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Forum

Common Fatty Markers in Diseases with Dysregulated Lipogenesis

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Recent studies have reported the upregulation of a subgroup of triacylglycerides as markers of different diseases with dysregulated lipogenesis, which means that these markers are not selective. This observation has a deep impact on their use as diagnostic tools in clinical practice (e.g., markers of risk of type 2 diabetes).

Dysregulated lipogenesis in the liver has been associated with the risk and status of different diseases, for example, type 2 diabetes, non-alcoholic fatty liver disease, and hepatocellular carcinoma. This increase of lipogenesis in the liver is reflected in both liver tissue and very-low-density lipoproteins in plasma [1]. Consequently, recent studies have used lipidomics as a new diagnostic tool for these diseases. These studies have reported different lipids as markers of disease (e.g., glycerophospholipids and sphingolipids [2]), however, we have found a common pattern in triacylglycerides (TGs) as markers. This pattern carries meaning in biochemical interpretation and also has implications in the use of these triacylglycerides as predictors of disease risk and status.

Razquin *et al*. have recently reported a unique lipid profile in plasma in relation to type 2 diabetes: TG(50:1) was the triacylglyceride that best correlated with disease risk with [2]. From this starting point, we searched in the literature for lipidomics studies that have found specific upregulation of triacylglycerides in relation to type 2 diabetes. Suvitaival *et al*. have reported that, among triacylglycerides, TG(50:0), TG(48:0), TG(52:0), and TG(54:1) presented the highest levels in progressors [3]. By using different cohorts, Mamtani *et al*. have selected the upregulation of TG(52:1) (with palmitic, stearic, and oleic acids) as a predictor of type 2 diabetes risk [4]. Mousa *et al*. have also found in plasma that TG (48:0) with palmitic acid, TG(50:0) with stearic acid, TG(50:1) with oleic acid, and TG(52:1) with stearic acid are inversely associated with insulin sensitivity in overweight or obese nondiabetic individuals [5]. In addition, other diseases associated with dysregulated lipogenesis present a similar profile of triacylglycerides. Regarding non-alcoholic fatty liver disease, Orešič *et al*. have found that TG(52:2) and TG(50:1) (with palmitic and oleic acids) were the most upregulated triacylglycerides in serum [6]. In relation to cardiovascular incidents, Stegemann *et al*. have found that TG (50:1), TG(50:2), TG(52:1), and TG(54:2) in plasma were positively correlated with disease risk [7]. With regard to hepatocellular carcinoma, Yang *et al*. have reported the upregulation of TG(50:2) in plasma as a disease marker [8]. In addition, Li *et al*. have studied the triacylglyceride profile in the liver tissue from hepatocellular carcinoma and have found that TG(52:0) and TG(50:0) presented the highest increases in abundance [9]. From this bibliographic search, we observed a new pattern. All of these triacylglycerides have two common structural characteristics: (i) the sum of the acyl carbons is between 48 and 54; and (ii) the sum of the acyl unsaturations is between zero and two. While some of these studies did not identify the fatty acids esterified in the triacylglycerides, all of them are commonly associated with combinations of palmitic, palmitoleic, stearic, and oleic acids [10]. We call this ensemble of triacylglycerides the 'common fatty markers'.

At this point, we wondered: does enzyme selectivity explain why the common fatty markers are more upregulated than other triacylglycerides in dysregulated lipogenesis? The answer is affirmative. Dysregulated lipogenesis affects the synthesis of triacylglycerides by increasing the *de novo* synthesis of fatty acids and by regulating the acyltransferases involved in the *de novo* synthesis of triacylglycerides. Regarding the *de novo* synthesis of fatty acids, fatty acid synthase (FAS) preferentially synthesizes palmitic acid, but it also yields stearic acid. In addition, stearoyl-CoA desaturase (SCD)-1 synthesizes palmitoleic and oleic acids from palmitic and stearic acids – which are the main products of FAS. Consequently, the upregulation of FAS and SCD-1 increases the availability of palmitic, palmitoleic, stearic, and oleic acids for the acyltransferases in the *de novo* synthesis of triacylglycerides. Among these acyltransferases, glycerol-3-phosphate acyltransferase (GPAT)-1 shows strong preference for saturated fatty acids, especially palmitic acid [11]. While dysregulated lipogenesis in the liver affects other enzymes and lipids, the combined upregulation of FAS, SCD-1, and GPAT-1 explains the profile of triacylglycerides in these diseases: those with palmitic, palmitoleic, stearic, and oleic acids (the common fatty markers) show the highest increases.

We wondered again: is there a signaling pathway relating the upregulation of FAS, SCD-1, and GPAT-1 and the diseases that present dysregulated lipogenesis? Again, the answer is affirmative. At a transcriptional level, sterol regulatory element-binding protein (SREBP)-1c upregulates FAS, SCD-1, and GPAT-1; and carbohydrate-responsive element-binding protein (ChREBP) upregulates

FAS and SCD-1. Furthermore, the upregulation of liver X receptors (LXRs) upregulates both SREBP-1c and ChREBP [12,13]. In addition, LXRs directly upregulate SCD-1 and FAS [12]. Extensive research in recent years has associated the upregulation of LXRs, SREBP1-c, and ChREBP with the risk and status of the diseases that we reviewed (type 2 diabetes, non-alcoholic fatty liver disease, cardiovascular incidents, and hepatocellular carcinoma) [1,12,13]. At a ligand level, other lipids and hormones (e.g., insulin) regulate the LXR pathway. Consequently, the LXR pathway is influenced in a complex manner by diet, exercise, hormonal state, and genetic predisposition to induce dysregulated lipogenesis in the liver. Figure 1 schematically summarizes the biochemical relationships among lipogenesis, LXRs, SREBP-1c, ChREBP, GPAT-1, SCD-1, FAS, and the common fatty markers. Because of this common pathway of signaling and synthesis, the common fatty markers appear statistically associated with the risk and status of the diseases that present dysregulated lipogenesis in their onset and/or development. This common synthetic regulation has an important consequence in their use as markers of disease risk and status. We cannot consider them as selective markers of a specific disease, but markers of dysregulated lipogenesis in the liver, which is common to many diseases.

Is diet a confounding factor for the common fatty acids? In addition to the LXR-mediated *de novo* lipogenesis in the liver, diet can also change the profile of triacylglycerides in liver and plasma. On the one hand, a diet high in carbohydrates – a risk factor for dysregulated lipogenesis – also entails the activation of SREPB-1c by insulin and ChREBP. These upregulations would increase the common fatty markers in the liver and plasma after food intake. On the other hand, dietary fats affect the profile of triacylglycerides in both the liver and plasma after food intake. From diet, triacylglycerides contribute to the plasma lipidome by chylomicrons. However, after uptake by the liver, dietary fats can also affect the profile

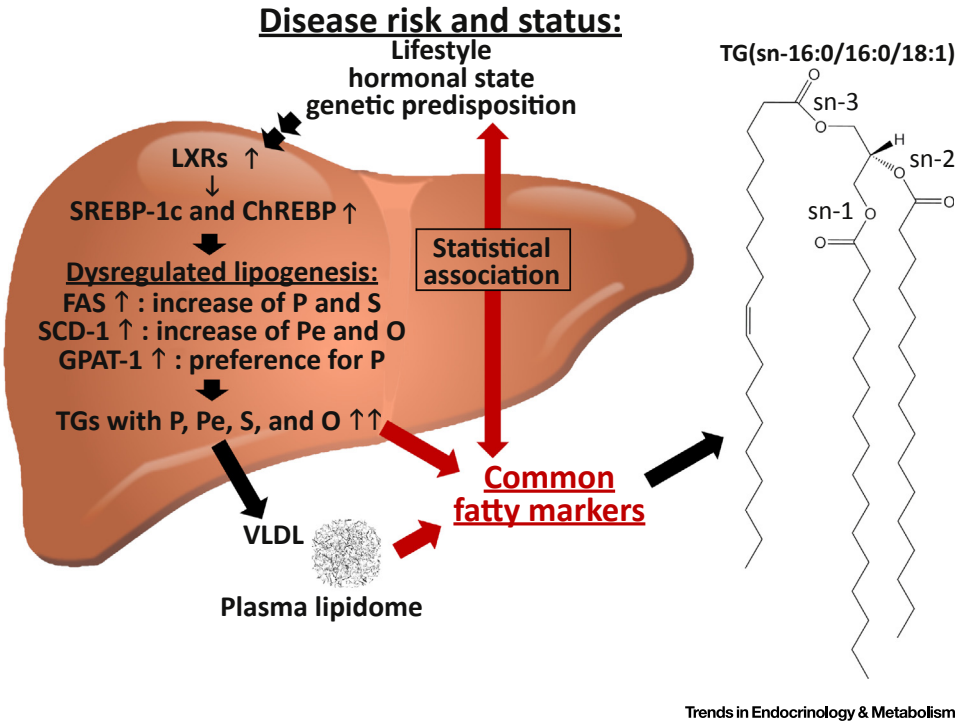

Figure 1. Summarized Relationship between the Common Fatty Markers and Diseases with Dysregulated Lipogenesis. Diet and exercise habits, hormonal state, and genetic factors integrate into dysregulation of lipogenesis in the liver via regulation of liver X receptors (LXRs), sterol regulatory element-binding transcription factor 1c (SREBP-1c), and carbohydrate-responsive element-binding protein (ChREBP). Among other enzymes, this dysregulation upregulates fatty acid synthase (FAS), stearoyl-CoA desaturase 1 (SCD-1), and glycerol-3-phosphate acyltransferase 1 (GPAT-1). This upregulation leads to an increase of triacylglycerides (TGs) with palmitic (P), palmitoleic (Pe), stearic (S), and oleic (O) fatty acids. Consequently, the upregulation of these triacylglycerides in the liver is significantly associated with the risk and status of diseases with dysregulated lipogenesis. This statistical association can be found in the liver tissue and the plasma lipidome by the contribution of very-low-density lipoproteins (VLDL). Because of the selectivity of the enzymes in this pathway, we highlight triacylglyceride TG (sn-16:0/16:0/18:1) as an example of the triacylglycerides among the common fatty markers. The liver image was adapted from an image in the DataBase Center for Life Science (Creative Commons Attribution 4.0 International license).

of the *de novo* synthesis of triacylglycerides. For example, a diet rich in palm or olive oils could affect and confound the profile of the common fatty markers. Consequently, diet can be a confounding effect for the common fatty markers, especially in postprandial periods. Nevertheless, the different studies that we reviewed collected blood during fasting and consistently found the upregulation of the common fatty markers. This fact suggests that upregulation during fasting of the common fatty markers in patients with dysregulated lipogenesis is robust against diet. Consequently, while diet can affect the common fatty markers in the postprandial period, their repeated appearance in different studies suggests that the potential confounding effect of diet is minimized during fasting.

Finally, considering the regulation and enzyme selectivity of lipogenesis in the liver, we wondered: what is the level of characterization in lipid profiling required to improve the biological information in studies with dysregulated lipogenesis? In a first level of characterization, we consider that many lipid-profiling studies only identify the total number of carbons and unsaturations [e.g., TG(50:1) for TG(sn-16:0/16:0/18:1)], despite the fact that the type of fatty acids in triacylglycerides carry biological information. Consequently, we strongly suggest that lipid profiling related to diseases with dysregulated lipogenesis requires the identification of esterified fatty acids. On a second level of characterization, we consider the position of the fatty acids in the glycerol backbone, that is, the enantiomeric and regioisomeric composition of the triacylglycerides. The *de novo* synthesis of triacylglycerides is initiated by GPATs [11]. These enzymes transfer a fatty acid to the sn-1 position of glycerol-3-phosphate. Despite other GPATs not seeming to be regulated and not showing preference for palmitic acid, GPAT-1 is upregulated in the LXR pathway and shows preference for palmitic acid [11]. Hence, the upregulation of the lipogenic LXR pathway is expected to increase the content of palmitic acid in the sn-1 position of triacylglycerides. Consequently, we propose that analysis of triacylglycerides with palmitic acid in the sn-1 position is of special interest [e.g., TG(sn-16:0/16:0/18:1); Figure 1]. Traditional analyses of regioisomers of triacylglycerides by lipases are cumbersome and not feasible for lipid profiling in clinical analysis. However, recent advances in mass spectrometry lipidomics have enabled the regioisomeric analysis of the common fatty markers [14]. We suggest that the application of the latest developments in profiling the positional isomers of triacylglycerides is recommendable in new studies in which dysregulated lipogenesis is involved.

In conclusion, we propose that the upregulation in the liver and plasma of triacylglycerides with palmitic, palmitoleic, stearic, and oleic acids – the common fatty markers – is a fingerprint of LXR-mediated lipogenesis in the liver. The increase of these triacylglycerides indicates the same biological process: dysregulated lipogenesis in the liver. This relationship has deep implications in the use of triacylglycerides as diagnostic tools in clinical practice: lipidomic studies cannot consider them as selective markers of a specific disease, but markers of dysregulated lipogenesis in the liver. To achieve selectivity in the statistical prediction of disease risk and status, we suggest the use of studies with multiple diseases. In these studies, the quantitative analysis of these triacylglycerides, together with the use of other markers (metabolic or clinical), might yield selective tools for prediction of disease risk and status. As a general lesson, to improve and delimit the use of metabolic markers in clinical practice, the field should not only focus on finding statistically significant changes between two groups, but also on the metabolic and medical significance of these changes.

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