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Metabolic effects of *L*-citrulline in type 2 diabetes

Short title: L-citrulline and type 2 diabetes

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Abbreviations

AC, Acylcarnitine; AT, Adipose tissue; ATGL, Adipose triglyceride lipase; AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; ASL, Argininosuccinate lyase; ASS, Argininosuccinate synthetase; BAT, Brown adipose tissue; Car, Carnitine; CAT, Carnitine-acylcarnitine translocase; CoA, Coenzyme A; CPT, Carnitine palmitoyltransferase; cNOS, Constitutive nitric oxide (NO) synthase (NOS); cAMP, cyclic adenosine monophosphate; CREB1, cAMP response element-binding protein 1; cGMP, Cyclic guanosine monophosphate; DAG, Diacylglyceride; eNOS, Endothelial NOS; FFA, Free fatty acid; GSIS, Glucose-stimulated insulin secretion; GLUT4, Glucose transporter type 4; GLN, Glutaminase; GyK, Glycerol kinase; G3P, Glycerol-3-phosphate; HbA1c, Glycated hemoglobin; HDL-C, High-density lipoprotein-cholesterol; HFD, High-fat diet; HSL, Hormone-sensitive lipase; HOMA-IR, Homeostasis model assessment of insulin resistance; iNOS, Inducible NOS; IL-6, Interleukin-6; KB, Ketone bodies; Arg, *L*-arginine; Cit, *L*-citrulline; LDL-C, Low-density lipoprotein-cholesterol; MDA, Malondialdehyde; MAG, Monoacylglycerol; MGL, Monoglyceride lipase; MCP-1, Monocyte chemoattractant protein-1; nNOS, Neural NOS; NMMA, N^G-monomethyl-*L*-arginine; *L*-NAME, N^ω-nitro-*L*-arginine methyl ester; NO, Nitric oxide; NOx, NO metabolites; NEFA, Non-esterified fatty acid; Nrf1, Nuclear respiratory factor 1; OAT, Ornithine aminotransferase; OTC, Ornithine transcarbamylase; OAA, Oxaloacetate; P5CS, Proline 5 carboxylate synthase; POX, Proline oxidase; P5C, Pyrroline 5 carboxylate; AMPK, 5' adenosine monophosphate-activated protein kinase; PGC-1α, Peroxisome proliferator-activated receptor-γ (PPAR-γ) co-activator 1α; PKG, Protein kinase G; PKA, Protein kinase A; PPARα, Peroxisome proliferator-activated receptor α; PEP, phosphoenolpyruvate; PEPCK, phosphoenolpyruvate carboxykinase; RET, Retroperitoneal; sGC, Soluble guanylate cyclase; SNP, Sodium nitroprusside; TG, Triglycerides; TLR-4, Toll-like

receptor 4; TAC, Total antioxidant capacity; T2D, Type 2 diabetes; UCP1, Uncoupling protein 1; VDCC, Voltage-dependent *L*-type Ca^{2+} channel; WAT, White adipose tissue; ZDF rat, Zucker diabetic fatty rat.

Glossary

AMP-activated protein kinase (AMPK): An energy sensor that is activated by nutrient deficiency and stimulates glucose uptake and lipid oxidation to produce energy. AMPK regulates cellular metabolism.

Argininosuccinate lyase (ASL): One of the enzymes of the urea cycle and citrulline-arginine cycle that catalyzes the reversible cleavage of *L*-argininosuccinate to fumarate and *L*-arginine; an intermediate step reaction for *de novo L*-arginine synthesis in non-hepatic tissues.

Argininosuccinate synthase (ASS): One of the enzymes of the urea cycle and citrulline-arginine cycle that catalyzes the formation of argininosuccinate from aspartate, citrulline, and ATP, and together with ASL, it is responsible for the biosynthesis of arginine in most body tissues.

Cyclic guanosine monophosphate (cGMP): A cyclic nucleotide produced from guanosine triphosphate (GTP) by guanylyl cyclase and acts as a second messenger.

Glucose transporter 2 (GLUT2): A transmembrane carrier protein that facilitates glucose transport across membranes. It helps glucose uptake by the hepatocytes for glycolysis and glycogenesis and by pancreatic β-cells for stimulating insulin secretion. It also regulates the release of glucose from the liver cells into circulation during gluconeogenesis.

Glucose transporter 4 (GLUT4): An insulin-regulated glucose transporter founds primarily in the adipose tissues and skeletal muscle.

cGMP-dependent protein kinase G (PKG): A serine/threonine protein kinase activated by cGMP.

Interleukin (IL)-6: An interleukin produced at the site of inflammation and plays a key role in the acute phase response.

K_{ATP} channel: An ATP-sensitive potassium channel and metabolic sensor that couples cellular metabolism to electrical activity. It regulates glucose-stimulated insulin secretion (GSIS) in the pancreatic β-cells and is a target for sulfonylurea antidiabetic drugs.

Nitric oxide (NO): A colorless and odorless gas soluble in aqueous and organic solvents and acts as a biological molecule.

Nitric oxide synthase (NOS): Enzyme that produces NO for *L*-arginine. In mammals, NO is generated by three different isoforms of the enzyme NO synthase, viz., neuronal nNOS (NOS-I), inducible iNOS (NOS-II), and endothelial eNOS (NOS-III).

NO synthase (NOS) inhibitors: Pharmacologically active substances that inhibit NOS enzymes and, thus, NO production. The most common NOS inhibitors are *L*-arginine analogs [e.g., N^G nitro-*L*-arginine methyl ester (*L*-NAME), *L*-N^G-monomethyl *L*-arginine (L-NMMA), and nitro-*L*-arginine (*L*-NNA)], which are competitive and nonselective inhibitors of NOS. Some NOS inhibitors are selective; for example, 7-nitroindazole (7-NI) is a specific nNOS inhibitor, and aminoguanidine is a selective iNOS inhibitor.

S-Nitrosylation: A post translational modification refers to the addition of NO to a reactant (e.g., cysteine residues of a protein).

Soluble guanylyl cyclase (sGC): A heterodimeric (α and β subunits) heme protein that acts as the NO receptor; NO activates sGC and produces 3′,5′-cGMP, which actives PKG (NO-sGC-PKG signaling pathway).

Voltage-dependent *L*-type Ca^{2+} channels (VDCCs): A subset of voltage-gated ion channels found in the membrane of excitable cells (e.g., pancreatic β-cells) providing permeability to calcium ions.

Abstract

The prevalence of type 2 diabetes (T2D) is increasing worldwide. Decreased nitric oxide (NO) bioavailability is involved in the pathophysiology of T2D and its complications. *L*-citrulline (Cit), a precursor of NO production, has been suggested as a novel therapeutic agent for T2D. Available data from human and animal studies indicate that Cit supplementation in T2D increases circulating levels of Cit and *L*-arginine while decreasing circulating glucose and free fatty acids and improving dyslipidemia. The underlying mechanisms for these beneficial effects of Cit include increased insulin secretion from the pancreatic β-cells, increased glucose uptake by the skeletal muscle, as well as increased lipolysis and β-oxidation, and decreased glyceroneogenesis in the adipose tissue. Thus, Cit has antihyperglycemic, antidyslipidemic, and antioxidant effects and has the potential to be used as a new therapeutic agent in the management of T2D. This review summarizes available literature from human and animal studies to explore the effects of Cit on metabolic parameters in T2D. It also discusses the possible mechanisms underlying Cit-induced improved metabolic parameters in T2D.

Keywords: Carbohydrate metabolism, *L*-citrulline, nitric oxide, pancreatic β-cell, skeletal muscle, type 2 diabetes.

1. Introduction

The prevalence of diabetes mellitus in the adult population has increased from 151 to 537 million during the first two decades of the 21st century and is estimated to reach 783 million by the year 2045.[1] About 90–95% of all people with diabetes have type 2 diabetes (T2D), characterized by insulin resistance and β-cell dysfunction.[2] Currently, various treatments are available for T2D, but unfortunately, most have insufficient efficacy.[3] For example, the effectiveness of antidiabetic drugs for achieving glycemic control is only 41%, emphasizing the need for further investigations to provide more efficient treatments.[4] In addition, it has been suggested that the treatment approach to T2D needs to be changed from only a glycemic control to a pathophysiological-based approach, which also includes managing lipids, blood pressure, and obesity.[5]

Endothelial dysfunction, mainly characterized by decreased nitric oxide (NO) bioavailability, is involved in the pathophysiology of T2D.[6] NO is produced from the *L*-arginine (Arg)-NO synthase (NOS) and the nitrate-nitrite-NO pathways.[7] Decreased endothelial NOS (eNOS)-derived NO, increased inducible NOS (iNOS)-derived NO,[8,9] and impaired nitrate-nitrite-NO pathway[10] have been reported in T2D. Results from human studies on polymorphisms in NOS genes, genetically altered animals, and pharmacological studies support the involvement of disturbed NO homeostasis in developing T2D.[10] Obtained data indicate that NO produced by constitutive isoforms of NOS (cNOS), i.e., eNOS and neural NOS (nNOS), increases insulin secretion and sensitivity[10], increases skeletal muscle glucose uptake,[11] and decreases hepatic glucose output.[12] In contrast, iNOS-derived NO increases insulin resistance and leads to the development of T2D.[9] In addition, NO-releasing drugs can improve carbohydrate metabolism in T2D, and the NO system partially mediates favorable metabolic effects of some antidiabetic medications (e.g., metformin).[13,14] Therefore, boosting the NO system may have therapeutic effects on T2D.[15-17] One

strategy for NO boosting is enhancing endogenous NO synthesis,[18] which can be achieved by administration of Arg,[19,20] nitrate/nitrite,[21,22] and *L*-citrulline (Cit).[23]

Results of clinical and experimental studies indicate that Arg, an NO precursor, has beneficial effects on T2D[19,24-28] and improves endothelial[20,24] and β-cell[29,30] function and glucose tolerance.[31] However, Arg does not affect fasting glucose and hemoglobin A1c (HbA1c) levels in patients with T2D.[32,33] In addition, it has undesirable side effects, including induction of arginase activity,[34] enhancing inflammatory and immunologic responses,[35-37] and increasing mortality in patients with myocardial infarction.[38]

Inorganic nitrate and nitrite supplementation to boost the nitrate-nitrite-NO pathway have shown promising metabolic effects in animal models of T2D.[10,39-41] These anions increase insulin secretion from the β-cells[15,42,43] and improve glucose utilization at the periphery.[44-48] However, this has not been the case in human studies where nitrate or nitrite was ineffective in improving metabolic disorders;[49,50] for details, see a recent review.[51] In addition, a high intake of nitrate and nitrite may increase the risk of β-cell autoimmunity and type 1 diabetes.[52]

Cit, a precursor of Arg *de novo* synthesis and NO production,[53] has a highly efficient intestinal absorption rate, low first-pass metabolism, and high renal reabsorption. These characteristics that make Cit a good candidate for NO boosting in NO-disrupted conditions, including diabetes.[54] In addition, it is needed to manage other comorbidities, including obesity, dyslipidemia, and hypertension in patients with T2D.[55] This increases the prevalence of polypharmacy, using greater than 5 drugs/day, as reported to be 57% to 84% in patients with T2D.[56,57] Polypharmacy is associated with increased drug side-effects[58] (e.g., higher risk of bone fracture and depression[59]), lower quality of life, and higher healthcare costs.[60] The therapeutic effects of Cit against oxidative stress, hyperglycemia, hypertension, hypertriglyceridemia, hypercholesterolemia, and insulin

resistance have been reported in experimental[61-64] and clinical studies[65-71] in both male[61-64,66-71] and females.[67-71] Therefore, Cit can potentially act as a one-drug-multi-target agent in patients with T2D. In this review, we first summarize the effects of Cit on metabolic parameters in T2D and then discuss the possible mechanisms that underlie such improved outcomes.

2. *L*-citrulline metabolism

Cit is a non-essential and non-protein amino acid in humans;[54] it was extracted in 1914 from watermelon by Koga and named in 1930 by Wada according to the Latin name of watermelon, i.e., *Citrullus Colocynthis.*[72] Watermelon is the main dietary source of Cit; the concentration of Cit in watermelon ranges between 0.7 and 3.6 g/kg of fresh weight.[73,74] However, diet is a poor source of Cit, and the main source (60–80%) of the human body Cit is endogenous synthesis from glutamine[75,76] (Table 1). The main site of Cit synthesis (60-90%) in humans is the small intestinal enterocytes.[77,78] Arg (20-40%) and proline (~3%) have lower contributions in Cit synthesis in the human enterocytes. As shown in Figure 1, human enterocytes take up precursor amino acids for Cit biosynthesis via apical and basolateral membranes. Within the enterocytes, glutamine is converted to glutamate by glutaminase (GLN); pyrroline 5 carboxylate synthase (P5CS) and proline oxidase (POX) convert glutamine and proline to pyrroline 5 carboxylate (P5C), which is then converted to ornithine by ornithine aminotransferase (OAT). Arginase converts Arg to ornithine, which is converted to Cit by ornithine transcarbamylase (OTC); Cit then enters the circulation and can be taken up by other cells.[79,80] Kidneys are the main site of Cit metabolism in the human body, metabolizing up to 80% of the Cit produced in the small intestinal enterocytes. Kidneys take up about 1.5 g of Cit/day from the circulation,[81] and high plasma Cit levels may reflect renal disorders.[82] Cit bypasses splanchnic extraction,[83] and its first-pass metabolism is negligible;[84] thus, the intestine and the liver have no major contribution to Cit metabolism.

Cit metabolism shows sex- and species differences (Table 1). Overall, the mean plasma Cit concentration is 20–50 μM in healthy adults.[85-89] However, adult women have slightly lower circulating Cit than men,[88,89] which is attributed to higher glucose-mediated insulin output in women.[89] In addition, compared to men, women have lower Cit plasma flux, lower Arg/Cit and higher Cit/nitrite ratios in RBC and serum,[90] higher NOS-dependent NO production,[90] and higher whole-body NO biosynthesis.[91] Furthermore, the systolic- and diastolic blood pressure-lowering effect of Cit is higher in women,[92,93] which is partially explained by the higher activity of the enzymatic machinery involved in Cit metabolism. In men, following administration of Cit (2-15 g), it is distributed in a volume of 15.2-17.2 L, and its plasma concentration rapidly (T_{max}=38-56 minutes) reaches a maximum level (C_{max}=515-3849 μM).[94] In women, C_{max} (386-1069 μM) of Cit is lower, but it's T_{max} (60 minutes) is slightly higher than in men, as measured after administration of 7 g Cit.[95] However, some pharmacokinetic parameters of Cit metabolism have not been reported in women, an issue that warrants further investigation, as highlighted previously.[96]

Female mice have higher serum Cit concentration than males;[97,98] this is due to higher endogenous Arg synthesis (by ~42%)[97] and flux (by ~6%)[97] as well as higher serum (147±17 vs. 106±14 μM) ,[99] kidney (440±79 vs. 263±88 μmol/kg)[99] and skeletal muscle (728±220 vs. 367±37 μmol/kg)[99] Arg concentrations in females. In addition, compared to males, Cit plasma flux,[97,100] Cit renal clearance,[97] rate of Cit conversion to Arg,[97] and rate of Arg conversion to Cit[97] as well as arginase activity in the pancreas (0.43±0.04 vs. 0.34±0.1 μmol/mg/min)[97] and kidney (0.49±0.04 vs. 1.1±0.08 μmol/mg/min)[97] are higher in female mice. Female mice also produce more NO than males.[97,101]

Considering species differences in Cit metabolism (Table 1), the main source of Cit production is glutamine in humans[79,100] but proline in rodents.[102-104] In addition, compared to humans, values of

endogenous production,[85,104] plasma concentration,[88,89,97] plasma flux,[85,97,100,105,106] renal clearance,[97,107] and half-life of Cit[78,94] are higher in rodents. Furthermore, rates of Cit conversion to Arg[97,105,106,108] and Arg conversion to Cit[97,105,106] are also higher in rodents. Differences have also been reported between rats and mice; male rats have higher serum Cit (64.6±7.8[109] and 70.0±8.0[110] vs. 40±5[97] μM) and plasma Cit flux (197±11[100] vs. 95.4±29.1[97] and 81.1±4.7[100] μmol/kg/h) than males mice. Furthermore, a higher rate of NO production was reported in mice (7.68±1.47 μmol/kg/h) than in rats (0.55±0.05 μmol/kg/h).[111]

2.1. Cit-Arg cycle

The only known fate of Cit is its conversion to Arg via Cit-Arg cycle,[77] and the therapeutic applications of Cit are mainly based on the capacity to increase Arg availability for NO production.[112] In the Cit-Arg cycle, Cit is metabolized to argininosuccinate by argininosuccinate synthetase (ASS), which is then metabolized to Arg by argininosuccinate lyase (ASL). Finally, different isoforms of the NOS enzymes (eNOS, nNOS, and iNOS) metabolize Arg to NO and Cit.[113] Arginase, which metabolizes Arg to urea and ornithine, competes directly with NOS for Arg; hence, increased arginase activity can decrease Arg levels, reducing its availability to NOS and, thus, decreasing NO production in T2D.[114] Then, ornithine is converted back to Cit by OTC to start the cycle again. The enzymatic machinery necessary for the Cit-Arg cycle is found in most cells,[83] including pancreatic β-cells,[115] skeletal muscle cells,[116] and adipose cells[117] of rats[115,117] and humans[116] (Figure 1).

Expressions of ASS and ASL have been documented in the β-cells of male Wistar rats,[115] diaphragm and gastrocnemius muscles of male[118,119] and female[119] Sprague-Dawley rats, and retroperitoneal (RET) adipose tissue (AT) of young (but not old[120]) male Sprague-Dawley rats.[117] All three NOS isoforms are expressed in the pancreatic β-cells; eNOS,[115,121-124] nNOS,[115,121,125-128]

and iNOS[126,129] expressed in islet β-cells of humans,[127] Wister rats,[122,125] Sprague–Dawley rats,[126,129] Zucker diabetic fatty (ZDF) rats,[127] db/db mice,[123] NMRI mice,[130] INS-1E cell line,[128] Min6 cell line,[124] and HIT T15 cells, an insulinoma cell line.[121] Although controvesioal,[131] it seems that nNOS and, to a lesser extent, eNOS play the major role in regulating insulin secretion in the pancreatic β-cells.[10] In the skeletal muscle cells, nNOS is the predominant source of NO[116] and the most relevant NOS isoform involved in glucose uptake.[132] The predominant or probably the only cNOS isoform expressed in white adipose tissue (WAT) and brown adipose tissue (BAT) seems to be Enos.[133,134] In BAT, eNOS is located in the cytoplasm of adipocytes.[135] The iNOS isoform is found in both BAT and WAT[136] and is localized in adipocytes and other cells, such as proinflammatory macrophages.[137]

Little is known about the kinetic of the enzymes involved in the Cit-Arg cycle; however, in the rat liver, V_{max} (μmol/min/mg protein) and Km (mM) for ASS, ASL, and eNOS have been reported to be 0.009 and 1.25,[138] 0.009 and 0.24,[139] and 1 and 0.003, respectively.[140]

In addition to synthesis in cells, Cit may enter the cells from circulation. Neutral amino acids such as Cit use distinct transport systems in different cells,[141] including a saturable but nonselective neutral carrier in macrophages,[142] a Na^+-independent transport system in vascular smooth muscle cells,[143] a Na^+-dependent uptake mechanism via the system $B^{0,+}$ in human intestinal epithelial cells,[144] and large neutral amino acid transporter 1 in the brain capillary endothelial cells.[141] However, no evidence indicates the presence of a Cit-specific transport in β-cells, skeletal muscle, and AT.[83]

3. Circulating concentrations of *L*-citrulline in T2D

Data on circulating levels of Cit in patients with T2D is not consistent, as both increased[90,145-148] and decreased[149-152] levels have been reported (Table 2). Data presented in Table 2 favor increased

mean Cit concentrations (~15%) in patients with T2D compared to healthy subjects (36.4 μM vs. 31.8 μM). High circulating Cit in patients with T2D is attributed to small intestine hyperplasia and hypertrophy in the presence of insulin resistance,[146,147] considering that about 80-90% of the Cit is derived from enterocytes.[153] On the other hand, low circulating Cit levels in patients with T2D may be due to increased arginase activity[150] and decreased eNOS activity,[149,150] resulting in the lower conversion of Arg to Cit.[149,150] Data on circulating Cit concentrations in animals is also scarce, and lower plasma concentration (60±7.3 *vs.* 47±4.1 μM, $p<0.05$) has been reported in male ZDF rats.[154]

4. Metabolic effects of *L*-citrulline in T2D

Cit increases insulin secretion from the pancreatic β-cells,[115] improves insulin sensitivity,[155] and has antihypertensives,[61-63] antihyperglycemic,[61] antidyslipidemic,[61,64] and antioxidant[65] effects. *In vitro* studies indicate that Cit (100 μM) increases insulin release in the presence of 8.3 mM glucose from isolated islets of Wistar rats.[115]

In vivo animal studies indicate that Cit administration in T2D increases circulating NO metabolites (NOx), Cit, and Arg concentrations,[156,157] increases plasma insulin levels,[158] decreases circulating glucose[61,156] and free fatty acid (FFA),[156] and improves dyslipidemia[61] (Table 3). Administration of watermelon juice, a rich source of Cit, to male ZDF rats for 4 weeks decreased serum glucose by ~22% (410.8 ± 8.1 *vs.* 320.7 ± 10.3 mg/dL, $P<0.05$) and serum FFA by ~18% (1.53±0.07 *vs.* 1.26±0.08 mM, $P<0.05$).[156] Cit administration to type 2 diabetic male Wistar rats at doses of 200, 400, and 800 mg/kg, significantly lowered fasting serum glucose from 210.5±3.9, 214.5±7.6, and 201.3±1.5 on day 0 to 192.5±3.4, 181.8±1.2, and 174.8±2.8 mg/dL on day 22, respectively.[61] In addition, Cit (400 mg/kg for 21 days) markedly decreased concentrations of total cholesterol (TC, 149.8±2.7 *vs.* 57.8±1.2 mg/dL $P<0.05$), triglycerides (TG, 141.8±2.3 *vs.* 13.2±0.6 mg/dL, $P<0.05$),

low-density lipoprotein-cholesterol (LDL-C, 85.3±1.7 *vs.* 26.1±1.0 mg/dL, P<0.05), and increased high-density lipoprotein-cholesterol (HDL-C, 24.6±1.1 vs. 58.8±0.5 mg/dL, P<0.05) levels in type 2 diabetic male Wistar rats.[61] Kudo et al. showed that Cit supplementation (500 mg/kg for 11 weeks) to high-fat diet (HFD)-fed male Sprague Dawley rats decreased TC (58.2±5.6 *vs.* 40.1±8.2 mg/dL, P<0.01), insulin (19.8±3.5 *vs.*11.7±2.1 ng/mL, P<0.05), and homeostasis model assessment of insulin resistance (HOMA-IR, 8.0±1.5 *vs.* 4.3±1.4, P<0.05) but does not affect fasting serum glucose, TG, and FFA levels[155], suggesting improvements in insulin sensitivity.[155]

Regarding human studies (Table 3), results of a randomized, double-blind, placebo-controlled clinical trial of 45 patients (males and females) with T2D indicated that Cit supplementation (3000 mg/day for 60 days) increased serum Cit concentrations by more than 2-fold (13.4±8.6 *vs.* 30.5±12.4 μM, P<0.001) and decreased fasting serum glucose (157.9±41.7 *vs.* 134.9±32.2 mg/dL, P<0.001) and HbA1c (7.2±1.2 *vs.* 6.6±1.35%, P=0.003) but had no effects on serum insulin, interleukin-6 (IL-6), serum monocyte chemoattractant protein-1 (MCP-1), and serum toll-like receptor 4 (TLR-4).[159] In addition, in these patients, Cit decreased serum malondialdehyde (MDA, 1.98±0.66 *vs.* 1.48±0.47 μM, P<0.001) and increased serum total antioxidant capacity (TAC, 1.26±0.17 *vs.* 1.54±0.18 mM, P<0.001) and NOx (1.76±0.63 *vs.* 2.25±0.70 μM, P<0.001).[65] Cit (2, 5, 10, and 15 g) did not affect plasma insulin levels in eight healthy young men.[94,160] In addition, Cit supplementation (2000 mg/day for 30 days) decreased arginase activity by 21% and increased plasma NO levels by 38% in men and women with T2D. Additionally, these patients had a modest but not statistically significant improvement in HbA1c levels.[161]

4.1. Dose and safety of Cit

Cit has been administrated in doses ranging from 2.7-15 g/day in humans and 0.05-5.7 g/kg in rodents. These clinical and experimental studies showed that Cit has antihyperglycemic,[61,162]

antidyslipidemic,[61] anti-obesity,[155] anti-inflammatory,[163] anti-oxidative stress,[159] and renoprotective[163] effects as well as increases serum insulin,[162] improves glucose tolerance,[164] and increases protein synthesis in the skeletal muscle.[158] In human studies, doses of 2.7,[68] 3,[67,71,165] 3.4,[166] 6,[66,69,167,168] 8,[169] 10,[107] and 15 g/day have been used as a single bolus dose[94] or for durations of 7,[71,165] 8,[169] 14,[166,168] 28,[66] 42,[68,69] and 56[67,167] days. However, high doses of Cit (15 g/day) have a lower fractional absorption rate because of the saturation of the Cit transporters and lower conversion of Cit to Arg.[112] Hence, Cit at 10 g/day has been suggested for clinical use.[94] In addition, for increasing circulating Arg concentrations, doses of Cit as low as 3 g/day are effective.[53] Thus, it has been suggested that the minimum and maximum effective doses of Cit for treating cardiometabolic disorders in humans are ~3 and 10 g/day, respectively.[112] In rodents, doses of 0.05,[163] 0.2,[61] 0.3,[170] 0.4,[61] 0.5,[155] 0.6,[164,170] 0.8,[61] 0.9,[170-172] 1,[155] 5,[158] and 5.7[173] g/kg have been used for durations of 7,[158,170-172] 14,[163] 21,[61] 28,[156,162] 56,[169] 63,[155,173] 77,[155] and 105[164] days in male mice,[155,163,164,173] male rats.[61,155,156,158,170-172]

Literature indicates that doses as high as 15 g/day[94] and 5.7 g/kg[173] are well tolerated in humans and animals, respectively. In a clinical trial, Cit (15 g/day for two weeks) was used for restoring NO bioavailability of airways in obese subjects with asthma; some side effects, including mild to severe nausea (12-41%), headache (17-44%), lightheadedness (3-20%), and diarrhea (13%) have been reported. All of these side effects lasted for 2-3 days after the initial use.[174] However, regarding Cit safety, some points deserve further attention. First, administration of Cit (2 g/kg/day for 16 days) in rats during the pre-weaning stage disturbed lipid profiles during adulthood.[175] Thus, the safety of using Cit during the early stages of life needs to be further investigated. The second point about the safety of Cit is the short-term duration of Cit administration in both human (up to 56 days[67,167]) and animal (up to 105 days[164]) studies. Therefore, long-term studies are warranted.

Finally, Cit may interact with some drugs used for managing hypertension, T2D, and cardiovascular diseases; for example, metformin affects Cit metabolism[176-178] so that, following metformin therapy, plasma Cit concentrations are lower in diabetic patients and mice.[176]

5. Mechanisms underlying beneficial effects of *L*-citrulline in T2D

5.1. Increased insulin secretion

As shown in Figure 2, the possible mechanisms explaining the stimulatory effect of Cit on glucose-stimulated insulin secretion (GSIS) include (1) activation of voltage-dependent *L*-type Ca^{2+} channels (VDCCs) via (a) inhibition of K_{ATP} channels and (b) increases in intracellular cyclic guanosine monophosphate (cGMP); (2) stimulation of insulin gene promoter; (3) S-nitrosylation of glucokinase; and (4) promotion of mitochondrial ATP production by the coupling of the Cit-Arg cycle to the Krebs cycle via the malate-aspartate shuttle.

Cit causes an NO-induced increase in $[Ca^{2+}]_i$ in β-cells; in support of this notation, it has been shown that exposing Wistar rat β-cells to Cit (100 μM) increases $[Ca^{2+}]_i$ by 77%, which is inhibited by NOS inhibition[121] and in Ca^{2+}-free conditions.[115] In addition, exposing rat β-cells to Cit (100 μM) increases insulin secretion by 58% in the presence of 8.3 mM glucose that is completely blocked by N^G-monomethyl-*L*-arginine (NMMA).[115] NO increases $[Ca^{2+}]_i$ levels through inhibition of K_{ATP} channels and subsequent membrane depolarization, leading to the opening of *L*-type VDCCs.[179] In addition, NO activates soluble guanylate cyclase (sGC) and increases intracellular cGMP,[180] which causes Ca^{2+} influx through VDCC as increases in the $[Ca^{2+}]_i$ is abolished by Ca^{2+} channel blockers.[181]

In addition to increasing $[Ca^{2+}]_i$, NO stimulates insulin gene promoter and insulin mRNA expression in the pancreatic β-cells.[182] NO increases endogenous insulin gene expression in Min6

cells and isolated pancreatic islets of rats.[182] We previously reported that nitrite, an NO donor, increases insulin mRNA expression[43] and insulin content[15] in the isolated pancreatic islets of male T2D Wistar rats and thus increases GSIS. In addition, S-nitrosylation of glucokinase (at cysteine-371) is another mechanism by which NO facilitates GSIS.[183] In this line, it has been reported that nitrite increases glucokinase mRNA expression in isolated islets in male T2D Wistar rats.[43]

It has been suggested that the Cit-Arg cycle is coupled to the Krebs cycle via the malate-aspartate shuttle.[121] The conversion of *L*-argininosuccinate to Arg is associated with the formation of fumarate in the cytosol; fumarate enters the Krebs cycle in the mitochondrion, where it is converted into malate by the enzyme fumarase and then participates in ATP production.[121] Fumarate (from the Cit-Arg cycle) and pyruvate (from glycolysis of phosphorylated glucose) increase ATP production in the Krebs cycle, which closes K_{ATP} channels and increases insulin secretion from pancreatic β-cells. In β-cells, K_{ATP} channels are the primary determinants of membrane depolarization and the subsequent activation of *L*-type Ca^{2+} channels. This results in the elevation of $[Ca^{2+}]_i$, followed by insulin release into the circulation.[184]

Overall, most stimulatory effects of Cit on insulin secretion are reported to be NO-dependent, but little is known about its NO-independent effect; this issue warrants further study. NO-independent effects of Arg, including improved atherosclerotic cardiovascular disease[185-187], immune function[188,189], wound healing[190,191], and decreased carcinogenesis and tumor growth[192,193] have been reported in humans previously.

5.2. Improved peripheral glucose metabolism

5.2.1. Skeletal muscle

Cit increases glucose uptake in the skeletal muscle by several NO-dependent mechanisms (Figure 3): (1) increased gene expression of glucose transporter type 4 (GLUT4) by phosphorylation of 5'

adenosine monophosphate-activated protein kinase (AMPK), (2) enhanced GLUT4 translocation from the cytosol to the cell membrane by (a) its phosphorylation and (b) S-nitrosylation, (3) increased mitochondrial biogenesis by increasing mRNA expression of peroxisome proliferator-activated receptor-γ (PPAR-γ) co-activator 1α (PGC-1α), a transcription factor for mitochondrial biogenesis, and nuclear respiratory factor 1 (Nrf1).

In the skeletal muscle, NO binds to the heme group on sGC, produces cGMP, and subsequently activates protein kinase G (PKG) (sGC–cGMP-PKG signaling pathway).[194] PKG phosphorylates AMPK and the phosphorylated AMPK translocate to the nucleus and increases mRNA expression of GLUT4.[15,16,44,195] NOS inhibitor, N^{ω}-nitro-*L*-arginine methyl ester (*L*-NAME), prevents 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR)-induced AMPK activation and GLUT4 mRNA expression by 38% and 92%, respectively in rat L6 myoblasts; this implies that NOS activity is required upstream to AMPK for Cit-induced glucose uptake in the skeletal muscle.[195] In addition, sodium nitroprusside (SNP), an NO donor, raises cGMP levels and increases glucose uptake,[196] whereas LY-835, an sGC inhibitor, prevents the increase in cGMP and glucose uptake.[194] PKG also phosphorylates GLUT4 and increases GLUT4 translocation from the cytosol to the plasma membrane.[197] NO-mediated translocation of GLUT4 and glucose uptake in the skeletal muscle also occurs through a cGMP-independent pathway[10] by S-nitrosylation of proteins involved in GLUT4 translocation.[197]

Cit also improves carbohydrate metabolism in the skeletal muscle by increasing mitochondrial biogenesis. Administration of Cit for 15 days at a dose of 250 mg/kg dissolved in water increased PGC1-α mRNA and protein expressions in the hind and forelimb muscles in mice.[198] This effect appears to be NO-dependent as Cit (50 μM)-induced upregulation of PGC-1α expression in C2C12 myotubes was suppressed by 98% in the presence of the NOS inhibitor (*L*-NAME, 100 μM).[198]

NO activates sGC to generate cGMP, which activates protein kinase A (PKA).[199] PKA phosphorylates Cyclic adenosine monophosphate (cAMP) response element-binding protein 1 (CREB1), allowing its nuclear translocation and activation of the PGC1-α and Nrf1 gene expression.[200] Increased expression of PGC1-α and Nrf1 increases mitochondrial biogenesis.[201] 6-day treatment of rat L6 myoblasts with NO donor, (Z)-1-[2-(2-aminoethyl)-N-(2-ammonioethyl)amino]diazen1-ium-1,2 diolate, (50 μM) increased mRNA expression of PGC-1α and Nrf1; this effect is mediated via the sGC-cGMP pathway since 8 Br-cGMP (3 mM) mimicked NO effects and 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one (1 μM), an sGC inhibitor, abolished it.[199]

5.2.2. Adipose tissue

As shown in Figure 4, the effects of Cit on AT include: (1) stimulating lipolysis by increasing phosphorylation of hormone-sensitive lipase (HSL), (2) inducing fatty acid β-oxidation by increasing expression of carnitine palmitoyl-transferase 1 (CPT1), (3) decreasing glyceroneogenesis by reducing expression of cytosolic phosphoenolpyruvate carboxykinase (PEPCK), and (4) inducing thermogenesis by increasing the uncoupling protein 1 (UCP1) expression.

Cit increases lipolysis in AT by increasing the phosphorylation of HSL. In mammalian lipolysis, three lipases act in sequence with the concomitant release of one FFA in each step; adipose triglyceride lipase (ATGL) converts TAG to diacylglyceride (DAG),[202] which is hydrolyzed to monoacylglycerol (MAG) by HSL; monoglyceride lipase (MGL) cleaves MAG into glycerol and FFA.[203] Cit (2.5 mM) promotes HSL phosphorylation and induces non-esterified fatty acid (NEFA) release by about twofold in the incubation medium,[117] i.e., it increases lipolysis.[117,120] This effect is NO-dependent since when RET AT was pretreated with *L*-NAME (1 mM for 30 minutes),

Cit-induced HSL phosphorylation and NEFA release were significantly reduced.[117] Cit also increases glycerol kinase (GyK) expression by threefold in Cit (2.5 mM)-treated RET AT from HFD-fed male Sprague Dawley rats.[117] However, GyK probably would not be active in the mobilization of adipose triglyceride stores in the normal animals.[204,205]

Fatty acids are activated for β-oxidation via conjugation with coenzyme A (CoA) in the cytosol.[206] The long-chain fatty-acyl-CoA is esterified with carnitine (Car) to produce acylcarnitine (AC) by the enzyme CPT1, which resides on the mitochondrial outer membrane.[207,208] AC is then transported across the mitochondrial inner membrane by carnitine-acylcarnitine translocase (CAT); then CPT2, located on the inner aspect of the mitochondrial inner membrane, converts the long-chain AC back to long-chain fatty acyl-CoA and carnitine.[209] The acyl-CoA then goes through β-oxidation to produce acetyl-CoA and carnitine; the latter is transported out.[206] If the flux of acetyl-CoA is greater than what the TCA cycle can handle, ketone bodies (KB) accumulate, a condition often seen in uncontrolled diabetes. Cit (2.5 mM) increases CPT1-b gene expression in the RET AT of HFD-fed male Sprague Dawley rats[117] and 3T3-F442A adipocytes.[120] This effect, at least in part, is mediated by an increase in Cit-induced PPARα gene expression as reported in RET AT and 3T3-FAA2A adipocytes;[120] the evidence for this notion is that Cit-induced CPT1 gene expression decreases in the PPARα knockout mice.[210,211] PPARα activates the transcription of genes involved in fatty acid transport and mitochondrial β-oxidation.[212]

Regarding the effects of Cit on CPT1-b,[117,120] it should be noted that the authors only measured gene expression, which does not necessarily translate into protein expression and enzyme activity. The increase in FA oxidation observed could be due to peroxisomal FA oxidation, a possibility that was not considered. Furthermore, the authors worked with frozen tissue, thus exposing both CPT1 and CPT2 in their preperations.[209]

Cit decreases glyceroneogenesis, the *de novo* synthesis of glycerol-3-phosphate (G3P) from precursors other than glucose and glycerol (i.e., pyruvate and lactate), by decreasing gene expression of PEPCK. Cit (2.5 mM) attenuated glyceroneogenesis flux (80%) in RET AT of HFD-fed male Sprague Dawley rats.[117] Glyceroneogenesis has been suggested as a potential pathway for G3P formation in AT.[213] G3P is required as a substrate for fatty acid re-esterification into triacylglycerol.[214] During fasting, about 30-50% of NEFAs derived from lipolysis are immediately re-esterified into newly synthesized triacylglycerol in AT of male Sprague Dawley rats and humans.[215,216] PEPCK, which catalyzes the GTP-dependent oxaloacetate (OAA) decarboxylation to phosphoenolpyruvate (PEP), is a key regulatory enzyme in glyceroneogenesis.[217] Cit-induced decrease in glyceroneogenesis is done by increasing NO, which decreases gene expression of PPARγ.[218,219] A 24-hour exposure to Cit reduced PEPCK protein by 48% in rat AT in an NO-dependent manner as *L*-NAME (10 mM) abolished it.[117] PPARγ is the main transcriptional activator of PEPCK expression in adipocytes, so the decrease in PPARγ decreases PEPCK expression and glyceroneogenesis.[220] In support, Cit (2.5 mM) decreases PPARγ gene expression (86%) in RET of male Sprague Dawley rats.[221] In summary, Cit decreases glyceroneogenesis by promoting NO synthesis, decreasing PPARγ and, thereby, PEPCK.

Cit increases thermogenesis in adipocytes by increasing the UCP1 expression, which uncouples oxidative phosphorylation and ATP production, dissipating energy as heat.[222] Following a 24h Cit exposure (2.5 mM), UCP1 protein was upregulated by 53% in RET AT of male Sprague Dawley rats.[221] Cit effect on UCP1 gene expression is supposedly transcriptional through a PPARα/PGC-1α -process since this couple was demonstrated to act as an inducer of the UCP1 gene in rodents and humans.[223,224] PPARα, as a direct activator of PGC-1α, is necessary for full thermogenic activation of PGC-1α gene transcription in BAT.[223,225] PGC-1α is a transcriptional co-activator

involved in controlling energy metabolism in BAT that plays a critical role in inducing UCP1 gene expression.[226] Cit (2 mM) induces PGC-1α and PPARα gene expressions in RET AT of male Sprague Dawley rats,[221] leading to a rise in the thermogenesis.[223] Thus, Cit may act as a mediator of WAT mass reduction, WAT browning, and increased energy consumption by inducing UCP1.[221]

Of note, some *in vitro* studies[117,120,221] conducted on the effects of Cit on glucose metabolism in AT used high doses of Cit (2500 μM) that are ~50-250 folds higher than normal circulating Cit concentration (10-50 μM). All of these studies were conducted in the same laboratory, and the authors mentioned that a Cit concentration of 2500 μM mimics the plasma concentration of Cit after administration of Cit at a dose of 5 g/kg/day for 7 days in rats acording to a previous study.[158]

6. Conclusions and perspectives

Cit improves insulin sensitivity and has antihyperglycemic, antidyslipidemic, and antioxidant effects. The mechanisms underlying these effects include: (1) increased GSIS in pancreatic β-cells by activating VDCCs, stimulation of insulin gene promoter, and promotion of mitochondrial ATP production, (2) increased glucose uptake in the skeletal muscle by promoting the expression and translocation of the GLUT4 and increasing mitochondrial biogenesis, (3) increased lipolysis, β-oxidation, and thermogenesis as well as decreased glyceroneogenesis in adipocytes. These effects are mainly NO-dependent and occur through the Cit-Arg cycle and NO production.

Some points should be considered about the beneficial metabolic effects of Cit in T2D. First, a large body of evidence has been provided from *in vitro* or animal studies. Because Cit metabolism shows species differences, extrapolation of these findings to humans needs caution. Second, Cit metabolism is different between males and females; for example, the blood pressure-lowering

effects of Cit are more significant in women than men;[92,93] these findings highlight the importance of considering sex differences on the impact of Cit when carbohydrate metabolism is evaluated. Third, most reported animal and human studies on the metabolic effects of Cit are from short-term studies, with concerns remaining about their long-term potential adverse effects.[112] Finally, most of the beneficial metabolic effects of Cit are attributed to NO, and further studies are needed to explore the NO-independent metabolic effects of Cit.

All in all, Cit administration can be considered a promising treatment for T2D, but it needs to be investigated in randomized clinical trials.

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Conflict of intrest

The authors have declared no conflicts of interest.

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Table 1. Sex- and species-dependent metabolism of *L*-citrulline (Cit)

Parameter		Human		Rodent	
		Men	**Women**	**Males**	**Females**
Main site of endogenous synthesis		Small intestine (60-90%)[77]		Small intestine (60-90%)[83]	
Main sources of endogenous synthesis (%)	Glutamine	60-80[79,100]	60-80[100]	~20[103]	NA
	Arginine	20-40[79,100]	20-40[100]	<1[102,104]	NA
	Proline	~3[79,100]	~3[100]	~80[102,104]	NA
Rate of endogenous production (μmol/kg/h)		6-15[85]	6-15[85]	95-154[97,104]	141[97]
Plasma flux (μmol/kg/h)		5.5-10.6[85,100,105,106]	5.5-8.9[85,106]	81-95[97,100]	141±28[97]
Plasma concentration (μM)		26-37[88,89]	22-35[88,89]	40±5[97]	51±5[97]
Rate of Cit conversion to arginine (μmol/kg/h)		9-12[105,106,108]	11.9-12[106,108]	73±23[97]	120±28[97]
Rate of arginine conversion to Cit (μmol/kg/h)		0.36-0.96[105,106]	0.36±0.1[106]	2.9±0.9[97]	4.2±1.6[97]
Plasma flux (μmol/kg/h)		5.5-10.6[85,100,105,106]	5.5-8.9[85,106]	81-95[97,100]	141±28[97]
Renal clearance (mL/min)		0.27±0.12[107]	NA	39.6±8.3[97]	45.0±3.3[97]
Half-life (min)		40-70[94]	NA	180-240[78]	NA

NA, Not available.

Table 2. Circulating concentrations of Cit (μM) in patients with type 2 diabetes

Study	n	Control	Type 2 diabetes*	**Change**	
				μM	**%**
Tessari et al.[145]	18	31±6.4	53±8.4	+4	+11.4
Froukje et al.[146]	70	26.0±1.4	35.0±2.1	+9	+25.7
Zhou et al.[147]	226	35.7±7.9	49.8±8.7	+14.1	+28.3
Saleem et al.[148]	60	43.0±1.3	61.2±5.7	+18.3	+29.9
Lee et al.[149]	109	33.1±6.3	30.3±5.8	-2.8	-8.5
Kövamees et al.[150]	25	30.3±7.4	22.7±7.3	-7.6	-25.1
Tosur et al.[151]	12	27.8±1.7	17.8±1.2	-10	-35.9
Hsu et al.[152]	12	27.2±1.3	21.6±2.2	-5.6	-20.6

*All values are significantly different from controls.

Table 3. Animal and human studies related to the effects of *L*-citrulline (Cit) on type 2 diabetes (T2D)

Study models	Animal/patients	Model of T2D	Treatment	Duration (days)	Outcomes				Ref.
					Serum glucose	Serum insulin	Serum lipid profile	others	
Animal studies	Male rats (n=30)	Zucker diabetic fatty (ZDF)	63% watermelon juice (2014 mg/L Cit) in drinking water	28	↓	↔	↓FFA	Serum: ↑ Arg; ↑ Cit; ↓ *L*-ornithine	156
	Male rats (n=10)	HFD+Dex (1 mg/kg, intraperitoneal)	Oral Cit, 200, 400, 800 mg/kg	21	↓	NR	↓ TC; ↓ TG; ↓ LDL-C; ↑HDL-C	NR	61
	Male rats (n=12)	Zucker fatty diabetes mellitus (ZFDM)	2.0% Cit in drinking water	28	NR	NR	NR	↑ Plasma NOx; ↑ Cit; ↑ Arg	157
Human studies	T2D (n=25)	—	2000 mg/day	30	NR	NR	NR	↓Plasma arginase activity; ↑ Plasma nitrites	161
	T2D (n=45)	—	3000 mg/day	60	↓	↔	↓ TG ↑HDL-C	↓ HOMA-IR; ↑ HbA1c	159
	T2D (n=45)	—	3000 mg/day	60	NR	NR	NR	Serum: ↑NO metabolites; ↑ TAC; ↓ MDA	65

Arg, *L*-arginine; Dex, dexamethasone; FFA, free fatty acid; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein-cholesterol; HFD, high-fat diet; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein-cholesterol; MDA, malondialdehyde; NO, nitric oxide; TAC, total antioxidant capacity; TC, total cholesterol; TG, triglycerides; T2D, type 2 diabetes; ZFDM rats are derived from Zucker fatty rats and were generated by the repeated mating of male fatty (fa/fa) and female lean (fa/+) rats; NR, not reported.

Legend to Figures:

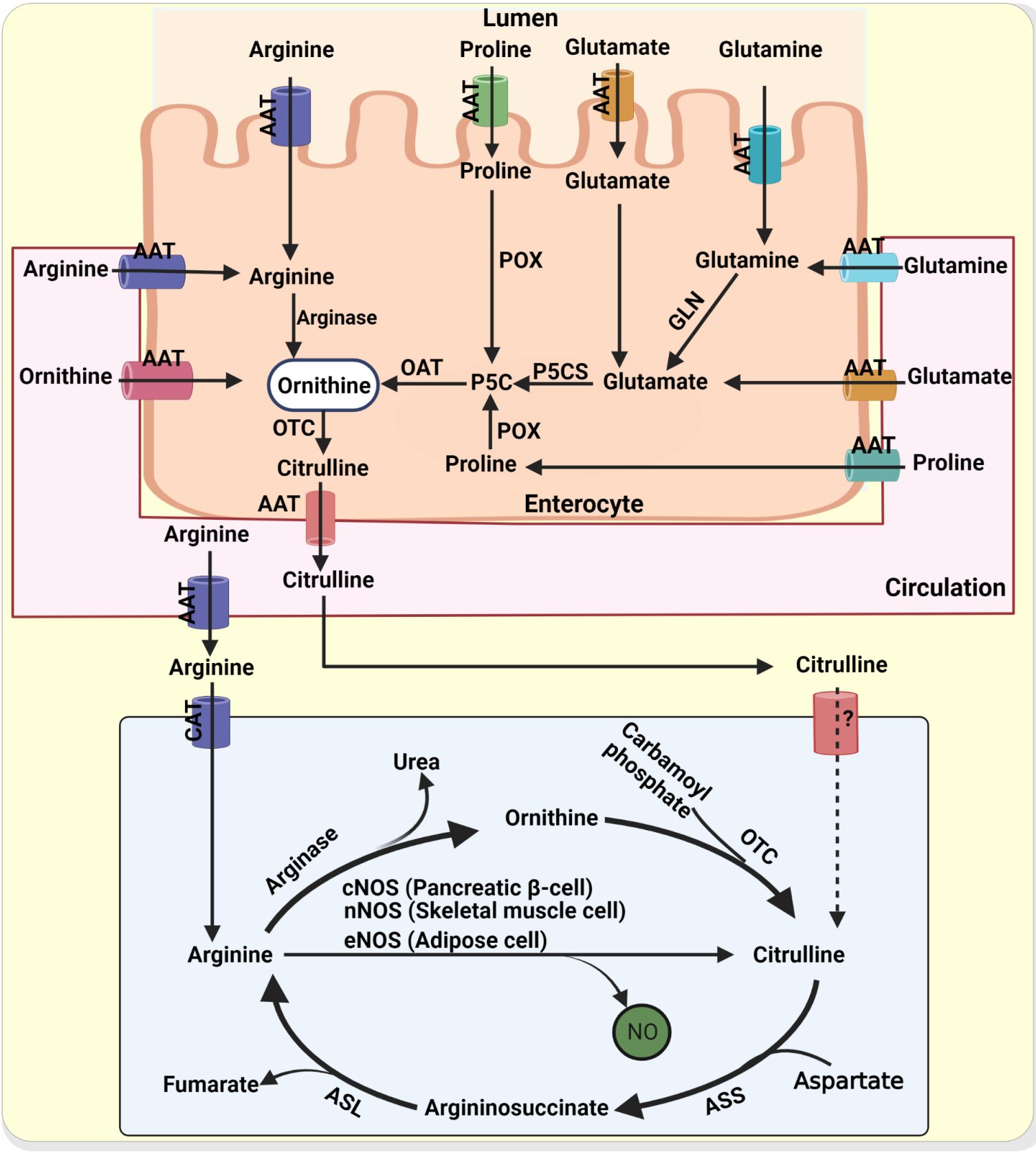
Figure 1. *L*-citrulline (Cit) synthesis in small intestine enterocytes and the enzymatic machinery for the Cit–arginine (Arg) cycle in β-cells, skeletal muscle, and adipose tissue. AAT, amino acid transporter; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; CAT, cationic amino acid transporter; cNOS, constitutive nitric oxide (NO) synthase; eNOS, endothelial NO synthase; GLN, glutaminase; nNOS, neuronal NO synthase; OAT, ornithine aminotransferase; OTC, ornithine transcarbamylase; P5C, pyrroline 5 carboxylate; P5CS, pyrroline 5 carboxylate synthase; POX, proline oxidase. *Created with BioRender.com*

Figure 2. Proposed mechanisms by which *L*-citrulline (Cit) stimulates glucose-stimulated insulin secretion in the pancreatic β-cells. These include (1) activation of voltage-dependent Ca^{2+} channels (VDCCs) via (a) inhibition of adenosine triphosphate (ATP)-sensitive K^+ channels (K_{ATP} channels) and (b) increase in intracellular cyclic guanosine monophosphate (cGMP); (2) stimulation of insulin gene promoter; (3) S-nitrosylation of glucokinase (GK); and (4) promotion of ATP production in the mitochondrion through the coupling of the Cit-Arg cycle to the Krebs cycle via the malate-aspartate shuttle. ADP, adenosine diphosphate; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; cNOS, constitutive nitric oxide (NO) synthase; ER, endoplasmic reticulum; ETC, electron transport chain; GLUT2, glucose transporter type 2; G6P, glucose 6-phosphate; GTP, guanosine triphosphate; mCAT2A, mouse cationic amino acid transporter 2A; OAA, oxaloacetate; sGC, soluble guanylate cyclase; TCA cycle, tricarboxylic acid cycle. *Created with BioRender.com*

Figure 3. Possible nitric oxide (NO)-dependent mechanisms by which *L*-citrulline (Cit) stimulates glucose uptake in the skeletal muscle. (1) increasing glucose transporter type 4 (GLUT4) gene expression by 5' adenosine monophosphate-activated protein kinase (AMPK) phosphorylation, (2) stimulating GLUT4 translocation from the cytosol to the cell membrane by (a) phosphorylation and (b) S-nitrosylation of GLUT4, and (3) increasing peroxisome proliferator-activated receptor γ (PPARγ) co-activator 1α (PGC-1α) and nuclear respiratory factor 1 (Nrf1) mRNA expression by phosphorylation of cyclic adenosine monophosphate (cAMP) response element-binding protein 1 (CREB1). ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; cGMP, cyclic guanosine monophosphate; IRS1, insulin receptor substrate-1; nNOS, neuronal NO synthase; PI3K, phosphoinositide 3-kinases; PKG, protein kinase G; sGC, soluble guanylate cyclase. *Created with BioRender.com*

Figure 4. Possible mechanisms of *L*-citrulline (Cit) metabolic effects in adipose tissue: (1) stimulating lipolysis by increasing phosphorylation of hormone-sensitive lipase (HSL), (2) inducing β-oxidation by increasing expression of carnitine palmitoyl-transferase1 (CPT1), (3) reducing glyceroneogenesis by decreasing expression of cytosolic phosphoenolpyruvate (PEP), and carboxykinase (PEPCK), (4) inducing thermogenesis by increasing the uncoupling protein 1 (UCP1) expression. AC, acylcarnitine; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; ATGL, adipose triglyceride lipase; ATP, adenosine triphosphate; Car, carnitine; CAT, carnitine-acylcarnitine translocase; Cit-Arg cycle, citrulline-arginine cycle; CPT2, carnitine palmitoyl-transferase2; DAG, diacylglycerol; DHAP, dihydroxyacetone phosphate; eNOS, endothelial nitric oxide (NO) synthase; FA-CoA, Fatty Acyl-CoA; GLUT4, glucose

transporter type 4; G3P, glycerol 3-phosphate; G6P, glycerol 6-phosphate; GyK, glycerol kinase; KB, ketone bodies; MAG, monoacylglycerol; MGL, monoglycerol lipase; NEFA, nonesterified fatty acid; OAA, oxaloacetate; PGC-1α, peroxisome proliferator-activated receptor γ (PPARγ) co-activator 1α; PPARα, peroxisome proliferator-activated receptor-alpha; TAG, triacylglycerol; TCA cycle, tricarboxylic acid cycle. *Created with BioRender.com*



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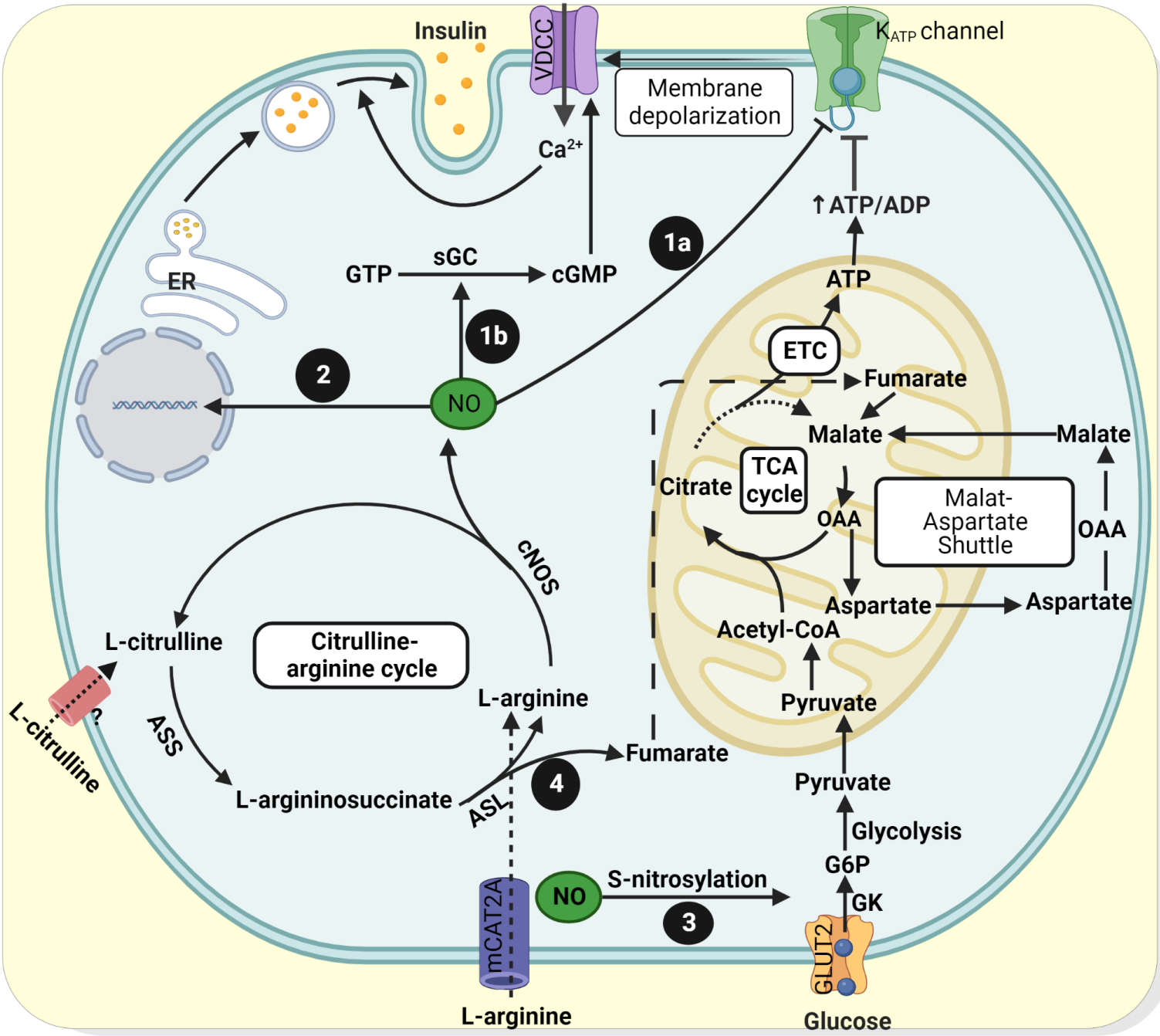

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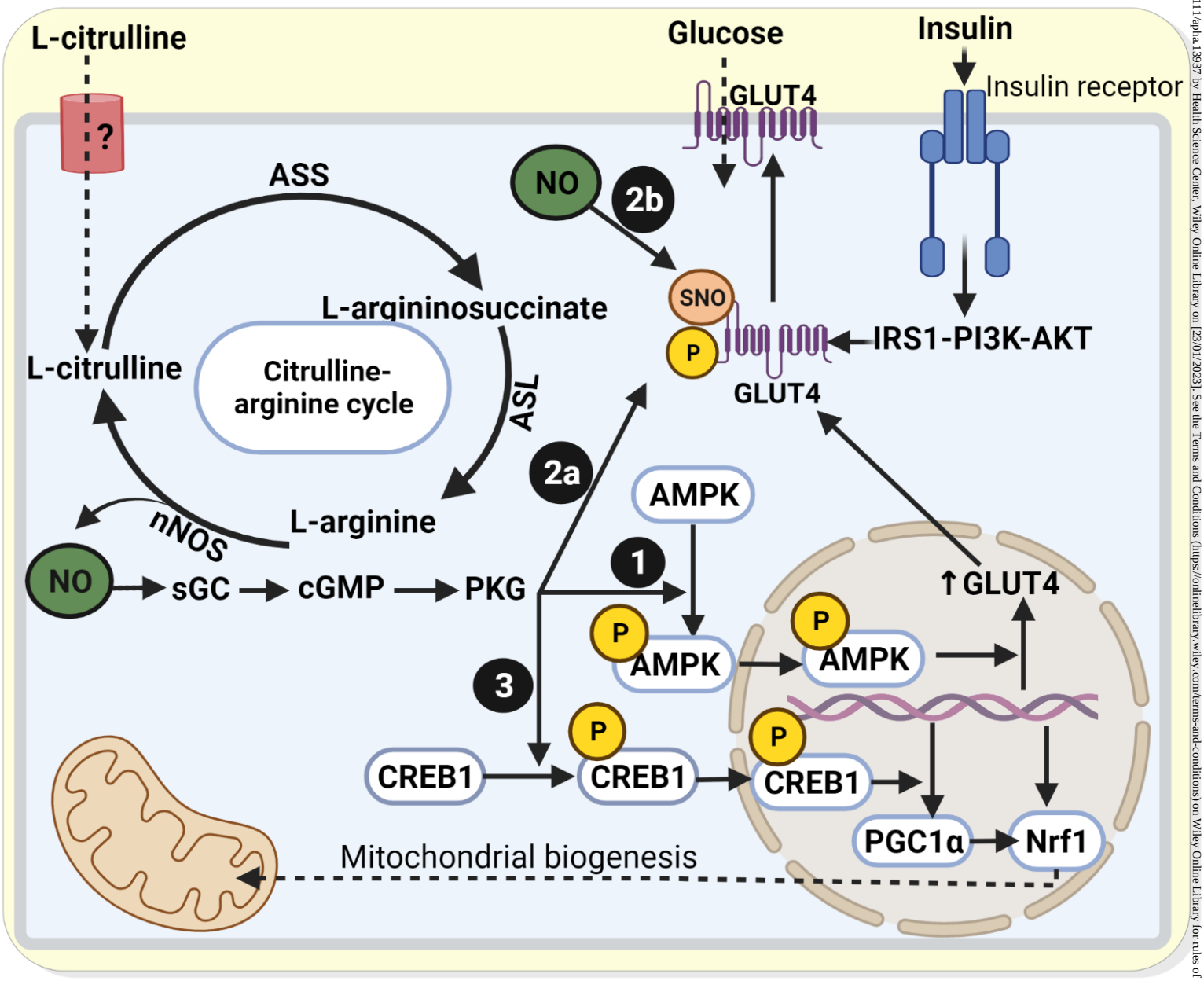

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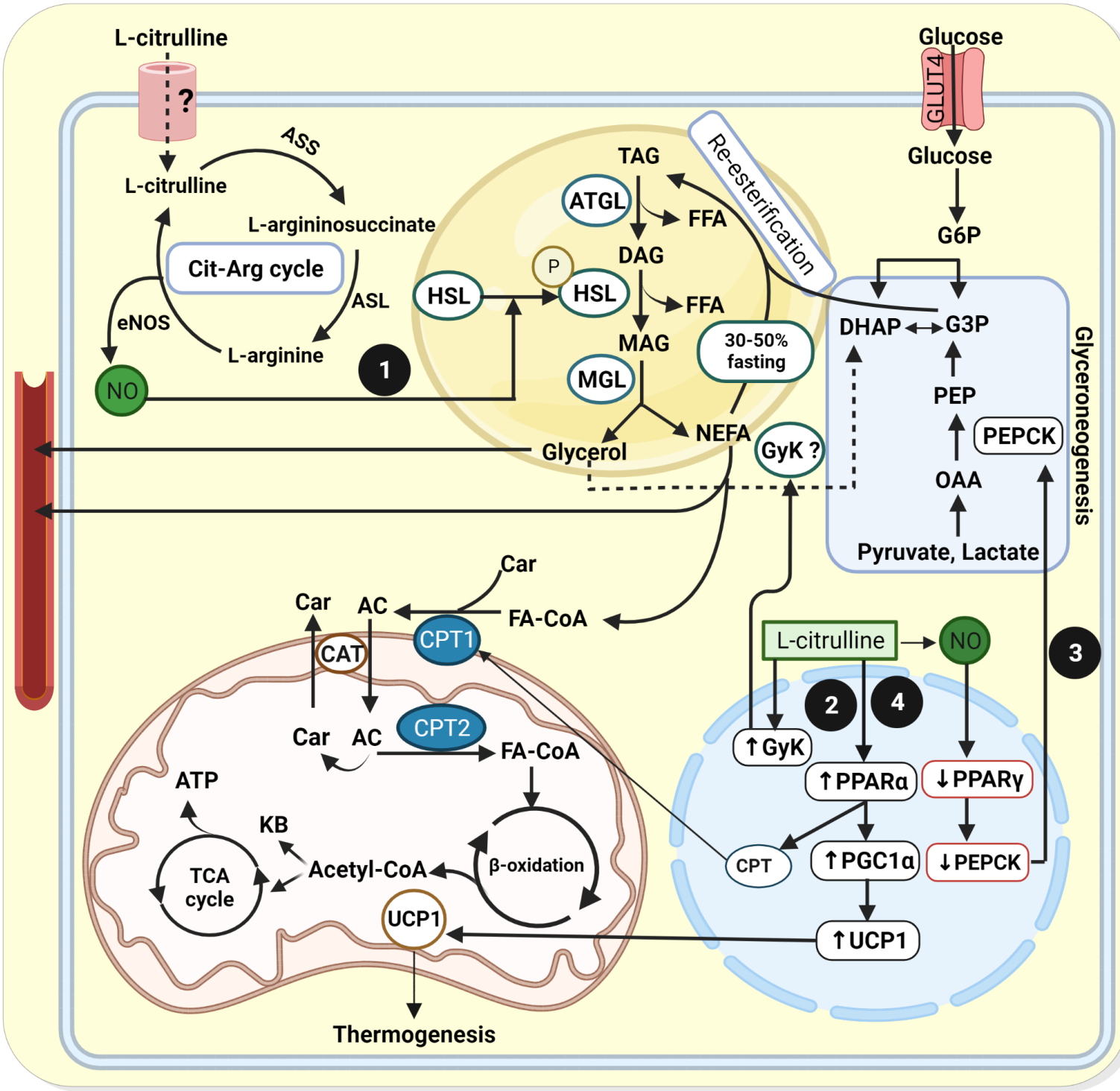

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