Form(ul)ation of adipocytes by lipids

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ABSTRACT

Lipids have the potential to serve as bio-markers, which allow us to analyze and to identify cells under various experimental settings, and to serve as a clinical diagnostic tool. For example, diagnosis according to specific lipids that are associated with diabetes and obesity. The rapid development of mass-spectrometry techniques enables identification and profiling of multiple types of lipid species. Together, lipid profiling and data interpretation forge the new field of lipidomics. Lipidomics can be used to characterize physiologic and pathophysiologic processes in adipocytes, since lipid metabolism is at the core of adipocyte physiology and energy homeostasis. A significant bulk of lipids are stored in adipocytes, which can be released and used to produce energy, used to build membranes, or used as signaling molecules that regulate metabolism. In this review, we discuss how exhaust of lipidomes can be used to study adipocyte differentiation, physiology and pathophysiology.

Introduction

Lipids are important components of cells, and high lipid content is especially evident in adipocytes. Adipocytes display dynamic lipid metabolism that includes synthesis, modification and breakdown. Thus, the identity and composition of lipids in adipocytes projects a snapshot of ongoing metabolic pathways. This projected lipid metabolism does not only characterize adipocytes per se, but also reflects a systemic response to energetic demands and metabolic adaptions. A “systems biology” approach to characterize physiologic and pathophysiologic processes, based on lipid profiles (or lipidomes), is feasible in a similar fashion to genomic and proteomic analyses. Termed lipidomics, this technical and analytic methodology is discussed in this review as an application to define adipocyte cell differentiation, physiology and pathophysiology (Fig. 1). The versatile roles of lipids in adipocytes (Fig. 2) are broadly reviewed in references.

Adipose tissue-resident stem cells are capable of differentiation into lipid-laden adipocytes. Lipid droplets (LD) are a prominent morphological feature of adipocytes that is maintained throughout their life-cycle. Histology and lipid staining techniques show the formation of LDs in differentiating and mature adipocytes. Lipids are accumulated in adipocytes via fatty acid uptake or via lipogenesis. De novo lipogenesis begins with fatty acid biosynthesis and may lead to storage of triglycerides (TG) in LDs. Fatty acid biosynthesis is undertaken by successive enzymatic reactions, which include key enzymes, such as ATP-citrate Lyase (ACL), Acetyl-CoA Carboxylase (ACC), the Fatty Acid Synthase (FAS) complex, fatty acid elongases (Elovl) and Stearoyl-CoA desaturases (SCD). Esterification of fatty acid acyl chains together with glycerol-3-phosphate into TG is enzymatically controlled by a group of enzymes called acyltransferases. The breakdown of lipids is termed lipolysis, in which TG undergo hydrolysis to release fatty acids. Lipolysis is mediated by the activity of extracellular lipases, such as lipoprotein lipase (LPL), or cytosolic lipases, including adipose-triglyceride lipase (ATGL), hormone-sensitive lipase (HSL) and mono-acylglycerol lipase (MGL). Fatty acids can be further broken down via oxidation. β-oxidation is a catabolic process by which fatty acids are transferred to the mitochondria, and are broken down to generate precursors for ATP generation (reviewed in reference). Of note, fatty acids are direct or indirect precursors for nearly all lipid types in addition to TG, including phospholipids (PL, e.g. phosphatidylserines), sphingolipids (SL, e.g., ceramides), eicosanoids (e.g., prostaglandins) and sterols (e.g., cholesterol).
Differences in lipid composition and lipid metabolic pathways can distinguish between cell types of adipocytes. For example, mature white adipocytes predominantly store TG as an energy reserve, whereas brown/beige adipocytes primarily dissipate energy, as part of their function in thermogenesis and energy expenditure. While classical brown adipose tissue (BAT) is intrascapular, white adipose tissue (WAT) is anatomically composed of 2 broad compartments - subcutaneous WAT (SWAT) and visceral WAT (VWAT). Those distinct anatomic adipose depots demonstrate metabolic differences that are also reflected in lipid composition. For example, as compared with SWAT, VWAT displays higher lipid turnover and its expansion in obese subjects is associated with metabolic dysfunction. Thus, the respective lipid profiles are useful not only to characterize normal metabolism in adipocytes, but also to characterize abnormal metabolism in a disease setting. The dynamic changes in lipid composition in adipocytes present a methodological challenge of how to detect, analyze and decipher the lipid profiles. Thus, tandem/shotgun mass-spectrometry (MS/MS) technologies and the emergent discipline of lipidomics can address biochemical/physiologic questions that previously have not been analytically addressed in a systematical manner.

**Lipidomics – technology and analytical principles**

MS/MS-led lipidomic analysis is a powerful methodology to quantify changes in lipid composition based on tissue type, experimental settings and pathological condition. The lipidome of a tissue represents thousands of different lipid species, which can be classified into several classes (see Lipid MAPS consortium and glossary). High-throughput and accurate detection of many lipid species at low concentrations in a heterogeneous crude tissue sample can be enabled using chemical lipid extraction and tandem/shotgun MS/MS technologies. The MS/MS analysis is coupled with algorithms that can identify lipids based on parameters, such as mass and charge of the ionized molecules. It should be emphasized that these values are relative, therefore a measurement of absolute concentrations of lipids requires synthetic standards to compare with. Various MS/MS techniques and strategies for the study of lipidomes as well as general lipid metabolic pathways are thoroughly discussed in references. Previous technical approaches focused on measuring the concentrations of specific lipids, while new technical approaches take into account global changes in lipid composition. This requires rigorous statistical and computational analyses of lipidome databases, which generate systemic lipid profiles and outputs. Popular
data presentations include: 1) Principal Component Analysis that separates experimental groups according to statistical variance; 2) Identification of bio-markers that correlate to physiologic/pathophysiological readouts (e.g., insulin resistance and drug therapy); 3) Interpretation of effects on metabolic pathways that can be validated genetically or biochemically (e.g., quantitative gene expression, chromatography-based analysis and enzymatic assays); 4) Multidimensional charts, such as heat and correlation maps that cluster together a set of individual lipids according to fold-changes / changes in composition; 5) Assessment of global changes in lipids profiles or in specific features (e.g., a subclass of lipids, acyl-chain length, number of double bonds, etc...). The outputs presented in these figures may vary and depend on the lipid extraction technique, the mass spec technology and its sensitivity. Taken together, lipidomics combines a biologic/clinical experiment, a chemical analysis technology and bio-informatic tools to characterize alterations in physiologic and pathophysiologic lipid metabolism (Fig. 1).

**Lipidomics as a clinical diagnostic tool**

Lipid profiles undergo substantial change, upon cell differentiation, as an adaptation to environmental cues (e.g., diet, exercise, drugs) or under a pathological condition (e.g., diabetes type II, hepatic steatosis). There are even promising lipidomic studies on diseases that do not belong to the metabolic syndrome family, such as Alzheimer disease, demonstrating the power of lipidomics. Thus, lipids can serve as diagnostic bio-markers, and can be correlated with various prognostic factors, such as insulin resistance or ectopic fat accumulation. While it is possible to obtain tissue biopsies from patients, plasma or serum samples are quite easy to obtain and analyze. The blood is rich in numerous lipid species, and can be represented in part as a projection of an integrated lipid physiology. Since adipose tissue is a major hub of lipid metabolism, lipid storage and release, changes in the lipidome of the adipose tissue may be reflected in the blood (supported by our yet unpublished data in mouse models).

Lipidomics of blood samples has been performed in clinical studies of obesity and diabetes. On one hand, clinical insulin resistance and diabetes are associated with higher plasma diglycerides (DG) and cholesteryl esters (CE), which are abundant in low-density lipoproteins. On the other hand, they are associated with lower plasma levels of lysophosphatidylcholines (LyPC), which are abundant in high-density lipoproteins.
Childhood obesity is also associated with lower LyPC.\textsuperscript{18} Generally, obesity is associated with higher plasma TG,\textsuperscript{14} which reflect increased energy intake and TG storage in adipocytes of obese individuals. In addition to total plasma TG levels, the plasma TG composition may represent a diabetes signature. This diabetes lipid signature is associated with TG that display higher saturation (i.e., low number of double bonds) and shorter acyl chains (i.e., low number of carbons).\textsuperscript{15,19} While the mechanism remains poorly understood, it is indicative of decreased activity of fatty acid desaturases (FADS) in patients with diabetes.\textsuperscript{20} These patterns are reflected by predictive combinatorial bio-markers of diabetes risk. For example, high TG50:0 / low TG58:1 or high TG54:2 / (low TG36:0).\textsuperscript{21} These patterns are represented by predictive combinatorial bio-markers of diabetes risk. For example, high TG50:0 / low TG58:1 or high TG54:2 / (low TG36:0).

Adipocytes of obese individuals. In addition to total plasma TG levels, the plasma TG composition may represent a diabetes signature. This diabetes lipid signature is associated with TG that display higher saturation (i.e., low number of double bonds) and shorter acyl chains (i.e., low number of carbons).\textsuperscript{15,19} While the mechanism remains poorly understood, it is indicative of decreased activity of fatty acid desaturases (FADS) in patients with diabetes.\textsuperscript{20} These patterns are reflected by predictive combinatorial bio-markers of diabetes risk. For example, high TG50:0 / low TG58:1 or high TG54:2 / (low TG36:0).

Lipidomics of blood samples has been also performed following regimens of weight loss by diet, exercise, bariatric surgery or drug therapy.\textsuperscript{19,22,23} The results demonstrate that weight loss produces a characteristic lipid signature that is in part opposite to the characteristic lipid signature of patients with diabetes. The plasma signature of weight loss is characterized by TG with longer and desaturated acyl chains,\textsuperscript{19,22} and by a decrease in total plasma levels of TG, CE and ceramides.\textsuperscript{22,23} In summary, lipidomics is a useful diagnostic tool to discover bio-markers associated with metabolic syndrome with its various manifestations, relying on global changes in systemic lipid metabolism. To understand the biologic significance that underlies these lipid signatures, lipidomics may be used as a new scientific avenue to decipher lipid profiles of tissues, including adipocytes, in humans and animal models.

**Lipidomics of adipocyte differentiation**

The lipid composition of the adipose lineage substantially changes from the stem cell stage to the differentiated adipocyte. For example, the lipidomes of cultured undifferentiated cells are strikingly different than the lipidomes of the respective differentiated cells, and include characteristic bio-markers for each stage of the lineage.\textsuperscript{24} Despite general differences between cell lines and primary cells, PCA plots indicate a similar trajectory in how lipid profiles change during differentiation.\textsuperscript{24} Interestingly, comparison of the lipidomes of in vitro differentiated adipocytes to dissected mouse adipose tissues show similarities together with dissimilarities. For example, TG composition of in vitro differentiated white cells is similar to that of WAT, whereas their PL composition shows no resemblance.\textsuperscript{24} Another example is that in vitro differentiation of brown-like cells is relatively similar to BAT in terms of SM composition.\textsuperscript{24} However, the lipidomes of adipocytes differentiated from pre-adipocyte cell lines are quite different in comparison to the lipidomes of adipocytes differentiated from mesenchymal stem cells.\textsuperscript{24} Collectively, cultured adipocytes do not exactly resemble a freshly obtained adipose tissue. Those differences in lipid profiles might be a result of diverse signals driving lipogenesis in vivo, the presence of other cell types in the tissue apart from adipocytes, and the different fatty acids supplemented in vitro as compared with the fatty acids obtained by food intake. Indeed, de novo differentiation of primary adipocytes in vitro is affected by the presence of dietary fatty acids; for example, arachidonic acid drives formation of large LDs, whereas eicosapentaenoic acid drives formation of small LDs.\textsuperscript{25} Lipidomics as a comprehensive discriminatory tool for stages of cell differentiation is yet to be developed.

**Lipidomics of the adipose tissue**

Lipidomics can also distinguish between different types of tissues, such as mouse white and brown adipose tissues. TG/DG-rich WAT and BAT cluster together in PCA plots and are distinct from other organs in terms of their characteristic lipidomes.\textsuperscript{26} Yet, WAT and BAT do not closely cluster together according to a parallel gene expression analysis. This exemplifies how lipidomes may correspond to the common metabolic aspects of organs, rather than to their genetic and developmental aspects.\textsuperscript{26} PCA plots also show that BAT is distinct from closely-associated clusters of SWAT and VWAT, and that BAT lipidomes additionally display sexual dimorphism between males and females.\textsuperscript{27} Of note, BAT, as compared with WAT, consists of more saturated TG,\textsuperscript{26} theoretically because saturated fatty acids (SFA) generate more ATP than polyunsaturated fatty acids (PUFA) as an efficient energy source. In addition, BAT consists of higher levels of cardiolipins and PL,\textsuperscript{27} which reflect their relative abundance in the mitochondria. Mitochondria biogenesis is high in brown adipocytes and is increased following stimuli, such as cold exposure.\textsuperscript{9} Strikingly, cold-exposure leads to elongation of TG acyl chain length and alterations in PL composition (e.g., reduction in monosaturated species), both of which are biochemical adaptations to cold.\textsuperscript{28} These cold adaptions involve mitochondria biogenesis and facilitated fatty acid catabolism, which are in alignment with elevated gene expression of fatty acid elongases and enzymes involved in PL
metabolism. These 2 mechanisms modify brown adipocytes for an enhanced energy expenditure capacity. Another feature of an enhanced energy expenditure in activated BAT is the observation of elevated CE, a byproduct associated with the increase of lipoprotein-derived TG clearance from the circulation in response to cold. Not only cold exposure, but also exercise training alters the BAT lipidome; this include altered PL metabolism and a reduction in TG content, which were observed in SWAT as well. It is not surprising that some of the observed lipidomic changes are common to physical activity and cold-exposure, since both are known to increase BAT activity and to induce beiging of WAT.

Obesity is associated with metabolic dysfunction, WAT expansion and low energy expenditure. In mice, high-fat diets (HFD) lead to the development of obesity and diabetes. Diet-induced obesity is accompanied by lower concentrations of PUFA and higher concentrations of monounsaturated fatty acids (MUFA) in VWAT. The lower PUFA/MUFA ratio is hypothesized to be associated with inhibition of de novo lipogenesis and an expression of fatty acid desaturases that selectively generate MUFA over PUFA. On the other hand, a lipidomic clinical study of twins discordant for acquired obesity describes PL with increased levels of PUFA and reduced levels of MUFA and SFA. The authors hypothesize that increased PL desaturation and length are an adaptation to augment membrane fluidity and thickness due to the increased cell size of adipocytes in obese subjects.

Lipidomics does not only teach us about biophysical properties of adipocytes, but can also be applied to discover and to study genetic pathways that are involved in pathophysiological settings. For example, analysis of the WAT lipidome of mice under HFD, reveals a substantial downregulation of a sub-class of eicosanoids, termed epoxy-fatty acids. Epoxy-fatty acids are generated via the cytochrome p450 epoxygenase pathway, and therefore it is of no surprise that implicated enzymes are also downregulated in the WAT of obese mice. While the roles of these enzymes in adipocyte physiology are still poorly understood, it exemplifies how lipidomics can be used as a reverse approach to identify genetic and metabolic pathways that can be further investigated in mouse models. Altogether, these findings demonstrate how deciphered lipidomes of the adipose tissue allow comparison between different cell types, and reflect changes in lipid metabolism under different experimental settings and pathological conditions. The dynamics of lipid metabolism in adipocytes highlights the biologic significance of lipids in adipocyte differentiation, physiology and pathophysiology.

**Adipocyte differentiation - lipid build-up**

The limited information of how LDs are typically formed during adipocyte differentiation is primarily based on a culture setting. The cultured adipocytes are differentiated from committed pre-adipocytes, such as the 3T3-L1 cell line, or from adipogenic stromal vascular cells, which are directly obtained from the dissected WAT. Lipid accumulation rates depend upon sufficient supplementation of dietary fatty acids, and on the degree by which fatty acid biosynthesis compensates for their deficiency. Under normal conditions, de novo lipogenesis is more important in progenitor cell differentiation than in mature adipocytes. All cells are capable of fatty acid bio-synthesis from a citrate precursor, however, differentiating adipocytes display a robust lipogenesis. This process is central for adipocyte differentiation, in which fatty acid biosynthesis is followed by incorporation of fatty acids in TG. For example, inhibition of acyltransferase activity, which mediates TG formation from fatty acids, impairs pre-adipocyte differentiation. Notably, apart from de novo fatty acid synthesis, fatty acids are imported by differentiating adipocytes, with the assistance of lipid transporters and chaperons, such as fatty acid binding proteins (FABP). Once inside the cells, the fatty acids are converted into TG. TG are stored in LDs – dynamic organelles surrounded by an endoplasmic reticulum (ER)-derived membranes. LDs are regulated by lipid binding proteins with hydrophobic fatty acid-binding domains, such as perillipins that allow influx and efflux of fatty acids from LDs. Reagents that cause abnormal LD formation and remodeling also lead to impaired lipogenesis and differentiation in vitro. While lipogenesis during in vitro adipocyte differentiation has been extensively studied, lipidomics has not been applied yet to study these processes. Various genes that have been shown to be involved in fatty acid biosynthesis and metabolism can be knocked-down or overexpressed in cells, and the adaptive phenotype of differentiating adipocytes can be characterized by studying the lipidome. Changes in lipid profiles could be very informative and complementary to more common tools, such as gene expression profiles.

**Adipocyte differentiation - regulation by lipids**

The lipogenesis process is governed by a transcriptional program upon activation of adipocyte differentiation. Transcription factors that are essential for the formation of mature adipocytes include the nuclear receptor peroxisome proliferator-activated receptor-γ (PPARγ), CCAAT/enhancer-binding proteins (C/EBP) and sterol responsive element-binding protein1 (SREBP1). These transcription factors induce the expression of enzymes that participate in fatty acid biosynthesis, transport and
incorporation of fatty acids into TGs. Although TGs serve as an energy reserve and comprise most of lipids in mature white adipocytes, there are other lipids that are crucial for adipocyte differentiation and physiology. An example for such lipids are several types of fatty acids and eicosanoids that are known to bind PPARγ. In turn, activated PPARγ translocates to the nucleus and heterodimerizes with retinoid X receptor α (RXRα). Together with other transcription factors, such as C/EBPα, PPARγ induce expression of a multitude of genes involved in fatty acid and glucose uptake, lipogenesis and lipid storage, collectively required for adipocyte differentiation. PPARγ-dependent adipocyte formation is regulated by prostaglandins (PG), a family of endogenous bioactive lipids that signal via G-protein-coupled receptors. Overexpression of cyclooxygenases (COX), which catalyze the rate-limiting step of PG synthesis, in pre-adipocytes, suppresses subsequent differentiation. Members of the PG family have versatile effects on adipocyte formation. For example, PGJ2 activates adipose progenitors to promote differentiation, PGE2 and PGF2α are early-phase negative regulators of differentiation, while PGD2 and derivatives of PGJ2 are late-phase positive regulators of differentiation. One of the key hormones that promote adipocyte differentiation and lipogenesis is insulin. Fatty acids are capable of regulating insulin sensitivity in differentiating adipocytes. For example, PUFAs increase insulin sensitivity in differentiating adipocytes, whereas ceramides and high-dose of SFAs reduce insulin sensitivity. Free fatty acid receptors (FFAR), which signal in response to fatty acid binding, play an important role in the regulation of insulin sensitivity of adipocytes. Lipidomics can be used to discover pro-adipogenic and anti-adipogenic bio-active lipids. For example, a recent study profiled the lipidome of WAT in mice treated with β3-adrenergic receptor agonist that triggers beige/brown adipocyte activation. The search led to an unexpected observation – an elevated expression of pro-adipogenic eicosanoids in infiltrating macrophages rather than in the stimulated adipocytes. Moreover, a supplement of these eicosanoids in vitro promoted in vitro beige adipocyte differentiation. Taken together, fatty acids and other types of lipids endogenously induce or inhibit lipogenesis and lipid uptake, and consequently play a major part in the positive/negative feedback loop of adipocyte differentiation.

**Lipids in adipocytes – a fuel source and structural components**

Glucose and fatty acids are essential fuel sources in various tissues. WAT is the primary organ that stores fatty acids in the form of TG, while the liver is the primary organ that stores glucose in the form of glycogen. De novo lipogenesis from carbohydrates mostly takes place in the liver, followed by the WAT, despite the fact that this process is essential for adipocyte formation. However, unlike adipocytes, hepatocytes release newly synthesized fatty acids and TG to the circulation in the form of plasma lipoprotein particles. Hepatocytes do not normally store TG in large LDs. Thus, robust esterification of 3 acyl chains of fatty acids with glycerol to form TG, which are stored in large LDs, is a hallmark of adipocytes.

Adipose tissues are also capable of extensive lipolysis – breakdown of TG into free fatty acids. This 2-way traffic of fatty acids suggests that hepatocytes and adipocytes are efficient in fatty acid uptake and release upon demand, rendering them the gateway cells that mediate systemic lipid trafficking. This dynamic process is rapidly responsive to environmental cues, for instance, an increase in fatty acid uptake following feeding and an increase in fatty acid release following fasting or exercise. While non-esterified free fatty acids, carried by albumin in the blood, can basically diffuse through the adipocyte plasma membrane, there is a panel of fatty acid transporters and binding proteins that facilitate uptake. Well-characterized proteins that participate in fatty acid import include fatty acid translocase (also known as CD36), FABP, fatty acid transport proteins (FATP) and Acyl-CoA synthetases. Nonetheless, most circulating fatty acids cannot be directly imported, since they exist in an esterified form as TG carried by lipoproteins. TG, carried by circulating lipoproteins in the capillaries of adipose tissue, are hydrolyzed by LPL to produce fatty acids. In turn, the released fatty acids are imported to the cells. LPL is therefore important for fatty acid uptake as an energy source, particularly evident in activated muscle fibers and in brown/beige adipose adipocytes. In addition to extracellular LPL, white and brown adipocytes express cytosolic lipases. These lipases sequentially catalyze intracellular TG hydrolysis, and thus participate in lipolysis rather than in lipid uptake, as LPL does. Imported or lipolysis-released fatty acids serve as an energy source primarily through the β-oxidation pathway, which takes place at varied rates in all cell types (further reviewed in reference). Of note, fatty acid β-oxidation and enzymes that shuttle fatty acids to the mitochondria (e.g., carnitine acyltransferases) are crucial for BAT-dependent thermogenesis.

Multilocular brown/beige adipocytes are distinct from unilocular white adipocytes. The brown/beige lineage form multiple smaller LD and are rich in mitochondria (therefore the eponymous brownish color). Mitochondria in brown adipocytes are characterized by their own
set of lipids, which include different types of PL and the 4-acyl chain cardiolipins.24,27 The latter are abundant in mitochondrial lamellar crista architecture. Inner mitochondrial membranes of brown/beige adipocytes are marked by the expression of uncoupling protein-1 (UCP-1), which can disrupt the proton gradient generated in mitochondrial oxidative phosphorylation to generate heat.9,49 UCP-1 is stabilized by cardiolipins and is activated by certain fatty acids.49,50 The exact composition of lipids in adipocytes, such as TG in LDs and PL in the plasma membrane and mitochondria, depends on the types of fats in the diet and on the physiologic/metabolic state. In other words, adipocyte physiologic states may be reflected by the identity of dietary and newly synthesized lipid species. For example, elongation of fatty acids takes place in stimulated brown adipocytes, as part of mitochondrial biogenesis.51,52 Another example is that higher SFA/PUFA ratio in the diet is correlated with adipocyte hypertrophy (i.e., excessive lipid accumulation) and insulin resistance.53 Hence, lipidomics can be potentially applied to study energetic and structural aspects of adipocyte biology. For example, the preference to utilize short and medium acyl chains of TG in response to exercise,36 and the altered PL composition in BAT in response to cold exposure.28 The enzymatic pathways that mediate these changes and their biologic significance are yet to be determined. Taken together, lipids are at the core of the energetic, structural and functional facets of adipocytes.

**Lipids as indicators of physiology and pathophysiology**

Lipidomics can serve as a clinical diagnostic tool, but can also identify functional molecules in basic and pre-clinical research. Indeed, this approach has been harnessed recently for the identification of novel bio-active lipids in the adipose tissues of mouse models. Lipidomics of the adipose tissue in genetically modified mice has led recently to the discovery of novel lipokines – lipids that act as hormones. Mice deficient for the lipid chaperones FABP4 and FABP5 exhibit increased de novo lipogenesis, enhanced insulin sensitivity and protection against diet-induced obesity.54,55 Remarkably, WAT lipidomics supported previous observations that these mutant mice are obesity-resistant by displaying lipidomes in PCA plots.54,55 The lipidomes of mutant mice were clustered together independently of diet, whereas the lipidomes of wild-type mice were dissociated once normal chow and high-fat diet are compared.55 In other words, diet had limited effects over the lipid profile of the WAT in mutant mice. Furthermore, the authors detected an elevation in the production of the fatty acid palmitoleate (MUFA C16:1n7) in the WAT of mutant mice.55 Palmitoleate is released to the plasma, and in turn increases insulin sensitivity in the muscle and de novo lipogenesis in the liver.55 A novel class of insulin-sensitizing lipokines, namely fatty acid esters of hydroxy fatty acids (FAHFA), was recently discovered using mass-spectra based lipidomics in transgenic animals.56 Several FAHFA species are produced by adipocytes, and show a marked elevation in mice overexpressing glucose transporter type-4 (GLUT4) selectively in WAT56; these transgenic mice are known to be highly insulin sensitive.57 In wild-type mice, fasting increases FAHFA bio-synthesis, while HFD reduces FAHFA bio-synthesis.56 Moreover, administration of FAHFA improves the metabolism of insulin-resistant mice, and lower levels of FAHFA are detected in diabetic patients.56 Based on these observations, FAHFA have the potential to develop into an anti-diabetic drug. It is not unlikely that new bio-active lipokines are yet to be discovered, some of which may have positive metabolic effects, and some may have negative metabolic effects.

**Concluding remarks and future perspective**

In conclusion, lipidomics is a powerful tool that enables comprehensive characterization of specific types of cells and their physiology, identification of novel lipids in basic and clinical research, along with the search for specific diagnostic bio-markers (summarized in Fig. 1). In addition, we discussed the versatile roles of lipids in adipocytes – in regulation of adipocyte differentiation, as an energy source and as signaling molecules (summarized in Fig. 2). Thus, lipidomics is not only a tool to decipher lipid composition in a certain experimental/clinical setting, but it is also a tool capable of identifying bio-active lipids and actual changes in lipid metabolism. Lipidomics may be used in the near future to identify a specific cell type, according to tissue origin, age, sex, differentiation stage or a pathological state - the same way surface antigens are examined using flow cytometry in the immunology field or gene expression arrays are examined in the cancer research field. Lipidomics has potential future applications for personalized healthcare. According to the characteristic lipidome of a patient, the physician can make decisions which therapies might be beneficial based on reported data. This is in parallel to the utilization of genomics and proteomics as already established diagnostic and prognostic tools.

**Disclosure of potential conflicts of interest**

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**Glossary (Box)**

**Free fatty acids (FFA)** – Non-esterified carboxylic acids with an aliphatic acyl chain (in mammals usually 14–22 carbons). Nomenclature of FFA - CX:YnZ, where X = carbon number, Y = number of double bonds, Z = location of the first double bond in relation to the acyl chain terminus. For example, C16:1n7 represents palmitoleate, one of the fatty acids discussed in this review. FFA are major fuel sources and precursors for many types of cellular lipids. FFA can be elongated using enzymes, such as elongases (ELOVL) or broken down via \( \beta \)-oxidation.

**Saturation / desaturation of fatty acids** – Saturated fatty acids (SFA) do not have double bonds, monounsaturated fatty acids (MUFA) have one double bond, and polyunsaturated fatty acids (PUFA) have multiple double bonds. PUFA are abundant in fish, vegetables and seeds. The relative low abundancy of PUFA in meat is explained by the fact that mammals have limited capacity to desaturate fatty acids on specific locations. Nevertheless, mammals express several fatty acid desaturases that generate MUFA (the SCD family) and PUFA (the FADS family).

**Triacylglycerols (triglycerides, TG)** – Three acyl chains of FFA are esterified together with glycerol-3-phosphate to form TG. TG are a very common form of energy reserve, typically observed in lipid droplets (LD) of adipocytes.

**Lipogenesis** – Biosynthesis of fatty acids and TG. During adipocyte differentiation, lipogenesis is required to build-up LDs. Moreover, lipogenesis is an ongoing dynamic process in mature adipocytes that are very sensitive to energy homeostasis demands. **De novo** lipogenesis refers to fatty acid biosynthesis directly from carbohydrates precursors (i.e. glucose -> citrate -> acetyl-coA -> fatty acid) under conditions of unavailable dietary fatty acids. In turn, FA form TG.

**Plasma lipoprotein particles** – Circulating particles that transport TG and cholesterol. These lipoprotein particles are synthesized by the gut and the liver. While all cells can basically take up lipids from lipoproteins, the TG-rich adipose tissue is highly active in extracting fatty acids from lipoproteins, based on extracellular lipase activity. The extracted fatty acids can be re-formed into TG or oxidized for energy.

**Lipolysis** – Hydrolysis of TG into glycerol and FFA, using intracellular lipase activity. In turn, FFA are released to the circulation as an energy source or used directly by the adipocytes.

**\( \beta \)-oxidation** – Generation of energy via oxidation of fatty acids. FFA are transported from the cytosol to the mitochondria, and then are broken down in a step-wise of 2 carbons to generate ATP and acetyl-coA. Long FFA can be also oxidized in peroxisomes, where energy production is not directly coupled to mitochondrial respiration, also termed \( \alpha \)-oxidation.

**Diaclyglycerols (diglycerides, DG)** – A glycerol with 2 acyl chains, either a product of TG hydrolysis or a precursor for TG synthesis. They also serve as signaling molecules.

**Monoacylglycerols (monoglycerides, MG)** – A glycerol with one acyl chain, either a product of DG/TG hydrolysis or a precursor for DG/TG synthesis. They also serve as signaling molecules.

**Cholesterol esters (CE)** – A fatty acid esterified to a cholesterol, a component of membranes and a precursor for steroid hormones.

**Glycerophospholipids or Phospholipids (PL)** – PL are synthesized in the endoplasmic reticulum or the peroxisome, and consist of a glycerol, 2 acyl chains and a polar head group (phosphate modified or not by an alcohol). Due to their amphipathic nature, PL serve as a building block for the membrane bi-layer. Examples for common PL subclasses: phosphatidic acids, phosphatidylethanolamines, phosphatidylcholines, phosphatidylserines, phosphatidylglycerol, phosphatidylinositols and cardiolipins (also termed diphosphatidylglycerol).

**Lysosphospholipids (LyPL)** – Derivatives of PL, in which one or both acyl chains have been hydrolyzed. They also serve as signaling molecules.

**Sphingolipids (SL)** – Components of membranes and signaling lipids that are based on a sphingosine backbone (i.e., amino alcohol hydrocarbon chain). These can be further modified in various enzymatic reactions to synthesize other SL. For example, ceramides have an extra fatty acid residue, and sphingomyelin (SM) is a ceramide with a polar head group.

**Eicosanoids** – Signaling molecules that are generated by oxidation of PUFA, mostly arachidonic acid C20:4n6 and Eicosapentaenoic acid C20:5n3. Eicosanoids have diverse functions, including regulation of inflammation and cell growth. The pro-/anti-adipogenic Prostaglandins (PG) and epoxy-fatty acids, discussed in this review, belong to this class of bio-active lipids.

**Plasmalogens** – Vinyl-ether PL, which consist of one acyl chain and one alkyl chain as compared with the 2 acyl chains form of glycerophospholipids. They serve as modulators of membrane dynamics.

**Fatty Acid Hydroxy Fatty Acids (FAHFA)** – Branched fatty acids, in which a hydroxy fatty acid backbone is linked to a second acyl chain. FAHFA are newly discovered, endogenously synthesized, bio-active lipids.

**Lipidomics** – The study of lipid profiles (or lipidomes) using principles of analytical chemistry and computational/statistical analysis. This scientific approach typically relies on tandem/shotgun MS/MS technology, in which lipids are ionized, undergo fragmentation, and are characterized according to mass and charge. Algorithms are used to detect and measure a multitude of lipid species. The MS/MS spectrometer may be coupled to liquid chromatography, using standards, to measure absolute lipid concentrations. Lipidomics is a powerful tool to study lipid metabolism and to detect bio-markers in an experimental/clinical setting.

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