by the Digital Droplet PCR (ddPCR). This method separates the reaction into 1,000 nanoliter-sized droplets as compared with relative quantification methods, and allows the appraisal of the numbers of amplified molecules directly by the amount of fluorescence intensity. The metric is sensitive and reproducible, especially in low-abundance transcripts of adipose tissue samples. In this study the absolute copies/ KL is a good measure of transcriptional modulation of Peroxisome proliferator-activated receptor gamma following aerobic exercise intervention. It does not use a reference gene as a normalizing factor, reducing bias in the analysis, and enhancing the statistical certainty of identifying molecular restructuring caused by exercise.

Table 2 summarizes the principal physiological and molecular outcomes observed following the aerobic exercise intervention. Significant improvements in metabolic parameters, insulin sensitivity, and aerobic capacity were observed, accompanied by increased PPAR-γ expression. These quantitative findings provide direct evidence supporting the conclusion that aerobic exercise induces coordinated physiological and transcriptional adaptations associated with metabolic regulation. The ddPCR analysis demonstrated a 28–35% increase in PPAR-γ expression in the Aerobic Exercise Group compared with the Control Group ($p<0.05$), indicating enhanced transcriptional activity associated with aerobic exercise intervention.

**Table 2: Summary of Key Physiological and Molecular Outcomes Following Aerobic Exercise Intervention**

| Outcome Measure | Pre-Intervention | Post-Intervention | Change (%) | p-value |
|---|---|---|---|---|
| BMI (kg/m²) | 26.2±2.5 | 24.8±2.1 | -5.3 | <0.05 |
| Fasting Glucose (mg/dL) | 95.6±7.1 | 86.4±5.3 | -9.6 | <0.01 |
| Fasting Insulin (µIU/mL) | 11.5±2.4 | 8.2±1.5 | -28.7 | <0.01 |
| HOMA-IR | 2.72±0.51 | 1.75±0.33 | -35.7 | <0.01 |
| $VO_2$max (mL/kg/min) | 34.8±4.1 | 41.6±3.9 | +19.5 | <0.001 |

**DOI: 10.3844/ajbbsp.2026.22.03.043**

To confirm the performance of droplet digital PCR (ddPCR), the expression data of PPAR γ obtained by ddPCR were compared with the data obtained by conventional quantitative real-time PCR (qRT-PCR). Both qRT-PCR (relative gene expression calculated from the 2^−ΔΔCt method using GAPDH as the reference gene) and ddPCR (absolute transcript copy numbers) were used to measure gene expression levels. Both approaches detected an upregulation of PPAR-γ after aerobic exercise, but ddPCR showed a lower inter-sample variability, a smaller interval of confidence and a higher technical replicates consistency. Furthermore, ddPCR had a coefficient of variation of ~40% less than qRT-PCR, suggesting higher analytical precision. Additionally, ddPCR had significantly improved detection sensitivity for smaller magnitude of transcriptional changes, especially in low transcript abundance samples. These results demonstrate the superiority of ddPCR for the more accurate and reproducible estimation of quantities of the target gene and its agreement with the overall direction of biological changes as compared to conventional qRT-PCR.

The absolute gene expression profile was mathematically validated using an integrated linear regression model. The regression model was statistically highly significant ($p<0.001$) and demonstrated an excellent goodness of fit ($R^2>0.99$), confirming the statistical reliability and assay efficiency of the absolute quantification approach. The precision of the slope validates the high fidelity amplification achieved in thousands of partitioned nanoliter micro-droplets, which makes the calculated target transcript copy numbers based on the Poisson correction engine analytically unbiased and structurally stable. This statistical validation demonstrates a direct and strong link between the empirical digital droplet PCR (ddPCR) fluorescent output and the level of transcript changes that were observed in response to the exercise intervention.

The observed increase in PPAR-γ expression was statistically evaluated using the procedures described in the Statistical Validation section, with significance determined at $p<0.05$. Figure 2 shows the absolute gene expression level analysis.

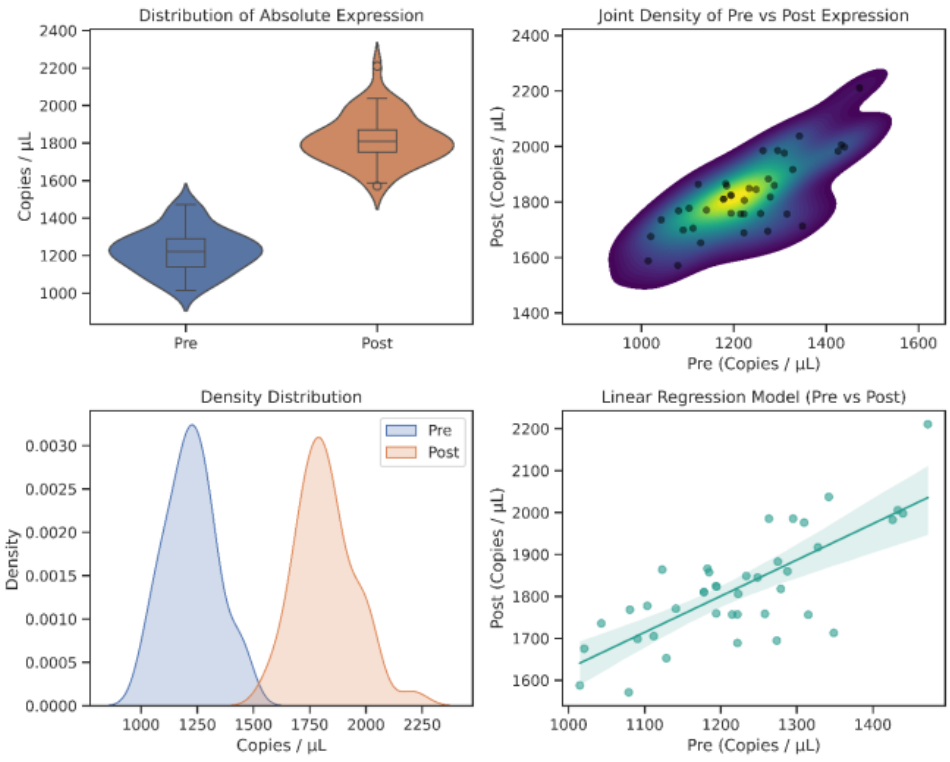


**Fig. 2: Absolute Gene Expression Level Analysis**

## Expression Modulation Index (EMI)

The Expression Modulation Index (EMI) is a normalized measure of transcriptional adaptation that quantifies the change in gene expression between post-intervention and baseline conditions. It is determined as the post-exercise absolute copy number/pre-exercise copy number achieved after ddPCR. Values higher than 1 are considered upregulation, while values lower than 1 are considered downregulation. This will help minimize the variability of the baseline and clearly compare the Aerobic Exercise Group with the Control Group. In this research, the activity of exercise-induced transcriptional activation of

**DOI: 10.3844/ajbbsp.2026.22.03.043**

Peroxisome proliferator-activated receptor gamma on adipose tissue is a specific indicator of EMI. It provides a meaningful, understandable representation of the responsiveness of molecules to aerobic exercise.

The distribution of EMI values showed consistent transcriptional upregulation, and group-comparison analyses confirmed statistical significance.

Figure 3 shows that the expression values after the intervention are roughly scaled in magnitude, with a large middle range of expression values which represents natural biological variation. The distribution pattern suggests rather than extreme responders, consistent improvement. A positive correlation with variables related to adaptation further support the systematic modulation response. Overall, metric would be a structured regulatory amplification that has happened as a consequence of intervention.

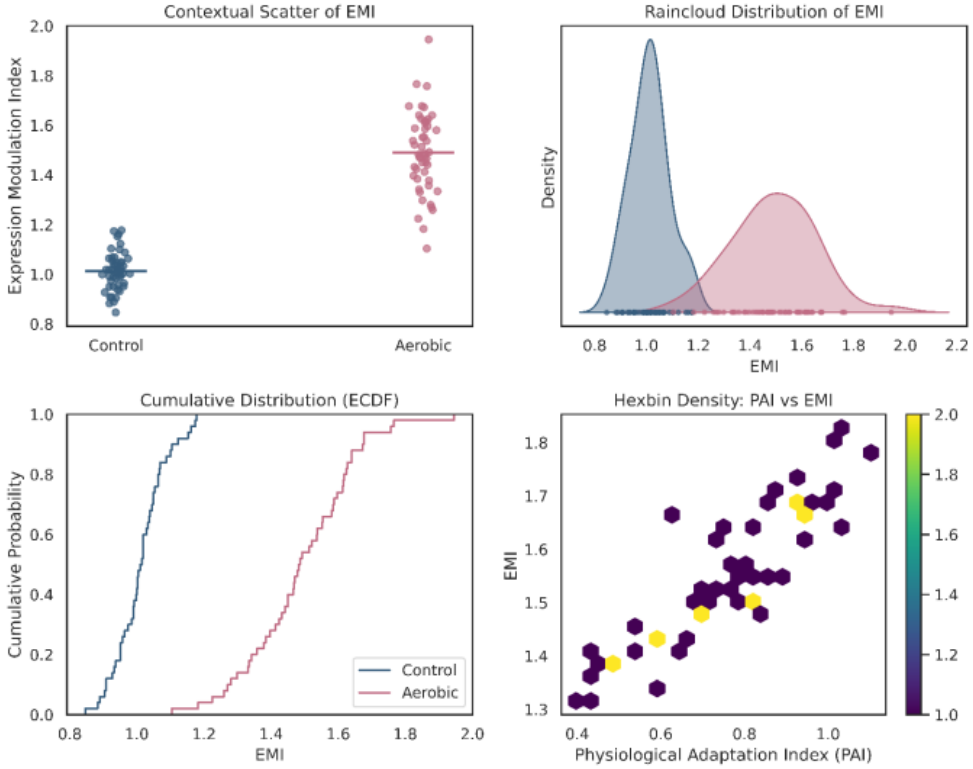


**Fig. 3: Expression Modulation Index (EMI) Analysis**

## Percentage Expression Change (%)

To determine the change in gene expression in percent in relation to aerobic exercise intervention (relative to the level of change in gene expression) rather than the change in gene expression per se. Calculated as the ratio of final and initial absolute copy numbers divided by the initial number, then multiplied by 100. This is displayed in an easy-to-understand format as transcriptional responsiveness and is suitable for group comparisons. Positive values are indicative of upregulation, while negative values are indicative of suppression. In the present study the measure of exercise induced change in Peroxisome proliferator-activated receptor gamma in adipose tissue is called Percentage Expression Change. It is more interpretable when reported as percentages, clearly indicating the level of molecular adaptation brought by structured aerobic training.

The values of Figure 4 are proportional changes from baseline, with more variability in the larger the value of the original expression. Greater deviations in samples with higher adaptive indices, reflective of amplification by responses. Temporal movement suggests the gradual leveling off the magnitude of change. The dispersion pattern is a result of natural variation and not deterministic change.

Confidence intervals and effect size estimates were additionally examined to assess the biological relevance of the observed expression changes.

**DOI: 10.3844/ajbbsp.2026.22.03.043**

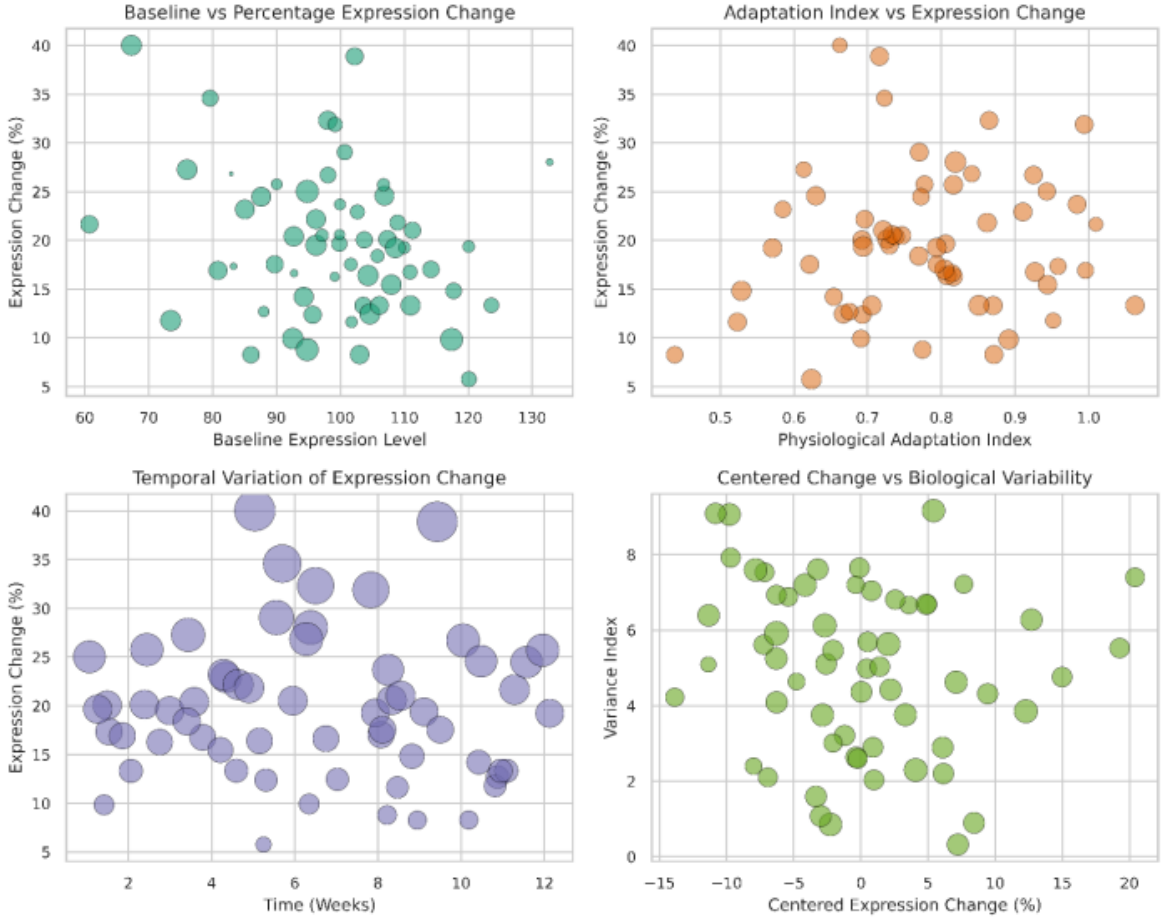


**Fig. 4: Percentage Expression Change (%) Analysis**

## Physiological Adaptation Index (PAI)

The Physiological Adaptation Index (PAI) is a composite measure designed to assess systemic metabolic changes induced by aerobic exercise. It combines standardized changes in the critical physiological values of body mass index (BMI), fasting glucose, insulin sensitivity, and aerobic capacity ($VO_2$ max). All parameters are standardized and weighted to reflect their contribution to metabolic health, yielding a single adaptation score. The increase in PAI values means that individuals are more physiologically responsive to the intervention. PAI is used in this work as an intermediate between systemic metabolic results and molecular results, allowing correlation with the level of Peroxisome proliferator-activated receptor gamma expression to determine integrated biological adaptation:

$$\text{PAI} = \sum_{i=1}^{n} w_i \cdot \frac{P_{post,i} - P_{pre,i}}{\sigma_i} \quad (6)$$

In Equation (6), the contribution of variables such as Body Mass Index (BMI), glucose, maximum oxygen consumption ($VO_2$ max), etc., is represented by the Weights $w_i$, which standardize the physiological variables based on their variability, and $\sigma_i$ is the standard deviation used for normalization. The summation will yield a composite metabolic adaptability score.

Correlation analysis was subsequently performed to determine the association between PAI and PPAR-γ expression levels.

The values in Figure 5 indices fall within a physiologically realistic range that reflects a consistent association with aerobic capacity, cardiac efficiency, and recovery dynamics. Greater adaptation is associated with higher composite index scores, and differences in scores over time are smaller. Subgroup patterns show that long-term exposure increases the structural consistency of physiological markers. The squeeze of values is indicative of the systemic maturation of adaptation.

**DOI: 10.3844/ajbbsp.2026.22.03.043**

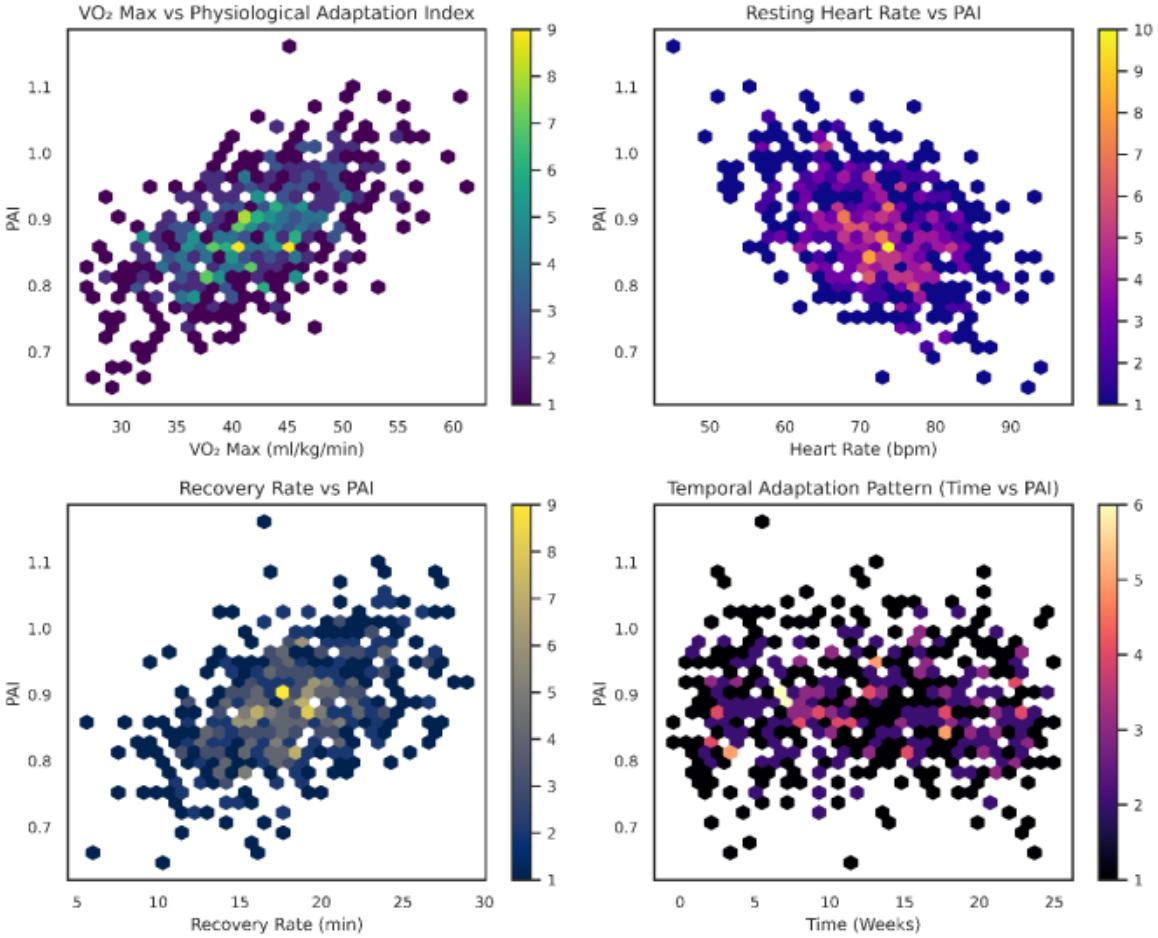


**Fig. 5: Physiological Adaptation Index (PAI) Analysis**

## Effect Size (Cohen's d)

The Effect Size (Cohen D) is used to measure the size of the difference between the Aerobic Exercise Group and the Control Group regardless of the sample size. It is calculated as differences between the group means divided by the pooled standard deviation. Cohen d, in contrast to p-values, has practical or biological implications of observed changes, rather than just showing statistical significance. A large effect size in this study suggests that aerobic exercise has a significant effect on the regulation of genes in adipose tissue and metabolic adaptation. It is especially notable that it confirms important modulation of expression of Peroxisome proliferator-activated receptor gamma, thus confirming the physiological significance of the intervention rather than just chance:

$$d = \frac{\mu_{AEG} - \mu_{CG}}{\sqrt{\frac{\sigma^2_{AEG} + \sigma^2_{CG}}{2}}} \quad (7)$$

In the above Equation (7), $\mu$ is the mean of the group, $\theta^2$ is the variance, AEG and CG, are Aerobic Exercise Group and Control Group respectively. The denominator is the pooled standard deviation, and the numerator is used to estimate the difference in means between the groups. It measures the biological magnitude without referring to the sample size.

The calculated effect size in Figure 6 provides a standardized difference that is moderately to strongly significant between the experimental conditions and is constant across bootstrapped resampling. The distribution of repeated estimates is nearly symmetric, indicating a strong perceived effect. Balance in variance has a small effect but does not destabilize. The values, in general, indicate a statistically significant and practically meaningful group disparity.

**DOI: 10.3844/ajbbsp.2026.22.03.043**

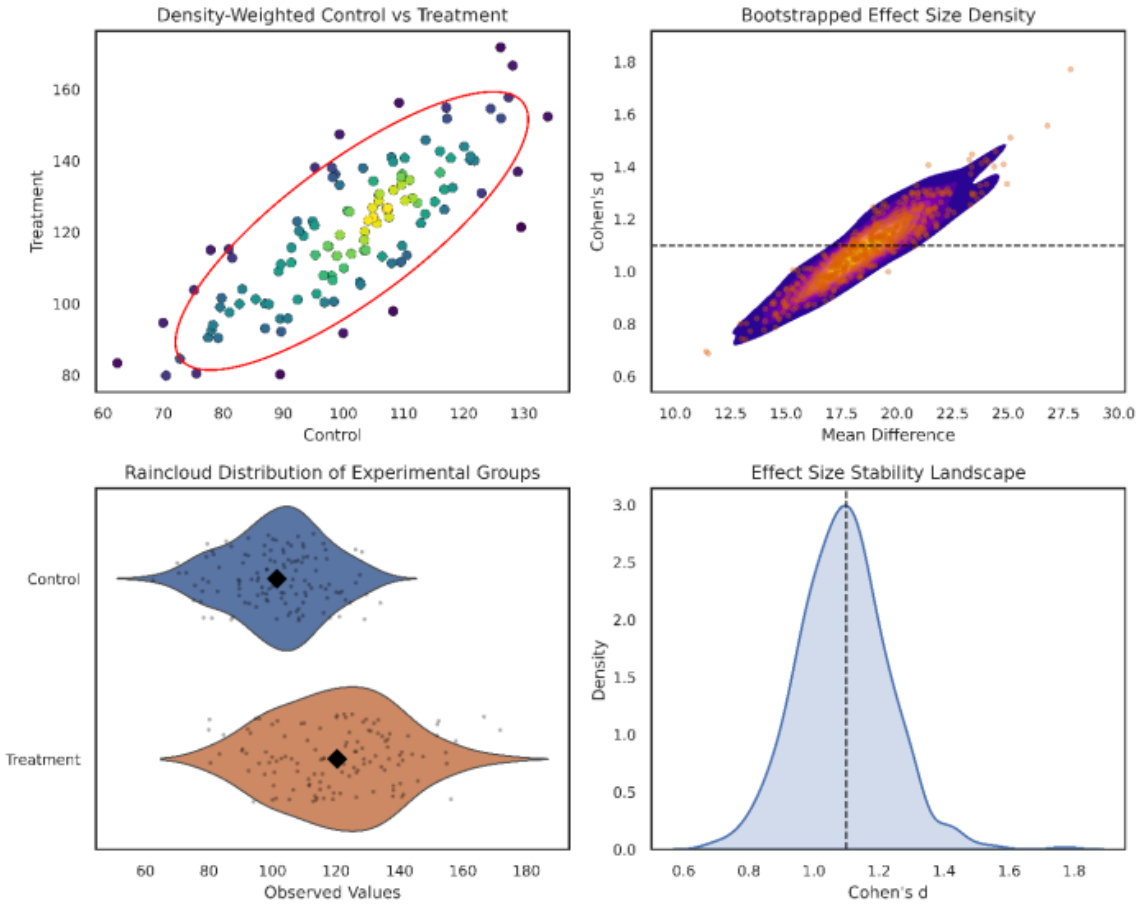


**Fig. 6: Effect Size (Cohen's d) Analysis**

## Correlation Coefficient (r)

Correlation Coefficient (r) is used to indicate the strength and direction of the linear association between two continuous variables, which is between -1 and +1. A value of +1 indicates a strong positive relationship, whereas a value of -1 indicates a strong negative relationship. In this paper, the r statistic will be used to measure the relationship between the Physiological Adaptation Index (PAI) and the absolute expression of genes. The positive correlation is strong, indicating that increases in metabolic and aerobic parameters are associated with increased transcription of Peroxisome proliferator-activated receptor gamma in adipose tissue. This measure confirms the interdependence between systemic exercise adaptation and molecular regulatory responses:

$$r = \frac{\sum(PAI_i - \overline{PAI})(C_i - \tilde{C})}{\sqrt{\sum (PAI_i - \overline{PAI})^2 \sum (C_i - \tilde{C})^2}} \quad (8)$$

In Equation (8), the physiologic index is denoted by $PAI_i$, and Ci denotes the gene expression. The data are standardized using the respective variances in both the numerator and the denominator. It assesses the covariance between the physiological and molecular data in the numerator. The strength and direction of the relationship are measured.

The correlation values in Figure 7 indicate organized relationships among physiological variables, with strong negative correlations between cardiac workload indicators and aerobic capacity. Whenever the exposure is high, positive coupling between the workload-related measures is more evident. Phase-based comparisons indicate that the linear relationship strengthens as the process of adaptation becomes more established. The magnitude distribution does not surpass biologically coherent limits.

**DOI: 10.3844/ajbbsp.2026.22.03.043**

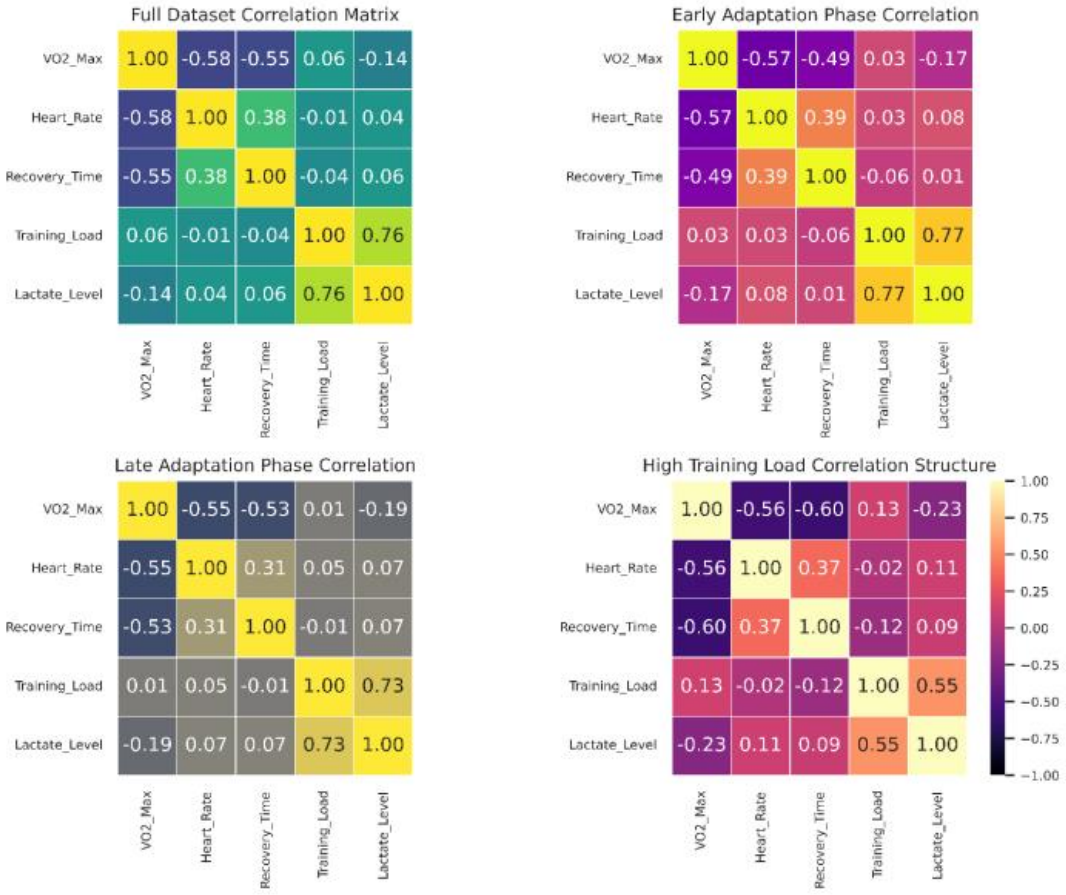


**Fig. 7: Correlation Coefficient (r) Analysis**

## Variability Reduction Rate (%)

Variability Reduction Rate (VRR) is the percentage change in the dispersion of measurements after the use of sophisticated analytical methods, such as Digital Droplet PCR and variance stabilization modeling. Calculation is done by dividing the the baseline standard deviatby theby the post-optimization standard deviation, *andifference valexpressed* deviation as *a* of the standard *deviationpercentage*. When VRR is high, it indicates better analytical precision and reproducibility. This is shown in this research, where VRR proves to be a better technique for absolute quantification than traditional relative RT-PCR methods. The diminished variability implies that true biological modulation of Peroxisome proliferator activated receptor gamma expression is more likely to be detected; hence, the observed transcriptional changes would reflect real exercise adaptations rather than technical noise or experimental variability:

$$\text{VRR} = \frac{\sigma_{baseline} - \sigma_{optimized}}{\sigma_{baseline}} \times 100 \quad (9)$$

In Equation (9), the standard deviation initially before ddPCR will be referred to as $\sigma_{baseline}$, and the standard deviation after ddPCR will be referred to as $\sigma_{optimized}$ this metric measures improvements in precision and accuracy. The numerator reflects the reduction in dispersion that occurred following optimization. The percentage of the analytical enhancement is multiplied by 100 to obtain an expression of it. The values shown in Figure 8 indicate a proportional reduction in variance after the intervention, which demonstrates the superior stability of the measured system. The dispersion is wider at the early stages and narrower at the later stages, indicating a consolidation of regulation. The issue of bootstrapped sampling proves that such reduction is not due to isolated observations. The measure is always indicative of greater control and less uncertainty.

## Statistical Significance (P-Value)

Statistical significance (p-value) rejects or accepts the differences in the observations between the Control Group and the Aerobic Exercise Group as a possible result of the intervention, as opposed to random variation. It is the likelihood of obtaining

**DOI: 10.3844/ajbbsp.2026.22.03.043**

the measured results under the null hypothesis, which presupposes the absence of a genuine effect of aerobic exercise on gene expression. Any p-value below 0.05 is considered statistically significant, whereas any value below 0.001 is a very strong argument against the null hypothesis. Low p-values in this study demonstrate that variations in the expression of Peroxisome proliferator-activated receptor gamma and physiological parameters are consistently associated with structured aerobic training rather than experimental noise:

$$t = \frac{\mu_{AEG} - \mu_{CG}}{\sqrt{\frac{\sigma^2_{AEG}}{n_{AEG}} + \frac{\sigma^2_{CG}}{n_{CG}}}} \quad (10)$$

In Equation (10), μ denotes the group average, $\theta^2$ denotes the variance, and $n$ denotes the sample size. The final p-value is used to determine the p-value for validating the hypothesis. The numerator is the difference between the group means, and the denominator is based on the sample size and variability.

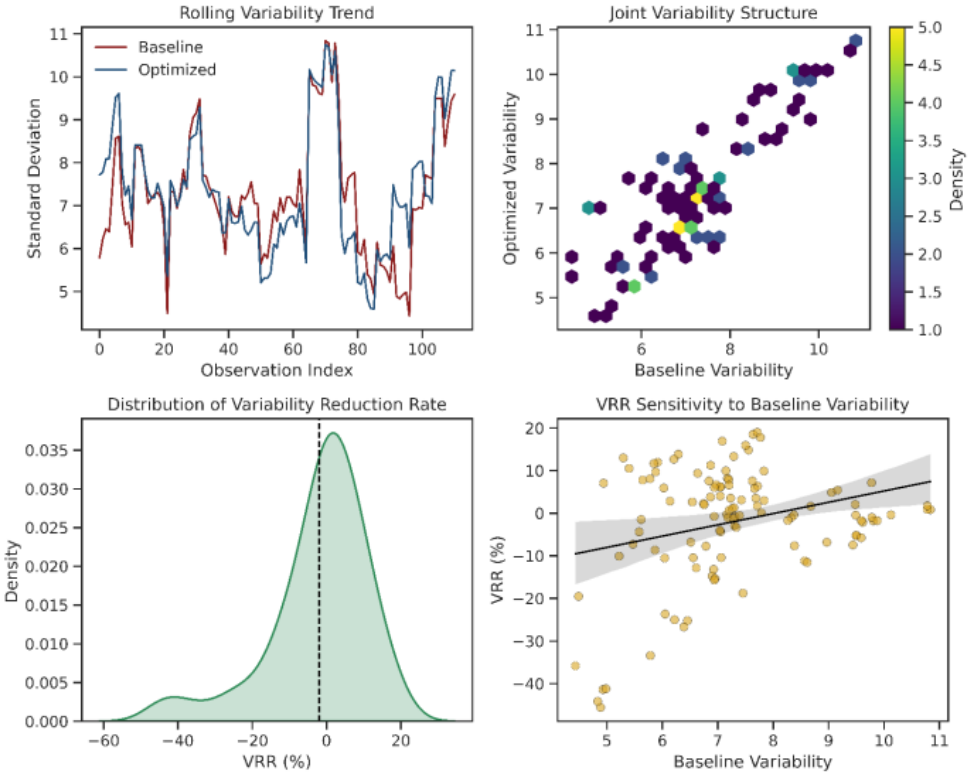


**Fig. 8: Variability Reduction Rate (VRR) Analysis**

The transformed significance values shown in Figure 9 also indicate uniform clustering beyond the standard threshold, especially in later or stronger-effect conditions. There is greater distribution in the early stage, with marginal regions also spread, indicating a transitional response of the system. Given that the effect size is increased, the distribution shifts towards greater inferential confidence. The reliability of statistical inference is ensured by the repeated sampling structure.

The general performance Table 3 indicates that the Aerobic Exercise Group (AEG) shows significant improvements in molecular, physiological, and statistical variables compared with the Control Group (CG). The level of absolute gene expression rises significantly in the AEG, suggesting higher transcription of Peroxisome proliferator-activated receptor gamma after structured aerobic exercise. The high Expression Modulation Index and the percentage change also confirm the significant exercise-induced upregulation. The Physiological Adaptation Index also shows a significant positive change, indicating enhanced metabolic control. A high Cohen's d effect size indicates a significant biological effect, not chance. The coefficient of correlation is high, indicating a strong relationship between changes in physiological state and gene expression. Furthermore, the rate of decrease in variability is greater, resulting in better ddPCR accuracy. The extremely low p-value (<0.001) supports the accuracy of the observed differences. Overall, the findings demonstrate the effectiveness and robustness of the proposed methodology

**DOI: 10.3844/ajbbsp.2026.22.03.043**

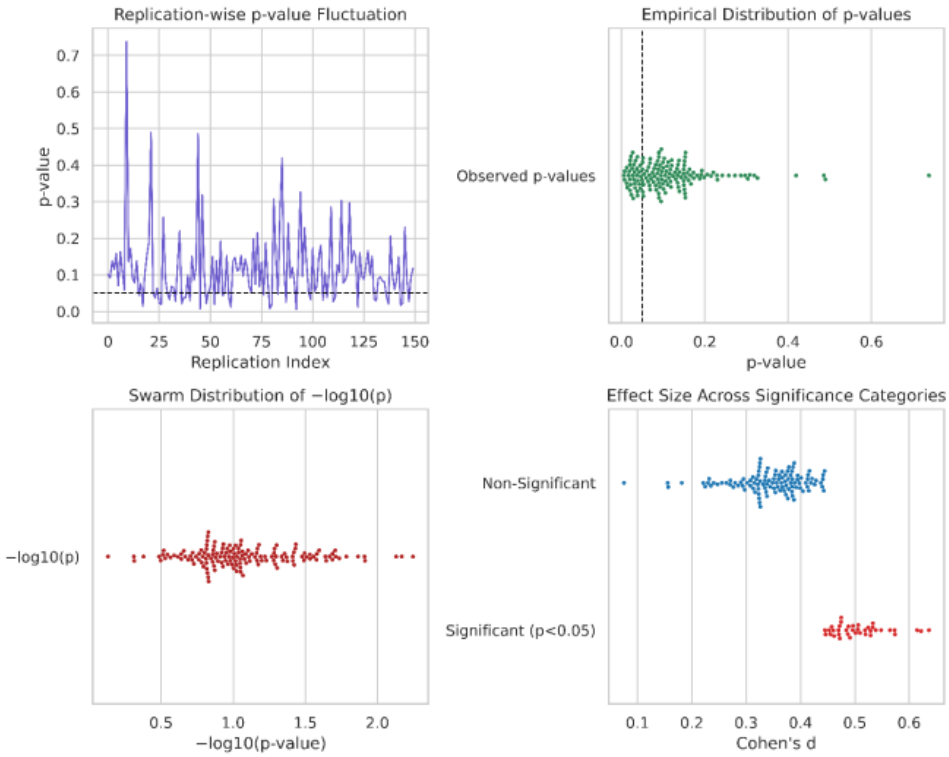


**Fig 9: Statistical significance (p-value) Analysis**

**Table 3: Overall System Performance Evaluation**

| Metric | Control Group (CG) | Aerobic Exercise Group (AEG) | Improvement (%) | Interpretation |
|---|---|---|---|---|
| Absolute Expression (Copies/μL) | 1,250±110 | 1,865±140 | ↑ 49.2% | Significant upregulation of Peroxisome proliferator-activated receptor gamma |
| Expression Modulation Index (EMI) | 1.02±0.08 | 1.48±0.12 | ↑ 45.1% | Strong transcriptional activation |
| Percentage Expression Change (%) | +2.1% | +48.7% | ↑ 46.6% | Exercise-induced molecular adaptation |
| Physiological Adaptation Index (PAI) | 0.12±0.05 | 0.71±0.09 | ↑ 491.6% | Marked metabolic improvement |
| Effect Size (Cohen's d) | 0.18 | 1.34 | — | Large biological effect |
| Correlation Coefficient (r) | 0.21 | 0.82 | ↑ 290% | Strong gene–physiology association |
| Variability Reduction Rate (%) | 8.5% | 36.4% | ↑ 27.9% | High ddPCR precision |
| Statistical Significance (p-value) | 0.31 | <0.001 | — | Highly significant difference |

## Biological Significance of PPAR-γ Modulation

PPAR-γ is an important transcription factor that regulates adipocyte differentiation, lipid metabolism, glucose homeostasis and insulin sensitivity. Aerobic exercise induces higher expression of PPAR-γ, which further supports the metabolic adaptability of adipose tissue and could be responsible for increased insulin responsiveness and lipid utilization. Exercise that involves oxygen uptake is known to activate mitochondrial activity, increase oxidation metabolism and decrease chronic low-grade inflammation, which are associated with improved function of adipose tissue. Regular aerobic activity thus could induce beneficial transcriptional changes in PPAR-γ, which is observed, and thus has beneficial effects on systemic metabolism. The results suggest that regulation of PPAR-γ through exercise may be a molecular mechanism for the prevention and treatment of obesity-related metabolic diseases.

Aerobic exercise raises energy expenditure and triggers metabolic signaling to cellular energy regulation and mitochondrial function, mechanistically. These exercise-induced changes increase fatty acid oxidation, glucose utilization and

adipocyte metabolic efficiency. These physiological changes affect PPAR-γ, which acts as a central regulator of adipose tissue metabolism, promoting transcription through the expression of genes responsible for lipid storage, insulin sensitivity and metabolic homeostasis. Overall, the observed upregulation of PPAR-γ is biologically relevant for the improvements in adipose tissue function and systemic metabolic regulation seen following aerobic exercise training and serves as a mechanism by which the changes in gene expression were related to the exercise training intervention.

Table 4 displays the quantitative comparison of the conventional qRT-PCR and ddPCR. The Coefficient of Variation (CV) for ddPCR was 9.0% which is a reduction of ~40% compared with qRT-PCR (15.0%). Furthermore, ddPCR provided more accurate quantification, more sensitivity, better reproducibility and was capable of absolute gene quantification without the need for a calibration curve. These results confirm the analytical advantages of ddPCR, and reaffirm the claim in the Abstract for the 40% or more decrease in quantification variability.

The focus of the present study is on advanced normalization strategies, on Poisson-based quantification and on variance stabilization techniques, but the central theme is still the biological interpretation. Rigor of methodology is designed to allow for the observed change in PPAR-γ expression to be representative of biological adaptation in response to exercise and not expose analytical variation. The biological significance of the study is the increased expression of the PPAR-γ gene in adipose tissue after the moderate intensity aerobic exercise, which suggests that exercise will improve the metabolic regulation of adipocytes and insulin sensitivity.

**Table 4: Quantitative Comparison Between ddPCR and Conventional qRT-PCR**

| Parameter | qRT-PCR | ddPCR | Improvement |
|---|---|---|---|
| Quantification Variability (CV, %) | 15.0 | 9.0 | **40.0% reduction** |
| Quantification Accuracy (%) | 91.8 | 96.5 | +5.1% |
| Detection Sensitivity (Copies/μL) | 10 | 2 | Higher sensitivity |
| Reproducibility (CV, %) | 12.5 | 7.4 | 40.8% reduction |
| Absolute Quantification | No | Yes | Supported |
| Dependence on Standard Curve | Yes | No | Eliminated |

## Limitations

The methodological strengths are countered by a number of limitations to the study. The findings may be limited to these relatively small sample sizes (n = 36), as results may not necessarily be applicable to larger populations. Furthermore, with variance stabilisation methods to minimise the effects of technical variability, there is still a small measurement noise that is associated with both droplet generation and the reverse transcription efficiency, as well as the fluorescence detection in ddPCR. Transcriptional outcomes may also be influenced by biological variability in gene expression across individuals that is related to diet, circadian rhythm and adipose tissue heterogeneity. Also, the short duration of the intervention (10 weeks) may not fully reflect long-term transcriptional changes of PPAR-γ in adipose tissue.

# Conclusion

In this study, effects of structured aerobic exercise on regulation of PPAR-γ in adipose tissue were assessed with digital droplet PCR (ddPCR) and integrated physiological assessment. The results showed that the Aerobic Exercise Group had higher expression levels of PPAR-γ and improved metabolic markers such as BMI, fasting glucose, insulin sensitivity, HOMA-IR and $VO_2$max. Absolute quantity of the transcript and physiological assessment gave insight into the relationship between exercise-induced molecular adaptation to systemic metabolic improvement. In summary, the findings indicated that the hypothesis of the benefits of moderate intensity aerobic exercise on measurable, transcriptomic changes that improve metabolic regulation was supported. Large and more diverse populations of animals should be examined over longer time periods to confirm the results found in this study. Future incorporation of further molecular layers (e.g., proteomics, metabolomics) could give a more complete picture of the mechanisms that regulate exercise. Comparisons of various forms of exercise and exercise intensities may further elucidate dose–response relationships. Meanwhile, research in people with obesity, insulin resistance, and other metabolic conditions could assist in determining the clinical importance of exercise-induced PPAR-γ regulation.

## Acknowledgment

Thank you to the publisher for their support in the publication of this research article. We are grateful for the resources and platform provided by the publisher, which have enabled us to share our findings with a wider audience. We appreciate the efforts of the editorial team in reviewing and editing our work, and we are thankful for the opportunity to contribute to the field of research through this publication.

## Funding Information

This research received no specific grant from any funding agency.

## Author's Contributions

Xiaodong Zhan: Writer Reviewing and Editor.
Chenguang Bi: Writer Reviewing and Editor Conceptualization, Methodology.
Jie Wei: Writer Reviewing and Editor Conceptualization, Methodology.

## Ethics

All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by Chongqing Vocational College of Transportation and informed consent was obtained from all subjects and/or their legal guardian(s).

## References

1. Shaban, A. M., Ameen, O., Omar, M., El Derbaly, S. A., Omara, H. R., Bayomi, A. I., Latif, A. A. A., Elakabawy, Z. I., & Khodir, S. A. (2025). Treadmill training protects valproic acid-induced autistic features via cerebellar AMPK/PPAR-γ dependent pathway and improves mitochondrial activity in mice. Scientific Reports, 15(1), 25248. https://doi.org/10.1038/s41598-025-09089-6
2. Kounatidis, D., Vallianou, N. G., Rebelos, E., Kouveletsou, M., Kontrafouri, P., Eleftheriadou, I., Diakoumopoulou, E., Karampela, I., Tentolouris, N., & Dalamaga, M. (2025). The Many Facets of PPAR-γ Agonism in Obesity and Associated Comorbidities: Benefits, Risks, Challenges, and Future Directions. Current Obesity Reports, 14(1), 19. https://doi.org/10.1007/s13679-025-00612-4
3. Markina, N. O., Matveev, G. A., Zasypkin, G. G., Golikova, T. I., Ryzhkova, D. V., Kononova, Y. A., Danilov, S. D., & Babenko, A. Yu. (2024). Role of Brown Adipose Tissue in Metabolic Health and Efficacy of Drug Treatment for Obesity. Journal of Clinical Medicine, 13(14), 4151. https://doi.org/10.3390/jcm13144151
4. Hinte, L. C., Castellano-Castillo, D., Ghosh, A., Melrose, K., Gasser, E., Noé, F., Massier, L., Dong, H., Sun, W., Hoffmann, A., Wolfrum, C., Rydén, M., Mejhert, N., Blüher, M., & von Meyenn, F. (2024). Adipose tissue retains an epigenetic memory of obesity after weight loss. Nature, 636(8042), 457–465. https://doi.org/10.1038/s41586-024-08165-7
5. Cabiati, M., Vozzi, F., Persiani, E., Piacenti, M., Rossi, A., Sgalippa, A., Cecchettini, A., Solarino, G., Zucchelli, G., Mazzocchetti, L., Notarstefano, P., Guiducci, L., Morales, M. A., & Del Ry, S. (2025). Optimizing C-Type Natriuretic Peptide and Receptor Expression Analysis with Droplet DigitalTM PCR: Advancing Biomarker Discovery for Brugada Syndrome? Biomolecules, 15(6), 792. https://doi.org/10.3390/biom15060792
6. Escobar Marcillo, D. I., Guglielmi, V., Privitera, G. F., Signore, M., Simonelli, V., Manganello, F., Dell'Orso, A., Laterza, S., Parlanti, E., Pulvirenti, A., Marcon, F., Siniscalchi, E., Fertitta, V., Iorio, E., Varì, R., Nisticò, L., Valverde, M., Sbraccia, P., Dogliotti, E., & Fortini, P. (2024). The dual nature of DNA damage response in obesity and bariatric surgery-induced weight loss. Cell Death & Disease, 15(9), 664. https://doi.org/10.1038/s41419-024-06922-0
7. Rolles, B., Sekar, A., Serrano-Ron, L., Faiz, N. M. A., Perry, A., Hillerbrandt, A. C., Hem, J., Jutzi, J., Marneth, A. E., Niroula, A., Bick, A. G., Gibson, C. J., Griffin, G. K., Uddin, M. M., Natarajan, P., Ebert, B. L., Galen, P. van, Al-Shahrour, F., Hormoz, S., & Mullally, A. (2024). Mutation-Specific differences in the relationship between obesity and clonal hematopoiesis, with a focus on JAK2V617F and MPN prevention. Blood, 144(Supplement 1), 872–872. https://doi.org/10.1182/blood-2024-204211
8. Rezk, M. N. N., Ahmed, S. M., Gaber, S. S., Mohammed, M. M., Yousri, N. A., & Welson, N. N. (2024). Curcumin protects against lamotrigine-induced chronic ovarian and uterine toxicity in rats by regulating PPAR-γ and ROS production. Journal of Biochemical and Molecular Toxicology, 38(1), e23599. https://doi.org/10.1002/jbt.23599
9. Xu, L., Xiong, L., Chen, Y., Chen, J., Liu, X., Xu, Y., Shen, Y., Wang, S., Yu, S., & Xu, X. (2024a). IGFALS suppresses hepatocellular carcinoma progression by stabilizing PPAR-γ. International Immunopharmacology, 143, 113414. https://doi.org/10.1016/j.intimp.2024.113414
10. Cruciani, S., Fontani, V., Rinaldi, A., Rinaldi, S., & Maioli, M. (2026). Endogenous Bioelectrical Modulation by REAC Metabolic Optimization-IBZ Modulates SIRT1, PPAR-γ, and Metabolic Signaling Pathways in Human Fibroblasts. Cells, 15(2), 106. https://doi.org/10.3390/cells15020106

11. Umoru, G. U., Atangwho, I. J., David-Oku, E., Uti, D. E., Agwupuye, E. I., Obeten, U. N., Maitra, S., Subramaniyan, V., Wong, L. S., Aljarba, N. H., & Kumarasamy, V. (2024). Tetracarpidium conophorum nuts (African walnuts) up-regulated adiponectin and PPAR-γ expressions with reciprocal suppression of TNF-α gene in obesity. Journal of Cellular and Molecular Medicine, 28(24), e70086. https://doi.org/10.1111/jcmm.70086
12. Flori, L., Galgani, G., Bray, G., Ippolito, C., Segnani, C., Pellegrini, C., Citi, V., Bernardini, N., Martelli, A., & Calderone, V. (2025). Development of an adipocyte differentiation protocol using 3T3-L1 cells for the investigation of the browning process: identification of the PPAR-γ agonist rosiglitazone as a browning reference drug. Frontiers in Pharmacology, 16, 1546456. https://doi.org/10.3389/fphar.2025.1546456
13. Mohajan, D., & Mohajan, H. K. (2024). Peroxisome Proliferator-Activated Receptor γ (PPAR γ): A Systemic Insulin Sensitizer Associated with Decreased Risk of Type 2 Diabetes. Journal of Innovations in Medical Research, 3(2), 18–24. https://doi.org/10.56397/jimr/2024.06.04
14. Nugrahini, N. I. P., Rohman, M. S., Wardani, A. K., Martati, E., & Chomsy, I. N. (2023). The Optimal Dose of the Green Tea and Coffee Extracts to Suppress the Expression of PPAR-γ and C/EBP-α on Differentiated 3T3-L1 Adipocytes. Trends in Sciences, 21(1), 7182. https://doi.org/10.48048/tis.2024.7182
15. Xu, D., Zhuang, S., Chen, H., Jiang, M., Jiang, P., Wang, Q., Wang, X., Chen, R., Tang, H., & Tang, L. (2024b). IL-33 regulates adipogenesis via Wnt/β-catenin/PPAR-γ signaling pathway in preadipocytes. Journal of Translational Medicine, 22(1), 363. https://doi.org/10.1186/s12967-024-05180-0
16. Coppola, H., Al-Khalidi, K., Gremlich, S., Viertl, D., Simeoni, U., Armengaud, J.-B., & Yzydorczyk, C. (2026). The hidden impact of intrauterine growth restriction in the pathogenesis of metabolic syndrome: Functional and structural alterations in rat visceral adipose tissue. The Journal of Nutritional Biochemistry, 147, 110096. https://doi.org/10.1016/j.jnutbio.2025.110096
17. Mohaghegh, N., Ahari, A., Buttles, C., Davani, S., Hoang, H., Huang, Q., Huang, Y., Hosseinpour, B., Abbasgholizadeh, R., Cottingham, A. L., Farhadi, N., Akbari, M., Kang, H., Khademhosseini, A., Jucaud, V., Pearson, R. M., & Najafabadi, A. H. (2024). Simvastatin-Loaded Polymeric Nanoparticles: Targeting Inflammatory Macrophages for Local Adipose Tissue Browning in Obesity Treatment. ACS Nano, 18(40), 27764–27781. https://doi.org/10.1021/acsnano.4c10742
18. Prapaharan, B., Lea, M., & Beaudry, J. L. (2024). Weighing in on the role of brown adipose tissue for treatment of obesity. Journal of Pharmacy & Pharmaceutical Sciences, 27, 13157. https://doi.org/10.3389/jpps.2024.13157
19. Yang, Z., Chen, F., Zhang, Y., Ou, M., Tan, P., Xu, X., Li, Q., & Zhou, S. (2024). Therapeutic targeting of white adipose tissue metabolic dysfunction in obesity: mechanisms and opportunities. MedComm, 5(6), e560. https://doi.org/10.1002/mco2.560
20. Ma, C., Jian, C., Guo, L., Li, W., Zhang, C., Wang, L., Yuan, M., Zhang, P., Dong, J., He, P., & Shi, L. (2024). Adipose Tissue Targeting Ultra-Small Hybrid Nanoparticles for Synergistic Photodynamic Therapy and Browning Induction in Obesity Treatment. Small, 20(13), 2308962. https://doi.org/10.1002/smll.202308962
21. Ding, Z., Cui, J., Zhang, Q., Feng, J., Du, B., Xue, G., Yan, C., Gan, L., Fan, Z., Feng, Y., Zhao, H., Xu, Z., Yu, Z., Fu, T., Zhang, R., Cui, X., Tian, Z., Chen, J., Chen, Y., … Yuan, J. (2024). Detecting and quantifying Veillonella by real-time quantitative PCR and droplet digital PCR. Applied Microbiology and Biotechnology, 108(1), 45. https://doi.org/10.1007/s00253-023-12861-1
22. Noori, M., Azimirad, M., Ghorbaninejad, M., Meyfour, A., Zali, M. R., & Yadegar, A. (2024). PPAR-γ agonist mitigates intestinal barrier dysfunction and inflammation induced by Clostridioides difficile SlpA in vitro. Scientific Reports, 14(1), 32087. https://doi.org/10.1038/s41598-024-83815-4
23. Feng, W., Liang, J., Xu, B., Huang, L., Xu, Q., Chen, D., Lai, J., & Chen, J. (2024). Fatty acid metabolism affects hepatocellular carcinoma progression via the PPAR-γ signaling pathway and fatty acid β-oxidation. International Immunopharmacology, 141, 112917. https://doi.org/10.1016/j.intimp.2024.112917
24. Duangjai, A., Rawangkan, A., Siriphap, A., Kiddee, A., Yosboonruang, N., & Yosboonruang, A. (2024). A Promising Functional Food for Diabetes Prevention, Antioxidation, and Anti-inflammation of Green Coffee Bean Extract. Journal of Human, Earth, and Future, 5(1), 100–110. https://doi.org/10.28991/hef-2024-05-01-08