RAD Conference Proceedings, vol. 9, pp. 31-36, 2025
ISSN 2466-4626 (online) | DOI: 10.21175/RadProc.2025.06
www.rad-proceedings.org

FREE FATTY ACIDS AND INFLAMMATION AS RISK FACTORS OF TYPE 2 DIABETES

Šaćira Mandal*

University of Sarajevo-Faculty of Pharmacy, Department of Chemistry in Pharmacy, Bosnia and Herzegovina

Abstract. *Type 2 diabetes (T2D) as a common type of diabetes mellitus is influenced by various factors such as genetics and environment. One of the most important risk factors for the development of T2D is insulin resistance (IR), the underlying mechanisms of which include impaired free fatty acid (FFA) metabolism and inflammation. Chronically elevated levels of plasma free fatty acids appear to disrupt normal glucose homeostasis, while increased levels of a key inflammatory marker, C-reactive protein (CRP), lead to endothelial dysfunction and other T2D complications. The aim of this study was to examine the relationship between plasma FFA concentration and CRP as an inflammatory marker in patients with diabetes. The research included 82 participants, 42 of whom were patients with type 2 diabetes and 40 healthy individuals as a control group, aged 40 to 65 years. Clinical and biochemical characteristics including body mass index (BMI), fasting glucose levels, HbA1c, insulin, CRP and lipid profile were determined using standard clinical methods, while the levels of individual FFAs were measured by gas chromatography. The results showed a positive correlation between myristic acid (C14:0) and CRP levels, as well as a negative association of docosapentaenoic acid (C22:5) with CRP levels ($p<0.05$) in control subjects. In the group of diabetics, a negative correlation of gamma-linolenic acid (C18:3) with CRP levels was observed ($p<0.001$). These findings suggest that concentrations of individual free fatty acids, with different chain lengths and degrees of saturation, such as C14:0 and C18:3, can be used as potential markers of low-grade inflammation and disease prognosis in T2D patients.*

Keywords: *free fatty acids, C reactive protein, inflammation, type 2 diabetes*

1. INTRODUCTION

Type 2 diabetes mellitus (T2D), the most common type of diabetes, currently represents a major global health problem. [1]. This chronic metabolic disorder is characterized by hyperglycemia due to defects in insulin secretion and insulin bioavailability, or both. The pathogenesis of T2D is complex, with progressive insulin resistance (IR) in peripheral and other tissues, and beta-cell dysfunction with impaired insulin secretion and elevated glucose levels (Figure 1). [2]-[4]. Studies have shown that the main underlying mechanisms of IR and increased adiposity involve impaired biosynthesis and metabolism of free fatty acids (FFA), followed by varying degrees of inflammation. [5], [6].

The exact physiological processes that lead to the initiation of the inflammatory response in obesity and IR are still not fully elucidated. [7]. A sedentary lifestyle, overeating and low physical activity, accompanied by elevated circulating FFA concentrations, appear to be main risk factors for the development of IR and T2D [4]. Changes in concentrations of FFA with different chain lengths and degrees of saturation in T2D patients are complex and not yet fully resolved. A recent metabolomic study found that of 42 potential biomarkers for type 2 diabetes, 12 were different plasma FFAs. [8]. Furthermore, it appears that certain fatty acids, such as saturated fatty acids and some of the unsaturated fatty acids, can directly activate proinflammatory molecules in endothelial cells and adipocytes, thus developing systemic inflammation. [9]-[12]. High-sensitivity C-reactive

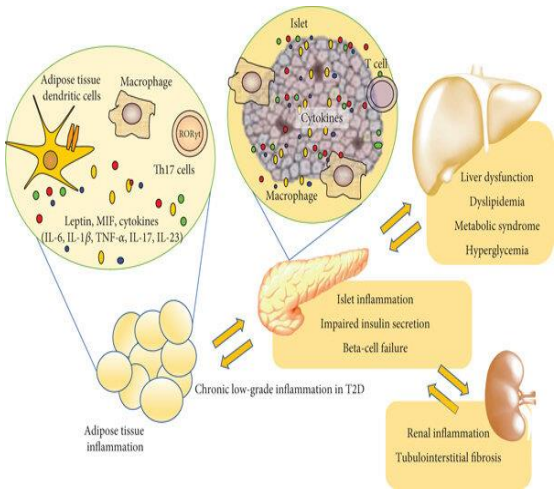

Figure 1. Chronic low-grade inflammation in Type 2 diabetes in the pancreas, adipose tissue, liver, and kidney [18].

protein (hsCRP), produced by the liver, is a key inflammatory marker and studies have shown that its presence in various inflammatory states is associated with changes in the lipid profile including FFAs. [13]-[17].

Therefore, the aim of this study was to analyze the association between the concentrations of specific

* E-mail of the corresponding author: shakira.mandal@gmail.com

free fatty acids as proinflammatory molecules and high-sensitivity C-reactive protein levels as an important inflammatory marker for the development of insulin resistance and increased risk of diabetes and its complications.

2. Experimental

2.1. Patients

This study included 42 patients diagnosed with type 2 diabetes and 40 healthy controls, aged 40 to 65. All of them gave and signed informed consent to participate in the study. Also, all analyses and material derived from human subjects in this study was done in accordance with ethical principles outlined in World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects (initiated in June 1964, last amendment in October 2000).

Subjects were enrolled according to the guidelines of the according to definitions of International Diabetes Federation (IDF) and were divided into two groups. [1]. Healthy controls were those with fasting blood glucose levels below 6.0 mmol/L, while the diabetic group was composed of individuals with fasting blood glucose levels above 7.0 mmol/L or non-fasting blood glucose levels above 11.1 mmol/L (when fasting samples were not available) and patients based on the presence of a history of type 2 diabetes for more than five years, without complications of the disease.

2.2. Methods

Venous blood samples were collected after an overnight fast and immediately centrifuged, and serum samples were stored at -70 °C until analysis. Laboratory measurements including hsCRP were performed on a VITROS 350 Chemistry System (Ortho-Clinical Diagnostic, Rochester, New York, USA) using standard IFCC (International Federation of Clinical Chemistry) methods, while individual FFA levels were determined by gas chromatography analysis. Hemoglobin A1c (HbA1c) was determined in the whole blood with EDTA by a standard chromatographic affinity separation method. IR was assessed by the homeostasis model assessment insulin resistance index (HOMA-IR) that was calculated by the formula: fasting insulin (mU/L) x fasting glucose (mmol/L) /22.5. Body Mass Index (BMI) was calculated as weight/height^2 (kg/m^2) ratio. [19], [20].

Fatty acid analysis done after lipid extraction with chloroform-methanol 2:1 (vol/vol) [21], and methylation of the samples by self-modifying method [22]. Fatty acid methyl esters (FAMEs) were prepared and analyzed on a Shimadzu 17A GC/FID gas chromatograph equipped with flame ionization detector (Kyoto, Japan), and capillary column Resterkorp OPTIMA® 120 (30m x 0.32 x 0.25µm film thickness) (Macherey-Nagel, Düren, Germany). The identification of each fatty acid peak was obtained by comparing the retention time of the peak with the retention times of referent standards with known fatty acids composition. The concentration of individual and different types of FFAs was calculated by using known concentrations of corresponding FAME standards.

2.3. Statistical analysis

The normality test of data distribution was done using Kolmogorov-Smirnov test, and the data showed a non-normal distribution. Therefore, statistical analysis was performed by using the nonparametric Mann-Whitney U test and for tested FFAs and hsCRP associations with clinical and biochemical parameters. Spearman's correlation coefficient was calculated to analyze the association between the clinical parameters, while the independent samples *t*-test was used to compare the mean values of biochemical parameters between diabetic and control groups. Data are expressed as mean ± standard error of the mean (SEM). All analyses were carried out by employing the SPSS (version 23.0 for Windows, SPSS Inc.; Chicago, IL, USA). A $p < 0.05$ was considered to be significant.

3. Results

The characteristics of type 2 diabetic patients and control subjects are presented in Table 1.

Table 1. Anthropometric and biochemical characteristics of patients with the type 2 diabetes and nondiabetic subjects*

Characteristic	T2D patients (n = 42)	Controls (n = 40)
Age	60 ± 1.63	44 ± 1.72
Males/Females	19/23	9/31
FPG, mmol/L	9.78 ± 0.42	5.26 ± 0.10
Fasting insulin, mU/L	12.28 ± 1.03	13.33 ± 2.31
HOMA-IR	5.40 ± 0.46	2.81 ± 0.41
HbA1c (%)	6.78 ± 0.16	4.52 ± 0.06
Total cholesterol, mmol/L	5.36 ± 0.13	5.81 ± 0.17
HDL, mmol/L	1.09 ± 0.06	1.72 ± 0.05
LDL, mmol/L	3.01 ± 0.14	3.05 ± 0.17
Triglycerides, mmol/L	2.59±0.21	2.32 ± 0.18
BMI (kg/m^2)	34 ± 1.85	30 ± 1.33
hsCRP (mg/L)	6.27 ± 0.44	5.55 ± 0.28

Values represent as mean ± standard error mean (SEM). BMI – body mass index; HOMA–IR – homeostasis model assessment insulin resistance index; LDL – low-density lipoprotein; HDL – high-density lipoprotein; hsCRP – high-sensitivity C-reactive protein; FPG – Fasting plasma glucose. *Significance of difference in Student *t*-test.

The measured anthropometric and metabolic parameters were significantly different between patients with T2D and healthy subjects as controls (Table 1). According to the latest classification and BMI reference values, it was indicated that the control subjects were overweight (BMI range 25-30) while the diabetics were in the obese class I group (BMI range 30-35). The HOMA-IR index as indicator of insulin resistance and pancreatic function can vary depending on the patient´s BMI, and normal levels for adults are between 0.5 and 1.4, levels of ≥1.9

indicate early IR, while levels of ≥2.9 indicate IR. In controls, HOMA-IR showed a levels of 2.8 as a consequence of high BMI in these individuals. HOMA-IR levels in patients with diabetes were 5.4, indicating that these individuals are insulin resistant (Table 1). An hsCRP reference interval for adults of less than 0.3 mg/L represents normal levels, levels between 0.3-1.0 mg/L are normal or mildly elevated, levels in the of range 1.0-10.0 mg/L are moderately elevated, while levels greater than 10.0 mg/L represent significantly elevated hsCRP. In both study groups, hsCRP levels were significantly increased, but still moderately elevated. These higher hsCRP values are caused by hormonal and genetic factors and lifestyle factors in healthy people, while in diabetics, in addition to the mentioned factors, they are also the result of obesity and metabolic dysfunction in diabetes.

The composition and levels of FFAs are shown in Table 2. Since the levels of arachidic acid, behenic acid, docosapentaenoic acid (DPA) (C22:5), and docosahexaenoic acid (DHA) were detected in very low concentrations in diabetic patients, it was difficult to assess differences in the levels of these fatty acids in this population sample.

Table 2. The composition and concentrations of FFAs in patients with the type 2 diabetes and control subjects*.

Fatty acid (μmol/L)	Cases (n=42)	Controls (n=40)	*p**
SFA			
Myristic acid, C14:0	7.07 ± 0.00	7.13 ± 0.00	NS
Palmitic acid, C16:0	148.55 ± 0.02	103.40 ± 0.01	NS
Stearic acid, C18:0	29.88 ± 0.00	29.28 ± 0.00	NS
Arachidic acid, C20:0	NA	2.12 ± 0.00	-
Behenic acid, C22:0	NA	2.20 ± 0.00	-
MUFA			
Myristoleic acid, C14:1	4.63 ± 0.00	4.55 ± 0.00	NS
Palmitoleic acid, C16:1	14.78 ± 0.00	10.75 ± 0.00	NS
Oleic acid, C18:1	94.02 ± 0.01	76.91 ± 0.01	NS
PUFA			
Linoleic acid, C18:2	143.55 ± 0.01	134.79 ± 0.01	NS
γ-linolenic acid, C18:3	3.10 ± 0.00	6.50 ± 0.00	**0.042**
DGLA, C20:3	23.48 ± 0.00	22.56 ± 0.00	NS
DTA C22:4	4.4 ± 0.00	3.00 ± 0.00	NS
DPA C22:5	NA	4.06 ± 0.00	-
DHA C22:6	NA	4.24 ± 0.00	-
Sum of SFA	182.76 ± 0.02	137.55 ± 0.01	**0.041**
Sum of MUFA	105.71 ± 0.01	85.35 ± 0.01	NS
Sum of PUFA	166.80 ± 0.01	158.77 ± 0.01	NS
Total of FFAs	455.27 ± 0.03	381.67 ± 0.02	NS

Values represent as mean ± standard error mean (SEM). DGLA– Dihomo-γ-linoleic acid, C20:3; DTA– docosatetraenoic acid, C22:4; DPA – docosapentaenoic acid, C22:5; DHA – docosahexaenoic acid, C22:6; SFA – saturated fatty acid; MUFA - monounsaturated fatty acid; PUFA - polyunsaturated fatty acid; FFA – free fatty acid; NA – not analyzed, NS – not significant. *Significance of difference in Student *t*-test.

As shown in Table 2, gamma-linolenic acid (γ-C18:3) levels were decreased in diabetics compared to nondiabetics (p=0.042). A significant difference between these two studied groups was also shown in the levels of total SFA, which were increased in patients with T2D (p=0.041). The association between MUFA (C16:1 and C18:1) and PUFA (C22:4) was strong but not significant (Table 2).

The relationship between FFA and baseline characteristics was investigated using Spearman's correlation. In both studied groups, a significant association was found between individual fatty acids (C16:0, C16:1, C18:0, C18:1, C18:2,), SFA, MUFA, PUFA, and total of FFAs concentrations with hsCRP (rho=0.317, p=0.004; rho=0.288, p=0.028 rho=0.318, p=0.004 rho=0.271, p=0.014 rho=0.258, p=0.020 rho=0.335, p=0.002 rho=0.274, p=0.013 rho=0.254, p=0.022 rho=0.326, p=0.003; respectively). Also, a positive correlation was found between fasting glucose and HbA1c with hsCRP levels (rho=0.219, p=0.049; rho=0.217, p=0.039, respectively).

As shown in Figure 2, a negative correlation was demonstrated between gamma-linolenic acid (γ-C18:3) and hsCRP levels (rho=-0.341, p=0.001), as well as a positive association between BMI and hsCRP levels in diabetics (rho= 0.483, p=0.020).

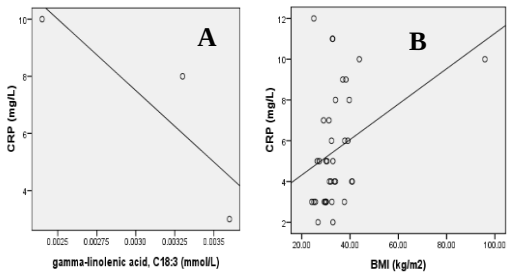

Figure 2. Spearman's correlation between levels of gamma-linolenic acid, GLA (rho = -0.341, p = 0.001) (A) and BMI (rho = 0.483, p = 0.020) with hsCRP levels in T2D patients (B).

In healthy subjects, a positive association was observed between myristic acid, C14:0 and hsCRP levels ((rho=0.414, p=0.050) and a negative association between docosapentaenoic acid (DPA) C22:5, and hsCRP levels (rho= -0.894, p=0.041) (Figure 3A and 3B).

HbA1c levels were assessed as a marker of glycemic control, and significant associations with fasting plasma glucose, HOMA-IR, hsCRP, specific FFAs (C16:0, C18:1, DTA), SFA, MUFA and age were demonstrated in all study participants (rho = 0.916, p = 0.001; rho = 0.497, p = 0.001; rho = 0.217, p = 0.039; rho = 0.307, p = 0.005; rho = 0.227, p = 0.040; rho = 0.989, p = 0.010; rho = 0.248, p = 0.025; rho = 0.226, p = 0.041; rho = 0.674, p = 0.001, respectively).

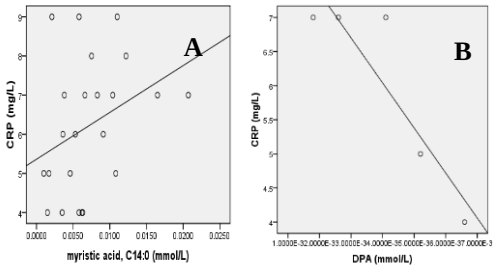

Figure 3. Spearman's correlation between levels of myristic acid (rho = 0.414, p = 0.050) (A) and DPA (rho = -0.894, p = 0.041) with hsCRP levels in control subjects (B).

4. Discussion

Changes in the metabolism of FFA's are accepted as one of the main factors leading to insulin resistance. Many studies in patients with T2D suggest that chronic exposure to high glucose and/or high FFA levels impairs pancreatic beta-cells function. Another valuable and important factor in the development of IR, obesity, and T2D appears to be adipose tissue inflammation. Hyperglycemia appears to be involved in the stimulation of cytokine release, leading to the production and secretion of acute-phase proteins in adipose tissue cells. Previous studies reported that increased hsCRP levels coincided with elevated fasting glucose levels, while other studies found no such association [23]-[26].

The results of this study showed an association between the levels of specific types of FFAs (with different carbon chain length and degree of saturation) and hsCRP levels in patients with T2D. Also, SFA levels were shown to be increased compared to non-diabetic subjects This is consistent with a number of previous studies, which have reported that the most common pattern observed in patients with T2D was an increase in saturated FFAs, while the evidence regarding other FFAs is often inconclusive. [27]-[29].

Recent studies have highlighted the important role of ethnicity and gender in the association between inflammation and lipid levels in selected study populations [30], [31]. Furthermore, it has recently been suggested that the pro- and anti-inflammatory effects of fatty acids depend on their chemical structure such as chain length and the number and location of double bonds. Therefore, specific fatty acids may be helpful in understanding the role of these biomolecules in disorders, such as T2D and IR. Previously published data indicate that inflammation in diabetics can be associated with the activation of a range of cytokines by shorter and longer SFA, MUFA or PUFA. The association between different FFAs and hsCRP levels shown in the results of this study may indicate a potential role of FFAs as an important modulator in inflammatory processes and potential mechanisms of association with hsCRP as a markrer of low-grade inflammation [18], [29], [31]. According to currently published data, specific fatty acids of different types (i.e. SFA, MUFA or PUFA) can show a distinct association with inflammatory markers, where the most abundant SFA in human body, palmitic acid (C16:0), and its abnormal increase within cells can reduce insulin signaling, leading to an important role in the development of IR and inflammation. [30], [32], [33]. However, the results regarding MUFA are contradictory as some studies have documented that they have a similar pro-inflammatory response to SFA, while in others thex have no effect or have a beneficial effect on inflammation. Previous findings reported that MUFA in healthy individuals reduce hsCRP, while showing no effect on hsCRP in patients with T2D. Also, recent studies suggest that dietary MUFA can alleviate IR and inflammation. [32] Similarly, there are conflicting data reporting the stimulatory or inhibitory effects of a diet enriched in n-6 PUFA on inflammation [5], [10], [34]-[37]. In Spearman´s analysis of the association between certain individual FFAs, as well as SFA, MUFA, PUFA, and total FFAs with hsCRP levels, a positive significant association of C16:0, C16:1, C18:1, C18:2, SFA, MUFA, PUFA, total FFAs and hsCRP was found in all participants, which is consistent with the results of other researchers. [15]- [17], [32], [33], [35].

In the diabetic group, a negative association was found between hsCRP and γ-C18:3, while in the control group, a significant association was observed between C14:0 (Figure 2A) and a negative association of C22:5 with hsCRP (Figure 2B). Investigations have shown that C14:0 exhibits low pro-inflammatory activity, while γ-C18:3 can reduce inflammation. Kaska *et al.* in their study involving morbidly obese women showed that both, total SFA and MUFA positively correlated with plasma hsCRP levels, while total n-6 and n-3 PUFAs, showed an inverse correlation with hsCRP levels. Also, their findings suggest that pro-inflammatory effect of SFA depends on their chain length. [38]. Similarly, some of the studies have shown that the potential pro-inflammatory effects of MUFA and the anti-inflammatory actions of PUFA may depend on their chain length and the presence and localization of double bonds [39], [40].

Previous studies have documented a relationship between hyperglycemia, as well as glycemic control, and inflammation, and in this study, a relationship was found between hsCRP and HbA1c in both groups of participants (rho=0.219 p=0.049). The researchers also showed that the gender of study population influences the incidence and development of T2D. [5], [41]. The factors for gender differences still remain unclear, and possible causes include differences in physiology, i.e. hormonal pathophysiology, disease treatment, and others. Similar effects of gender on FFAs especially SFAs and PUFAs, were shown in a previous study by authors, conducted in patients with prediabetes and T2D and a trend of increased risk for IR and T2D with aging was observed. [42]-[45], Moreover, the cause of the differences between healthy controls and diabetics is not only gender, but also genetics, ethnicity, race, age, dietary habits as well as other environmental factors. [17], [28], [35], [40], [46].

The main limitation of this study is related to the relatively small number of participants, especially with regard to the age and gender of the patients and healthy subjects. Future research is needed in a larger population to clarify the roles of FFAs and other inflammatory markers in the risk of developing of T2D and its complications.

5. Conclusion

In summary, in this case-control study results demonstrated the association of specific saturated and polyunsaturated fatty acid levels with hsCRP as a marker of low-grade inflammation in Type 2 diabetic patients, which might contribute to pathogenesis of disease. Thus, observed data suggest monitoring levels of individual FFAs, particularly C14:0, C16:0 and C18:3, in diabetic patients as potential novel prognostic biomarkers of T2D development.

References

1. International Diabetes Federation. Diabetes Atlas — 10th Edition, Diabetes Atlas, 2021.
2. G.S. Hotamisligil, "Inflammation and metabolic disorders", *Nature*, vol. 14, no. 7121, pp. 860–867, 2006. https://doi.org/10.1038/nature05485
3. C. de Luca, J.M. Olefsky, "Inflammation and insulin resistance", *FEBS Letters*, vol. 582, no. 1, pp. 97–105, 2008. https://doi.org/10.1016/j.febslet.2007.11.057
4. M.C. Calle, M.L. Fernandez, "Inflammation and type 2 diabetes", *Diabetes Metab*., vol. 38, no. 3, pp. 183–191, 2012. https://doi.org/10.1016/j.diabet.2011.11.006
5. Š. Mandal, "Association of free fatty acid concentrations with glucose levels in Bosnian subjects", *RAD Conf. Proc*, vol. 7, 2023, pp. 47–51. http://dx.doi.org/10.21175/RadProc.2023.09
6. A. Pan, Y. Wang, J.M. Yuan, W.P. Koh, "High-sensitive C-reactive protein and risk of incident type 2 diabetes: a case-control study nested within the Singapore Chinese Health Study," *BMC Endocr Disord*., vol. 17, no. 1, pp. 1–8, 2017. https://doi.org/10.1186/s12902-017-0159-5
7. V.T. Samuel, G.I. Shulman, "The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux", *J. Clin. Invest*., vol. 126, no. 1, pp. 12–22, 2016. https://doi.org/10.1172/JCI77812
8. A.I.S. Sobczak, C.A. Blindauer, A.J. Stewart, "Changes in plasma free fatty acids associated with type 2 diabetes", *Nutrients*, vol. 11, no. 9, pp. 1–42, 2019. https://doi.org/10.3390/nu11092022
9. C. Menni, E. Fauman, I. Erte, J.R.B. Perry, G. Kastenmuller, S.Y. Shin, A.K. Petersen, C. Hyde, M. Psatha, K.J. Ward, et al., "Biomarkers for type 2 diabetes and impaired fasting glucose using an ontargeted metabolomics approach," *Diabetes*, vol. 62, no. 12, pp. 4270–4276, 2013. https://doi.org/10.2337/db13-0570
10. D. Tripathy, P. Mohanty, S. Dhindsa, T. Syed, H. Ghanim, A. Aljada, et al., "Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects", *Diabetes*, vol., no. 12, pp. 2882–2887, 2003. https://doi.org/10.2337/diabetes.52.12.2882
11. J. Stanimirovic, J. Radovanovic, K. Banjac, M. Obradovic, M. Essack, S. Zafirovic, Z. Gluvic, T. Gojobori, E. R. Isenovic, "Role of C-reactive protein in diabetic inflammation", *Mediators Inflamm*., vol. 2022, 3706508- 1-15, 2022. https://doi.org/10.1155/2022/3706508
12. M.S. Barbalho, J.R. Toffano, D.M. Bechara, R.K. Quesada, M. Oshiiwa, G.C. Mendes, R.M. Gabaldi, "Association between hyperglycemia, C-reactive protein and other risk factors in patients at cardiovascular risk", *Journal of Diabetes Mellitus,* vol. 6, no. 1, pp. 16–24, 2016. http://dx.doi.org/10.4236/jdm.2016.61003
13. K. Eguchi, I. Manabe, "Islet inflammation in type 2 diabetes and physiology", *J Clin Invest*., vol. 127, no. 1, pp. 14–23, 2017. https://doi.org/10.1172/jci88877
14. C. Löwbeer, L. Mårtensson, E. Berg, H. Wallinder, "Associations between C-reactive protein and apolipoproteins, lipoprotein(a) and conventional serum lipids in outpatients: correlations and time trends", *Open Journal of Clinical Diagnostics*, vol. 5, no. 2, pp. 33–40, 2015. http://dx.doi.org/10.4236/ojcd.2015.52006
15. A.P. Simopoulos, "An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity", *Nutrients*, vol. 8, no. 3, pp. 1–17, 2016. https://doi.org/10.3390/nu8030128
16. K. Eguchi, I. Manabe, "Macrophages and islet inflammation in type 2 diabetes," *Diabetes, Obesity and Metabolism*, vol. 15 (Suppl 3), pp. 152–158, 2013. https://doi.org/10.1111/dom.12168
17. M. Ebrahimi, A.R. Heidari-Bakavoli, S. Shoeibi, S.R. Mirhafez, M. Moohebati, H. Esmaily, et al., "Association of serum hs-CRP levels with the presence of obesity, diabetes mellitus, and other cardiovascular risk factors", *Journal of Clinical Laboratory Analysis*, vol. 30, pp. 672-676, 2016. https://doi.org/10.1002/jcla.21920
18. T.V. Velikova, P.P. Kabakchieva, Y.S. Assyov, T.A. Georgiev, "Targeting Inflammatory Cytokines to Improve Type 2 Diabetes Control," *Hindawi BioMed Res. Inter*., vol. 2021, Article ID 7297419, 12 pages, 2021. https://doi.org/10.1155/2021/7297419
19. D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, "Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man", *Diabetologia*, vol. 28, no. 7, pp. 412–19, 1985. https://doi.org/10.1007/bf00280883
20. WHO. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. World Health Organ Tech Rep Sep 1995, 854, 1–452.
21. J. Folch, M. Lees, G.H.S. Stanley, "A simple method for the isolation and purification of total lipids from animal tissues", *J Biol Chem*., vol. 226, no. 1, pp. 497–509, 1957. https://pubmed.ncbi.nlm.nih.gov/13428781
22. G. Lepage, C.C. Roy, "Specific methylation of plasma nonesterified fatty acids in a one-step reaction", *J Lipid Res*., vol. 29, no. 2, pp. 227–235, 1988. https://pubmed.ncbi.nlm.nih.gov/3367090
23. L.I. Rachek, "Free fatty acids and skeletal muscle insulin resistance", *Prog Mol Biol Transl Sci*., vol. 121, pp. 267–292, 2014. https://doi.org/10.1016/b978-0-12-800101-1.00008-9
24. A.T. Kharroubi, H.M. Darwish, "Diabetes mellitus: The epidemic of the century", *World J Diabetes*, vol. 6, no. 6, pp. 850-867, 2015.

https://doi.org/10.4239/wjd.v6.i6.850
25. Y.A. Nasykhova, Y.A. Barbitoff, E.A. Serebryakova, D.S. Katserov, A.S. Glotov, "Recent advances and perspectives in next generation sequencing application to the genetic research of type 2 diabetes", *World J Diabetes*, vol. 10, no. 7, pp. 376–396, 2019. https://doi.org/10.4239/wjd.v10.i7.376
26. D.E. King, A.G. 3rd Mainous, T.A. Buchanan, W.S. Pearson, "C-reactive protein and glycemic control in adults with diabetes", *Diabetes Care*, vol. 26, no. 5, pp. 1535–9, 2003. https://doi.org/10.2337/diacare.26.5.1535
27. C. Klein-Platat, J. Drai, M. Oujaa, J.-L. Schlienger, C. Simon, "Plasma fatty acid composition is associated with the metabolic syndrome and low-grade inflammation in overweight adolescents", *Am J Clin Nutr*., vol. 82, no. 6, pp. 1178–84, 2005. https://doi.org/10.1093/ajcn/82.6.1178
28. H. Petersson, L. Lind, J. Hulthe, A. Elmgren, T. Cederholm, U. Riserus, "Relationships between serum fatty acid composition and multiple markers of inflammation and endothelial function in an elderly population," *Atherosclerosis*, vol. 203, no. 1, pp. 298–303, 2009. https://doi.org/10.1016/j.atherosclerosis.2008.06.020
29. N. Rajkovic, M. Zamaklar, K. Lalic, A. Jotic, Lj. Lukic, T. Milicic, et al., "Relationship between obesity, adipocytokines and inflammatory markers in type 2 diabetes: relevance for cardiovascular risk prevention", *Int. J. Environ. Res. Public Health*, vol. 11, no. 4, pp. 4049–4065, 2014. https://doi.org/10.3390/ijerph110404049
30. S. Santos, A. Oliveira, C. Lopes, "Systemic review saturated fatty acids on inflammation and circulating levels of adipokines", *Nutr. Res*., vol. 33, no. 9, pp. 687–695, 2013. https://doi.org/10.1016/j.nutres.2013.07.002
31. C. Nienaber-Rousseau, B. Swanepoel, C.R. Dolman, M. Pieters, R.K. Conradie, G.W. Towers, "Interactions between C-reactive protein genotypes with markers of nutritional status in relation to inflammation", *Nutrients*, vol. 6, no. 11, pp. 5034–5050, 2014. https://doi.org/10.3390/nu6115034
32. M. Perreault, K. Roke, A. Badawi, D.E. Nielsen, S.A. Abdelmagid, A. El-Sohemy, et al., "Plasma levels of 14:0, 16:0, 16:1 n-7, and 20:3 n-6 are positively associated, but 18:0 and 18:2 n-6 are inversely associated with markers of inflammation in young healthy adults", *Lipids*, vol. 49, no. 3, pp. 255–263, 2014. https://doi.org/10.1007/s11745-013-3874-3
33. D. Wu, J. Liu, X. Pang, S. Wang, J. Zhao, X. Zhang, L. Feng, "Palmitic acid exerts proinflammatory effects on vascular smooth muscle cells by inducing the expression of C reactive protein, inducible nitric oxide synthase and tumor necrosis factor α", *International Journal of Molecular Medicine*, vol. 34, no. 6, pp. 1706–1712, 2014. https://doi.org/10.3892/ijmm.2014.1942
34. I.D. Santaren, S.M. Watkins, A.D. Liese, L.E. Wagenknecht, M.J. Rewers, S.M. Haffner, et al., "Individual serum saturated fatty acids and markers of chronic subclinical inflammation: The Insulin Resistance Atherosclerosis Study (IRAS)", *J. Lipid Res*., vol. 58, no. 11, pp. 2171–2179, 2017. https://doi.org/10.1194/jlr.p076836
35. S.A. Khan, R.T. Jackson, "Polyunsaturated fatty acids, inflammation, and metabolic syndrome in South Asian Americans in Maryland", *Food Sci Nutr*., vol. 6, no. 6, pp. 1575–1581, 2018. https://doi.org/10.1002/fsn3.698
36. A.Z. Lalia, M.L. Johnson, M.D. Jensen, K.C. Hames, J.D. Port, I.R. Lanza, "Effects of dietary n-3 fatty acids on hepatic and peripheral insulin sensitivity in insulin-resistant humans", *Diabetes Care*, vol. 38, no. 7, pp. 1228–1237, 2015. https://doi.org/10.2337/dc14-3101
37. C. Itsiopoulos, W. Marx, H.L. Mayr, O.A. Tatucu-Babet, S.R. Dash, E.S. George, et al., "The role of omega-3 polyunsaturated fatty acid supplementation in the management of type 2 diabetes mellitus: A narrative review", *J. Nutr. Intermed. Metab*., vol.14, pp. 42–51, 2018. https://doi.org/10.1016/j.jnim.2018.02.002
38. L. Kaska, A. Mika, P. Stepnowski, M. Proczko, K. Ratnicki-Sklucki, T. Sledzinski, et al., "The relationship between specific fatty acids of serum lipids and serum high sensitivity C- reactive protein levels in morbidly obese women", *Cell Physiol Biochem*, vol. 34, no. 4, pp. 1101–1108, 2014. https://doi.org/10.1159/000366324
39. E. Alvarez-Curto, G. Milligan, "Metabolism meets immunity: The role of free fatty acid receptors in the immune system", *Biochem. Pharmacol*., vol. 114, pp. 3–13, 2016. https://doi.org/10.1016/j.bcp.2016.03.017
40. A. Coope, A.S. Torsoni, L.A. Velloso, "Metabolic and inflammatory pathways on the pathogenesis of type 2 diabetes", *Eur. J. Endocrinol*., vol. 174, no.5, pp. R175–R187, 2016. https://doi.org/10.1530/eje-15-1065
41. Š. Mandal, A. Čaušević, "The correlation between C-reactive protein and regulation of glycemia in type-2 diabetic patients", *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina*, vol. 48, pp. 5–8, 2017. https://hemija.pmf.unsa.ba/glasnik/index.php/2014-03-20-22-03-11/issue-48
42. K.M. Dinh, K.A. Kaspersen, S. Mikkelsen, O.B. Pedersen, M.S. Petersen, L.W. Thørner, H. Hjalgrim, K. Rostgaard, H. Ullum, C. Erikstrup, "Low-grade inflammation is negatively associated with physical health-related quality of life in healthy individuals: results from The Danish Blood Donor Study (DBDS)", *PLoS One*., vol. 14, no. 5, pp. e0216339-1-16, 2019. https://doi.org/10.1371/journal.pone.0214468
43. S. Kanmani, M. Kwon, M.K. Shin, et al., "Association of C-reactive protein with risk of developing type 2 diabetes mellitus, and role of obesity and hypertension: a large population-based Korean cohort study", *Sci Rep*., vol. 9, 4573-1-8, 2019. https://doi.org/10.1038/s41598-019-40987-8
44. Š. Mandal, "Association of marker of inflammation, hepatic enzymes and lipid profile in Type 2 diabetes", *RAD Conf. Proc*, vol. 6, 2022, pp. 119–123. http://doi.org/10.21175/RadProc.2022.21
45. S. Mandal, A. Causevic, H. Dzudzevic-Cancar, S. Semiz, "Free fatty acid profile in type 2 diabetic subjects with different control of glycemia," *IFMBE Proceedings*. vol. 62, pp. 781–786, 2017. https://link.springer.com/book/10.1007/978-981-10-4166-2
46. M. Poreba, P. Rostoff, A. Siniarski, M. Mostowik, R. Golebiowska-Wiatrak, J. Nessler, et al., "Relationship between polyunsaturated fatty acid composition in serum phospholipids, systemic low-grade inflammation, and glycemic control in patients with type 2 diabetes and atherosclerotic cardiovascular disease," *Cardiovasc Diabetol*., vol. 17, no. 1:29, pp, 1-11, 2018. https://doi.org/10.1186/s12933-018-0672-5