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Decreased Expression of Peroxisome Proliferator-activated Receptor α Gene as an Indicator of Metabolic Disorders in Stunting Toddler

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DAFTAR ISI

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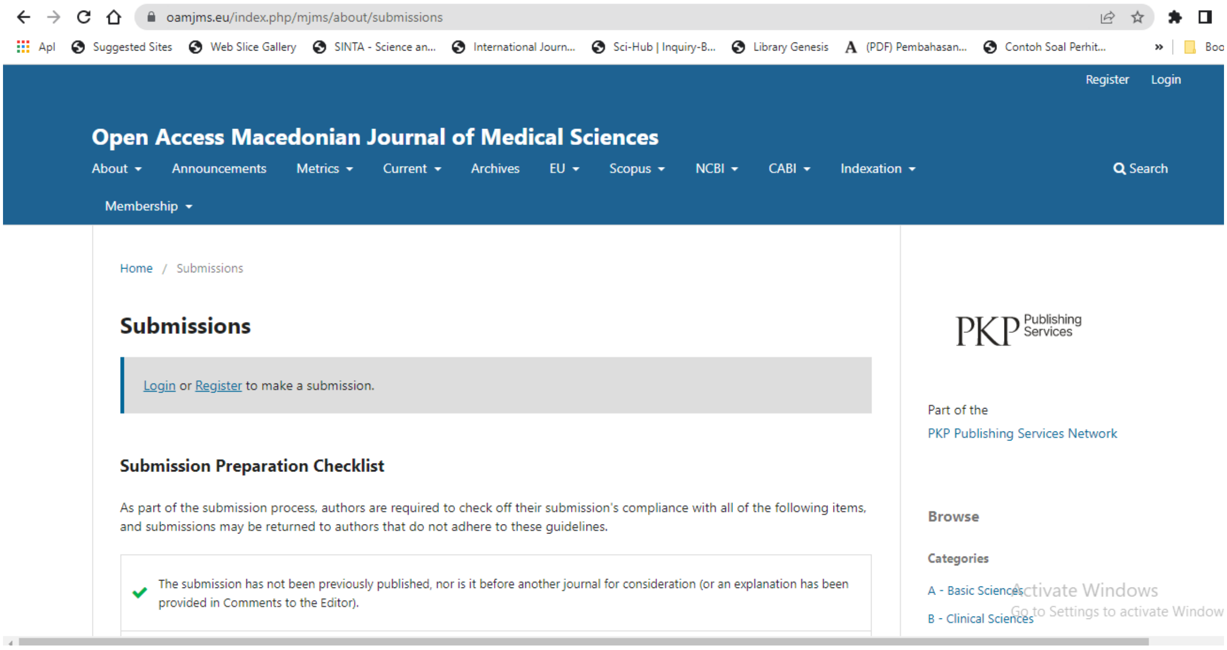

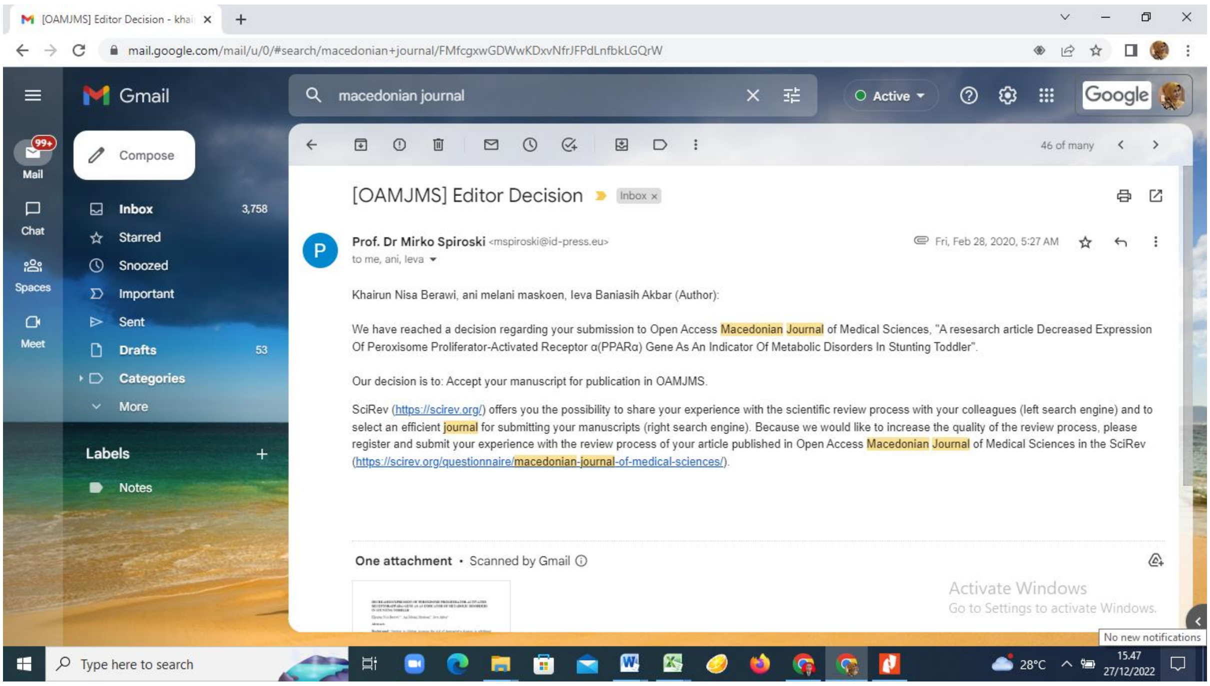

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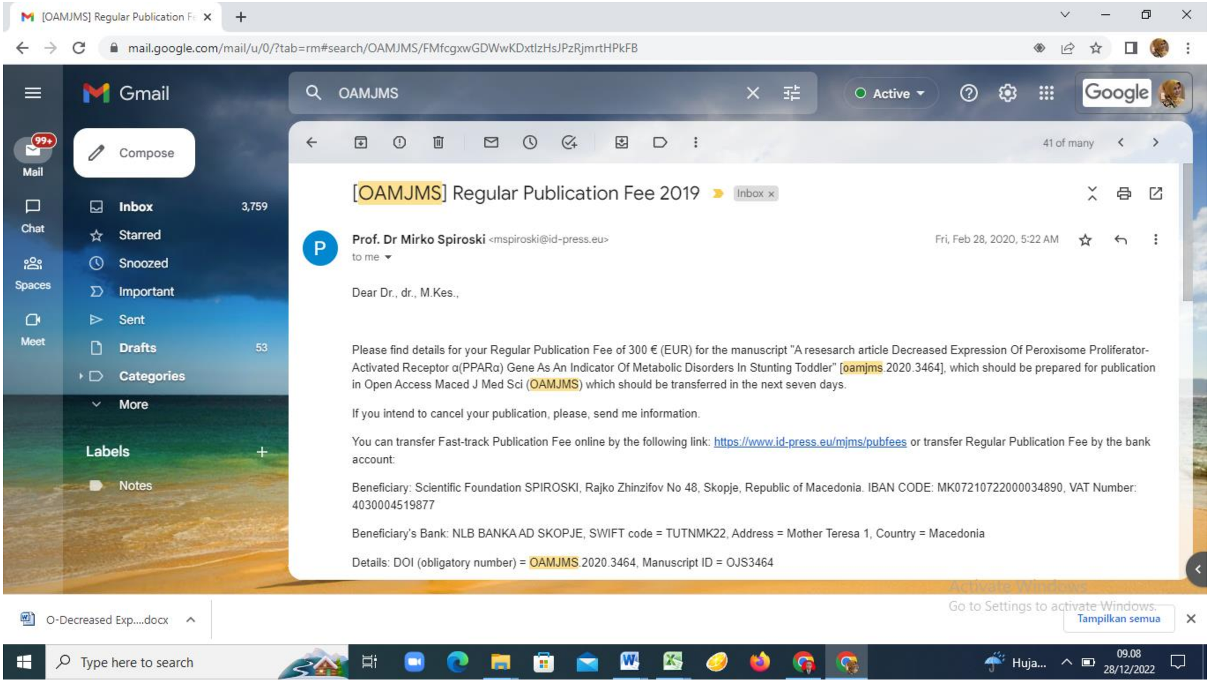

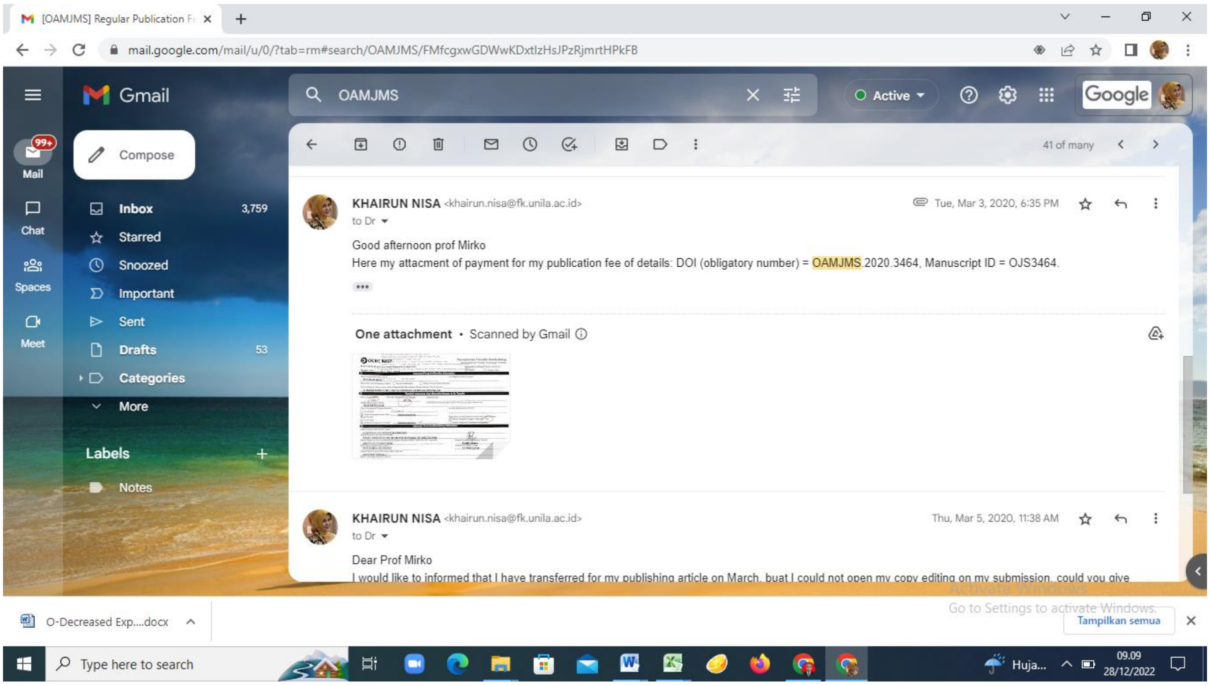

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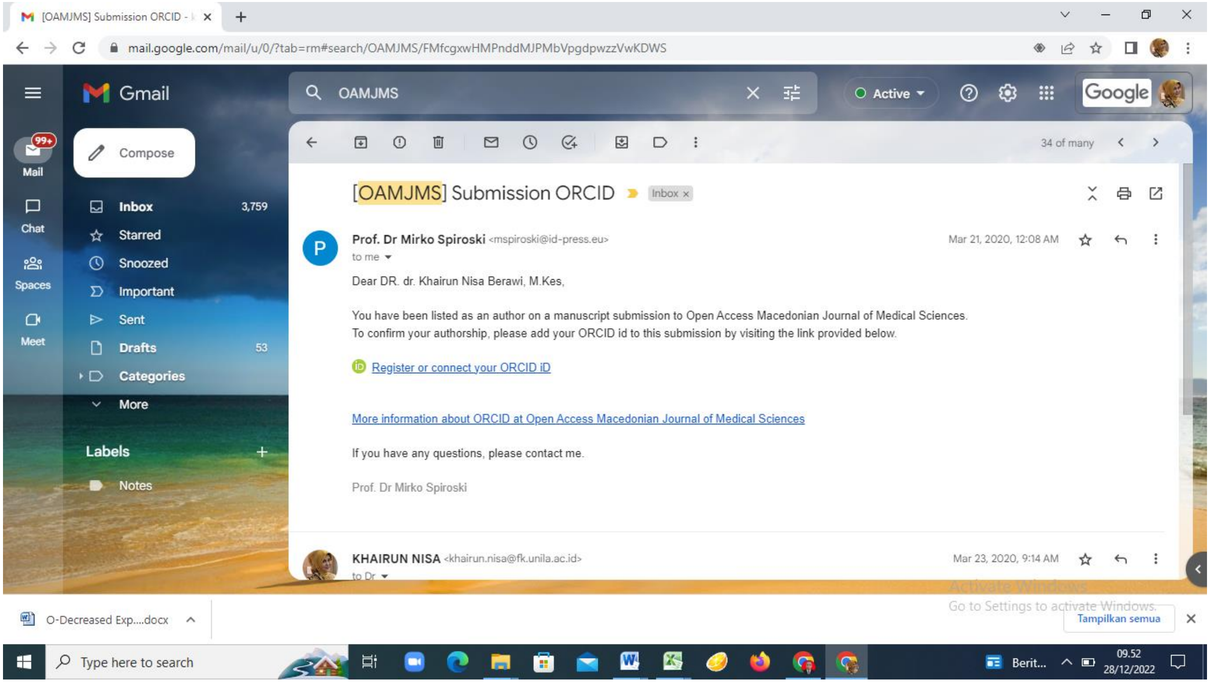

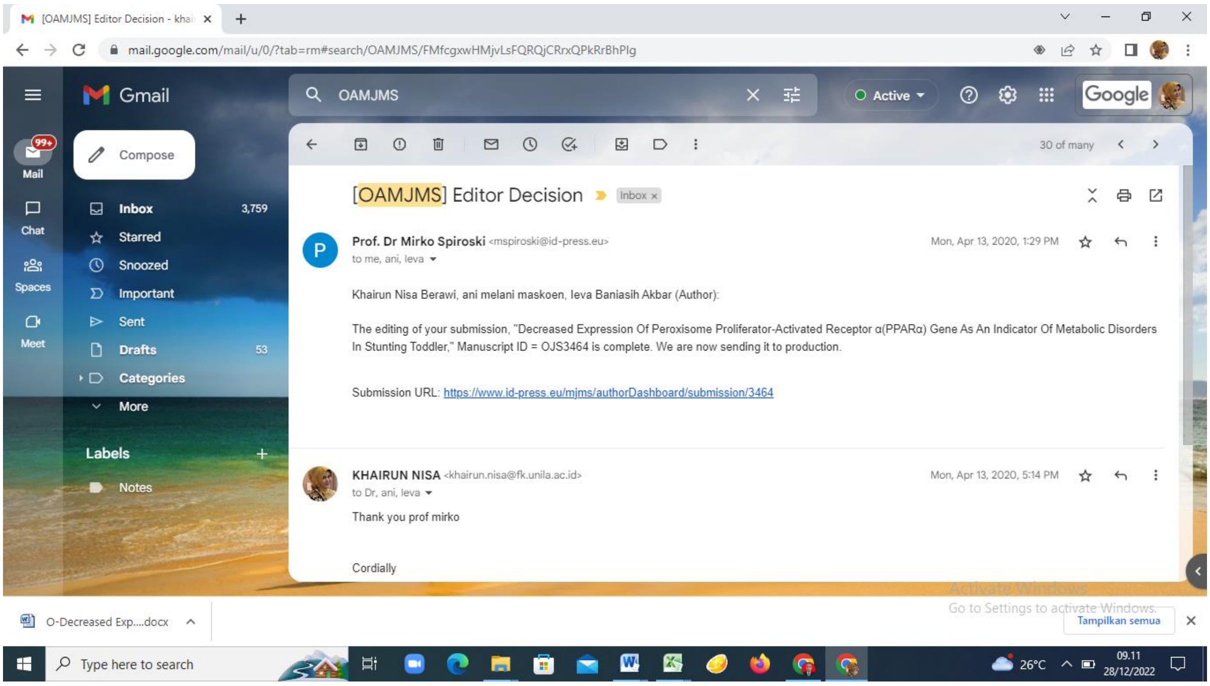

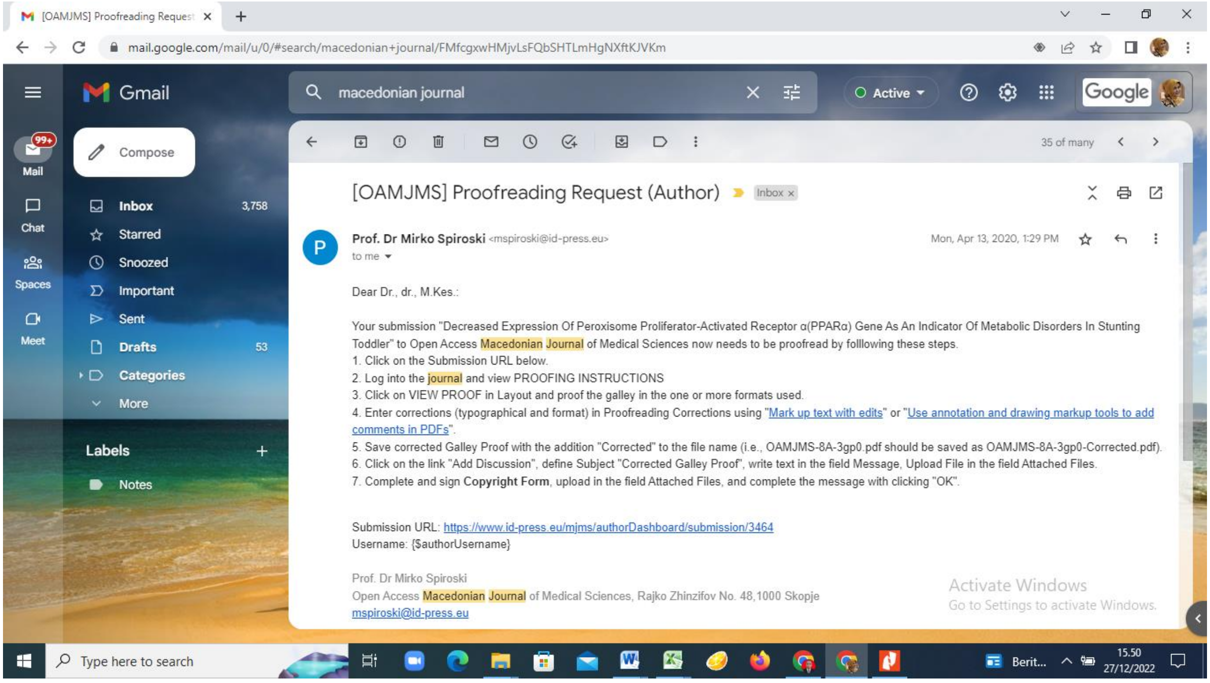

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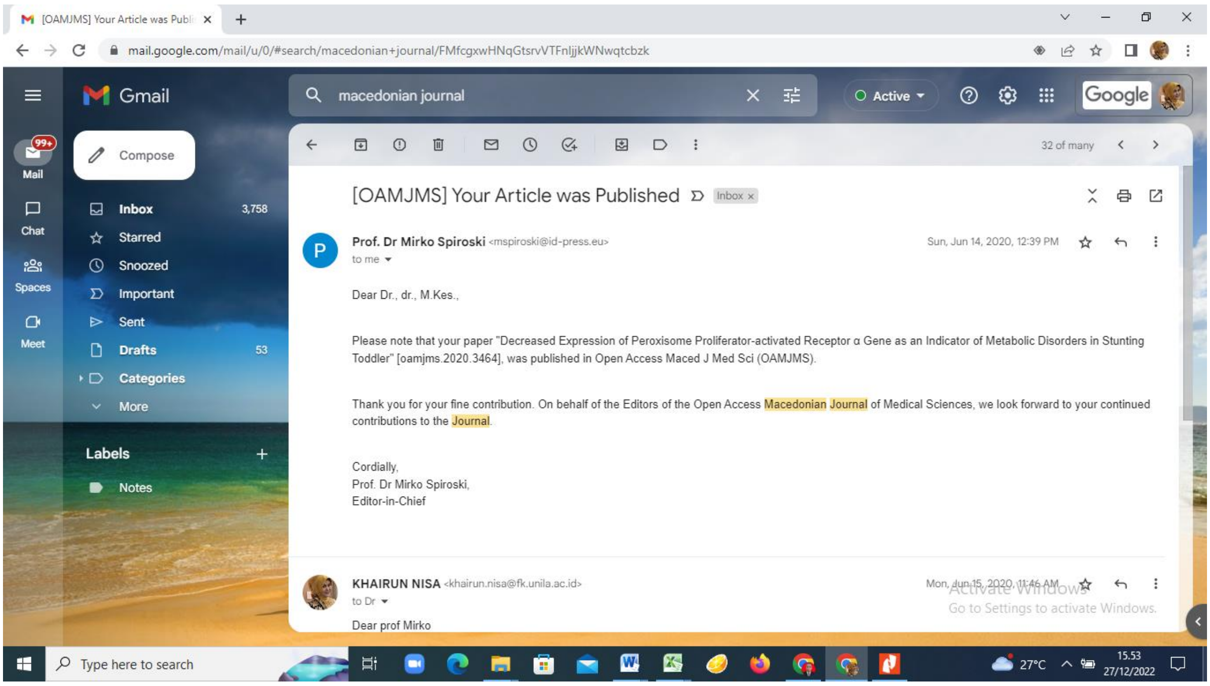

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Decreased Expression of Peroxisome Proliferator-activated Receptor α Gene as an Indicator of Metabolic Disorders in Stunting Toddler

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Abstract

BACKGROUND: Stunting in children increases the risk of degenerative diseases in adulthood, including dyslipidemia, obesity, type 2 diabetes mellitus, and cardiovascular disease. This is based on the result of metabolic changes that may be caused by chronic malnutrition and experienced by stunting children. Stunting in children is associated with metabolic disorders that are based on impaired fat oxidation, a trigger factor for obesity in adulthood. The peroxisome proliferator-activated receptor (PPAR) α gene is a transcriptional factor that regulates fat, carbohydrate, and amino acid metabolism whose genetic variants are linked to the development of dyslipidemia and cardiovascular disease.

AIM: The study assessed the effect of metabolic changes in stunting toddler on PPARα gene expression.

MATERIALS AND METHODS: An analytical-observational laboratory was done using 41 blood samples, coming from 23 stunting toddlers, and 18 not-stunting toddlers. In all research subjects, anthropometric measurements and examination of PPARα gene mRNA expression were carried out. Analysis of PPARα gene mRNA expression using one-step quantitative reverse transcriptase-polymerase chain reaction using specific primers, as a comparison of gene expression using the GAPDH gene. The relative expression of the PPARα mRNA gene was analyzed using the LIVAK formula.

RESULTS: The study obtained a mean of ∆CT in stunting toddlers of 5.81, whereas in stunting toddlers at 5.082. Analysis with LIVAK 2 ^ - formula (∆CT stunting -∆CT not stunting) obtained PPARα mRNA gene expression of 0.6.

CONCLUSION: We conclude that there is a decrease in PPARα gene expression in stunting toddlers.

Introduction

Stunting is a condition of failure to thrive in children under 5 years due to chronic malnutrition so that the child becomes shorter than his age which can be seen after the child is already 2 years old. The prevalence of stunting toddlers in the world based on data from Joint Child Malnutrition Estimates, 2018, experienced a downward trend from 2010 at 26.1%, decreasing in 2017 to 23.2% [1]. Stunting is assessed based on height index according to age (TB/U) with threshold (z-score) <-2 child growth standard (SD) [2], [3]. Stunting can occur due to a lack of nutrients that occur in 1000 days after conception until the first 2 years of life [4], [5], [6]. Stunting relates to many factors, including socioeconomics, nutritional intake of pregnant women and infants, infections, maternal nutritional status, infectious diseases, micronutrient, and environmental deficiencies [1], [5], [7], [8]. Research on stunting toddlers indicates that growth is not optimal; the occurrence of metabolic disorders and trigger cognitive development that is not optimal also decreases the child's body survival against diseases and infections. In adulthood, children will suffer from adulthood with productivity that is not optimal and vulnerable to the development of various degenerative diseases such as obesity, diabetes mellitus, and cardiovascular disease [3], [9], [10], [11], [12], [13].

The peroxisome proliferators-activated receptor (PPAR) isotypes, PPARα, PPARδ/β, and PPARγ are ligand-activated nuclear transcription factors, which modulate the expression of an array of genes that play a central role in regulating glucose, lipid, and cholesterol metabolism, where imbalance can lead to obesity, type 2 diabetes mellitus, and cardiovascular disease [13], [14], [15], [16], [17]. PPARα located on chromosome 22q12-q131 is transcriptional factors from core hormone receptors that regulate several genes involved in metabolic processes, especially fat metabolism. PPARα is activated under conditions of decreased energy (under-nutrients) and is important in the process of ketogenesis, a key adaptive response to long-term fasting. Activation of PPARα increases the uptake, use, and catabolism of lipid acids by upregulating genes involved in lipid acid transport, binding and activation of lipids, and beta lipid acid oxidation in peroxisomes and mitochondria [18], [19]. The expression of the PPARα gene can be activated by ligand which can be an external/synthetic factor such as dietary intake containing polyunsaturated fatty acids (SFA) and fibrate drugs used for dyslipidemia [13], [15], [16], [17]. Endogenous ligands include fatty acids such as arachidonic acid and their metabolism. Certain nutritional conditions, such as protein restriction diets for a long time (malnutrition), which are found in stunting toddlers, produce long-term effects on PPARα gene expression through modification in the methylation of specific loci surrounding the PPARα gene related to the development of several diseases in the body, such as dyslipidemia, diabetes, and obesity [16], [20], [21], [22], [23]. This proves the PPAR-a gene as a mediator of metabolic adaptation responses to nutritional and environmental factors [15], [18], [19]. As a transcriptional factor, PPARα refers in transcriptional factors of various regulatory genes that play a role in metabolism such as fatty acid metabolism, bile acid synthesis, synthesis and degradation of ketone objects, and metabolic glycerophospholipid as well as its interaction with various PPAR superfamily genes and core receptors [13], [16], [17], [24], [25].

Research on the relationship of metabolic disorders that occur with stunting children to the expression of PPARα gene which is a mediator metabolic gene for adaptation response to nutrirional and environtmental factors in Indonesian children, have never been established yet, mainly in 2018 when the research was done.

Materials and Methods

This research is an observational laboratory analytic study. The assessment was performed on 41 toddlers with 23 stunting toddlers and 18 toddlers without stunting.

Anthropometric examination and venous blood extraction

Venous blood extraction was performed to measure PPARα gene expression and compare the decline between stunting and non-stunting children. Anthropometric measurements using the WHO global database on child growth and malnutrition for a Z score cut off point of <-2 SD to height for age in stunting subject. Not stunting subject if point range beween -2 sd to +2 SD [19]

Examination of PPARα gene by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)

Analytical-observational laboratory of PPARα gene expression was carried out by examining qPCR (real-time PCR) in blood ethylenediaminetetraacetic acid toddlers. There are four steps that were done, namely, primary design, RNA isolation, NanoDrop, and quantification of gene expression using qPCR.

The primary design

This study found that the primary base sequence of the PPARα gene refers to previous research and after checking with BLAST according to the target gene that you want to examine. The primary sequence of the PPARα gene is as follows: [12]

Forward primer: 5 ‘-TGCAGATCTCAAATCTCTGG-3’
Reverse primer: 5’-ATCACAGAAGACAGCATGGC-3 ‘.

RNA isolation using NanoDrop

Isolation and measurement of RNA concentration using NanoDrop. RNA was extracted using QIAamp RNA Blood Mini Kit (Qiagen 52304) following the manual kit procedure. The RNA concentration obtained was then measured using a NanoDrop 2000 Spectrophotometer from Thermo Scientific.

Measurement of PPARα gene expression using qRT-PCR

Measurement of PPARα gene expression using one-step qRT-PCR. The primers used are as follows: Primary forward: 5’-TGCAGATCTCAAATCTCTGG-3 ‘and primary reverse: 5’-ATCACAGAAGACAGCATGGC-3’ [26] 2–10 ul RNA template (sample isolated) added PCR master-mix containing primer. The PCR cycle conditions for genes included an initial denaturation step of 94°C for 3 min, followed by an amplification cycle consisting of denaturation at 94°C for 45 s, annealing at 58°C for 45 s, extension 72°C for 45 s, and final extension at 72°C for 10 min.

The measurement of the PPARα gene concentration is using the relative quantification method. Measuring the cycle threshold of the PPARα gene compared to the GADPH gene as new housekeeping compared the mean between the two groups and the mean level of decrease in gene expression.

$$\Delta C_{T\ stunting} = C_{T\ stunting\ target} - C_{Tstuunting\ housekeeping}$$
$$\Delta C_{T\ Non\ stunting} = C_{T\ Non\ stunting\ target} - C_{Non\ stunting\ housekeeping}$$
$$\Delta\Delta C_{T\ experiment} = \Delta C_{T\ stunting} - \Delta C_{non\ stunting}$$

The comparison of gene expression levels = $2^{\Delta\Delta CT}$. The measurement of concentration is by using LightCycler® software program referred to LIVAK formula (concentration in picogram size).

This research has received ethical research approval from the ethics committee of the Faculty of Medicine, University of Lampung in 2018, and in collaboration with the Molecular Genetics Laboratory, Faculty of Medicine, University of Padjadjaran, Bandung, Indonesia.

Results

Sample characteristics

Toddlers are the sample of this study. The research were held with 41 children under five (Tables 1 and 2), consisting 14 boys and 27 girls. Under-five children are aged between 24 months and 60 months, with an average age of 41.85 months. A total of 23 stunting toddlers and 18 children under five were not stunting. They came from one study area in Central Lampung District, Lampung Province. Blood samples were taken from all toddlers in the research subject and then being grouped between stunting toddlers and toddlers not stunting.

Table 1: qPCR optimization of the PPARα gene and the main GADPH for infants who are not stunted by growth

Subject	CT GADPH	CT PPARa	∆CT
A	28.28	32.68	4.4
B	27.46	31.41	3.95
C	27.61	32.24	4.63
D	24.02	28.97	4.95
E	30.56	36.59	6.03
F	30.08	37.97	7.89
G	31.47	34.39	2.92
H	30.23	34.55	4.32
I	30.12	38.54	8.42
J	29.83	32.32	2.49
K	29.48	34.51	5.03
L	25.61	29.07	3.46
M	23.19	28.11	4.92
N	27.86	33.51	5.65
O	26.75	30.39	3.64
P	20.62	28.55	7.93
Q	20.62	28.55	0.57
		Average	5.0

PPAR: Peroxisome proliferator-activated receptors, qPCR: Quantitative polymerase chain reaction.

Table 2: qPCR tables primary optimization of the PPARα gene and GADPH stunting toddlers

NAMA	CT GADPH	CT PPARa	∆CT
A	22.44	30.44	8
B	20.55	27.79	7.24
C	21.74	28.35	6.61
D	20.51	26.49	5.98
E	23.88	29.63	5.75
F	18.79	26.92	8.13
G	18.8	26.05	7.25
H	27.18	31.99	4.81
J	20.34	28.5	8.16
K	20.78	29.68	8.9
L	31.52	32.79	1.27
M	22.73	30.7	7.97
N	32.49	33.5	1.01
O	19.49	27.23	7.74
P	26.38	30.72	4.34
Q	27.18	31.99	4.81
R	30.8	30.75	-0.05
S	29.48	34.51	5.03
T	23.19	28.11	4.92
U	22.35	28.64	6.29
V	25.61	29.07	3.46
W	30.08	37.97	7.89
X	26.7	29.13	2.43
		Average	5.817727273

PPAR: Peroxisome proliferator-activated receptors, qPCR: Quantitative reverse transcriptase-polymerase chain reaction.

Optimization of real-time PCR of PPARα and GADPH genes

The results showed threshold cycle PPARα gene expression (CT PPARα) in non-stunting toddlers with an average (∆CT) of 5.0 (Table 1 and Figure 1).

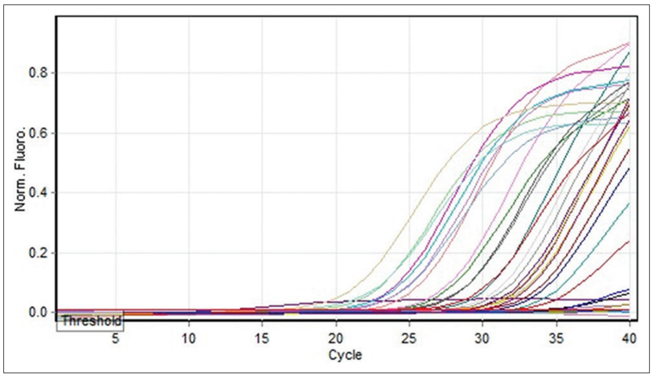

Figure 1: The main optimization chart of the peroxisome proliferator-activated receptors α gene and GADPH qPCR for toddlers who are not stunted by growth

The results showed threshold cycle PPARα gene expression (CT PPARα) in non-stunting toddlers with an average (∆CT) of 5.81 (Table 2 and Figure 2).

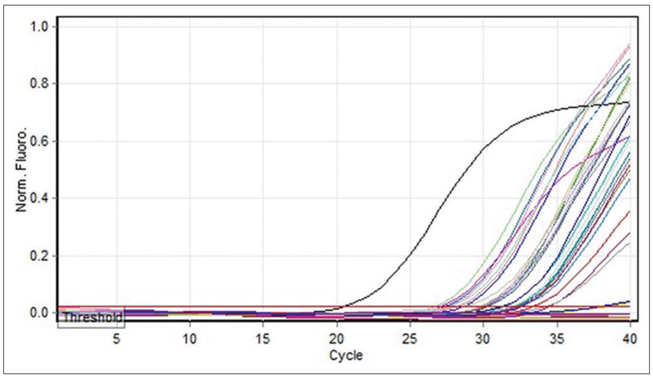

Figure 2: Quantitative polymerase chain reaction graph primary optimization of the peroxisome proliferator-activated receptors α gene and GADPH stunting toddlers

Decrease expression in PPARα stunting toddlers

The results showed stunting toddlers threshold cycle PPARα gene expression (CT PPARα) in stunting toddlers with a mean (∆CT) of 5.81 compared to non-stunting toddlers of 5.0. For normalization we use hausekeeping genes, the GADPH gene. Comparison of expressions was used to compare groups of toddlers who are not stunting. The measurement results showed that the higher CT is, the lower gene expression was measured, so the difference in threshold cycle average (∆∆CT) between stunting toddlers and non-stunting toddlers was 0.81, and the PPARα mRNA gene expression based on LIVAK 2 formula ^ - (∆∆CT) is 0.6. It means that if there is no change in expression between stunting and non-stunting, the value is 1, while the value obtained is 0.6 means a decrease in expression in non-stunting toddlers.

Discussion

Factors that cause stunting are accumulative chronic processes that can occur starting from maternal nutritional factors from before and during pregnancy that affects the growth of children in the fetus (in the womb), infancy and throughout the first 1000 days of life [9]. The state of malnutrition that occurs in women of reproductive age during pregnancy will cause a disruption in fetal growth and development (Fetal Growth Retardation = FGR) which contribute to the occurrence of stunting in childhood. Children who experience stunting are found to have an increased risk of recurrent infectious diseases and an increased risk of metabolic disorders due to impaired energy used by the body of the affected child [6]. Even a number of studies and analysis that have been done previously suggests a relationship between shortness of obesity in childhood and adulthood. The analysis conducted in five countries of Arabian found that short children have a greater risk than children who are not short to fat [3].

The results showed stunting toddlers' threshold cycle PPARα gene expression (CT PPARα) in stunting toddlers with a mean (∆CT) of 5.81 compared to non-stunting toddlers of 5.0 as housekeeping genes used the GADPH gene (CT GADPH). Comparison of expressions was used to compare groups of toddlers who are not stunting. The measurement results showed that the higher CT is, the lower gene expression was measured, so the difference in threshold cycle average (∆∆CT) between stunting toddlers and non-stunting toddlers was 0.81, so the PPARα mRNA gene expression based on LIVAK 2 formula ^ - (∆∆CT) is 0.6. This result shows that if there is no change in expression between stunting and non-stunting, the value is 1, while the value obtained is 0.6 means a decrease in the expression of non-stunting toddlers.

The PPARA gene is a transcriptional factor that regulates target gene proteins that are widely expressed in tissues with high levels of beta fatty acid oxidation such as the liver and muscles also regulating the target genes involved in the transport and oxidation of fatty acids. The PPARA gene was activated due to its binding with ligand. Endogenous ligands such as long-chain fatty acids (long-chain fatty acids) from SFA, unsaturated fatty acids, and eicosanoids, or exogenous ligands of hypolipidemic drugs such as fibrates, fenofibrates, and NSAIDs can even be both [4], [8] (Alsaleh *et al*., 2012). The expression of the PPAR-α gene induces the expression of lipoprotein lipase, which releases fatty acids from triglycerides and blocks ApoC-III, a LPL inhibitor which decreases triglyceride synthesis. PPAR-α expression also plays a role in limiting the vascular cell inflammatory response through inhibition of the expression of adhesion molecules and limiting the initial inflammatory mediators such as NF-kB and cytokine expression so that the normal expression of PPAR-α has an atheroprotective effect (Desvergne and Wahli, 2015, Robitaille *et al*., 2004).

Decreasing PPARα gene expression will lead to a decrease in lipoprotein lipase expression, thus reducing triglyceride synthesis from lipoprotein particles can cause a disruption of metabolism energy, especially from fatty acids in stunting children. This decrease in PPARα gene expression also triggers an inflammatory response such as NF-kB and cytokines which develop chronic inflammatory processes in stunting children. Children who experience stunting are found to have an increased risk of recurrent infectious diseases and an increased risk of metabolic disorders due to the disruption of energy used by the affected body.

Conclusion

The results showed a decrease in PPARα gene expression in stunting toddlers. PPAR-α gene expression takes a role in limiting the vascular cell inflammatory response through inhibition of the expression of adhesive molecules and limiting the initial inflammatory mediators such as NF-kB and cytokine expression so that it will have an atheroprotective effect in normal condition. Decreasing of PPARA gene expression in stunting children will develop metabolic disorders, including dyslipidemia, atherosclerosis, and diabetes mellitus in adulthood.

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Declaration

Ethics approval and consent to participate: Ethical approval was given by the Health Research Ethics Committee of Lampung University Medical School with the number No. 3698/UN26.18/PP/05.02.00/2018.

Authors' contributions

KNB and AMM contributed equally to this work. KNB carried out the molecular genetic studies and drafted the manuscript. AMM carried out the molecular genetic studies and also in the sequential alignment. IBA participated in the design of the study and performed the statistical analysis and participated in its design and coordination and also helped to draft the manuscript. All authors read and approved the final manuscript.

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