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Fatty acid transport proteins: targeting FATP2 as a gatekeeper involved in the transport of exogenous fatty acids†

Paul N. Black,*ab Constance Ahowesso,a David Montefusco,a Nipun Sainia and Concetta C. DiRusso*ac

The fatty acid transport proteins (FATP) are classified as members of the solute carrier 27 (Slc27) family of proteins based on their ability to function in the transport of exogenous fatty acids. These proteins, when localized to the plasma membrane or at intracellular membrane junctions with the endoplasmic reticulum, function as a gate in the regulated transport of fatty acids and thus represent a therapeutic target to delimit the acquisition of fatty acids that contribute to disease as in the case of fatty acid overload. To date, FATP1, FATP2, and FATP4 have been used as targets in the selection of small molecule inhibitors with the goal of treating insulin resistance and attenuating dietary absorption of fatty acids. Several studies targeting FATP1 and FATP4 for drug development were based on the intrinsic acyl CoA synthetase activity of these proteins and not on transport directly. While several classes of compounds were identified as potential inhibitors of fatty acid transport, in vivo studies using a mouse model failed to provide evidence these compounds were effective in blocking or attenuating fatty acid transport. Our studies targeting FATP2 employed a naturally occurring splice variant, FATP2b, which lacks intrinsic acyl CoA synthetase due to the deletion of exon 3, yet is fully functional in fatty acid transport. These studies identified two compounds, 5′-bromo-5-phenyl-spiro[3H-1,3,4-thiadiazole-2,3′-indoline]-2′-one), now referred to as Lipofermata, and 2-benzyl-3-(4-chlorophenyl)-5-(4-nitrophenyl)pyrazolo[1,5-a]pyrimidin-7(4H)-one, now called Grassofermata, that are effective fatty acid transport inhibitors both in vitro using a series of model cell lines and in vivo using a mouse model.

Fatty acids and lipotoxic disease

The obesity epidemic is fueled by intake of calorie dense foods in excess of that needed for growth and development and to maintain health. Among the nutritional components correlated with increased disease risk are fats, particularly saturated and trans-unsaturated fatty acids. Few drugs are currently available to treat obesity, which is now classified as a disease affecting 34.9 percent of adults and 16.9 percent of children and adolescents.3 Of these, most drugs generally seek to suppress appetite or decrease fat digestion to limit absorption in the intestine.2–6 A member of the first class is phenteramine, which is also accompanied by adverse cardiovascular events.7–9 Orlistat is a member of the second class of drugs.10,11 This drug acts to inhibit pancreatic lipase and is associated with gastrointestinal disturbances. In addition, the use of orlistat for one year only results in modest weight reduction. Given the health imperative and economic burden of the obesity epidemic and the lack of treatment options, Paul N. Black is the Charles Bessey Professor of Biological Chemistry and Chair of the Department of Biochemistry at the University of Nebraska-Lincoln where he leads a research program addressing fatty acid transport and trafficking. He was selected as an Established Investigator of the American Heart Association for his early studies describing the mechanistic details of coupled fatty acid transport and activation (vectorial acylation) in bacteria. This research has been extended to unravel the mechanistic details of how several members of the fatty acid transport protein family function in fatty acid transport using cell-based and animal model systems.
alternative methods must be found to decrease the prevalence of obesity, limit the toxic effects of fatty acids, or interrupt the molecular mechanisms that link excessive accumulation in fatty acids to debilitating diseases.

Chronic imbalances in lipid flux and metabolism often cause a variety of metabolic abnormalities and pathologies, including hyperlipidemia, type two diabetes (T2D), nonalcoholic fatty liver disease (NAFLD), cardiovascular disease (CVD), and some cancers.12–14 A name given to the cellular dysfunction caused by high levels of intracellular fatty acids is lipotoxicity.15–21 For most tissues, the excess fatty acids are derived from an extracellular source (i.e., diet) when blood levels of triglycerides and free fatty acids are chronically elevated. However, de novo synthesis in liver of fatty acids and triglycerides can also add to the burden of systemic lipid overload when dietary carbohydrate is consumed chronically at levels above the amount required to meet daily energy demands. Apart from adipocytes, most cells have a limited capacity for lipid storage and when this capacity is exceeded, cell death may result from lipotoxicity characterized by ER stress, mitochondrial and nuclear dysfunction.19,22,23 Obesity is a common initiating condition for these diseases due to chronic hyperlipidemia and elevated free fatty acids. Current evidence indicates free fatty acids and/or their metabolites mediate cell death, although the exact mechanism is poorly understood.22,24–26 Lipid overload in muscle can lead to insulin resistance and in pancreatic β-cells may lead to dysregulation of insulin secretion and apoptotic cell death, both of which may contribute to the genesis of the diabetic state.27–29 Lipoapoptosis is also observed in the heart, which leads to the development of heart failure.30–32 In liver, chronically elevated fat deposits result in NAFLD, which can lead to steatohepatitis (NASH) and, eventually, to non-reversible hepatic cirrhosis.25,33 Therefore, there is much interest in preventing abnormal lipid accumulation, which can lead to lipotoxicity, metabolic dysfunction,
Fatty acid transport through vectorial acylation

Like glucose transport that is coupled to phosphorylation, one mechanism that drives fatty acid transport into cells is coupled to esterification with coenzyme A (vectorial acylation). This hypothesis of vectorial acylation, concomitant fatty acid transport and activation, was put forth by Overath and co-workers in 1969 and arose from their studies in *E. coli*. This seminal work demonstrated that a long chain acyl CoA synthetase (Acsl) is required to target the fatty acids for metabolism in *E. coli*. Importantly, these studies demonstrated that free fatty acids did not accumulate in cells where fatty acids were provided as the sole carbon and energy source, but rather acyl CoA derivatives were detected. *E. coli* cells lacking the structural gene encoding the Acsl, *fadD*, are unable to transport exogenous fatty acids further supporting this concept. Mechanistically, it is thought the bacterial Acsl partitions into the inner membrane in response to exogenous long chain fatty acids where this enzyme abstracts the fatty acid substrate concomitant with catalysis to produce fatty acyl CoA. The resultant acyl-CoA is the ligand of the transcription factor FadR. The FadR-acyl-CoA complex cannot bind DNA, thus inducing the expression of genes encoding fatty acid degrading enzymes and the fatty acid transporter FadL, while also reducing the expression of genes encoding those genes required for unsaturated fatty acid biosynthesis. This foundational work in bacteria describing the process of vectorial acylation, the generation of long chain acyl CoA and transcriptional regulatory network has established the basis for addressing this process in eukaryotic systems.

In the yeast *Saccharomyces cerevisiae*, the process of vectorial acylation is operational, but with increased complexity. The yeast Fat1 protein (Fat1p; YBR041W) was identified by structural and functional similarities to members of the FATP family. Deletion of the yeast *FAT1* gene, encoding Fat1p, disrupts fatty acid transport measured using both radioactive substrates and the fluorescent fatty acid analogue, C1-BODIPY-C12. The reduction in fatty acid transport in *fat1Δ* strains is also associated with inability of yeast cells to grow under conditions where cells are auxotrophic for long chain fatty acids such as during hypoxia when the fatty acyl CoA Δ9 desaturase (Ole1p) is inactive. Fat1p-dependent fatty acid uptake is specific for long chain fatty acids (C16–C20).

Like several mammalian FATPs, Fat1p is a dual functional protein with acyl CoA synthetase activity specific for very long chain fatty acids (>C20; Acsvl). Deletion of *FAT1* reduces activation of lignoceric acid (C24:0) and results in the accumulation of very long chain fatty acids. Importantly, alanine scanning mutagenesis of the yeast *FAT1* generated altered forms of Fat1p that have activities that separate fatty acid transport from fatty acid activation. Three additional lines of evidence further support the conclusion the transport and activation activities in different members of the FATP protein family distinct. First, the expression of Fat1p, 2 and 4 in yeast results in increased Acsvl (i.e., C24:0 or C20:4) activity but has no affect on Acsl activity using long chain fatty acid substrates (i.e., C14:0 or C18:1). FATP 5 and 6 do not function in this capacity, nor do they complement transport deficiencies in yeast *fat1Δ* strains. Second, the characterization of five different murine FATP1, 4, and 6 protein chimeras distinguished fatty acid transport and activation. And third, two splice variants of human FATP2 (2a and 2b) are expressed in various tissues. Variant 2b is missing exon 3 encoding the ATP/AMP binding site and functions in fatty acid transport but not fatty acid activation. The fundamentals of vectorial acylation in yeast and mammals are shown in Fig. 1 where the formation of acyl CoA drives transport. While there is evidence that in the case for FATP4, it may function alone to drive this process, the genetic data and the identification of a catalytically deficient form of FATP2 detailed above supports the proposal that a cognate acyl CoA
synthetase must function in concert with an FATP to drive this process.

**FATP/Scl27 family members as drug targets**

As summarized above, the fatty acid transport proteins (FATP) are classified as members of the solute carrier 27 (Scl27) family of proteins and have been evaluated for their ability to promote the transport of exogenous fatty acids in a number of different cell types. The first fatty acid transport protein, FATP1, as well as Acsl1, was identified using expression cloning from a cDNA library prepared from murine 3T3-L1 adipocytes directly demonstrating its physiological role in the net accumulation of fatty acids. A number of different isoforms of FATP have subsequently been identified experimentally in mice, rats, humans, C. elegans, Drosophila and yeast. In mammals there are six isoforms that differ based on their tissue and cell type distribution, regulation and preferred substrate. While the expression of each of these proteins has been correlated with increased levels of fatty acid transport, several have also been described as very long chain acyl CoA synthetases. Indeed prior to the classification of this family of proteins as members of the Scl27 family, several were first described as very long chain acyl CoA synthetases, and based on their sequence encoding an ATP binding domain clearly fall into the larger family of adenylate-forming enzymes. One member of this family, FATP4, plays a major role in maintaining the barrier function of the skin through ceramide metabolism and contributes to the epidermal barrier function. Another, FATP5, has activation activity towards bile acids and is involved in secondary bile acid metabolism. Finally, there is evidence these proteins are localized to the endoplasmic reticulum in addition to the plasma membrane, which may occur at regions of juxtaposition between the two membranes (e.g., see ref. 94 and 95). None-the-less, both knockdown and over expression studies of several members of the FATP family are consistent with the conclusion they function in the process governing the transport of exogenous fatty acids into the cell in a highly regulated manner.

Given their important roles in fatty acid transport, the FATP proteins are viewed as viable targets to prevent aberrant accumulation of fat in cells and tissues that leads to lipotoxic disease. FATP1 and FATP4 have been used to select inhibitors by high throughput screening. In each screen, acyl CoA synthetase activity was targeted and not fatty acid transport directly. It was rationalized that the Acsl activity was essential for transport activity due to coupled activation and transport. However, from our work in yeast, the activation and transport activities are separable. FATP1 was particularly chosen as a therapeutic target for the treatment of insulin resistance and various arylpiperazines were selected as inhibitors. However, upon testing in mice, the selected compound imidazolepyridine did not limit the accumulation of triglyceride in muscle when mice were fed a high fat diet leading to the conclusion that inhibition of FATP1 alone is insufficient to block fatty acid transport. FATP4 was originally described as the major FATP in the intestine and the rationale for its selection as a therapeutic target was to block the absorption of dietary fatty acids. In this case screening studies identified dihydropyrimidinones as FATP4-specific inhibitors. Again, in subsequent in vivo studies these compounds were ineffective as inhibitors of fatty acid absorption. These results informed our choice to focus on FATP2 and to limit our screening design to fatty acid transport rather than Acsl activity.

**Fatty acid transport protein 2 (FATP2)**

Recent work in our laboratories has focused on FATP2 (Scl27a2), which is expressed in specific tissue types, particularly the small intestine, pancreas and liver. FATP2 was originally defined as a very long chain acyl CoA synthetase (Acsvl) with an indirect role in the pathophysiology of X-linked adrenoleukodystrophy (X-ALD). However, while the FATP2/Acsvl knockout mouse (fatp2/-) has reduced very long chain acyl CoA synthetase (Acsvl) and peroxisomal very long chain FA oxidation activities in liver, it does not accumulate very long chain fatty acids in blood, a hallmark of X-ALD. The role of FATP2 in fatty acid transport was subsequently demonstrated in both yeast and mammalian cells. Of particular importance was the identification of a splice variant of FATP2 (FATP2b) that lacks acyl CoA synthetase activity yet is fully functional in fatty acid transport demonstrating the transport activity is distinct from fatty acid activation.
FATP2 is highly expressed in small intestine, liver, kidney, pancreas and placenta and to a lesser extent in brain, heart, and colon; expression is not detectable in white adipose tissue}\(^{18}\) (see http://www.proteinatlas.org/). FATP2 expression in the small intestinal mucosa has been confirmed and moreover was shown to significantly increase in mice fed a high fat diet.\(^{99,100}\) FATP2 is the predominant FATP isoform expressed in Caco-2 cells, a human epithelial colorectal adenocarcinoma cell line.\(^{48}\) Expression of FATP2 in liver is also consistent with a key role in fatty acid transport and metabolism. In primary rat hepatocytes, the development of lipotoxicity by palmitate (C\(_{16:0}\)) is correlated with increased FATP2 expression, plasma membrane localization, and the development of insulin resistance.\(^{47}\) Increased expression is linked with the progression of nonalcoholic fatty liver disease (NAFLD)\(^{18}\), while decreased expression using adenoviral-directed fatp2 shRNA reduces fatty acid transport and protects mice from diet-induced NAFLD.\(^{45}\) FATP2 levels increase in isolated human pancreatic islets under high glucose conditions supporting a mechanistic basis of this protein in fatty acid transport in \(\beta\) cells.\(^{101}\) FATP2 is the predominant isoform in INS-1E cells, a rat pancreatic \(\beta\) cell line.\(^{102}\)

The regulation of FATP2 expression is complex. In the small intestine, FATP2 expression levels are increased four- to five-fold in response to the PPAR\(\alpha\) agonists Wy12643 and GW7647.\(^{103}\) In PPAR\(\alpha\) null mice, the expression of FATP2 is decreased two-fold; the expression of FATP2 remains unchanged in PPAR\(\alpha\) null mice treated with both agonists.\(^{103}\) There is evidence the winged helix/forkhead box (Fox) transcription factor Foxa1 regulates the expression of FATP2 in liver. As noted above, the increased expression of Foxa1 in HepG2 cells and primary human hepatocytes decreases FATP2 expression, which is correlated with approximately two-fold lower levels of fatty acid transport.\(^{16}\) In this regard Foxa1 expression appears to function, in part, to protect liver from steatosis through the regulation of FATP2. The increased expression of FATP2 in liver is also associated with activation of the insulin receptor substrates IRS-1 and IRS-2, which is coincident with increased lipogenesis. Given that exogenous fatty acids directly contribute to NAFLD, we suggest FATP2 is a key determinant in the development of a chronic fatty liver.\(^{49,50,104,105}\) Finally, and of specific interest in the context of obesity, FATP2 expression in peripheral blood mononuclear cells of rats fed a hyperlipidemic cafeteria diet was 33-fold higher than rats fed a lower fat normal caloric diet, suggesting this protein may be an ideal marker for overweight development and lipotoxicity.\(^{43}\) With this information as a backdrop, the challenge at present is to target FATP2 to prevent and resolve lipotoxicity using novel small compound inhibitors to prevent associated pathologies.

Collectively, FATP2 is an attractive target to develop inhibitors that block fatty acid transport for several reasons. First, expression of FATP2 is specific to a limited number of tissues, but is highly expressed in intestine and liver.\(^{41}\) Thus, inhibition is predicted to affect absorption, distribution, and over-accumulation of fatty acids in liver. Second, studies in animals and cells, as well as in human clinical studies, indicate depression of FATP2 levels in the liver is associated with decreased fatty acid uptake and is protective for NAFLD. FATP2 has been suggested as a biomarker for the development of overweight and obesity.\(^{43,44,46}\) Third, a complete germline deletion of FATP2 in mice did not affect development, growth, or normal functions,\(^{98}\) while liver-targeted gene expression knock down was protective of development of fatty liver induced by high fat diet.\(^{45}\) Thus, FATP2 fulfills most of the requirements for a good drug target:\(^{106}\) [1] limited tissue expression and good specificity, [2] demonstrated target for NAFLD in animals, [3] long-term inhibition should not affect most normal physiological functions predicting limited side effects and good tolerability, [4] high throughput screening directed against the catalytically deficient form has yielded five candidate compounds and structural families,\(^{39}\) and [5] in vitro and cell-based assays demonstrate the selected FATP2 inhibitors have limited impact on cell viability and provided good protection from palmitate-induced cellular dysfunction and death.\(^{102}\)

Development of high throughput screens to identify compounds that block FATP2

To address this challenge and further understand the role of the FATP2 in fatty acid transport, activation and intracellular trafficking, we devised a screening method for small compound inhibitors that relied on the transport of the fluorescent long chain fatty acid C\(_{1}-\text{BODIPY-C12}\)\(^{107,108}\). The primary screening method employed a yeast strain deficient in fatty acid transport (fat1\(\Delta\)) and with reduced Acs1 activity (faa1\(\Delta\)). In this strain we expressed human FATP2b, which functions in fatty acid transport but is deficient in acyl CoA synthetase activity.\(^{107,108}\) The selected transport inhibitors are useful as [1] chemical probes to understand the biochemical mechanisms that govern fatty acid transport into cells, and [2] novel compounds of therapeutic value to treat pathological states resulting from, or exacerbated by, fatty acid accumulation in non-adipose tissue. Hits identified were scrutinized secondarily to eliminate those that were not specific to fatty acid transport but caused cellular toxicity, permeability, or because the compound acted as a fluorescence quenching agent toward the fluorescent fatty acid analogue C\(_{1}-\text{BODIPY-C12}\)\(^{107}\) used in the screening process.\(^{39}\) The final hit list contained 90 compounds that were categorized to five structural families. For each family, representatives that passed secondary screens were further characterized in two mammalian cell lines: Caco2 cells, a model for intestinal epithelia, and HepG2 cells, a model for hepatocytes. Dose response curves were employed to measure an apparent IC\(_{50}\) for fatty acid uptake selecting only those in the low micromolar range. The hit compounds were further tested in these cells and were shown to significantly depress long chain fatty acid transport using \([^{14}\text{C}]\)-oleate (C\(_{18:1}\)\(^{cis}\)), while not affecting the transport
of glucose. None of the selected compounds disrupted membrane barrier function tested measuring trans-epithelial electrical resistance (TEER) of the Caco2 cell line or decreased total Acs1 activity. The most effective member of each family (lowest IC50 in Caco2 and HepG2 cells) was then evaluated in 3T3L1 adipocytes. In fat cells, the selected compounds had a relatively high IC50. The rationale was that prevention of absorption across the intestine and accumulation of fatty acid in adipocytes would be preventive of lipotoxicity, while accumulation of fatty acid in adipocytes is viewed as protective and, therefore, should be preserved in any therapeutic approach to prevention and management of lipotoxic disease. Among the final hit list, two, CB16.2 called Lipofemata and CB5 called Grassofermata, were selected as lead hits since they had the best profiles for features indicative of a high affinity and specificity with low toxicity in all our screening assays (DiRusso, C. C. and Black, P. N., 2013, Patent US8431582 B2, Inhibitors of fatty acid uptake and methods of use covers Grassofermata US8263640 B2, covers Lipofermata) (Fig. 2).

### Lipofermata

The best-characterized inhibitor to date is Lipofermata, 5′-bromo-5-phenyl-spiro[3H-1,3,4-thiadiazole-2,3′-indoline]-2-one), which specifically attenuates FATP2-mediated fatty acid transport without impacting other cellular functions, including long chain acyl CoA synthetase activity. Lipofermata is highly effective in attenuating fatty acid transport and importantly prevents lipid accumulation in cells that are models for hepatocytes (HepG2), enterocytes (Caco-2), myocytes (C2C12) and pancreatic β cells (INS-1E). This compound is 10- to 50-fold less effective in inhibiting fatty acid transport in 3T3L1 adipocytes and human adipocytes, the preferred lipid storage site.

Lipofermata inhibits the transport of the fluorescent fatty acid analogues C1-BODIPY-C12 and BODIPY-FL-C16 through a non-competitive process with IC50s in the low micromolar demonstrating its utility in blocking the transport of long and very long chain fatty acids, respectively. These data are consistent with studies using FATP2 over-expressing and knock-down cell lines treated with isotopically labeled exogenous fatty acids, which demonstrated this protein contributes to fatty acid transport through vectorial acylation.

The saturated fatty acid, palmitate, is a major component of the western diet and high levels of dietary intake correlate with a number of lifestyle diseases including type 2 diabetes and NAFLD. Palmitic acid induces apoptosis in...
Fig. 3 Role of FATP2 in fatty acid transport and homeostasis. A. Normal fatty acid transport in hepatocytes coupled with cellular homeostasis at the levels of fatty acid oxidation, lipid synthesis and transcriptional regulation. B. Increased fatty acid transport and lipid overload as associated with cellular dysfunction, noted in red, and associated with mitochondrial dysfunction (increasing reactive oxygen species (ROS) and lost of cytochrome C); ER stress (reflected by increased levels of ROS and BiP); increased peroxisomal fatty acid oxidation and associated oxidative stress; dysregulation of transcription (associated with activation of CHOP; an increase in apoptotic proteins and loss of nuclear membrane integrity); all of which contribute to the activation of the caspase cascade leading to apoptosis. As detailed in the text, treatment of cells under conditions of fatty acid overload with either Lipofermata or Grassofermata attenuate or eliminate the cellular dysfunctions noted in B.
pancreatic β-cell lines as well as in hepatocytes.\textsuperscript{111,112} Lipofermata treatment blocks both palmitate-induced accumulation of lipid droplets and apoptotic cell death in a dose-dependent manner.\textsuperscript{102} Chronic exposure of both liver and β cells to high levels of saturated fatty acids has been shown to induce apoptosis and disrupt cellular function thereby contributing to the pathogenesis of fatty liver disease (modeled in Fig. 3)\textsuperscript{102,111,112} and type 2 diabetes.\textsuperscript{114} Lipofermata at relatively low concentrations (5–50 μM) promotes survival of HepG2 and INS-1E cells and protects against palmitic acid-induced lipotoxicity measured by evaluating ROS, GSH, BiP, CHOP and caspase-3 levels).\textsuperscript{102} On the basis of these findings, we propose Lipofermata is likely to be useful to attenuate cellular and organ lipotoxicity, which leads to disease and may be of therapeutic use in protecting β cells against lipotoxicity caused by diets high in saturated fat.

One of the predicted uses of Lipofermata is as an inhibitor of intestinal fatty acid absorption for use in mechanistic studies in animals and, perhaps eventually, as a therapeutic. Lipofermata attenuates fatty acid absorption across the gut by 80% in a temporal manner.\textsuperscript{102} There are no other compounds that inhibit fatty acid absorption specifically targeting fatty acid transport. Xenical (orlistat) is an approved drug for use in inhibiting fat absorption\textsuperscript{11} that function by inhibiting pancreatic lipase and thus triglyceride breakdown.\textsuperscript{114} In this case, since the fatty acids are not released from the complex lipid, they cannot be absorbed. It is worth speculating that Lipofermata may be used as a partner with orlistat, which will further inhibit free fatty acid transport across the intestinal epithelium. As reviewed above, selected FATP1 and FATP4 inhibitors do not prevent fatty acid absorption.\textsuperscript{96,97}

### Grassofermata

A second FATP2-inhibitor identified in our high throughput screen is Grassofermata, 2-benzyl-3-(4-chlorophenyl)-5-(4-nitrophenyl)pyrazolo[1,5-a]pyrimidin-7(4H)-one.\textsuperscript{39} Like Lipofermata, Grassofermata is inhibits the transport of fluorescent fatty acid-analog C1-BODIPY-C12 in various cell lines that are models for small intestine, liver, pancreatic islets, muscles and human adipocytes.\textsuperscript{102} Inhibition by Grassofermata is specific for long and very long chain fatty acid transport and is protective effects against palmitic acid induced lipotoxicosis.\textsuperscript{19,116,117} As predicted, treatment of cells with this compound does not alter medium chain fatty acid transport. Consistent with in vitro studies, Grassofermata, like Lipofermata when delivered orally, prevents intestinal absorption of \textsuperscript{13}C labeled olate.\textsuperscript{117} Pharmacokinetic data show this inhibitor is detectible in the blood within 30 minutes of dosing and for up to 6 hours indicating it is absorbed as well.\textsuperscript{117} Like Lipofermata, Grassofermata is a specific FATP2 fatty acid transport inhibitor that has valuable potential to add to our arsenal of pharmaceuticals to prevent and treat lipotoxic disease.

### Prospective

Exogenous long-chain fatty acids contribute to normal metabolic homeostasis that includes energy production, complex lipid synthesis for membranes and storage, and protein anchoring. Under normal conditions, excess fatty acids are esterified and stored as triglycerides in adipocyte stores that are dynamically responsive to systemic energy needs. Apart from adipocytes, most cells have a limited capacity for lipid storage and when this capacity is exceeded, as in the case of fatty acid overload, cell death may result from a process called lipotoxicity.\textsuperscript{17,19,116,117} The identification of Lipofermata (5’-bromo-5-phenyl-spiro[3H,1,3-thiadiazole-2,3’-indoline]-2’-one) and Grassofermata (2-benzyl-3-(4-chlorophenyl)-5-(4-nitrophenyl)pyrazolo[1,5-a]pyrimidin-7(4H)-one), as effective FATP2 fatty acid transport inhibitors, represents a key advance in targeting FATP2/Slc27a2 fatty acid transport protein to combat diseases associated with lipid overload.

### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Acsl</td>
<td>Long chain acyl CoA synthetase</td>
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<tr>
<td>BiP</td>
<td>Binding immunoglobulin protein (glucose regulated protein 78 (Grp78))</td>
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<tr>
<td>C1-BODIPY-C12</td>
<td>4,4-Difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoic acid</td>
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<tr>
<td>CHOP</td>
<td>ISR-regulated transcription factor</td>
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<td>CVD</td>
<td>Cardiovascular disease</td>
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<td>ER</td>
<td>Endoplasmic reticulum</td>
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<tr>
<td>FadR</td>
<td>Escherichia coli long chain acyl CoA-responsive transcription factor</td>
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<tr>
<td>FAT1</td>
<td>Saccharomyces cerevisiae Fat1p structural gene</td>
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<tr>
<td>Fat1p</td>
<td>Saccharomyces cerevisiae FATP orthologue</td>
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<tr>
<td>FATP</td>
<td>Fatty acid transport protein</td>
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<tr>
<td>GSH</td>
<td>Glutathione</td>
</tr>
<tr>
<td>Fox</td>
<td>Winged helix/forkhead box</td>
</tr>
<tr>
<td>IRS</td>
<td>Insulin receptor substrate NASH – nonalcoholic steatohepatitis</td>
</tr>
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<td>NAFLD</td>
<td>Nonalcoholic fatty liver disease</td>
</tr>
<tr>
<td>Ole1p</td>
<td>Saccharomyces cerevisiae fatty acyl CoA Δ9 desaturase</td>
</tr>
<tr>
<td>PPAR</td>
<td>Peroxisome proliferator-activated receptor</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>Slc27</td>
<td>Solute carrier family 27</td>
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<td>T2D</td>
<td>Type 2 diabetes</td>
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<tr>
<td>TEER</td>
<td>Trans-epithelial electrical resistance</td>
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<tr>
<td>Acsvl</td>
<td>Very long chain acyl CoA synthetase</td>
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<td>X-ALD</td>
<td>X-linked adrenoleukodystrophy</td>
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