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The relationship between concentration of triacylglycerols and free radicals caused by fatty acid composition of triacylglycerols in diabetic patients

Vecka M.[1], Tvrzická E.[1], Votruba M.[2]

[1]IV Department of medicine, Charles University in Prague, First faculty of medicine and general university hospital in Prague
[2]MI-VO-LA consulting, Prague

SUMMARY
Objective: The lipoperoxidation plays a causal role in the development of most important risk factors of cardiovascular disease. The lipoperoxidation results from the reaction of lipid molecules with free radicals, regardless of their origin. The relationship between triacylglycerols (TAG) and the free radicals (FR) amount is not clear. Thus, it can turn up that the hypertriacylglyceridaemia is accompanied with the low values of FR, or *vice versa*. One possible explanation of this phenomenon is that the degree of lipoperoxidation of TAG is dependent on the composition of fatty acids (FA) in their molecule. Therefore, the aim of this study was to analyze FA composition in TAG and its relationship to FR production in type 2 diabetes mellitus.
Design: observational study with internal control group
Material and Methods: 18 normotriacylglycerolemic (NTG) individuals (12M/6F) and 24 hypertriacylglycerolemic (HTG) patients (16M/8F). Patients in both groups were persons, suffering from type 2 diabetes mellitus. Fatty acid analysis of plasma triacylglycerols was performed by gas chromatography and free radicals were determined by direct spectrophotometric method. Statistical analyses (both parametric and non-parametric) were performed with the statistical software STATISTICA for Windows.
Results: We found lower content of myristic acid (14:0) (1.62 [1.23-2.07] vs. 1.90 [1.72-2.79], p = 0.022, [median (1st-3rd quartile)]) and higher content of alpha-linolenic acid (18:3n-3) (1.51 [1.21-1.86] vs. 1.16 [1.02-1.42], p = 0.021) in HTG group. The ratio of arachidonic/eicosapentaenoic acid was in HTG group lower (5.54 [3.62-7.98] vs. 7.25 [5.98-15.12], p = 0.027). When the HTG group was stratified into the tertiles by the FR concentrations, we observed lower values in the second tertile for ratios based on arachidonic acid (20:4n-6) and eicosapentaenoic acid (20:5n-3) (4.05 [2.66-4.67] vs. 7.64 [5.83-11.00], 1st tertile, and 6.21 [3.20-8.31], 3rd tertile, p = 0.019). In pooled groups, we proved negative correlation between MA and TAG (r = -0.3153, p = 0.045) and the link between FR and FA composition of TAG expressed as positive relationship of FR with content of stearic acid (p < 0.001) and negative correlation with some n-6 polyunsaturated fatty acids (18:3n-6, p < 0.05, 22:5n-6, p < 0.01).
Conclusion: The composition of FA in TAG molecules has undoubtedly some relationship to the FR generation. However, the other factors seem to play a role, since the relationship is not straightforward within entire FR range.
Key words: free radicals, fatty acids, triacylglycerols, type 2 diabetes mellitus

SOUHRN
Vecka M., Tvrzická E., Votruba M.: Vztah mezi koncentrací triacylgelcerolů a volných radikálů způsobený složením triacylglycerolů u diabetických pacientů
Cíl práce: Lipoperoxidace je dávána do příčinné souvislosti s rozvojem nejdůležitějších rizikových faktorů kardiovaskulárních onemocnění. Lipoperoxidační procesy jsou důsledkem reakcí lipidů s volnými radikály bez ohledu na jejich původ. Vztah mezi triacylglyceroly (TAG) a volnými radikály (VR) není zcela jasný. Může se dokonce stát, že hypertriacylglycerolémie je doprovázena nízkými koncentracemi VR a naopak. Jedno z možných vysvětlení spočívá ve vztahu lipoperoxidace a složení mastných kyselin v molekulách TAG. Cílem této studie bylo analyzovat vztah složení mastných kyselin v TAG a produkci VR u pacientů trpících diabetes mellitus 2. typu.
Typ studie: observační s kontrolní skupinou
Materiál a metody: Studie zahrnovala 18 normotriacylglycerolemických (NTG) jedinců (M/F 12/6) a 24 hypertriacylglycerolemických (HTG) jedinců (M/F 16/8). Pacienti v obou skupinách byli diabetici 2. typu. Analýzu mastných kyselin v triacylglycerolech plazmy jsme provedli kapilární plynovou chromatografií a koncentrace volných radikálů byly změřeny přímou spektrofotometrií. Statistické analýzy (parametrické i neparametrické) byly provedeny pomocí statistického software STATISTICA pro Windows.
Výsledky: Ve skupině HTG jsme pozorovali nižší zastoupení kyseliny myristové (14:0) (1.62 [1.23-2.07] vs. 1.90 [1.72-2.79], p = 0.022, [medián (1.-3. kvartil]) a vyšší poměr alfa-linolenové kyseliny (18:3n-3) (1.51 [1.21-1.86] vs 1.16 [1.02-1.42], p = 0.021). Poměr kyselin arachidonové/eikosapentaenové byl ve skupině HTG nižší (5.54 [3.62-7.98] vs 7.25 [5.98-15.12], p = 0.027). Když jsme HTG skupinu dále rozdělili podle koncentrací VR na tercily, prokázali jsme nižší hodnoty poměru kyseliny arachidonová/eikosapentaenová ve druhém tercilu (4.05 [2.66-4.67] vs 7.64 [5.83-11.00], 1. tercil, a 6.21 [3.20-8.31], 3. tercil p = 0.019). V spojených HTG+NTG skupinách jsme zaznamenali negativní korelaci mezi MA a TAG (r = -0.3153, p = 0.045). V triaclylglycerolech jsme prokázali pozitivní korelace VR a kyseliny stearové (p < 0.001) spolu s negativní korelací některých n-6 vícenenasycených mastných kyselin (18:3n-6, p < 0.05, 22:5n-6, p < 0.01).
Závěr: Zastoupení mastných kyselin v molekulách TAG je spojeno s tvorbou VR. Nicméně do tohoto vztahu vstupují i jiné faktory.
Klíčová slova: volné radikály, mastné kyseliny, triacylglyceroly, diabetes mellitus 2. typu

Introduction

The lipoperoxidation plays a causal role in the development of most important risk factors of cardiovascular disease. The lipoperoxidation results from the reaction of lipid molecules with free radicals, regardless of their origin. In all lipid classes (with the exception of triacylglycerols (TAG)), the linear dependency of their concentration to the free radicals (FR) amount is seen. Thus, it can turn up that the hypertriacylglyceridaemia is accompanied with the low values of FR, or *vice versa*. One possible explanation of this phenomenon is that the degree of lipoperoxidation of TAG is dependent on the composition of fatty acids (FA) in their molecule. The antioxidant properties of some FA were confirmed by recent studies [1] but practically nothing is known about the role of other FA which compose a TAG molecule. Besides, many papers describe the positive effect of supplementation of selected FA [2,3].

The FA composition in TAG has various ability to produce the FR in the dependency of their chemical structure. Saturation or unsaturation, the length of the chain, conjugation of double bonds and isomerization in molecule, all these factors can play the crucial role in the process of lipoperoxidation. This problem is interesting not only from the academic point of view. Oxidation of TAG in lipoprotein particles leads directly to the destruction of all lipoproteins, to the formation of small dense LDL fraction, to the rise in Apo-B lipoprotein concentration and other possible changes which can induce the process of atherosclerosis. All these findings are very important especially in case of diabetes mellitus 2. The aim of the study was to analyze FA composition in TAG and its relationship to FR production in type 2 diabetes mellitus patients.

Study group

Ambulatory patients enrolled into the study comprised of the group of normotriacylglycerolemic (NTG) 18 individuals (age 51[44-65] [median(1^{st}-3^{rd} quartile)] years, 12 men and 6 women) and 24 hypertriacylglycerolemic patients (HTG) (age 57[50-63] years, 16 men and 8 women). Patients in both groups were persons, suffering from type 2 diabetes mellitus who gave their informed consent with the study. The blood samples were obtained after overnight fasting and stored at -80°C till further analyses. We excluded from the study smokers, the patients with BMI > 30 kg/m^2, kidney disease, and inflammatory diseases (CRP > 10 mg/l), as these states could influence the concentrations of FR.

Materials and Methods

The routine biochemical parameters (total cholesterol, glucose, triacylglycerols, CRP) were analyzed with the Roche Hitachi 911 device using Bio-La-Test kits.

Fatty acid analysis. Total lipid was extracted from 1.0 ml of serum by the method of Folch and co-workers [4] using dichloromethane instead of chloroform. After the separation of lipid classes by thin-layer chromatography, TAG were transmethylated to fatty acid methyl esters using the previously described procedure [5]. Gas chromatography was performed with a Shimadzu GC-17 (Shimadzu Corp., Kyoto, Japan) gas chromatograph equipped with a capillary split/splitless injector and flame-ionization detector, combined with AOC-20i auto-sampler (Shimadzu). Analyses of methylesters were performed on the fused-silica capillary columns coated with chemically bonded stationary phase DB WAXETR (30 m x 0.32 mm I.D.) (J&W Scientific, USA). The oven temperature was programmed from 80 °C to 120 °C at 10°/min, to 200 °C at 2 °C/min, to 250 °C at 20 °/min, then isothermal 25 min. The injector and detector temperatures were 250 and 270 °C, respectively. Hydrogen carrier gas was maintained at a head pressure of 70 kPa and total flow 25 ml/min. Integration software Clarity for Windows® (Data Apex® Ltd., Prague) was used for data acquisition and handling [6].

Free radicals were determined by direct spectrophotometric method based on chlorophyllin reaction with FR. The assay is based on the chlorophyllin ability (sodium-copper salt of chlorophyll) to receive and provide electrons by simultaneous and stable change of its absorbance maximum. This effect is facilitated by alkaline pH of the reaction mixture and addition of the catalyst. The quantification of obtained values is ensured by the calibration that is based on the ability of Fe ion to spontaneously change its valence in alkaline solution from Fe^{2+} to Fe^{3+}. The concentration of calibrators is therefore expressed as mmol/l Fe^{2+} [7]. Statistical analyses were performed with the statistical software STATISTICA for Windows, v.8.3 (Stat Soft, Inc., Tulsa, U.S.A.). The variables were log-transformed where appropriate. Still, in some cases, we decided to use nonparametrical approach to the original data sets.

Results

The results of our measurements are shown in the presented tables. The Tables 2a,2b show individual fatty acid composition in TAG fraction of serum lipids. These tables illustrate first of all the lower content of myristic acid (14:0) in HTG group. The phenomenon can be of some importance and therefore it will be discussed further, as well as the ratio between linoleic (18:2n-6) and myristic acids. The following ratios mentioned in the Table 2b can have a connection with the metabolic role of myristic acid, too [8].

The results in Table 3 depict the relationship between TAG values and the level of FR. To have a closer look on this ratio, we divided the HTG group into tertiles, based on FR levels. As shown in Table 3, we found influence of FR on the FA indices, which is expressed as different values in the second tertile. First, the proportion of arachidonic acid to eicosapentaenoic acid (EPA, 20:5n-3) is the lowest in the second tertile in comparison to the first and third ones. Second, the concentration of AA decreases also in the second tertile. If this ratio is modified by further addition of linolenic acid to the numerator and

Table 1: Basic characteristics of the groups

Parameter	NTG (n = 18)	HTG (n = 24)	p *
M/F	12/6	16/8	1.000 **
age (years)	51 (44-65)	57 (49.5-62.5)	0.3443
BMI ($kg.m^{-2}$)	23.7 (22.5-24.9)	25.6 (24.6-27.9)	0.0035
TC (mmol/l)	4.95 (4.71-5.03)	5.15 (4.92-5.36)	0.0543
TAG (mmol/l)	1.45 (1.08-1.58)	3.55 (2.49-4.15)	0.0000
glucose (mmol/l)	5.61 (5.23-6.24)	6.02 (5.38-6.79)	0.1677
FR (mmol/l Fe^{2+})	16.92 (15.12-19.44)	16.77 (13.07-17.32)	0.0903

Data are presented as median (1st quartile-3rd quartile), FR – free radicals, TC - total cholesterol, TAG - triacylglycerols, NTG - normotriacylglycerolemic group, HTG - hypertriacylglycerolemic group; *- Mann-Whitney test, ** - Chi-squared with Yates´corr.

Table 2a: Fatty acid composition of serum TAG in NTG and HTG groups

Fatty acid	NTG (n = 18)	HTG (n = 24)	p *
myristic acid (14:0)**	1.90 (1.72-2.79)	1.62 (1.23-2.07)	0.0222
palmitic acid (16:0)	27.08 (25.12-29.49)	25.45 (23.70-27.54)	0.1210
16:1n-7	3.49 (2.73-3.81)	3.83 (2.96-4.34)	0.2423
stearic acid (18:0)	4.27 (3.21-5.11)	3.76 (3.10-4.60)	0.4160
oleic acid (18:1n-9)	40.55 (36.98-42.38)	40.66 (39.13-43.37)	0.2323
18:1n-7	3.15 (2.91-3.40)	3.33 (2.99-3.78)	0.3874
linoleic acid (18:2n-6)	14.05 (11.82-16.97)	15.13 (13.40-16.45)	0.7994
γ-linolenic acid (18:3n-6)	0.26 (0.20-0.34)	0.34 (0.18-0.45)	0.4766
α-linolenic acid (18:3n-3)	1.16 (1.02-1.42)	1.51 (1.21-1.86)	0.0221
20:2n-6	0.28 (0.25-0.36)	0.28 (0.23-0.30)	0.4454
20:3n-6	0.29 (0.26-0.32)	0.26 (0.24-0.30)	0.2970
arachidonic acid (20:4n-6)	0.96 (0.84-1.21)	1.04 (0.74-1.26)	0.8588
eicosapentaenoic acid (20:5n-3)	0.14 (0.06-0.18)	0.16 (0.13-0.28)	0.0599
22:5n-3	0.25 (0.20-0.30)	0.33 (0.19-0.43)	0.2224
docosahexaenoic acid (22:6n-3)	0.40 (0.35-0.44)	0.43 (0.28-0.76)	0.4766

Data are presented as median (1st quartile-3rd quartile). NTG - normotriacylglycerolemic group, HTG - hypertriacylglycerolemic group; The fatty acid composition of serum triacylglycerols is expressed as molar percentage, *- Mann-Whitney test ; ** - shorthand notation for fatty acids is in the A:Bn-x format, where A stands for total number of carbons in the molecule, B for number of double bonds and x for the position of the 1st double bond from the methyl end of the molecule

Table 2b: Derived parameters of fatty acid metabolism in NTG and HTG groups

Parameter	NTG (n = 18)	HTG (n = 24)	p *
Σsaturated fatty acids	33.50 (30.18-37.22)	30.87 (28.58-33.17)	0.1093
Σmonounsaturated fatty acids	48.72 (43.82-49.65)	48.50 (46.13-50.06)	0.2974
Σn-6 PUFA	15.94 (13.78-19.05)	17.44 (14.88-18.52)	0.8588
Σn-3 PUFA	2.14 (1.86-2.38)	2.47 (1.99-3.14)	0.0222
unsaturation index	90.33 (84.42-96.17)	95.46 (90.65-101.51)	0.1474
peroxidation index	24.46 (22.54-26.68)	27.30 (23.31-31.29)	0.3470
Σn3/Σn6 PUFA	0.13 (0.10-0.14)	0.15 (0.11-0.18)	0.0600
AA/LA	0.06 (0.05-0.09)	0.07 (0.05-0.09)	0.8788
AA/EPA	7.25 (5.98-15.12)	5.54 (3.62-7.98)	0.0270
AA/(EPA + DHA)	1.95 (1.61-2.31)	1.60 (1.03-2.11)	0.1210
AA/DHA	2.55 (1.81-3.04)	2.26 (1.51-2.97)	0.4926
LA/MA	6.75 (5.02-8.97)	8.95 (6.89-12.90)	0.0600
(AA+LA)/(EPA+DHA)	29.70 (22.42-36.69)	27.31 (15.45-35.36)	0.2858
AA/Σsaturated fatty acids	0.03 (0.03-0.04)	0.03 (0.02-0.04)	0.7220
AA/MA	0.49 (0.32-0.72)	0.58 (0.40-0.86)	0.2130

Data are presented as median (1st quartile-3rd quartile).The derived parameters are ratios (sums) of the data based on molar percentages, Σ- sum, unsaturation index -Σ fatty acid(i, mol%)*number of double bonds in fatty acid(i), peroxidation index - Σ fatty acid(i, mol%)*(number of double bonds in fatty acid(i)-1) *- Mann-Whitney test; PUFA - polyunsaturated fatty acids, AA - arachidonic acid (20:4n-6), LA - linoleic acid (18:2n-6), EPA - eicosapentaenoic acid (20:5n-3), DHA - docosahexaenoic acid (22:6n-3), MA - myristic acid (14:0)

Table 3: Derived parameters of fatty acid metabolism in hypetriacylglycerolemic group stratified by concentration of free radicals

	1st tertile	2nd tertile	3rd tertile	p *
TC (mmol/l)	5.06 (4.81-5.29)	5.20 (5.05-5.43)	5.24 (4.93-5.50)	0.4892
TAG (mmol/l)	2.69 (2.35-3.25)	2.71 (2.50-2.94)	4.50 (2.82-6.15)	0.1000
FR (mmol/l Fe^{2+})	12.69 (12.49-13.09)	14.72 (14.36-15.61)	18.23 (17.32-27.16)	0.0000
AA/LA	0.05 (0.05-0.07)	0.09 (0.07-0.10)	0.06 (0.05-0.09)	0.0975
AA/EPA	7.64 (5.83-11.00)	4.05 (2.66-4.67)	6.21 (3.20-8.31)	0.0194
AA/(EPA+DHA)	1.97 (1.57-2.17)	1.03 (0.76-1.60)	1.74 (1.36-2.41)	0.0497
AA/DHA	2.64 (2.01-2.79)	1.39 (1.08-2.21)	2.81 (2.06-3.38)	0.0935
LA/MA	10.23 (9.19-14.70)	7.78 (6.15-10.78)	8.02 (4.89-14.88)	0.1959
(AA+LA)/(EPA+DHA)	33.42 (28.29-47.78)	15.45 (11.24-21.18)	28.45 (21.84-37.89)	0.0084

Data are presented as median (1st quartile-3rd quartile), FR – free radicals,*- Kruskal-Wallis ANOVA; TC - total cholesterol, TAG - triacylglycerols, FR - free radicals, AA - arachidonic acid (20:4n-6), LA - linoleic acid (18:2n-6), EPA - eicosapentaenoic acid (20:5n-3), DHA - docosahexaenoic acid (22:6n-3), MA - myristic acid (14:0)

EPA to the denominator, the influence of arachidonic acid is much more lower. It is interesting that these phenomena can be found in the second tertile only. Patients in the group can be characterized by higher values of FR, but these values were not so extreme as those ones in the third tertile (Table 3).

Discussion

Results of this study point at two main issues. The first one is concerned with the specific property of myristic acid. Myristic acid (MA), the 14-carbon saturated fatty acid (14:0), usually accounts for small amounts (0.5%–1% weight of total fatty acids) in animal tissues. Since it is a relatively rare molecule in the cells, the specific properties and functional roles of myristic acid have not been fully studied and described. Like other two dietary saturated fatty acids (palmitic (16:0) and lauric (12:0) acid), this fatty acid is usually associated with negative consequences for human health. Nevertheless, one feature of myristoyl-CoA is its ability to be covalently linked to the N-terminal glycine residue of both eukaryotic and viral proteins. This reaction is called N-terminal myristoylation. Through the myristoylation of hundreds of proteins, MA can activate many physiological pathways [9].

As was shown by Legranda [8], this acid undergoes many metabolic conversions in humans: incorporation into lipids, beta-oxidation, elongation and desaturation. The most important for our study are the last two: elongation and desaturation. First step of myristic acid conversion is elongation into palmitic acid, in case myristic acid is not needed for myristoylation. High content of myristic acid drives the desaturation of palmitic and stearic acids and production of n-3 and n-6 PUFA by the activation of conversion of alpha-linolenic and linoleic acids towards longer chain derivatives. In pulse-chase studies, MA treatment resulted in reduced apoB-100 degradation, in agreement with promotion of its secretion. In triacylglycerol studies, its synthesis was stimulated equally by oleic acid (OA, 18:1n-9), MA, and docosahexaenoic acid (DHA, 22:6n-3), but TAG secretion was relatively decreased with MA and DHA. Compared with OA, myristic acid induces secretion of smaller, denser apo B-100 lipoproteins. Furthermore, the relative recruitment of newly synthesized TAG to lipoproteins was impaired with MA [10]. Consistently with this study, our HTG group (secreting more TAG), had lower MA content and content of MA negatively correlated with the concentration of TAG in serum ($r = -0.3153$, $p < 0.05$, Pearson corr. coeff.). Even in non-diabetics, lowering of TAG concentrations by fenofibrate increases the content of MA in TAG of LDL particles [11].

In HTG group, we have found elevated proportions of α-linolenic acid (18:3n-3). PUFA n-3 are known to lower plasma TAG [12], but they do not lower LDL (apoB) production [13]. However, it is known that the Inuits, the population with high intake of PUFA n-3, are more obese than the Caucasian population of Northern America, therefore some variations in TAG molecules might have protective effects. The results by Tremblay [14] suggest that a fatty acid content of TAG characterized by low palmitic acid (16:0) and high α-linolenic (18:3n-3) as well as γ-linolenic (18:3n-6) proportions helps visceral obese individuals to cope with the development of metabolic alterations of the insulin resistance syndrome. Additionally, the content of α-linolenic acid in total esterified fatty acid fraction did not predict the development of impaired fasting glucose or type 2 diabetes mellitus [15].

We did not find any special fatty acids composition, which can be responsible for abnormal oxidation due of FR. We can only show that higher FR concentration is connected in diabetic patients with higher content of stearic acid (18:0, $p < 0.001$) and lower content of some n-6 polyunsaturated fatty acids (18:3n-6, $p < 0.05$, 22:5n-6, $p < 0.01$, Pearson corr. coeff.). Nevertheless, our Table 3 shows that if the concentration of FR is too high, possible compensatory mechanisms in diabetic patients seems to be attenuated. Under diabetic conditions, chronic hyperglycemia and subsequent augmented formation of reactive oxygen species (ROS) deteriorate beta-cell function and increase insulin resistance which leads to the aggravation of type 2 diabetes. In addition, chronic hyperglycemia and ROS are also involved in the development of atherosclerosis which is often observed under diabetic con-

ditions [16]. Beneficial actions of polyunsaturated fatty acids describes in detail Das [3]. In his interpretation the most useful PUFA are: eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (AA), γ-linolenic acid (18:3n-6) and dihomo-γ-linolenic acid (20:3n-6). These acids are able to prevent cardiovascular disease, thrombosis and atherosclerosis, reduce cardiac arrhythmias, lower plasma cholesterol and TAG, lower high blood pressure and they can also suppress the production of free radicals and enhance nitric oxide generation. It is necessary to remark that many other PUFA, which can be oxidized very easily, occur in blood samples of diabetic patients. Remarkable finding, concerning the given amount of n-3 polyunsaturated FA, shows Visioli et al. [17] as a result of experiment on healthy persons. They found that the intake of a milk preparation providing low amounts of EPA+DHA to healthy individuals led to marked increases of n-3 polyunsaturated FA and vitamin E in plasma and associated favorable changes in HDL and TAG.

On the other hand, Halvorsen et al. [18] highlighted that monounsaturated fatty acids seemed to stimulate peroxisomal beta-oxidation and to increase plasma triacylglycerol, whereas the mitochondrial oxidation was slightly decreased. This finding is probably the first attempt to explain the observed disproportions between TAG and FR levels. In our group of patients, we observed only weak correlation between the content of oleic acid (18:1n-9) and concentration of TAG ($p = 0.05$, Pearson corr. coeff.) Also Sidossis and al. [19] found that hyperglycemia inhibited FR concentration, but at the same time, values of TAG increase. Another viewpoint presents Staprans et al. [20]. In diabetic subjects with poor glycaemic control, dietary oxidized lipids induce an exaggerated and sustained increase in the levels of oxidized lipids in chylomicrons when compared with either control subjects or diabetic patients with good glycemic control. These increased postprandial levels of potentially atherogenic oxidized lipids may contribute to the accelerated atherosclerosis associated with diabetes. In other words it is the level of glucose concentration which can regulate the oxidation of lipids in case of type 2 diabetes, independently of real concentration of FR in plasma, arising out from „classic" sources of FR. The second determining parameter have to be TAG, or, more precisely, their content in lipoproteins [21]. The increase of TAG concentration permits the degree of lipoprotein oxidation due to free radicals. So by the combination of these effects and other ones, which were not discussed, such as free fatty acid concentration or influence of special types of proteins, playing the important role in lipid metabolism, all this seems to be responsible for abovementioned discrepancy between FR and TAG concentration.

The limits of the study include not measured levels of glycated hemoglobin. With the respect to the fact that in some cases, authors question its analytical methodology and thus clinical relevance [22, 23], we considered low TAG concentrations as a criterion for diabetes control rather than glycated hemoglobin. The low concentrations of TAG in diabetics point at their positive adherence towards self-care management of one of the most important factors connected with diabetes, metabolic syndrome. Moreover, the GENFIEV study revealed that insulin resistance is most tightly linked to hypertriacylglycerolemia [24].

Conclusions

The composition of FA in TAG molecules has undoubtedly some association with the FR generation. However, the other factors seem to play a role, since the relationship is not straightforward within entire FR range.

Abbreviations

DHA	docosahexaenoic acid (22:6n-3)
EPA	eicosapentaenoic acid (20:5n-3)
FA	fatty acids
FR	free radicals
HDL	high density lipoproteins
HTG	hypertriacylglycerolemic
LDL	low density lipoproteins
MA	myristic acid (14:0)
NTG	normotriacylglycerolemic
OA	oleic acid (18:1n-9)
PA	palmitic acid (16:0)
PUFA	polyunsaturated fatty acids
ROS	reactive oxygen species
SA	stearic acid (18:0)
TAG	triacylglycerols

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Adresa pro korespondenci:
RNDr. Marek Vecka, Ph.D.
IV. interní klinika 1. LF UK a VFN
U Nemocnice 2
128 08 Praha 2
Email: marvec@volny.cz