International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:7, No:6, 2013

Implication and Genetic Variations on Lipid Profile of the Fasting Respondent

Rohayu Izanwati M. R., Muhamad Ridhwan M. R., Abbe Maleyki M. J., Ahmad Zubaidi A. L., Zahri M. K.

***Abstract*—**PPARs function as regulators of lipid and lipoprotein metabolism. The aim of the study was to compare the lipid profile between two phases of fasting and to examine the frequency and relationship of peroxisome proliferator-activated receptor, *PPARα* gene polymorphisms to lipid profile in fasting respondents. We conducted a case-control study protocol, which included 21 healthy volunteers without gender discrimination at the age of 18 years old. 3 ml of blood sample was drawn before the fasting phase and during the fasting phase (in Ramadhan month). 1ml of serum for the lipid profile was analyzed by using the automated chemistry analyser (Olympus, AU 400) and the data were analysed using the Paired T-Test (SPSS ver.20). DNA was extracted and PCR was conducted utilising 6 sets of primer. Primers were designed within 6 exons of interest in *PPARα* gene. Genetic and metabolic characteristics of fasting respondents and controls were estimated and compared. Fasting respondents were significantly have lowered the LDL levels ($p=0.03$). There were no polymorphisms detected except in exon 1 with 5% of this population study respectively. The polymorphisms in exon 1 of the *PPARα* gene were found in low frequency. Regarding the 1375G/T and 1386G/T polymorphisms in the exon 1 of the *PPARα* gene, the T-allele in fasting phase had no association with the decreased LDL levels (Fisher Exact Test). However this association is more promising when the sample size is larger in order to elucidate the precise impact of the polymorphisms on lipid profile in the population. In conclusion, the *PPARα* gene polymorphisms do not appear to affect the LDL of fasting respondents.

***Keywords*—**Fasting, LDL, Peroxisome proliferator activated receptor alpha (PPAR-α), Polymorphisms.

I. Introduction

LIPID profile is one of the metabolic parameters that have a major impact on health and diseases. Lipid profile is used as a diagnostic marker in predicting the cardiovascular disease [1] and others such as atherosclerosis [2], [3]. Moreover lipid profile changes are associated with others disorder such diabetes [4], [5]. In order to reduce the risk factors contributing and leading to disease progression, fasting is strongly recommended. Fasting in Ramadhan month is the one of the compulsory worship for Muslims as it is the third Islamic Pillars. Fasting stands for abstaining from any food and drink since dawn to dusk [5]. The fasting period may vary between 12-19 hours due to geographical location of the nation [6]. As proven by Rehman and Shafiq [5], fasting has influenced the carbohydrate and lipid metabolism as it resulted changes in blood chemistry. Even though the lipid profile changes were contradicted among gender however the Atherogenic Index showed significantly decreased in Kuwaiti population [7]. Studies have also shown Ramadhan fasting has significantly decreased the blood glucose, total cholesterol, LDL-c, urea and acid uric among the Iraqi population [4]. The potential of fasting in affecting the metabolic activity is underlies interactions between nutrition and the genetic influences as PPARα is responsible for a ligand-activated transcription factor that regulates the transcription of genes involved in fatty acid oxidation, lipid metabolism, tissue homeostasis and proteins. PPARα regulates the lipid and lipoprotein metabolism and play the important role in the regulation of inflammatory and oxidative pathways. The association of PPARα with fatty acid oxidation, lipid metabolism and inflammation has served the best platform to study the CVD [1]. A plethora of studies has demonstrated the variation in the gene encoding *PPARα* contributes the variability in lipid ranges and cardiac hypertrophy interindividualy. Reinhard [8] claimed that the common variations in the *PPARα* gene may influence the risk of myocardial infarction in a European population. In response to fenofibrate, LDL and interleukin-2 were found significantly associated with *PPARα* gene polymorphisms [9]. These findings become a marker in predicting the pathogenesis. Therefore, this study was carried out with the aim of exploring the correlation of *PPARα* polymorphisms with lipid profile changes subjected to Ramadhan fasting.

II. Methodology

A. Study Samples

Twenty one healthy Malay volunteers at the age of 18 years old were recruited at the Faculty of Medicine and Health Sciences, Universiti Sultan Zainal Abidin, Kuala Terengganu, Terengganu, Malaysia. Consent for inclusion in the study was obtained from the respondents. No dietary restriction applied during the fasting period in Ramadhan as well as before Ramadhan month. The research protocol was approved by the UniSZA Human Ethics Committee (UHREC), reference no: UniSZA.N/1/628-1 (1).

Rohayu Izanwati M. R is with the Faculty of Medicine and Health Sciences, Universiti Sutan Zainal Abidin, Kampus Kota, Jalan Sultan Mahmud 20400, Kuala Terengganu, Terengganu, Malaysia (phone: 609-627 5663; fax: 09-622 1707; e-mail: izanwati@unisza.edu.my).

Muhamad Ridhwan M. R., Abbe Maleyki M. J., and Ahmad Zubaidi A. L. are with the Faculty of Medicine and Health Sciences, Universiti Sutan Zainal Abidin, Kampus Kota, Jalan Sultan Mahmud 20400, Kuala Terengganu, Terengganu, Malaysia (e-mail: ridhwanripin@gmail.edu.my, maleyki@unisza.edu.my, azubaidi@unisza.edu.my).

Zahri M. K. is with the Faculty of Medicine and Health Sciences, Universiti Sutan Zainal Abidin, Kampus Kota, Jalan Sultan Mahmud, 20400 Kuala Terengganu, Terengganu, Malaysia (phone: 609-627 5697; fax: 09-622 1707; e-mail: khairizahri@unisza.edu.my).

B. Lipid Profiles

Three millilitres of blood were collected in a plain tube. The collected blood was centrifuged in order to obtain the serum. One millilitre of serum was analysed for lipid profile using the automated chemistry analyser (Olympus, AU 400). These procedures were applied twice for pre and during Ramadan fasting.

C. Genotyping

Genomic DNA was extracted from the whole blood with the use of the QiaAmp mini blood kit (Qiagen, Valencia, California, USA). Genotyping was carried out by polymerase chain reaction (PCR). *PPARα* genotyping was performed in a 25µl reaction mix containing 1X PCR buffer, 5µmol of each dNTP, 1.75mmol/L $MgCl_2$, 0.5 pmols/mL of each primer, and 0.5U Taq polymerase. PCR primers used by Vohl [13] were adopted with modification for the study. The primer sets were synthesized by 1st Base, Biosyntech Sdn. Bhd. and the sequences are listed in Table I. The PCR amplification generated fragments of 200 – 600 bp. The PCR products were resolved using 2-2.5% agarose gel electrophoresis.

TABLE I
PRIMER SEQUENCES

Forward Primer (5'-3')	Reverse Primer (5'-3')	Melting temperature (°C)	Product Size (base pairs)
PPARαex1. GCATCCAGAGAACA ACCGTAA	PPARαex1. CCATCTGGAAACAG TAAATTAAACC	59	169
PPARαex2. TCCATTCAAGCTGC TATAACAAAAT	PPARαex2. TAATGATAACAGAA TTCATCCACCA	59	213
PPARαex5. AGTAAAGCAAGTGC GCTGGT	PPARαex5. AAGGAAGGGGAACT GAGGAA	59	243
PPARαex6. CTCACTGCTCATGC CTGTGT	PPARαex6. CCAAGAGAACCCAG AACAGC	59	274
PPARαex7. GCATCCCACATCAC CTGAC	PPARαex7. TCAGTGACATGATA CCAGCAGA	60	530
PPARαex8. TGATAAGCAGTTCT TGGGTGA	PPARαex8. ACCATTGAGCATAA TTCGCC	60	566

D. Sequencing

The PCR products were purified using QIAQuick PCR purification kit (QiaGen Valencia CA. USA) and sequenced. The results were aligned using Bioedit software and compared with normal target sequences (NG_012204.1) using NCBI database (www.ncbi.com) in identifying the variations.

E. Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 20 for windows. The effect of fasting on lipid profile was analysed using Paired T-Test. The means were compared as a measure of significancy of the affected lipid components. Simple Logistic Regression analysis was carried out to test whether the distribution of *PPARα* polymorphisms is significantly associated with the LDL changes. A p-value <0.05 was considered statistically significant.

III. RESULTS

A. Lipid Profiles

The effect of Ramadan fasting on lipid profile was studied in 21 healthy volunteers. The blood parameters of the volunteers in the 1 week before and at the third week of the Ramadan month were further studied and compared. Lipid profile analysis as shown in Table II exhibited a significant drop of LDL-c value before and during Ramadhan fasting (p <0.05). However, other lipid profiles such as total cholesterol, triglycerides and HDL-c did show either reduction or increment but the difference was not statistically significant.

TABLE II
LIPID PROFILES

Lipid Profiles	Pre (Mean ± SD)	During (Mean ± SD)	p- Value
Total cholesterol (mmol/L)	4.97 ± 0.84	4.81 ± 0.93	0.18
Triglycerides (mmol/L)	0.73 ± 0.27	0.75 ± 0.27	0.75
HDL-c (mmol/L)	1.20 ± 0.21	1.27 ± 0.25	0.10
LDL-c (mmol/L)	3.62 ± 0.87	3.40 ± 0.89	0.03*

*Significant level at p<0.05

B. Detection of mutations by PCR and Sequencing

Polymerase chain reaction was done for the targeted exons of *PPARα* gene. After sequencing, we had detected two polymorphisms which are 1375G/T and 1386G/T with the percentage of 5%. No significant association was found between the declines of LDL level with the polymorphisms detected (Table III).

TABLE III
SUMMARY OF LOGISTIC REGRESSION FOR POLYMORPHISMS PREDICTING THE LEVEL OF LDL

Predictors	LDL levels			
	B	SE B	eB	Sig
Polymorphisms	-1.504	2.083	0.222	0.470
Constant	1.916	6.383	6.793	0.764

*Significant level at p<0.05

IV. DISCUSSIONS

Controlled intake of fat and energy is the first line treatment of hyperipidemia. Islam has offered a method in controlling the intake of food which is obligatory for all Muslims through the month of Ramadhan. This will enable Muslims to reflect the status of other people who are in the need of food. One of the advantages of fasting during Ramadhan is enable the Muslims to control the intake of food. Fasting had shown a promising factor in reducing LDL-c and increase HDL-c. Most of the previous studies showed Ramadhan fasting significantly increases HDL-c [5], [10]. Our study showed similar increment but not statistically significant. This proved fasting during the month of Ramadhan from dawn to dusk (approximately 8 hours) does have its advantages in health.

The various effects of fasting across the globe show

metabolism of lipid is crucial in controlling the development of hyperlipidemia. PPARs have shown new potential therapeutic target for the treatment of metabolic syndrome [11]. Polymorphisms tend to affect the metabolism of lipids. Study by Uthurralt et al. [12] showed the male caucasion who have SNP of L162V had reduced HDL and increased LDL. Significant decrease of LDL-c in our study showed promising function of two SNPs detected (1375G/T and 1386G/T) in reducing pathogenesis of hyperlipidemia that leads to CVD especially atherosclerosis.

Even there is no association found between the drop of LDL-c level and the polymorphisms detected, the variation among population may explain the strength of association between gene polymorphisms and disease or affected metabolism.

V. Conclusion

We conclude that even there is no association detected between *PPARα* variations with LDL changes in response to Ramadhan fasting, however it does affect the LDL level. The capability of fasting in reducing the disease risk is believed to be a baseline for personalized diet. It also helps to prevent disease in advance and maintaining the health in future. Fasting becomes one of the recommended dietary practices. The association study should be more promising when the sample size is larger in order to elucidate the precise impact of polymorphisms on the lipid profile in the population.

Acknowledgment

The study was study was funded by Center of Research, Development and Innovation (PPPI), Universiti Sultan Zainal Abidin (UniSZA), Terengganu, Malaysia. The authors and research team are thankful to all staff and respondents involved in this study.

References

[1] R. Bordet, T. Ouk, O. Petrault et al., "PPAR: A New Pharmacological Target for Neuroprotection in Stroke and Neurodegenerative Diseases." *Biochemical Society Transactions*, vol. 34, no. 6, pp. 1341–1346, 2006.

[2] D. M. Flavell, Y. Jamshidi, E. Hawe et al., "Peroxisome Proliferator-Activated Receptor *α* Gene Variants Influence Progression of Coronary Atherosclerosis and Risk of Coronary Artery Disease." *Circulation*, vol. 105, no. 12, pp. 1440–1445, 2002.

[3] I. C. Ozturk and A. A. Killeen, "An Overview of Genetic Factors Influencing Plasma Lipid Levels and Coronary Artery Disease Risk." *Archives of Pathology and Laboratory Medicine*, vol. 123, no. 12, pp. 1219–1222, 1999.

[4] A. M. Thannoun and E .S. Mahmoud, "Effect of Fasting in Ramadan on Blood Glucose and Lipid Profile." *Mesopotamia Journal of Agriculture*, vol. 38, no. 2, 2010.

[5] J. Rehman and M. Shafiq , "Changes in Blood Glucose and Lipid Profile during Ramadan Fasting." *J Ayub Med Coll Abbottabad*, vol. 12, no. 3, pp. 13-15, 2000.

[6] A. Aldouni et al., "Fasting during Ramadan Induces a Marked Increase in High- Density Lipoprotein Cholesterol and Decrease in Low-Density Lipoprotein Cholesterol." *Annals of Nutrition and Metabolism*, vol. 41, pp. 242-249, 1997.

[7] S. A. Saleh, A. Elsharouni, B. Cherian, and M. Mourou, "Effects of Ramadan Fasting on Waist Circumference, Blood Pressure, Lipid Profile, and Blood Sugar on a Sample of Healthy Kuwaiti Men and Women." *Mal Journal Nutrition*, vol. 11, no.2, pp. 143-150, 2005.

[8] Reinhard et al. "Association between *PPAR-Alpha* Gene Polymorphisms and Myocardial Infarction," *Clinical Science*, doi: 10.1042/CS2007039. 2010.

[9] A. C. Frazier-Wood, J. M. Ordovas, R. J. Straka, J. E. Hixson, I. B. Borecki, H. K. Tiwari and D. K. Arnett, "The PPAR Alpha Gene is Associated with Triglyceride, Low Density Cholesterol and Inflammation Marker Response to Fenofibrate Intervention: The GOLDN study." *The Pharmacogenomics Journal*, doi: 10.1038/tpj.2012.

[10] D. Qujeq, K. Bijani, K. Kalavi, J. Mohiti, H. Aliakbarpour. "Effects of Ramadan Fasting on Serum Low Density and High Density Lipoprotein Cholesterol Concentrations." *Ann Saudi Med,* vol. 22 (5-6), pp. 297-299, 2002.

[11] T. Coll, R. Rodriguez-Calvo, E. Barroso, L. Serrano, E. Eyre, X. Palomer, M. Vazquez-Carrera "Peoxisome Proliferator-Activated Receptor (PPAR)β/δ: A New Potential Therapeutic Target for the Treatment of Metabolic Syndrome." *Current Molecular Pharmacology,* vol. 2, pp. 46-55, 2009.

[12] J. Uthurralt, et al. "PPARα Underlies Variation in Serum Triglycerides and Subcutaneous Fat Volume in Young Males." *BMC Medical Genetics,* 8:55 doi:10.1 186/1471 -2350-8-55. 2007.

[13] Marie-Claude Vohl, et al. **"**Molecular Scanning of the Human PPARα Gene: Association of the L162V Mutation with hyperapobetalipoproteinemia". *Journal of Lipid Research* Vol 41, pp. 945-952, 2000.