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GLUT4 Expression in Adipocytes Regulates De Novo Lipogenesis and Levels of a Novel Class of Lipids With Antidiabetic and Anti-inflammatory Effects

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Adipose tissue (AT) regulates systemic insulin sensitivity through multiple mechanisms, and alterations in de novo lipogenesis appear to contribute. Mice overexpressing GLUT4 in adipocytes (AG4OX) have elevated AT lipogenesis and enhanced glucose tolerance despite being obese and having elevated circulating fatty acids. Lipidomic analysis of AT identified a structurally unique class of lipids, branched fatty acid esters of hydroxy–fatty acids (FAHFAs), which were elevated in AT and serum of AG4OX mice. Palmitic acid esters of hydroxy–stearic acids (PAHSAs) are among the most upregulated FAHFA families in AG4OX mice. Eight PAHSA isomers are present in mouse and human tissues. PAHSA levels are reduced in insulin resistant people, and levels correlate highly with insulin sensitivity. PAHSAs have beneficial metabolic effects. Treatment of obese mice with PAHSAs lowers glycemia and improves glucose tolerance while stimulating glucagon-like peptide 1 and insulin secretion. PAHSAs also reduce inflammatory cytokine production from immune cells and ameliorate adipose inflammation in obesity. PAHSA isomer concentrations are altered in physiological and pathophysiological conditions in a tissue- and isomer-specific manner. The mechanisms most likely involve changes in PAHSA biosynthesis, degradation, and secretion. The discovery of PAHSAs reveals the existence of previously unknown endogenous lipids and biochemical pathways involved in metabolism and inflammation, two fundamental physiological processes.

The number of overweight and obese people is increasing in epidemic proportions worldwide, which has rapidly increased the prevalence of type 2 diabetes (T2D) and metabolic syndrome (1,2). Obesity and T2D are affecting the pediatric and adolescent population, putting them at risk for cardiovascular (3) and immune-mediated complications early in life (4,5). Therefore, we need more effective and sustainable prevention and treatment strategies for these serious disorders. The major pathogenic factors for T2D are insulin resistance in peripheral tissues and dysregulated insulin secretion (6). In spite of advances in understanding the pathogenesis of T2D, major gaps exist in our knowledge of the molecular and cellular mechanisms underlying insulin resistance and pancreatic β-cell dysfunction, which limits our ability to develop fully effective and safe therapies for these diseases. In general, elevated fatty acids contribute to insulin resistance (7,8). However, increased de novo lipogenesis (fatty acid synthesis from glucose) in adipose tissue (AT) is associated with enhanced insulin sensitivity in rodents and humans (9,10). This suggests that AT might produce lipids that have beneficial effects on insulin sensitivity and insulin secretion. While many lipids have detrimental metabolic effects, some, such as n-3 fatty acids (11,12) and palmitoleate (13), have beneficial effects, illustrating the potential for lipids to improve metabolic health.

IDENTIFICATION AND TISSUE DISTRIBUTION OF BRANCHED FATTY ACID ESTERS OF HYDROXY–FATTY ACIDS

A key function of adipocytes is the regulation of lipid and glucose homeostasis. This regulation depends on GLUT4-mediated glucose uptake (14). GLUT4 is a facilitative diffusion

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glucose transporter and is the major insulin-regulated glucose transporter in skeletal muscle, heart, and adipocytes (14). GLUT4 translocates from intracellular storage vesicles to the plasma membrane in adipocytes and muscle in response to increased insulin secretion after eating (14). GLUT4 is downregulated in adipocytes of both humans and rodents with obesity or T2D, and this downregulation is one of the earliest events in the pathogenesis of insulin resistance and T2D (14,15). The importance of GLUT4-mediated glucose uptake in adipocytes for glucose homeostasis was highlighted by findings indicating that the knockdown of GLUT4 selectively in adipocytes results in insulin resistance (16) and that overexpression of GLUT4 in adipocytes (AG4OX) reduces fasting glycemia and improves glucose tolerance (17,18). These effects on glucose homeostasis that result from adipocyte-GLUT4 overexpression are due in large part to glucose-dependent induction of de novo lipogenesis in AT by carbohydrate-responsive element–binding protein (ChREBP) (10).

Fatty acids derived from de novo lipogenesis and their products may have important signaling functions that affect several key metabolic and physiologic processes. In humans, expression of lipogenic enzymes in AT strongly correlates with insulin sensitivity (9). In contrast, de novo lipogenesis in liver leads to increased steatosis and increased serum triglyceride levels and correlates with insulin resistance (19,20). Moreover, induction of ChREBP in AT leads to improved glucose homeostasis (10). Although the amount of de novo lipogenesis in AT in humans is thought to be relatively small, a direct comparison of de novo lipogenesis in liver and fat in people on different diets showed similar rates in the two tissues (21). Another study on effects of glucose infusion in people provided evidence leading to the conclusion that "adipose tissue in man may be an important site for glucose lipogenesis during abundance of glucose while in other metabolic situations the liver may well be of relatively greater importance for lipogenesis" (22). A beneficial role for de novo lipogenesis in adipocytes has also been demonstrated during caloric restriction (23,24). Calorie restriction augments life span in several species, including humans, and delays the appearance of age-related diseases, such as T2D and cardiovascular diseases (23). Calorie-restricted mice have increased de novo lipogenesis in AT (24). Also, de novo lipogenesis is coupled with increased fatty acid oxidation during browning of AT (25). Taken together, de novo lipogenesis may have a beneficial role in systemic glucose metabolism and possibly even in determination of life span.

Elevated free fatty acids (FFAs) appear to play a causative role in metabolic syndrome (8). Lipolysis is increased in obesity and results in elevated serum FFA concentrations (26). There is a strong association among increased serum FFA levels, ectopic lipid accumulation, and insulin resistance (6,8,26). AG4OX mice have increased adiposity and de novo lipogenesis in AT and elevated serum FFA levels. But they have markedly enhanced glucose tolerance and no ectopic lipid accumulation (10,17,18). Thus, there is discordance between obesity, elevated FFA, and insulin resistance. We hypothesized that overexpression of GLUT4 in AG4OX adipocytes may drive the synthesis and secretion of lipid species that have beneficial metabolic effects.

Using untargeted mass spectrometry, we performed lipidomic analysis to profile AT lipids in AG4OX (27). We discovered a new class of lipids, branched fatty acid esters of hydroxy–fatty acids (FAHFAs) (Fig. 1*A*), which consists

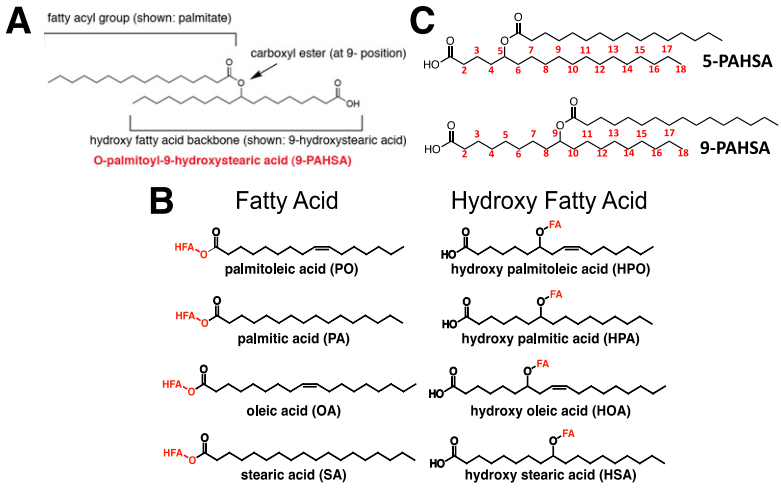

Figure 1—Structure of FAHFAs. *A*: FAHFAs are branched fatty acid esters of hydroxy-fatty acids. *B*: There are at least 16 different FAHFA family members in mouse serum, and these lipids arise from many possible combinations of common fatty acids and hydroxy–fatty acids. *C*: There are a number of different FAHFA isomers within each family. For example, 5- and 9-PAHSA combine palmitate as the fatty acyl group with a hydroxy–stearic acid backbone. But they differ in the position of the ester bond, which is 5 carbons away from the carboxylic acid group in 5-PAHSA and 9 carbons away in 9-PAHSA. Adapted with permission from Yore et al. (27).

of at least 16 different family members present in serum and many tissues in humans and rodents (27). The FAHFAs detected in serum and tissues consist of one of four different fatty acids and one of four distinct hydroxy–fatty acids in different combinations (Fig. 1*B*). The FAHFAs with palmitoleic acid, palmitic acid, or oleic acid as the fatty acid moiety and hydroxystearic acid or hydroxy palmitic acid as the hydroxy–fatty acid moiety are the most highly upregulated FAHFAs in AT of AG4OX mice (27). Recently, an in silico tandem mass spectrometric library was used to automatically annotate all potential FAHFA families (28). Several additional FAHFA families were identified in egg yolk, expanding the number of families in this class even further. We recently published a detailed methodology for measuring different FAHFA family members in mouse and human serum and tissues (29).

Palmitic acid esters of hydroxy–stearic acids (PAHSAs) are the most upregulated family member in AT of AG4OX mice. Targeted mass spectrometry showed that they are present in all tissues analyzed so far, including perigonadal (PG), subcutaneous (SQ), and brown AT (BAT); liver; pancreas; and kidney (27). In mouse tissues, a total of eight PAHSA isomers were identified (27). The isomers are composed of the same acyl chains but differ in the position of the ester bond relative to the carboxyl group (Fig. 1*C*). Mouse BAT, PG AT, and SQ AT have eight isomers, consisting of 5-, 7-, 8-, 9-, 10-, 11-, 12-, and 13-PAHSAs, and serum has a total of six isomers with the lack of 7- and 8-PAHSA isomers. Of note, the liver has only 9-, 12-, and 13-PAHSA isomers, indicating that the presence of PAHSAs in serum does not necessarily result in their accumulation in tissues. In humans, 5-, 9-, 10-, 11-, 12- and 13-PAHSAs are found in serum and SQ AT, whereas 7- and 8-isomers are not detected (27). Thus, the major PAHSA isomers that are present are the same in mouse and human serum and similar in mouse and human AT.

Evidence indicates that FAHFA levels and distribution are controlled by endogenous metabolism. Analysis of rodent diets revealed that the chow and high-fat diet (HFD) used in our studies have only five of the eight isomers present in mouse AT (27). Furthermore, 10-PAHSA is the predominant PAHSA in the mouse diets we used, and 5- and 7-PAHSAs were not detectable (27). But in mouse tissue samples, 9-PAHSA is the most abundant PAHSA, and 5- and 7-PAHSAs are present (27). Since the 7- and 5-PAHSA isomers are not present in mouse diets, these isomers must be synthesized in vivo. Furthermore, AG4OX mice have dramatically increased FAHFA levels even though they are on the same diet as wild-type mice (27). Together, these examples and additional data indicate the existence of biochemical pathways, controlled by uncharacterized genes, that are responsible for regulation of FAHFA levels in tissue and serum. Finding the enzymes that form and hydrolyze the ester linkage (Fig. 1) would identify the pathways necessary for FAHFA biosynthesis and catabolism, respectively.

FAHFAs can be broken down by hydrolysis of the ester linkage between the fatty acid and the hydroxy–fatty acid. This reaction can be monitored by measuring the amount of hydroxy–fatty acid generated upon incubation of FAHFAs with cell or tissue lysates or with pure enzymes. We tested this assay by incubating PAHSAs with tissue lysates, where we observed the enzymatic production of hydroxystearic acids. Lipid ester hydrolysis is mediated by lipases that typically contain a catalytic serine nucleophile that carries out the biochemistry (30).

In collaboration with the Cravatt and Saez laboratories, we began screening newly discovered atypical hydrolases for FAHFA hydrolysis activity (31). These experiments led to the serendipitous discovery that two related enzymes, androgen-induced gene 1 protein (AIG1) and androgen-dependent TFPI-regulating protein (ADTRP) (32,33), prefer FAHFAs as substrates over all the other major lipid classes tested (31). AIG1 and ADTRP are atypical because they use a threonine nucleophile instead of the typical serine nucleophile found in other serine hydrolases (31). The discovery of these hydrolases indicates the existence of enzymes that preferentially degrade FAHFAs. Confirmation of the endogenous activity and importance of these enzymes awaits mouse genetic experiments.

FAHFAs ARE SYNTHESIZED ENDOGENOUSLY

n-3 fatty acids are essential polyunsaturated fatty acids made in plant cells. For example, phytoplankton is the major source of n-3 polyunsaturated fatty acids in the marine food chain, which is the major source of n-3 fatty acids for humans (34,35). Mammals cannot synthesize n-3 fatty acids but can convert dietary n-3 fatty acids, such as linoleic acid and α-linolenic acid, into endogenous n-3 fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid (34). Three types of n-3 fatty acids, including α-linolenic acid (found in plant oils) and eicosapentaenoic acid and docosahexaenoic acid (commonly found in marine oils), are important in human physiology (34).

In contrast to n-3 fatty acids, PAHSAs are synthesized de novo in tissues of humans and other mammals (27). The known precursors for PAHSAs are fatty acids and hydroxy–fatty acids. Evidence for endogenous biosynthesis of PAHSAs comes from the observation that treatment of mice with the synthetic precursor, 9-hydroxy–heptadecanoic acid, by oral gavage results in the production of the FAHFA 9-palmitic acid hydroxy–heptadecanoic acid in vivo (27). 9-hydroxy–heptadecanoic acid and 9-palmitic acid hydroxy–heptadecanoic acid are not found in untreated mice, and these data indicate that biochemical pathways exist in vivo for the conversion of hydroxy–fatty acids into FAHFAs. Enzymatic activity in cell and tissue lysates produces PAHSAs via a reaction between palmitoyl-CoA and hydroxystearic acid. Because this process transfers a fatty acid (fatty acyl) onto a hydroxy–fatty acid, the enzyme responsible is likely to be a member of the lipid acyltransferase class of enzymes (36,37). We are currently investigating whether changes in PAHSA biosynthesis, degradation, or secretion account for the changes in PAHSA levels in altered metabolic states.

PAHSAs ARE REGULATED IN ALTERED METABOLIC STATES

PAHSA levels are regulated by physiological and pathophysiological alterations, such as fasting, refeeding, insulin resistance, and obesity (27). For example, PAHSA levels increase in SQ and PG AT with fasting (27) and rapidly return to normal with refeeding (I. Syed, A. Saghatelian, B.B. Kahn, unpublished results), indicating physiological regulation by biosynthetic or degradative enzymes and/or by release from the tissue. In contrast, levels of PAHSAs do not change in BAT or liver with fasting, and some PAHSA isomers are modestly decreased in serum with fasting (27). Thus, the pathways regulating PAHSA levels are tissue specific.

AG4OX mice have elevated PAHSA levels in serum and all fat depots, while in liver PAHSA isomers are reduced (27). This is another example of tissue-specific PAHSA isomer regulation. In addition, PAHSA isomers are differentially regulated with obesity in mice. All PAHSA isomers in SQ AT (5-, 7-, 8-, 9-, 10-, 11-, 12-, and 13-PAHSA) and in liver (9-, 12-, and 13-PAHSA) are reduced in HFD-fed mice. Similarly, in BAT, all isomers except 10-PAHSA are reduced. In contrast, in PG fat, levels of 7-, 8-, 9-, and 10-PAHSAs are elevated, whereas the level of 5-PAHSA is reduced. In serum, 5-, 12-, and 13-PAHSA levels are decreased in mice fed an HFD, whereas 9-, 10-, and 11-PAHSAs are not (27). This may reflect that more than one fat depot and other tissues may contribute to serum PAHSA levels. Thus, PAHSAs are differentially regulated in different tissues, which indicates tissue-specific function and control of their biosynthesis, degradation, uptake, and/or release.

A similar regulation is found in insulin resistant humans (27) in which serum levels of all PAHSAs except 9-PAHSA are reduced compared with those in insulin-sensitive people. Furthermore, serum PAHSA levels strongly correlate with insulin sensitivity by hyperinsulinemic-euglycemic clamp in people. In SQ AT of insulin resistant people, 5-, 9-, 10-, 12- and 13-PAHSA levels are reduced, and the levels of total PAHSAs and 5- and 9-PAHSA isomers in SQ AT correlate with insulin sensitivity (27). Serum PAHSA isomers levels do not correlate with serum fatty acid or triglyceride levels. Thus, PAHSA regulation in humans with insulin resistance is similar to that in mice with diet-induced obesity, indicating that PAHSA regulation in obesity and insulin resistance is conserved between mice and people.

PAHSAs ARE SIGNALING LIPIDS AND GPR120 MEDIATES PAHSA EFFECTS ON GLUCOSE TRANSPORT

The concentrations of PAHSAs in serum of mice and humans are similar to those of signaling lipids such as prostaglandins, prostacyclins, and steroids. Signaling lipids act as messengers, binding to protein targets/receptors, which mediate their effects. They cannot be stored in vesicles before release and need to be synthesized de novo (38). Signaling lipids have diverse biologic functions. For example, prostaglandins are produced by the cyclooxygenase or the lipoxygenase pathways from essential fatty acids (38), and they have several hormone-like functions affecting vasodilation, blood coagulation, and inflammation (38). Steroids, such as the sex hormones and glucocorticoids, are also signaling lipids with critical biologic effects, which are present in concentrations similar to PAHSAs in serum and tissues (39). Thus, we hypothesized that PAHSAs may have a role as signaling lipids, which could affect metabolism, inflammation, and other physiologic functions.

PAHSAs bind and activate GRP120, which mediates some of the metabolic effects (27) (Fig. 2). Knockdown of GPR120 in 3T3-L1 cells blocks the potentiation of insulin-stimulated glucose transport and GLUT4 translocation by PAHSAs (27). GPR120 was deorphanized in 2005 as a receptor for long-chain fatty acids in the intestine (40). Several n-3 polyunsaturated long-chain fatty acids can activate GPR120 (12). GPR120 is expressed in several regions of the gut and in AT, pancreas (delta cells), and immune cells (macrophages and dendritic cells) (11,12), suggesting that this receptor has multiple functions in the regulation of systemic metabolism and inflammation. Expression of GPR120 in adipocytes is increased in mice fed an HFD (12). Gene deficiency and dysfunction of GPR120 lead to obesity in both mice and humans (41). Similar to the effects of PAHSAs, n-3 fatty acids increase glucose transport in a GPR120-dependent manner (12) and improve glucose homeostasis in obese mice (11,12). Thus, some effects of PAHSAs and n-3 fatty acids may be mediated by the same receptors. But there are important differences between the mechanisms of action of these lipids. For example, a single oral dose of PAHSA improves glucose homeostasis, whereas the improvement of glucose homeostasis in HFD-fed mice treated with n-3 fatty acids appeared after 5 weeks. Moreover, n-3 fatty acids target peroxisome proliferator–activated receptor γ (42,43), but FAHFAs appear not to.

PAHSAs IMPROVE GLUCOSE TOLERANCE AND INSULIN SENSITIVITY AND STIMULATE INSULIN AND GLUCAGON-LIKE PEPTIDE 1 SECRETION

A single oral dose of PAHSAs markedly improves glucose tolerance in insulin resistant mice (27). This activity is most likely due to the acute effect of PAHSA to stimulate GLP-1 and insulin secretion in response to rising glucose levels (Fig. 3). More recent data from our laboratory show that chronic treatment of mice with 5- and 9-PAHSA improves insulin sensitivity as well as glucose tolerance. These improvements in insulin sensitivity are consistent with the effects of PAHSAs to augment insulin-stimulated glucose transport and GLUT4 translocation in adipocytes in vitro and their effects to reduce AT inflammation (27) (Fig. 3).

In human pancreatic islets in vitro PAHSAs augment insulin secretion in response to high glucose, but not low glucose, concentrations (27) (Fig. 3). PAHSAs also stimulate

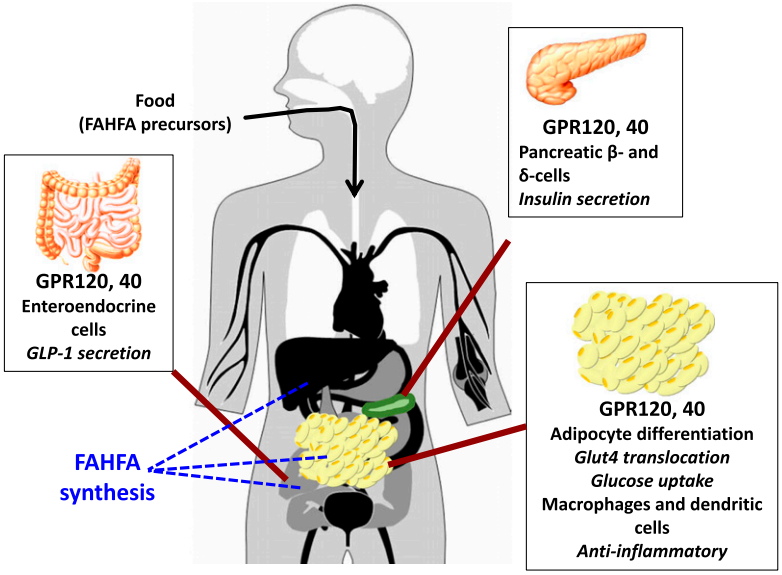

Figure 2—Proposed role of fatty acid receptors in the actions of FAHFAs in metabolic regulation. GPR120 and GPR40 regulate several physiologic processes. Activation of these receptors increases GLP-1 secretion from intestinal enteroendocrine cells and insulin secretion from pancreatic β-cells, stimulates glucose uptake and GLUT4 translocation to the plasma membrane in adipocytes, and inhibits inflammation (40,44,45). FAHFAs are synthesized in multiple tissues in vivo. They activate GPR120, which mediates the effect of FAHFAs to augment insulin-stimulated glucose transport and GLUT4 translocation in adipocytes. GPR120 may be involved in the effects of FAHFAs to stimulate GLP-1 secretion by enteroendocrine cells and possibly insulin secretion by pancreatic islets indirectly through effects on δ-cells. FAHFAs also inhibit dendritic cell activation in vitro and AT proinflammatory cytokine production in vivo. The receptors mediating the anti-inflammatory effects are not yet known.

GLP-1 secretion by enteroendocrine cells in vitro in a dose-dependent manner, with effects similar to those observed with n-3 fatty acids and a synthetic GPR120 agonist (27,40,44). Thus, PAHSAs directly enhance insulin and GLP-1 secretion. Also, the acute effects of PAHSAs to increase glucose-stimulated insulin secretion may be attributed to both direct effects on pancreatic islets and indirect effects through induction of GLP-1 secretion in the gut (Fig. 3).

In addition to GPR120-dependent stimulation of GLP-1 secretion (40), GPR40 appears to be required for the release of GLP-1 and gastric inhibitory polypeptide from enteroendocrine cells in the gut (45). Thus, effects of PAHSAs in intestinal cells to stimulate GLP-1 may be GPR40 and/or GPR120 dependent (Fig. 2).

PAHSAs also directly stimulate insulin secretion from pancreatic islets in a glucose-dependent manner (27). Since we see PAHSA effects specifically in β-cells where GPR40 is known to be involved in glucose-stimulated insulin secretion, GPR40 may mediate PAHSA effects on insulin secretion. In addition, PAHSAs may affect insulin secretion through GPR120 activation, since GPR120 inhibits somatostatin secretion by δ-cells and thereby indirectly stimulates insulin secretion from β-cells (44).

From a clinical perspective, PAHSAs could be used as, or facilitate the development of, coagonists that simultaneously activate multiple lipid-activated G-protein–coupled receptors, reducing the risks of ligand resistance and toxicity. Also, agents capable of increasing endogenous synthesis or reducing degradation of PAHSAs could have promising metabolic results.

PAHSAs ARE ANTI-INFLAMMATORY

The inflammatory response, which includes the activation of both innate (macrophages, dendritic cells, monocytes, neutrophils, innate lymphoid cells) and adaptive (B and T cells) immune systems, is triggered by AT expansion, adipokines (leptin, RBP4), increased lipolysis, cell death, and hypoxia (46–52), although the molecular mechanism that underlies this process is not fully understood. AT macrophages and dendritic cells can recognize danger signals through the activation of pattern-recognition receptors, such as Toll-like receptors (TLRs) (53). Fatty acids or their metabolites can initiate and perpetuate AT inflammation by activating TLRs (54). This process results in stimulation of downstream signaling pathways, including MAPKs (c-Jun N-terminal kinase, extracellular signal–related kinase, p38) and nuclear factor-κB pathways leading to production and secretion of inflammatory mediators (tumor necrosis factor [TNF], interleukin [IL]-1β, and IL-6) and subsequent impairment of insulin signaling (55). TLR4 activation by fatty acids results in upregulation of ceramides, which is associated with insulin resistance. Ceramides can activate the inflammasome, resulting in the production of IL-1β, perpetuating the AT inflammatory process (56).

Chronic inflammation is a key component of obesity-induced insulin resistance (47,57). Oral treatment of obese mice for 3 days with 9-PAHSA improves AT inflammation by reducing the levels of macrophages positive for the

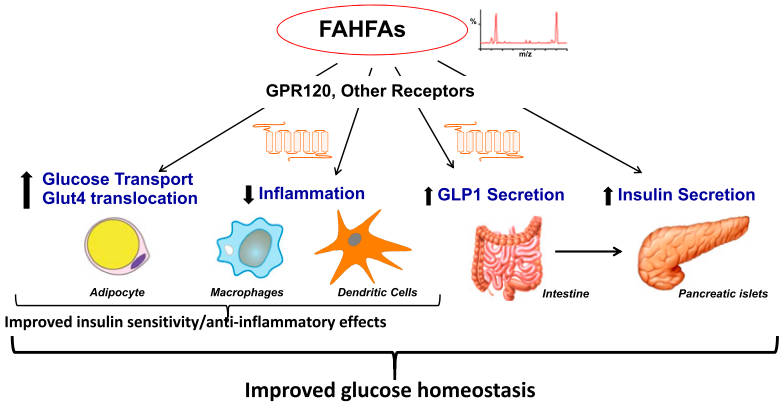

Figure 3—Regulation of glucose homeostasis by FAHFAs. FAHFAs exert a number of effects that result in improved glucose homeostasis. They activate GPR120 and other G-protein–coupled receptors, which mediate at least some of their local and systemic effects. FAHFAs have insulin-sensitizing effects, as evidenced by the fact that they augment insulin-stimulated glucose uptake in adipocytes by increasing GLUT4 translocation. FAHFAs are also anti-inflammatory. They reduce dendritic cell activation in vitro and AT inflammation in vivo including decreasing the production of proinflammatory cytokines by macrophages. FAHFAs also stimulate insulin secretion both directly and indirectly. They enhance insulin secretion indirectly by inducing GLP-1 secretion from enteroendocrine cells. FAHFAs also stimulate insulin secretion directly in human pancreatic islets. Together, the effects on insulin sensitivity, insulin secretion, and immune function improve systemic glucose homeostasis. Reprinted with permission from Yore et al. (27).

proinflammatory cytokines TNF and IL-1β (27). This indicates that part of the insulin-sensitizing effects of PAHSAs could be attributed to their anti-inflammatory properties (Fig. 3). 9-PAHSA inhibits lipopolysaccharide (LPS)-mediated activation of, and cytokine secretion from, bone marrow–derived dendritic cells (27). However, not all PAHSAs have anti-inflammatory roles. 5-PAHSA does not affect LPS-mediated macrophage or dendritic cell activation (27). This indicates that different isomers have differential roles, which are tissue and cell-type specific, and may involve different receptors.

The receptors mediating the anti-inflammatory effects of PAHSAs are not known. However, similar to the effects of PAHSAs, n-3 fatty acids reduce HFD-induced AT inflammation by inhibiting both TLR and TNF inflammatory signaling pathways (58). These effects appear to be GPR120 dependent. The mechanism of GPR120-mediated anti-inflammatory responses by n-3 fatty acids involves inhibition of c-Jun N-terminal kinase and nuclear factor-κB pathways through a β-arrestin2–dependent effect (58). This also inhibits inflammasome activation (58). GPR120 is expressed in macrophages and dendritic cells (11,12). Both natural and synthetic GPR120 agonists inhibit LPS-mediated inflammatory effects in wild-type but not GPR120 knockout macrophages indicating that GPR120 is involved in anti-inflammatory signaling in immune cells (12,58). It will be important to determine whether GPR120 mediates the anti-inflammatory effects of PAHSAs and if β-arrestin2 is involved.

The anti-inflammatory properties of PAHSAs appear to extend beyond obesity and T2D. 9-PAHSA oral treatment alleviates intestinal inflammation in a mouse model of colitis, and the mechanisms involve inhibition of immune cell activation (J. Lee, P. Moraes-Vieira, A. Castoldi, A. Saghatelian, B.B. Kahn, unpublished results). Thus, PAHSAs are promising anti-inflammatory lipids, which could have beneficial effects for the treatment of inflammatory diseases.

CONCLUSION

Although there are a number of antidiabetic agents in the clinic, there is still a large need for safe and highly effective insulin sensitizers. The fact that PAHSAs are insulin sensitizers as well as insulin secretagogues and have anti-inflammatory effects (Fig. 3) makes them unique candidates for drug development. Furthermore, the downregulation of PAHSAs in insulin resistant people with prediabetes (27) indicates that PAHSAs might be involved in the etiology of human T2D and also might hold promise as new biomarkers for insulin resistance and T2D risk. Restoring PAHSA levels may have beneficial effects on glucose control by ameliorating the deficiency of these lipids found in insulin resistant people. PAHSAs are endogenous molecules, and their concentrations fluctuate during normal physiology (e.g., fasting and refeeding). Therefore, restoring the levels in insulin resistant people is likely to be safe. These features make PAHSAs attractive candidates for further development. Given the unique combination of biologic activities, PAHSAs or PAHSA analogs have the potential to benefit millions of people worldwide with diabetes or inflammatory diseases.

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