REVIEW

Glutamine Metabolism in Macrophages: A Novel Target for Obesity/Type 2 Diabetes

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ABSTRACT

Obesity is a nutritional disorder resulting from a chronic imbalance between energy intake and expenditure. This disease is characterized by inflammation in multiple cell types, including macrophages. M1 macrophage responses are correlated with the progression of obesity or diabetes; therefore, strategies that induce repolarization of macrophages from an M1 to an M2 phenotype may be promising for the prevention of obesity- or diabetes-associated pathology. Glutamine (the most abundant amino acid in the plasma of humans and many other mammals including rats) is effective in inducing polarization of M2 macrophages through the glutamine–UDP-*N*-acetylglucosamine pathway and α-ketoglutarate produced via glutaminolysis, whereas succinate synthesized via glutamine-dependent anerplerosis or the γ-aminobutyric acid shunt promotes polarization of M1 macrophages. Interestingly, patients with obesity or diabetes show altered glutamine metabolism, including decreases in glutamine and α-ketoglutarate concentrations in serum but increases in succinate concentrations. Thus, manipulation of macrophage polarization through glutamine metabolism may provide a potential target for prevention of obesity- or diabetes-associated pathology. *Adv Nutr* 2019;10:321–330.

Keywords: α-ketoglutarate, diabetes, glutamine, obesity, macrophages, succinate

Introduction

As an essential component of the mononuclear phagocyte system, macrophages have a broad range of functions in the host, such as development, host defense, tissue homeostasis and repair, and pathology. Thus, macrophages show a high degree of plasticity and adapt their phenotypes to their microenvironments and their functional requirements (1). Macrophages are classified into 2 phenotypes: M1 macrophages (classically activated cells producing inflammatory cytokines) and M2 macrophages (alternatively activated and producing anti-inflammatory cytokines) (**Figure 1**), although this is an oversimplification of the consequences of macrophage activation. In response to stimulation from microbial products or proinflammatory cytokines (IFN-γ, TNF, or Toll-like receptor ligands, such as LPSs), macrophages are classically activated and have an M1 phenotype, including secretion of proinflammatory cytokines (e.g., IL-1β, IL-23) and reactive nitrogen and oxygen species (e.g., NO and reactive oxygen intermediates), high antigen presentation, as well as bactericidal and antitumor activities (2–5). Alternatively, upon stimulation with T-helper (Th) 2 (Th2) cytokines (e.g., IL-4, IL-10, and IL-13), macrophages acquire an anti-inflammatory M2 phenotype, characterized by upregulation of M2-specific markers (e.g., dectin-1, mannose receptor, and IL-10) and the production of ornithine and polyamines through the arginase–ornithine decarboxylase pathway, and the cells are also associated with immunosuppression, tissue remodeling, and tumor progression (6–10). At the cellular level, IFN-γ and LPS activate the interferon

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Abbreviations used: BMDM, bone-marrow–derived macrophage; CCL2, chemokine (C-C motif) ligand 2; FAO, fatty acid oxidation; GABA, γ-aminobutyric acid; GlcNAc, *N*-acetylglucosamine; GPR91, G protein–coupled receptor 91; GS, glutamine synthetase; HIF, hypoxia inducible factor; IKK, inhibitor of NF-κB kinase; IRF, interferon regulatory factor; OXPHOS, oxidative phosphorylation; PPP, pentose phosphate pathway; TCA, tricarboxylic acid; Th, T-helper.

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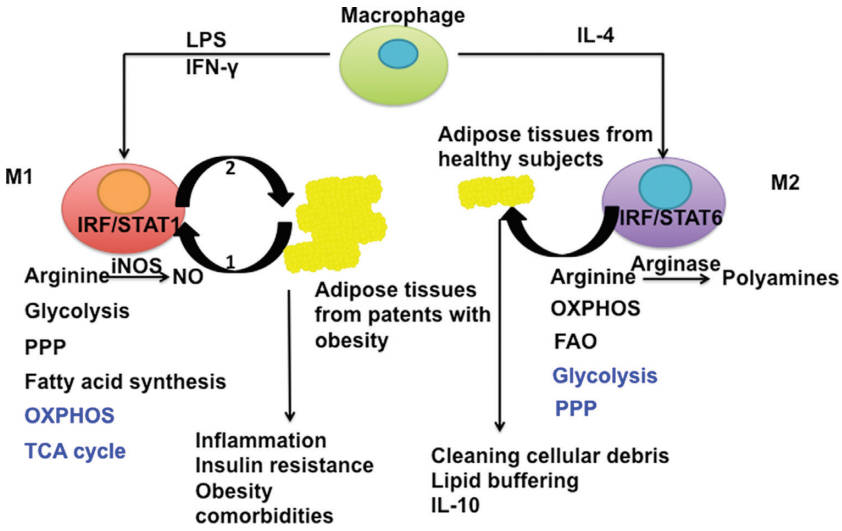

FIGURE 1 The relation between macrophages and obesity or diabetes. After activation of IRF/STAT-1 by stimulation with IFN-γ and LPS, macrophages convert to inflammatory macrophages (M1 macrophages), which are characterized by production of NO from arginine through iNOS activity, enhanced glycolysis, PPP flux, fatty acid synthesis, and impaired OXPHOS and TCA cycle activities. Anti-inflammatory macrophages (M2 macrophages, activation of IRF/STAT-6 with IL-4 stimulation) produce polyamines from arginine by arginase-1 and ornithine decarboxylase 1 and are characterized by OXPHOS, FAO, and less glycolysis and PPP. In lean adipose tissue, macrophages have an M2 phenotype and have critical roles in clearing cellular debris and lipid buffering, whereas macrophages in obese adipose tissue are M1 macrophages, which are activated by cytokines in the microenvironment of adipose tissues of obese individuals (1) and, in turn exacerbate inflammation in adipose tissue and trigger insulin resistance, as well as promote the development of metabolic syndrome associated with obesity (2). The blue pathway indicates activity in an impaired situation. FAO, fatty acid oxidation; iNOS, inducible NO synthase; IRF, interferon regulatory factor; OXPHOS, oxidative phosphorylation; PPP, pentose phosphate pathway; STAT, signal transducer and activator of transcription; TCA, tricarboxylic acid.

regulatory factor (IRF)/signal transducers and activators of transcription-1 signaling pathway to polarize macrophages toward the M1 phenotype, whereas IL-4 induces activation of IRF/signal transducers and activators of transcription-6 to skew macrophage functions toward an M2 phenotype (Figure 1) (4, 11, 12).

In addition to phenotypic and functional differences between M1 macrophages and M2 macrophages, there are metabolic characteristics that define subsets of macrophages. The established difference between M1 and M2 macrophages is the way in which they metabolize arginine. M1 macrophages convert arginine into NO through inducible NO synthase, whereas arginine is metabolized by arginase-1 for production of polyamines in M2 macrophages. In parallel with distinct differences in the metabolism of arginine, M1 macrophages are characterized by enhanced nonaerobic glycolysis, metabolic flux through the pentose phosphate pathway (PPP), fatty acid synthesis, and impaired mitochondrial oxidative phosphorylation (OXPHOS), as well as a truncated tricarboxylic acid (TCA) cycle, whereas M2 macrophages exhibit OXPHOS, fatty acid oxidation (FAO), decreased glycolysis, and less metabolism via the PPP (13–18) (Figure 1). These metabolic pathways not only provide energy but also regulate the phenotype and function of macrophages. For example, interference with glycolysis affects the activation and function of M1 macrophages and also regulates IL-4 responses in M2 macrophages (19–22). There are comprehensive reviews on glycolysis and FAO with respect to the polarization of macrophages (14–16).

Usually, in response to an infection, macrophages polarize initially to the M1 phenotype to orchestrate host immunity and then to the M2 phenotype to restrain proinflammatory responses during the repair of damaged tissues. However, deregulated activation of M1 macrophages, the prolonged restriction of M1 activation, and defects in the phenotypic switch in macrophages are associated with various diseases, such as obesity or diabetes (23–25). In the white adipose tissue of lean animals, macrophages have an M2 phenotype and have critical roles in adipose tissue homeostasis by clearing cellular debris and lipid buffering, whereas macrophages in the white adipose tissue of obese animals are M1 macrophages, producing proinflammatory cytokines to amplify inflammation and to contribute to obesity-induced insulin resistance, leading to type 2 diabetes (Figure 1) (16, 26, 27). The M1 macrophages are activated within the microenvironment of the white adipose tissue of obese individuals by lipids, lipoproteins, and cytokines; and then M1 macrophages exacerbate inflammation in adipose tissue, trigger insulin resistance, and promote the development of obesity-associated metabolic syndromes (Figure 1) (28–30). Thus, precise regulation of macrophage polarization may be helpful for the prevention of obesity- or diabetes-associated pathology. Notably, glutamine metabolism modulates the polarization of macrophages. For example, glutamine is

especially effective in inducing polarization of mouse M2 macrophages through induction of the glutamine–UDP-*N*-acetylglucosamine (GlcNAc) pathway and production of α-ketoglutarate, whereas succinate produced via glutamine-dependent anerplerosis or the γ-aminobutyric acid (GABA) shunt promotes polarization of mouse M1 macrophages (18, 31). This review highlights the recent understanding of regulatory functions of glutamine metabolism in macrophage polarization and consequences of the manipulation of glutamine metabolism in macrophages to prevent or ameliorate obesity or type 2 diabetes.

Macrophages in Obesity or Type 2 Diabetes

Macrophages in obesity

Alterations in macrophage function and polarization are associated with various human diseases. Exaggerated or prolonged M1 responses are involved in inflammatory diseases (e.g., sepsis, atherosclerosis, rheumatoid arthritis) and metabolic diseases (e.g., obesity or diabetes) (for reviews see references 32–34). For example, in the white adipose tissue from lean mice, the macrophages constitute <10% of the resident cells and macrophages resemble M2 macrophages; however, macrophages make up 50% of all cells in the white adipose tissue of obese mice and have an M1 phenotype (35, 36). Mechanistically, under metabolic stress associated with a high-fat diet, adipocytes produce greater amounts of TNF-α, FFAs, and chemokine (C-C motif) ligand 2 (CCL2; a chemotactic factor for monocytes), which recruit monocytes to white adipose tissue where they polarize to M1 macrophages in response to TNF-α and FFAs (for a review see reference 37). A mixture of glucose, insulin, and palmitate (conditions characteristic of metabolic syndrome) and obesity-induced hypoxia also activate macrophages (38, 39). Interestingly, although the metabolically activated macrophages produce proinflammatory cytokines (e.g., IL-1β and TNF-α), they also exhibit a phenotype that is different from classically activated macrophages in that they exhibit distinct expression patterns for cell surface proteins (e.g., CD38, CD319, and CD274) and activation of cellular signaling pathways (e.g., NF-κB) (38). Also, M1 macrophages in the white adipose tissues of obese individuals may derive from primitive yolk-sac progenitors and self-renew via proliferation (40).

Available evidence suggests that the metabolic microenvironment in white adipose tissue is mainly responsible for polarization of macrophages into an M1 phenotype. In turn, M1 macrophages in adipose tissue produce inflammatory cytokines such as TNF-α, IL-1β, and IL-6 to counteract the insulin-sensitizing action of adiponectin and leptin, resulting in insulin resistance and development of metabolic syndrome (for reviews see references 41 and 42). Thus, mice deficient in TNF-α, CCL2, or CCR2 (the CCL2 receptor) have fewer M1 macrophages and higher insulin sensitivity when fed a high-fat diet compared with wild-type control mice (43–45).

Collectively, macrophage infiltration and M1 macrophage polarization correlate with the degree of obesity; therefore, regulation of macrophage polarization may contribute to prevention or amelioration of obesity-associated pathology. Interestingly, there is evidence for alterations in iron metabolism in macrophages in a mouse model of obesity (46), suggesting that iron metabolism in macrophages may also be involved in the degree of obesity. In addition to pro- and anti-inflammatory macrophages, other types of immune cells are also detected in white adipose tissues from obese humans and mouse models of obesity (47–49), such as $CD4^+$ and $CD8^+$ T cells, dendritic cells, B cells, neutrophils, and NK cells. This suggests that the immune responses in obesity involve an array of immune cells. Thus, the roles of each type of immune cell in inflammation of adipose tissue from obese individuals require further investigations.

Macrophages in type 2 diabetes

Among diabetic patients, 95% have type 2 diabetes that is characterized by insulin resistance, β cell dysfunction, and obesity resulting from excess caloric intake and insufficient physical activity. Chronic tissue inflammation, especially from M1-polarized macrophages, may be a critical pathogenic mediator in the development of type 2 diabetes, insulin resistance, and β cell dysfunction (for reviews see references 50 and 51). Although M2 macrophages are essential for β cell proliferation, macrophages are polarized into M1 macrophages during inflammation of islets, which contributes to β cell dysfunction in mouse models of type 2 diabetes (52–55). M1 macrophages produce various inflammatory cytokines, like IL-1β, that result in local and systemic inflammation, dysfunction of pancreatic β cells, and insulin resistance in liver, adipose, and musculoskeletal tissues (50). Interference with the IL-1 pathway in animal models and even in humans can alleviate defects in β cells (56–59). Notably, the production of proinflammatory cytokines, chemokines, and proteases also promotes the accumulation of other inflammatory cells, as well as apoptosis, angiogenesis, and matrix protein remodeling. M1 macrophages are also associated with the complications of diabetes, such as nephropathy, neuropathy, retinopathy, and cardiovascular diseases (for a review see reference 51). The underlying mechanism for the accumulation of M1 macrophages in diabetic patients has not been established. Nevertheless, strategies to induce repolarization of tissue macrophages from an M1 to an M2 phenotype may be promising for the prevention or amelioration of obesity- or diabetes-associated pathology.

Glutamine Metabolism in Macrophage Polarization

Glutamine accumulation in M2 macrophages

Tavakoli et al. (60) found that M2 macrophages in mice accumulate glutamine and they determined intracellular concentrations of glutamine in response to IL-4, LPS or

IFN-γ plus TNF-α in vitro. IFN-γ plus TNF-α had little effect on the accumulation of intracellular glutamine, whereas LPS induced a decrease at 6 h, then a steady increase back to baseline levels at 24 h, and a 1.3-fold increase after 48 h, compared with the unstimulated macrophages. Interestingly, IL-4 induced the accumulation of glutamine over the period of 24 h relative to unstimulated macrophages. The accumulation of glutamine in macrophages may depend on glutamine uptake because IL-4 induces the expression of *Slc1a5* (*Solute carrier family 1 member 5*; a transporter for glutamine), and LPS increases the expression of *Slc3a2* (the heavy subunit of the large neutral amino acid transporter) at 48 h in macrophages (60). Further, LPS induces the expression of *Slc3a2* in mouse bone-marrow–derived macrophages (BMDMs) through the activation of NF-κB (31). Besides the uptake of glutamine by M2 macrophages, their accumulation of glutamine may also involve their synthesis of glutamine from glutamate and ammonia via glutamine synthetase (GS). GS is barely detectable in M1 macrophages but is highly expressed in M2 macrophages, particularly in response to IL-10 (61). Although intracellular glutamine is more abundant in IL-10–stimulated M2 than in control macrophages, methionine sulfoximine (a GS inhibitor) reduces the intracellular levels of glutamine in IL-10–stimulated macrophages (61). Collectively, glutamine likely accumulates in M2 macrophages owing to increased glutamine uptake and the synthesis of glutamine from glutamate.

Glutamine promotes M2 macrophage polarization

In mouse BMDMs, glutamine deprivation for 4 h before stimulation has a substantial effect on M2 polarization. This is evidenced by a reduction in the population of M2 macrophages by ~50% based on the expression of M2 activation markers (CD206, CD301, and Relm α); however, removal of glutamine had no effect on the capacity for M1 polarization based on the expression of NO synthase 2 (NOS2) in response to LPS and IFN-γ. Transcriptional analysis revealed that withdrawal of glutamine decreases expression of several M2-specific marker genes, including *Irf4*, *Ccl22*, and *Il4i1* and deprivation of glutamine in M2-polarized macrophages decreased the transcriptional signature of TCA cycle activity, compared with polarized M2 macrophages (62). However, this result does not eliminate other possible consequences of glutamine withdrawal on M2 macrophages, such as an increase in apoptosis of M2 macrophages. Another independent group also found that glutamine deprivation in vitro impairs expression of mRNAs for M2-specific markers after IL-4 stimulation, including *Arg1* (*Arginase 1*), *Ym1* (*Chitinase-like 3*), *Retnla* (*Resistin-like alpha 1*), and *Mrc1* (*Mannose receptor C type 1*), while increasing expression of M1-specific markers in response to LPS, including *Il1β*, *Tnf*, *Il6*, and *Il12*, compared with the mouse BMDMs activated in glutamine-replete culture medium (18). Thus, glutamine is essential for M2 polarization.

Glutamine promotes M2 macrophage polarization through the α-ketoglutarate and glutamine–UDP-GlcNAc pathways

α-Ketoglutarate derived from glutaminolysis promotes M2 macrophage polarization. Inhibition of glutaminase 1 (an enzyme for glutamine hydrolysis) decreases expression of the M2 phenotype in IL-4–treated mouse BMDMs, including expression of the M2 marker gene arginase 1. In contrast, dimethyl-αKG (DM-αKG), a cell-permeable analog of α-ketoglutarate, rescues the M2 phenotype, suggesting that α-ketoglutarate generated from glutaminolysis promotes the M2 phenotype. Mechanistically, α-ketoglutarate is essential for increasing OXPHOS and FAO in M2 macrophages (**Figure 2**). Meanwhile, α-ketoglutarate induces the M2 phenotype through Jmjd3 (Jumonji domain-containing 3, a key enzyme for demethylation of H3K27)-dependent demethylation of H3K27 in the promoter region of M2-specific marker genes (Figure 2) (18). Also, in LPS-stimulated mouse macrophages, α-ketoglutarate inhibits the activation of inhibitor of NF-κB kinase (IKK) via the prolyl hydroxylase domain, which inhibits activation of IKKβ through hydroxylation of IKKβ on P191 (Figure 2) (18, 63). Notably, M1 macrophages have a potential breakpoint in the metabolic flow of the TCA cycle at the isocitrate to α-ketoglutarate step, as evidenced by a higher ratio of isocitrate:α-ketoglutarate and lower expression of isocitrate dehydrogenase 1 (Idh1), which catalyzes oxidative decarboxylation of isocitrate to α-ketoglutarate, in M1 macrophages compared with M0 macrophages (Figure 2) (62).

The pathway for synthesis of UDP-GlcNAc is critical for M2 macrophage polarization because it is responsible for glycosylation of M2 marker proteins (e.g., macrophage mannose receptor and macrophage galactose binding lectin) (62). The pathway for UDP-GlcNAc synthesis is upregulated in mouse M2 macrophages, and tunicamycin (a *N*-glycosylation inhibitor) inhibits the expression of canonical M2 activation marker genes in macrophages stimulated with IL-4, including Relm α, CD206, and CD301, but has only a minor effect on expression of inducible NO synthase or major M1-specific cytokines (62). Although glucose is the major source of carbons in the synthesis of UDP-GlcNAc, more than one-half of the nitrogen in UDP-GlcNAc derives from glutamine (62). Indeed, glutamine is associated with UDP-GlcNAc synthesis via the hexosamine biosynthetic pathway. Collectively, glutamine promotes M2 macrophage polarization via the glutamine–UDP-GlcNAc pathway and α-ketoglutarate derived from glutaminolysis.

Succinate from glutamine-dependent anerplerosis promotes M1 polarization

In LPS-stimulated mouse macrophages, glutamine is used for the accumulation of succinate in macrophages (Figure 2). Through glutamine-dependent anerplerosis, LPS promotes the accumulation of succinate in macrophages, which stabilizes hypoxia inducible factor (HIF) 1α, resulting specifically in the regulation of expression of IL-1β and other

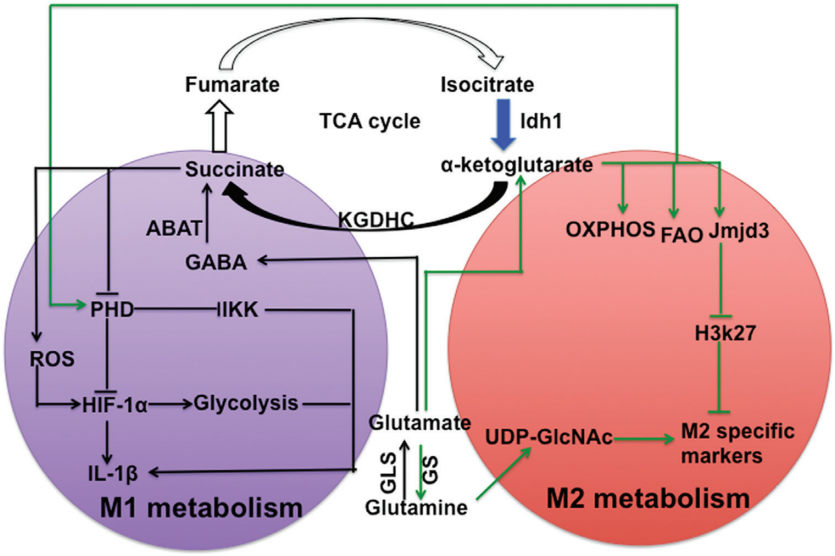

FIGURE 2 Glutamine metabolism and macrophage polarization. In M1 macrophages, succinate accumulates due to glutamine-dependent anerplerosis and the GABA shunt. Succinate stabilizes HIF-1α through inhibiting the enzymatic activities of PHD or ROS, resulting in specific regulation of expression of IL-1β and other HIF-1α–dependent genes, including enzymes required for glycolysis. In M2 macrophages, α-ketoglutarate generated from glutaminolysis is essential for OXPHOS and FAO and promotes an M2 phenotype through Jmjd3 (a key enzyme for demethylation of H3K27)-dependent demethylation of H3K27 on the promoters of M2-specific marker genes, as well as inhibition of the activation of IKK through PHD, which inhibits the activation of IKKβ through hydroxylation of IKKβ on P191. Glutamine also supports M2 macrophage polarization through the glutamine–UDP-GlcNAc pathway. Also, M2 macrophages have a potential isocitrate to α-ketoglutarate conversion breakpoint in the metabolic flow of the TCA cycle. Pathways in black are enhanced in M1 macrophages, the pathways in blue are impaired, and pathways in red enhance differentiation of M2 macrophages. ABAT, 4-aminobutyrate aminotransferase; FAO, fatty acid oxidation; GABA, γ-aminobutyric acid; GlcNAc, glutamine–UDP-*N*-acetylglucosamine; GLS; glutaminase; GS, glutamine synthetase; HIF-1α, hypoxia inducible factor 1α; Idh1, isocitrate dehydrogenase 1; IKK, inhibitor of NF-κB kinase; Jmjd3, Jumonji domain-containing 3; KGDHC, α-ketoglutarate dehydrogenase complex; OXPHOS, oxidative phosphorylation; PHD, prolyl hydroxylase domain; ROS, reactive oxygen species; TCA, tricarboxylic acid.

HIF-1α–dependent genes, including enzymes required for glycolysis (e.g., glucose transporter 1, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3, and monocarboxylate transporter 4), which are essential for the activation of LPS-stimulated macrophages, and the succinylation of proteins, like glyceraldehyde-3-phosphate dehydrogenase, glutamate carrier 1, and L-lactate dehydrogenase A chain (Figure 2) (31, 64). As for glutamine anerplerosis, a proportion of the increase in succinate in macrophages in response to LPS is derived from the GABA shunt (a bypass of the TCA cycle in which glutamine is used for synthesis of glutamate, GABA, succinic semialdehyde, and eventually succinate) (31). Vigabatrin (an irreversible inhibitor of the key GABA shunt enzyme, GABA transaminase) significantly reduces the concentration of succinate in macrophages, leading to reductions in LPS-induced stabilization of HIF-1α and secretion of IL-1β by macrophages (31). Succinate promotes the stabilization of HIF-1α by inhibiting the enzymatic activity of the prolyl hydroxylase domain, which mediates steady-state degradation of HIF-1α (31, 65). In addition, succinate promotes, indirectly, the stabilization of HIF-1α via the induction of reactive oxygen species (66). Inhibition of GS in IL-10–stimulated macrophages with methionine sulfoximine increases intracellular succinate, which promotes switching of macrophage polarization from an M2- to an M1-like phenotype through actions of HIF-1α (61). Collectively, succinate from glutaminolysis effects the polarization of M1 macrophages.

In summary, glutamine is highly effective in activating M2 macrophages, whereas succinate promotes the activation and function of M1 macrophages (Figure 2). Interestingly, there is evidence that intracellular metabolism of iron also regulates the polarization of macrophages, although its effects on classically activated macrophages and macrophages in adipose tissues are different (for a review, see reference 67). Whether glutamine affects macrophage polarization through glutamine-dependent purine metabolism or pyrimidine metabolism or glutamine-independent iron metabolism is unclear. The involvement of mammalian target of rapamycin signaling in mediating the regulatory roles of glutamine metabolism in the activation and function of macrophages is also largely unexplored, even though mammalian target of rapamycin signaling shapes T cell fate decision and macrophage polarization (12, 68–70). The function of other metabolites from glutaminolysis in modifying the phenotype of macrophages requires additional research. For example, fumarate resulting from glutaminolysis has critical roles in the induction of trained immunity by inhibiting KDM5

(lysine demethylase 5) histone demethylases to induce monocyte epigenetic reprogramming (71). Whether fumarate also shapes the macrophage phenotype through epigenetic reprogramming remains to be determined. Also, considering the heterogeneity of macrophages (2), it would be interesting to conduct comparative studies on the influence of glutamine metabolism on polarization of macrophages of different origins (e.g., embryonic origin compared with monocyte derivation) or macrophages from different tissues, such as bone (osteoclasts), lung (alveolar macrophages), central nervous system (microglial cells), connective tissue (histiocytes), liver (Kupffer cells), and skin (Langerhans cells).

Modulation of glutamine metabolism in macrophages for obesity or diabetes

As cellular metabolism of glutamine affects the polarization of macrophages, strategies to target and reverse macrophage polarization through glutamine metabolism may prevent or ameliorate obesity- or diabetes-associated pathology. This possibility deserves further study. However, because most investigations of effects of glutamine metabolism on macrophage polarization were conducted in vitro with mouse macrophages, it is not known if glutamine metabolism has similar effects in human macrophage polarization in vitro or in vivo. Also, macrophages were activated with LPS or IL-10 in published results, which makes it unclear if glutamine metabolism also shapes the polarization of metabolically activated macrophages, especially considering that metabolically activated macrophages have some distinct phenotypes compared with classically activated macrophages. Indeed, there is a decrease in glutaminolysis in the subcutaneous adipose tissue of obese compared with lean subjects (72).

In a study comparing metabolites in the serum of 80 children with obesity and 40 normal-weight children between 6 and 15 y of age using an MS-based metabolomics approach, obese children had lower concentrations of glutamine in their serum than did normal-weight children (73). Also, there are strong associations between insulin resistance and concentrations of glutamine or glutamate in plasma, as well as the ratio of glutamine to glutamate, indicating that a high ratio of glutamine to glutamate is associated with a lower risk of diabetes mellitus (74). However, in obese animals, the quantitative contributions of white adipose tissue to concentrations of glutamine in serum are not known. Skeletal muscle contributes to concentrations of glutamine in serum, and obese patients have less muscle mass, less muscle protein synthesis, and greater muscle proteolysis (75, 76); therefore, the reduction in muscle may account for decreases in serum concentrations of glutamine in obese individuals. Interestingly, patients with obesity also have higher concentrations of glutaminase (an enzyme that converts glutamine to glutamate) but lower concentrations of GS in the subcutaneous adipose tissues compared with the lean subjects (72). Analyses of glutamine and glutamate in the white adipose tissue of obese patients are needed to determine the degree to which glutamine influences the severity of obesity. Also, such studies would clarify the relevance of glutamine metabolism as a viable target for therapeutic intervention to prevent or ameliorate obesity or diabetes. In obese patients with type 2 diabetes or nondiabetic symptoms, glutamine administration increases the secretion of glucagon-like peptide 1, glucose-dependent insulinotropic polypeptide, and insulin (77). Similarly, glutamine uptake in patients with type 2 diabetes enhances the postprandial insulin response and glucagon-like peptide 1 response (78). However, whether glutamine influences the secretion of insulin in patients with type 2 diabetes by modulating macrophage polarization is unknown. Thus, further studies in animal models with clodronate liposome–mediated clearance of macrophages are needed. Glutamine has been reported to enhance pancreatic β cell function through macrophage-independent mechanisms. For example, dipeptide l-alanyl-l-glutamine significantly attenuates the decrease in chronic insulin secretion from β cells during treatment with inflammatory cytokines through coordinated effects on the glutamine–glutathione axis, heat shock protein pathway, and mitochondrial metabolism, which are essential for insulin release (79).

The Zucker obese rat (a model of type 2 diabetes) has lower amounts of α-ketoglutarate in urine than Wistar rats (80). However, the contribution of α-ketoglutarate in adipose tissue to its urinary excretion needs further investigation. Indeed, the expression of both glutamate pyruvate transaminase 1 and 2, which catalyze the conversion of glutamate to α-ketoglutarate in the cytosol and mitochondria, respectively, is downregulated in subcutaneous white adipose tissue of patients with obesity compared with lean subjects (72). Thus, α-ketoglutarate may be a potential candidate for therapeutic prevention of obesity- or diabetes-associated pathology. For example, α-ketoglutarate supplementation alleviates adipocyte inflammation and increases the ratio of M2 to M1 macrophages in the white adipose tissue of mice (81).

Succinate promotes the polarization of M1 macrophages, and thus it may also be a promising target for prevention of obesity- or diabetes-associated pathology. Interestingly, a high-fat diet increases the concentration of succinate in the serum of rats (82) and in the white adipose tissue of mice (83, 84). The concentration of succinate in plasma is significantly associated with BMI in humans (85), and concentrations of succinate in plasma are greater in patients with type 2 diabetes than in nondiabetic individuals (86). Succinate elicits secretion of IL-1β by mouse macrophages in a G protein–coupled receptor 91 (GPR91)–dependent manner (87). Likewise, GPR91-deficient mice have greater energy expenditure and glucose buffering and less white adipose tissue when fed an unpurified diet (88). Compellingly, a GPR91 deficiency protects mice fed a high-fat diet from obesity during the initial period (88), reduces the number of macrophages in mouse adipose tissues, and inhibits chemotaxis of mouse macrophages (86). Thus, the succinate–GPR91 axis may be modulated to prevent obesity.

Collectively, patients with obesity or diabetes have lower concentrations of glutamine and α-ketoglutarate but higher concentrations of succinate in their serum (**Figure 3**).

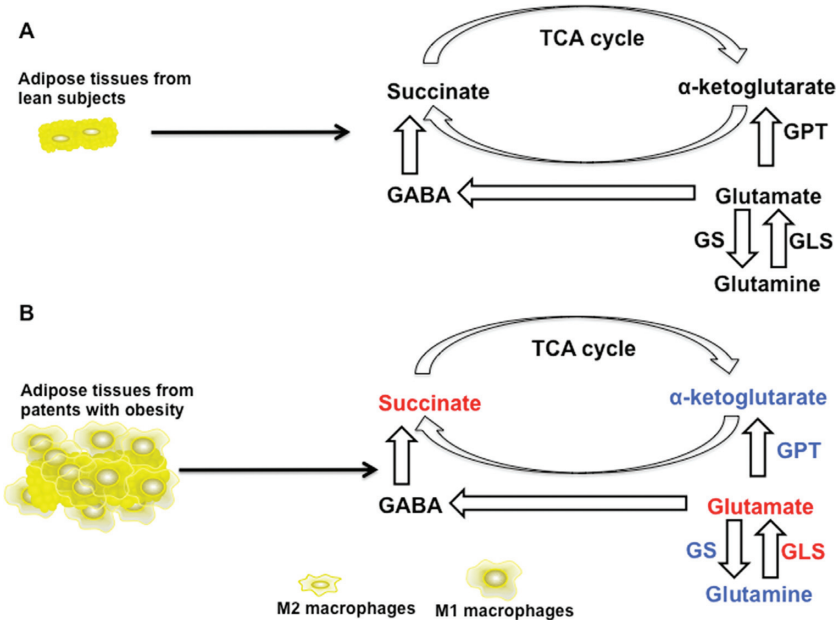

FIGURE 3 Altered glutamine metabolism in adipose tissues from individuals with obesity or type 2 diabetes. (A) In adipose tissue from healthy subjects, which has fewer M2 macrophages, glutamine is catalyzed for generation of succinate through glutamine-dependent anerplerosis and GABA shunt. (B) In adipose tissue from obese individuals, which has more M1 macrophages, glutamine and α-ketoglutarate decrease (blue), whereas glutamate and succinate increase (red). Mechanistically, the abundance of glutaminase (an enzyme that converts glutamine to glutamate) increases (red), but the abundance of GS (an enzyme that converts glutamate to glutamine) and GPT (an enzyme that catalyzes the conversion of glutamate to α-ketoglutarate) decreases (blue). GABA, γ-aminobutyric acid; GLS, glutaminase; GPT, glutamate pyruvate transaminase; GS, glutamine synthetase; TCA, tricarboxylic acid.

This is associated positively with the accumulation of M1 macrophages in obesity or diabetes. Thus, manipulation of macrophage polarization through these metabolites may be a promising strategy for the prevention of obesity- or diabetes-associated pathology. Considering that inflammation in obesity or type 2 diabetes may involve multiple immune cells and not only macrophages, repolarization of macrophages with glutamine metabolism only in the white adipose tissue is not sufficient to alleviate obesity or type 2 diabetes. Fortunately, there is increasing research on the importance of glutamine in other types of immune cells, such as T cells, B cells, and neutrophils. For example, glutamine is critical for the activation of T cells and the differentiation of mouse Th1 and Th17 cells (89). Compensatory changes and time variables in the pathogenesis of obesity or type 2 diabetes will be a challenge to those who wish to identify strategies to reverse the polarization of macrophages from those associated with diabetes to those associated with metabolically healthy individuals. Nevertheless, manipulation of glutamine metabolism may be a promising target for therapeutic candidates that may ameliorate obesity- or diabetes-associated pathology.

Concluding Remarks and Future Directions

Collectively, M1 macrophages are associated with the development of obesity or diabetes. Strategies to manipulate repolarization of tissue macrophages from an M1 to an M2 phenotype may be promising for the prevention or amelioration of obesity- or diabetes-associated pathology. Unfortunately, current knowledge of glutamine metabolism with respect to macrophage polarization is mainly from in vitro investigations with classically activated murine macrophages. The regulatory function of glutamine metabolism in human macrophage polarization in vivo is unknown; therefore, it is important that researchers determine the regulatory function of glutamine metabolism in metabolically activated human macrophages in white adipose tissue during the development of obesity and diabetes. Notably, glutamine and α-ketoglutarate are known to be beneficial in patients with obesity or diabetes and related animal models, although it is not known if they regulate the progression of obesity or diabetes through affecting macrophage polarization. Considering that other metabolic pathways also regulate macrophage polarization (90, 91), targeting glutamine metabolism with other metabolic pathways, such as glycolysis and PPP (92–94), will be a promising strategy for prevention of obesity- or diabetes-associated pathology. Unfortunately, it is not currently possible to specifically manipulate metabolic pathways [e.g., arginine metabolism and NO synthesis (95)] in macrophages of adipose tissue. But, manipulation of metabolic pathways in macrophages can be combined with traditional agents for modifying macrophage function, such as inhibition of recruitment of inflammatory macrophages through alterations in

CCR2–CCL2 signaling and C-X-C motif chemokine receptor 4 (CXCR4)–C-X-C motif chemokine ligand 12 (CXCL12) signaling (96).

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