Glutamine diet supplementation prevents obesity through inhibiting inflammation

Ying Yang
Department of Molecular Biology and Biochemistry; School of Biological Sciences, University of California, Irvine, Irvine, CA 92697, USA Correspondence: yingy33@uci.edu

Abstract
Obesity has become a serious health concern worldwide because it is associated with a higher risk of heart diseases, diabetes, and even certain types of cancers. Since diet and lifestyle adjustments could be very challenging, there has been growing interest in dietary supplements to promote bodyweight loss and decrease the relapse rate. Therefore, many different kinds of food supplementations are now popular in the United States; however, the effects and mechanisms of most supplementations are still understudied. It has been established that obesity is characterized by chronic inflammation in white adipose tissue. Interestingly, glutamine, which is normally used to boost the immune system has shown the function of decreasing body weight and composition of fat tissues in humans and animals by reducing obesity-related inflammation. However, the underlying mechanism of dietary glutamine’s effect on obesity is still far from clear. Here, in this review, we will discuss the current understanding of the use of glutamine supplementation as a treatment for obesity. The elucidation of the current dietary strategies using glutamine may highlight new approaches for obesity and diabetes treatment.

Keywords: Obesity, supplementations, glutamine, inflammation

Obesity has shown an unprecedented increase worldwide in the last several decades. According to recently published statistics, 39.8% of adults in the United States have obesity, which indicates more than 93 million people are suffering from it (1). Since obesity may increase the risks for other diseases such as cardiovascular disease, arthritis, hypertension, hyperlipidemia, non-alcoholic fatty liver disease, type 2 diabetes (T2D) and some types of cancers (2), it is considered a serious medical problem that affects the development of metabolic diseases (3). Specifically, obesity is the leading risk for T2D (4), which is evidenced by obese adults having three to seven times higher risk of developing T2D compared to those individuals with normal weight. Additionally, obesity is associated with up to 30% higher in medical costs than those with normal weight (5). Thus, obesity has attracted considerable attention to societies.

Besides surgery, the current medical treatments for obesity approved by the Food and Drug Administration (FDA) include Orlistat (Xenical), Lorcaserin (Belviq), Phentermine and topiramate (Qsymia), Bupropion/ naltrexone (Contrave), and Exenatide (Byetta). Unfortunately, the effects of these medicines may wane over time, and some may even have severe side effects. Meanwhile, it is common that people who lose weight regain the weight within a short time (6) no matter what treatment methods they try. This suggests that more effective and safe treatments are urgently needed for obese patients. This review mainly focuses on recently discovered diet supplementation interventions, with a specific focus on glutamine, and discusses the possible mechanisms of actions of glutamine supplementation on inflammation.

Dietary supplementation in treating obesity

According to the FDA, dietary supplements are products taken by mouth which contain a “dietary ingredient” such as vitamins, minerals, amino acids, herbs or some other substances in the diet. Usually, dietary supplements can be administered as tablets, capsules, powders, energy bars, and liquids. Compared to pharmacologic agents and surgery, dietary supplements represent alternatives to
traditional therapy because most of the ingredients have a low-toxicity profile and low cost. Since dietary supplements do not require premarket review or approval by the FDA, many ingredients are studied and have shown to promote weight loss and reduce body fat by different biological mechanisms (Table 1). So far, the data from scientific publications on each ingredient varies considerably and with limited information on the safety and efficacy. In addition, more studies tried to use supplement combinations partially due to the failures of a single dietary supplement.

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Possible Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bitter orange</td>
<td>Promote lipolysis; inhibit cyclic adenosine monophosphate (cAMP)</td>
<td>(7)</td>
</tr>
<tr>
<td>β-glucans</td>
<td>Non-digestible fiber contains linear β-1,3, 1-4-linkages</td>
<td>(8)</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>Increase peroxisome proliferator activated receptor gamma (PPAR-γ), uncoupling protein 1 (UCP-1), and peroxisome proliferator activated receptor gamma coactivator (PGC-1α)</td>
<td>(9)</td>
</tr>
<tr>
<td>Carnitine</td>
<td>Decrease the intramitochondrial acetyl-CoA/CoA ratio and activate pyruvate dehydrogenase complex (PDHC)</td>
<td>(10)</td>
</tr>
<tr>
<td>Chitosan</td>
<td>An insoluble fiber</td>
<td>(11)</td>
</tr>
<tr>
<td>Forskolin</td>
<td>Stimulate cAMP</td>
<td>(12)</td>
</tr>
<tr>
<td>Fucoxanthin</td>
<td>Decrease the expression of acetyl-CoA carboxylase; decrease the expression of PPAR-γ, CCAAT enhancer binding proteins (C-EBPα), sterol regulatory element binding protein 1 (SREBP-1c) at the late stages of adipocyte differentiation; increase the expression of PPAR-γ, C-EBPα, SREBP-1c, fatty acid binding proteins (FABP), lipoprotein lipase, and glucose transporter4 (GLUT4) at the early stages of adipocyte differentiation</td>
<td>(13)</td>
</tr>
<tr>
<td>Garcinia cambogia</td>
<td>Inhibit citrate lyase</td>
<td>(14)</td>
</tr>
<tr>
<td>Glucomannan</td>
<td>Human salivary and pancreatic amylase cannot split β-1,4-linkages</td>
<td>(15)</td>
</tr>
<tr>
<td>Green coffee</td>
<td>Inhibit fatty acid synthase, hydroxymethylglutaryl-CoA reductase, and acyl-CoA-cholesterol acyltransferase; promote PPAR-γ expression</td>
<td>(16)</td>
</tr>
<tr>
<td>Guar gum</td>
<td>β-1,4-linkages</td>
<td>(17)</td>
</tr>
<tr>
<td>Hoodia gordonii</td>
<td>Increase hypothalamic ATP production</td>
<td>(18)</td>
</tr>
<tr>
<td>Irvingia gabonensis</td>
<td>Inhibit the expression of PPAR-γ and leptin; increase adiponectin expression</td>
<td>(19)</td>
</tr>
<tr>
<td>Raspberry ketone</td>
<td>Decrease the expression of PPAR-γ, C-EBPα, FABP2, acetyl-CoA carboxylase 1, fatty acid synthase, and stearoyl-CoA desaturase 1; increase adipose triglyceride lipase, hormone-sensitive lipase and carnitine palmitoyl transferase</td>
<td>(20, 21)</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>Unclear</td>
<td>(22, 23)</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Reduce inflammation</td>
<td>(24)</td>
</tr>
<tr>
<td>White kidney bean</td>
<td>Inhibit pancreatic amylase</td>
<td>(25)</td>
</tr>
</tbody>
</table>

Table 1. Dietary supplementation in treating obesity
One possible mechanism of more than half of the ingredients in the most popular supplements is functioning through increasing peroxisome proliferator-activated receptor gamma (PPAR-γ) expression, which is a significant transcription factor regulating fatty acid storage and glucose metabolism. Interestingly, another possible mechanism is that some supplements have effectively inhibited pancreatic lipase activity, especially amylase, to stimulate the white and brown adipose (26). Alternatively, some ingredients are just soluble or insoluble fibers to inhibit fat absorption in the intestine.

Compared to supplements derived from food or product ingredients, there are limited studies on amino acid supplements except for branched-chain amino acids (leucine, isoleucine, and valine), which were shown to provide many physiological and metabolic benefits. Specifically, leucine is considered the main acting ingredient, in branched amino acid supplements, activating the mTOR signaling pathway and initiating protein synthesis (27). Amino acids are building blocks for proteins and are also essential factors in cellular development and repair, reactive oxygen species (ROS) homeostasis, the immune system, and the digestive system. Although amino acids dietary regimens have been comprehensively investigated for cancer therapies (28-30), their effect on obesity and diabetes remains understudied. Meanwhile, several groups consistently reported that the plasma amino acid profiles from obese people indicate a significant decrease in glutamine or its downstream intermediate glutamate (31-33). Therefore, the use of dietary glutamine supplementation to combat obesity has become an appealing approach for further studies in animal models and humans.

**Glutamine metabolism**

Glutamine, which occupies the central place in the biosynthesis of macromolecules, was considered a non-essential amino acid as the human body has the capability of synthesizing it. Theoretically, glutamine can be produced by glutamate dehydrogenase, which converts α-KG into glutamate, and glutamine synthetase which catalyzes glutamate into glutamine (34). During cell proliferation, glutamine, either from endogenous or exogenous, is an important intermediate to be used for producing amino acids, lipids and redox control molecules.

Four families of solute carrier (SLC)-type transporters are capable of transporting glutamine across the plasma membrane, which are SLC1, SLC6, SLC7 and SLC38. Generally, SLC38 is thought to be the major glutamine transporters (35). After entering cells, glutamine is deamidated to glutamate in mitochondria via glutaminase and then converted to α-KG which serves in replenishing the TCA cycle. During this process, NADH or NADPH are generated as high energy compounds and produce ATP through the electron transport chain (ETC) with oxidative phosphorylation (36). Then, during the conversion of glutamine to glutamate, the γ-nitrogen is provided for de novo synthesis of purine and pyrimidine through four rate-limiting enzymes including carbamoyl phosphate synthetase 2 (CAD), phosphoribosyl pyrophosphate amidotransferase (PPAT), and phosphoribosyl pyrophosphate synthetases 1 and 2 (PRPS1 and PRPS2) (37). Furthermore, mitochondrial glutamate is transported back to the cytosol where glutamate and cysteine are synthesized to gamma-glutamylcysteine by glutamate-cysteine ligase (GCL) followed by glycine binding to the C-terminal of gamma-glutamylcysteine which is catalyzed by glutathione synthetase. These adenosine triphosphate dependent steps are the main process of Glutathione biosynthesis (38).

**Glutamine supplement decreases fat tissue inflammation**

Obesity, which is defined by an excessive amount of body fat, results from a combination of genetics, overeating, psychological factors, personal diet, and exercise choices. Technically, not all body fat is closely linked to diseases (39), but fat accumulating around the central or
abdominal area has been associated with a statistically higher risk of disease including diabetes, heart disease, hypertension, and insulin resistance (40). Additionally, white adipose tissue (WAT) in the visceral fat was reported as a significant source of circulating free fatty acids (FFA) and a key factor for the occurrence of multiple risk changes (41). Recent discoveries suggest that there were increased inflammatory factors in the blood of obese subjects. Moreover, increased macrophages infiltration in WAT is often observed in patients and animals with obesity compared to their lean counterparts. These observations lead researchers to identify obesity as a low graded but chronic inflammatory state (42).

Physiologically, WAT contains not only adipocytes, but also macrophages, leukocytes, fibroblasts, cell progenitors, and endothelial cells (39). As new studies suggested, WAT is not just a passive energy reservoir but activates a chronic inflammatory response in obese humans and animals. The first observation linking obesity to inflammation was associated with increased secretion of pro-inflammatory mediators into the circulation system. Many cytokines and proteins are secreted to induce the inflammation in WAT, which include the interleukin family (such as IL-1β, IL-6, IL-8, and IL-10), inflammatory cytokines (such as TNFα, IFNγ, haptoglobin, and serum amyloid A (SAA) ), chemokines (such as monocyte chemotractant protein (MCP-1), angiopoietins, metallothioneins, and macrophage inflammatory protein-1α (MIP-1α) ), and some other factors like transforming growth factor-β (TGF- β) and retinol-binding protein-4 (RBP4) (43).

The current understanding of the progress on the inflammation in WAT is that, after long term accumulation of excess lipids in WAT, nuclear factor-κB (NF-κB), c-Jun N-terminal kinase (JNK), p44/42 mitogen activated kinase (ERK), nuclear subunit p65, and TLR target genes expression are activated in immune cells (44). Due to the activation of these pro-inflammatory signaling pathways, the production of chemokines, leukotrienes, adhesion molecules, and chemoattractant molecules are increased and released into the circulation. Then multiple proteins favor the recruitment of monocytes and other inflammatory cells to infiltrate the WAT (45). For instance, in adipose tissue, endothelial cells can release ICAM-1, VCAM-1, E-selectin, and P-selectin in response to increased free fatty acids. These molecules promote the adhesion of inflammatory T cells and monocytes as well as leukocytes. These hypotheses were confirmed as macrophage infiltration was inhibited by weight loss (46, 47).

Recent studies showed that most cells depend on extracellular input of glutamine because the endogenous biosynthetic pathway is not sufficient to meet the normal demands. Under some severe catabolic states such as trauma, sepsis or severe cellular stress, the body needs much more exogenous glutamine to counteract its increased consumption (48, 49). Thus, clinical studies have explored the benefits of using a glutamine supplement in cases of critical illnesses (49-51). Although glutamine’s effects on body weight, lipid synthesis, and glucose homeostasis have been investigated, how glutamine combats obesity is still unknown. Since glutamine level has been considered as a crucial factor for immune system functions, especially for neutrophils and macrophages (52, 53), researchers are trying to test the effect of glutamine supplementation on fat tissue inflammations, which was discovered commonly in obesity (Table 2).

<table>
<thead>
<tr>
<th>Glutamine</th>
<th>Samples</th>
<th>Method</th>
<th>Possible Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>30g per day</td>
<td>Human</td>
<td>Oral</td>
<td>Decrease lipopolysaccharides (LPS)</td>
<td>(33)</td>
</tr>
<tr>
<td>(0.4g/kg per day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30g per day</td>
<td>Human</td>
<td>Oral</td>
<td>Change gut microbiota</td>
<td>(54)</td>
</tr>
<tr>
<td></td>
<td>(0.4g/kg per day)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Glutamine supplementation on fat tissue inflammations

<table>
<thead>
<tr>
<th>Amount</th>
<th>Species</th>
<th>Route</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5g/kg per day</td>
<td>Female</td>
<td>Oral</td>
<td>-</td>
<td>(55)</td>
</tr>
<tr>
<td>30g per day</td>
<td>Human</td>
<td>Oral</td>
<td>-</td>
<td>(56)</td>
</tr>
<tr>
<td>2.4g/kg</td>
<td>Rat</td>
<td>Oral gavage and drinking water</td>
<td>Decrease LPS</td>
<td>(33)</td>
</tr>
<tr>
<td>-</td>
<td>Mice</td>
<td>Diet</td>
<td>Inhibit fatty acid oxidation</td>
<td>(57)</td>
</tr>
</tbody>
</table>

Preventing obesity using glutamine supplementation through inhibiting inflammation

Excluding the possibility that glutamine stimulates the release of the glucagon-like peptide 1 (58), which might induce the reduction of appetite upon the use of glutamine dietary supplements, the benefits of glutamine supplementation in combating obesity was revealed by several studies, yet, the exact mechanisms are still unclear. Since the growing focus on immunometabolism, which is the interaction of immunity and metabolism, several mechanisms to explain the effect of glutamine by reducing the inflammation in WAT will be discussed here (Figure 1).

**Figure 1. The model of glutamine mediated regulation of inflammatory response**

1. Glutamine reduces Lipopolysaccharides (LPS) induced inflammation
   A high amount of fat in the diet can alter the gut microbial composition, which leads to increased intestinal permeability (59). Because of that, LPS, a gut-derived endotoxin, enters circulation and activates both macrophages and adipocytes to trigger the secretion of a large number of cytokines. Some studies used LPS as a significant parameter to explain the effect of glutamine supplementation (Table 2) as glutamine can repair gut barrier dysfunction by fueling enterocytes and colonocytes. Thus, glutamine can inhibit LPS induced hypoxia-inducible factor (HIF) pathway, reactive oxygen species (ROS) production, and NF-κB pathway activation in WAT.

2. Glutamine promotes M2 macrophage polarization
In response to the increased amount of FFA in the microenvironment of WAT, macrophages are activated by multiple different signals to secrete pro-inflammatory cytokines and molecules. Macrophages are divided into two groups: M1, classically activated macrophages, and M2, alternatively activated macrophages. Based on current evidence, M2 macrophages induce the resolution phase of inflammation. Glutamine was shown to modulate the polarization of M2 macrophages in two ways. First, α-ketoglutarate (α-KG), a downstream metabolite of glutamine, is essential for increasing oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO) in M2 macrophages through histone modification regulated gene expression (60). The other pathway is mediated through the glutamine dependent UDP-N-acetylglucosamine (GlcNAc) pathway since the synthesis of UDP-GlcNAc is critical for the polarization of M2 macrophages. Therefore, glutamine may regulate the resolution phase of inflammation through M2 macrophages.

3. Glutamine promotes regulatory T and B cells activation
Emerging evidence suggested that regulatory T (Treg) (61) and B (Breg) cells (62) have significant roles in adipose tissue homeostasis. Compared to obese patients, Treg and Breg cells are predominant in the WAT in lean individuals (63), though the mechanisms of their contribution are still unclear. Treg cells suppress the activation and functions of effector T cells and negatively regulate immune responses (64). Especially, in diet-induced obesity mice model, Treg cells are dramatically reduced, which is consistent with that expanding Treg cells in HFD-fed mice improves metabolism (63). Recently, dietary glutamine was observed to be critical for the proliferation and activation of Treg cells (65). The underlying mechanism might be glutaminolysis is critical for replenishing the TCA intermediates and support Treg cells’ activation through mTORC1 signaling (66). Furthermore, due to elevated ERK signaling, Treg cells’ activation induces a great increase in glutamine import by activating the expression of glutamine transporters, such as SLC38A1 and SLC38A2, and critical enzymes, such as glutaminase and α-KG conversion-related enzymes (67).

Similar to Treg cells, IL-10 secreting regulatory B cells were discovered to suppress the inflammatory response and improve glucose sensitivity in obese mice. Due to their function of inducing Treg cells, Breg cells inhibit Th1/Th17 cells activation (68). This observation was consistent with the reports that Breg cells deficiency increased the infiltration of CD8+ T cells and M1 macrophages into adipose tissue of obese mice, and deleting Breg cells in lean mice leads to insulin resistance and limited fasting glucose clearance (69). Glutamine is a critical immunomodulatory nutrient for the function of B cells. In stimulated Breg cells, rapidly increased expression of glutamine transporter genes suggested that extracellular glutamine induced the activation response (70). Thus, both animal and human studies indicated an increased level of glutamine enhanced the activation of Treg and Breg cells (71), though the underlying mechanisms need to be clarified.

4. Glutamine alter inflammation-related gene expression by O-GlcNAcylation
The O-linked β-N-acetylglucosamine (O-GlcNAc) glycosylation is a dynamic post-translational modification that regulates various signaling pathways. O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA) are the only two enzymes that regulate O-GlcNAcylation. Diet-induced obesity increases the activity of OGT (72, 73) and a higher level of O-GlcNAcylation is considered to be significant in obesity statistically. Glutamine has been shown to lower the levels of O-GlcNAcylation in adipocytes through the inhibition of glycolysis and further decrease UDP-GlcNAc levels (74). Thus, the inflammation associated gene profile could be reversed by the supplementation of glutamine.

Conclusion
Obesity has become a serious consequence of the current fast life pace and the unhealthy western diet. Since dietary supplements are often easier to implement compared to strict dietary interventions and physical exercise, many studies focused on the development of new supplementations. Recently, new evidence demonstrated that glutamine dietary supplements can reduce body weight and fat mass and alleviate the inflammation in the adipose tissue associated with obesity. So far, some evidence showed that glutamine may reduce inflammation by reducing LPS, promoting M2 macrophage polarization, promoting regulatory T and B cells activation and regulating inflammation-related gene expression. But the mechanisms underlying dietary glutamine inhibits obesity is still unclear. Thus, it is imperative to study the signaling pathways affecting cell metabolism, especially in the adipose tissue upon the use of glutamine supplementation as a new avenue in the treatment of obesity.

Abbreviations list
T2D: type 2 diabetes; FDA: the Food and Drug Administration; cAMP: cyclic adenosine monophosphate; PPAR-γ: peroxisome proliferator activated receptor gamma; UCP-1: uncoupling protein 1; PGC-1α: peroxisome proliferator activated receptor gamma coactivator; PDHC: pyruvate dehydrogenase complex; C-EBPα: CCAAT enhancer binding proteins; SREBP-1c: sterol regulatory element binding protein 1; FABP: fatty acid binding proteins; GLUT4: glucose transporter type 4; mTOR: mammalian target of rapamycin; ROS: reactive oxygen species; WAT: white adipose tissue; FFA: free fatty acids; IL: interleukin; TNFα: tumor necrosis factor; IFNγ: type II interferon; SAA: serum amyloid; MCP-1: monocyte chemoattractant protein; MIP-1α: macrophage inflammatory protein-1α; TGF-β: transforming growth factor-β; RBP4: retinol-binding protein-4; NF-κB: nuclear factor-kB; JNK: c-Jun N-terminal kinase; ERK: p44/42 mitogen activated kinase; TLR: Toll-like receptor; SLC: solute carrier; α-KG: α-ketoglutarate; TCA: tricarboxylic acid cycle; NADH: nicotinamide adenine dinucleotide; NADPH: nicotinamide adenine dinucleotide phosphate; ETC: electron transport chain; CAD: carbamoylphosphate synthetase 2; PPAT: phosphoribosyl pyrophosphate amidotransferase; PRPS1 and 2: phosphoribosyl pyrophosphate synthetases 1 and 2; GCL: glutamate cysteine ligase; ICAM-1: intercellular adhesion molecule 1; VCAM-1: vascular cell adhesion protein 1; LPS: lipopolysaccharides; HIF: hypoxia induced factor; OXPHOS: oxidative phosphorylation; FAO: fatty acid oxidation; GlcNAc: N-acetylglucosamine; Treg: regulatory T cells; Breg: regulatory B cells; O-GlcNAc: O-linked β-N-acetylglucosamine; OGT: O-GlcNAc transferase; OGA: O-GlcNAcase.

References


64. Abul Abbas AHL, Shiv Pillai. Cellular and Molecular Immunology2014.


70. Shannon Heyse TC, Cheryl Doughty and Thomas Chiles. The regulation and role of L-glutamine in B-lymphocyte activation. The journal of immunology. 2015;194(1).


