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Emerging therapeutic potential of glycine in cardiometabolic diseases: dual benefits in lipid and glucose metabolism

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Therapeutic approaches with dual lipid-lowering and glucose-lowering properties are clinically advantageous for the treatment of cardiometabolic diseases, including cardiovascular diseases (CVD) and type 2 diabetes (T2D). Current lipid-lowering therapies are known to either increase (statins) or have neutral effects (proprotein convertase subtilisin/kexin type 9 inhibitors) on diabetes risk [1]. Although the association between cardiometabolic diseases with dyslipidaemia is well recognized, recent metabolomics-based studies have ignited the notion that dysregulated metabolism of specific amino acids may seemingly play a central role in cardiometabolic diseases [2]. Particularly, emerging clinical evidence has identified the lower plasma levels of glycine in patients with insulin resistance or T2D [3,4], acute myocardial infarction (AMI) risk [5], acute aortic dissection [6], nonalcoholic fatty liver disease (NAFLD) and obesity [7]. These recent reports highlight a key role for glycine metabolism in cardiometabolic diseases and are supported by mechanistic evidence [8,9,10,11,12,13,14,15], indicating the role of glycine in metabolic processes engaged in dual glucose and lipid metabolism as well as improving multiorgan metabolic and inflammatory status (e.g. liver and heart, Fig. 1).

Consistent with observational reports of lower plasma glycine levels in patients with T2D [3,4], recent studies indicate that glycine stimulates insulin secretion by acting on glycine receptor (GlyR) in islet β -cells [8,9]. Although glycine beneficial effects on glucose homeostasis have been characterized, its involvement in lipid metabolism and the implication to CVD are only beginning to be elucidated. Recent reports demonstrate that circulating glycine levels are associated with a favourable lipid and inflammatory plasma profile (higher HDL-cholesterol and apolipoprotein A1, lower triglycerides, apolipoprotein B and C-reactive protein), and inversely correlated with AMI risk [5,16]. Moreover, a recent prospective population-based study demonstrated an association between dietary amino acid pattern of higher glycine load and reduced risk of CVD events [17]. Accordingly, glycine attenuates cardiac hypertrophy and fibrosis in preclinical models by acting on

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Conflicts of interest

There are no conflicts of interest.

GlyR α 2 in cardiomyocytes [10]. As the underlying cause for most CVD is atherosclerosis, characterized by imbalanced lipid metabolism and inflammation, the above reports indicate a putative antiatherogenic role for glycine.

Glycine, the simplest of all amino acids, serves as substrate for multifaceted metabolic processes, including generation of glutathione, creatine, purines and heme [18], yet how glycine is utilized to exert favourable lipid metabolism is unknown. To address the gap of knowledge regarding the involvement of amino acids in atherosclerosis, we recently analysed the effects of all 20 amino acids on cellular lipid metabolism in macrophages [12,19], that play key roles during atherogenesis, with new insights into their proatherogenic crosstalk with adipocytes [20]. Among all amino acids, glycine exerts the most prominent lipid-lowering effects and efficiently inhibits cellular VLDL uptake and triglyceride biosynthesis rates. Glycine deficiency exacerbates Western diet-induced obesity, hypercholesterolaemia, hyperglycaemia and atherosclerosis, whereas glycine treatment yielding supraphysiological steady-state levels is atheroprotective. We are currently pursuing the metabolic and molecular mechanisms underlying the lipid-lowering and antiatherogenic properties of glycine, with indications of regulatory roles in hepatic cholesterol homeostasis.

Another metabolic disorder characterized by imbalanced lipid metabolism and inflammation is NAFLD, a leading cause of chronic liver disease with no currently approved pharmaceutical therapies [21]. Personalized genome-scale metabolic modelling in NAFLD patients, uncovered an inverse association between circulating levels of glycine and its main precursor serine with hepatic steatosis. Moreover, glycine was identified as the limiting substrate for de novo synthesis of the major endogenous antioxidant glutathione, with higher metabolic demand in patients with liver steatosis [13]. Restored glutathione supply by glycine also protects from diet-induced or ageing-induced vascular dysfunction and inflammation [11]. In accordance with recent reports of lower plasma glycine in NAFLD patients [7], glycine treatment has recently been shown to protect from NAFLD in preclinical settings. In genetically obese/diabetic KK-A^y mice, dietary glycine supplementation improves both glucose intolerance and hypertriglyceridaemia, preventing body weight gain, hepatic steatosis and inflammation via downregulation of lipogenic genes and improvement of hepatic innate immune microenvironment [14]. Similar effects were reported in Sprague–Dawley rats fed high-fat and sucrose diet, in which glycine supplementation protects from diet-induced liver damage via attenuation of apoptosis, oxidative stress and endoplasmic reticulum stress [15].

Overall, emerging evidence from observational and preclinical studies underscore a relevant role of glycine metabolism in cardiometabolic diseases. Human studies reveal an inverse association between plasma glycine levels and risk of T2D, AMI, NAFLD and obesity. In experimental models, glycine treatment improves glycaemic control, dyslipidaemia, cardiovascular function and steatohepatitis. As depicted in Fig. 1, glycine glucose-lowering and cardioprotective effects are mediated via its actions on GlyR α 1 in pancreatic islets and on GlyR α 2 in cardiomyocytes, respectively. The role of glycine in improving inflammation may be also explained, at least in part, by serving as a precursor for de novo glutathione synthesis. Dysregulated glycine metabolism is consistently identified as a metabolic biomarker in diabetes, yet additional evidence support inhibition of lipogenic pathways and

anti-inflammatory properties, both warrant further investigation. These emerging studies highlight the therapeutic potential of targeting glycine metabolism with relevance to some of the major clinical challenges observed in current cardiometabolic medicine, which include, first, identification of therapies with dual benefits in both glucose and lipid metabolism for the treatment of dyslipidaemia, T2D and CVD. Second, identification of novel therapeutic approaches against NAFLD. A multipronged approach including metabolomics, systems biology, experimental therapeutics, chemical and enzymatic biology is required to further our understating on the therapeutic potential of glycine and establish a glycine-based therapeutic approach. Current efforts are being actively pursued on the following aspects: first, to elucidate glycine antiatherogenic properties and its favourable impact on lipid metabolism and inflammation. Second, to identify chemical and/or enzymatic interactions, glycine mimetics or pharmacological/genetic approaches to leverage endogenous glycine metabolism for enhanced cardiometabolic protection. Third, clinically evaluate glycine abilities to improve glycaemic control and dyslipidaemia and subsequently, to reduce the risk of cardiometabolic diseases. Our studies underscore the integrative role of the simplest amino acid, glycine, as an example of metabolic convergence at the crossroad of lipid and glucose homeostasis.

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest

■ ■ of outstanding interest

- 1 ■ Sabatine MS, Leiter LA, Wiviott SD, et al. Cardiovascular safety and efficacy of the PCSK9 inhibitor evolocumab in patients with and without diabetes and the effect of evolocumab on glycaemia and risk of new-onset diabetes: a prespecified analysis of the FOURIER randomised controlled trial. *Lancet Diabetes Endocrinol* 2017; 5:941–950. [PubMed: 28927706] To date, the FOURIER trial is the largest study (27 564 patients with atherosclerotic disease) of clinical and glycaemic outcomes with a proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor. This study demonstrates that PCSK9 inhibition with evolocumab lowered LDL-cholesterol and significantly reduced cardiovascular risk with greater absolute risk reduction in patients with diabetes. Although previous studies reported that statins increase the risk of new-onset diabetes, in the FOURIER trial, PCSK9 inhibition did not worsen glycaemia over several years and did not increase the risk of new-onset diabetes.
- 2 ■ Newgard CB. Metabolomics and metabolic diseases: where do we stand? *Cell Metab* 2017; 25:43–56. [PubMed: 28094011] The review discusses the advances of using metabolomics to identify predictive biomarkers for cardiometabolic diseases and the importance of this approach for understating pathophysiologic mechanisms of such diseases. Emphasis is given to metabolomics-based studies that identified a signature of dysregulated metabolism of specific

amino acids such as branched-chain amino acids (BCAAs) and glycine in patients with various forms of cardiometabolic disease.

3. Floegel A, Stefan N, Yu Z, et al. Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. *Diabetes* 2013; 62:639–648. [PubMed: 23043162]
4. Gar C, Rottenkolber M, Prehn C, et al. Serum and plasma amino acids as markers of prediabetes, insulin resistance, and incident diabetes. *Crit Rev Clin Lab Sci* 2018; 55:21–32. [PubMed: 29239245]
- 5■■■. Ding Y, Svingen GF, Pedersen ER, et al. Plasma glycine and risk of acute myocardial infarction in patients with suspected stable angina pectoris. *J Am Heart Assoc* 2016; 5:e002621. The study of 4109 patients with suspected stable angina pectoris demonstrated, for the first time, that plasma glycine levels are inversely correlated with the risk of acute myocardial infarction (AMI). Significantly, plasma glycine levels are shown to be associated with a favourable lipid profile and lower prevalence of obesity, hypertension, and diabetes mellitus. These findings highlight the importance of elucidating the relationship between glycine metabolism and cholesterol metabolism, particularly in relation to atherosclerosis.
6. Wang L, Liu S, Yang W, et al. Plasma amino acid profile in patients with aortic dissection. *Sci Rep* 2017; 7:40146. [PubMed: 28071727]
- 7■. Gaggini M, Carli F, Rosso C, et al. Altered amino acid concentrations in NAFLD: impact of obesity and insulin resistance. *Hepatology* 2018; 67:145–158. [PubMed: 28802074] In this recent study, the plasma levels of amino acids were analysed in patients with nonalcoholic fatty liver disease (NAFLD) (with or without obesity) vs. healthy controls. Although the plasma concentrations of most amino acids were significantly higher in obese NAFLD patients, only glycine levels were reported to be significantly lower. Moreover, glycine plasma levels were shown to be significantly lower in the presence of increased liver damage as measured by hepatic ballooning and inflammation and inversely correlated with hepatic insulin resistance.
- 8■■■. Yan-Do R, Duong E, Manning Fox JE, et al. A glycine-insulin autocrine feedback loop enhances insulin secretion from human β -cells and is impaired in type 2 diabetes. *Diabetes* 2016; 65:2311–2321. [PubMed: 27207556] The study investigated the role of glycine in insulin secretion from human pancreatic islets and demonstrates that human β -cells express glycine receptor (GlyR) (mainly GlyR α 1), which mediates a depolarizing Cl[−] current that enhances action potential firing, Ca²⁺ entry, and insulin secretion from β -cells. In β -cells from donors without type 2 diabetes (T2D), but not in β -cells from donors with T2D, the GlyR-mediated current is enhanced by insulin. Significantly, GlyR α 1 protein expression and glycine-activated currents are shown to be downregulated in β -cells from donors with T2D. These findings provide a physiological basis for the observed beneficial effects of glycine on glycaemic control via its action on GlyR α 1 in pancreatic islets.
- 9■. Yan-Do R, MacDonald PE. Impaired ‘glycine’-mia in type 2 diabetes and potential mechanisms contributing to glucose homeostasis. *Endocrinology* 2017; 158:1064–1073. [PubMed: 28323968] A good review of the evidence supporting the role of glycine in glucose homeostasis via its central and peripheral actions. Studies demonstrating a negative correlation between plasma glycine levels and T2D, reports of increased plasma glycine levels in response to interventions such as exercise and bariatric surgery, as well as evidence for improved glycaemic control following glycine supplementation are discussed.
- 10■. Lu Y, Zhu X, Li J, et al. Glycine prevents pressure overload induced cardiac hypertrophy mediated by glycine receptor. *Biochem Pharmacol* 2017; 123:40–51. [PubMed: 27836671] The study demonstrates the protective effects of glycine pretreatment in murine cardiac hypertrophy and fibrosis models induced by transverse aortic constriction or by administration of angiotensin II. Glycine is shown to act via GlyR α 2 in cardiomyocytes and to inhibit extracellular signal-regulated kinase 1/2 phosphorylation and overexpression of transforming growth factor β , endothelin-1 and collagen I and III. These findings indicate a cardioprotective role for glycine via GlyR α 2.
11. Cieslik KA, Sekhar RV, Granillo A, et al. Improved cardiovascular function in old mice after *N*-acetyl cysteine and glycine supplemented diet: inflammation and mitochondrial factors. *J Gerontol A Biol Sci Med Sci* 2018 [Epub ahead of print]
- 12■■■. Rom O, Grajeda-Iglesias C, Najjar M, et al. Atherogenicity of amino acids in the lipid-laden macrophage model system in vitro and in atherosclerotic mice: a key role for triglyceride

metabolism. *J Nutr Biochem* 2017; 45:24–38. [PubMed: 28431321] Recently, we carried out a systematic analysis of all 20 amino acids in macrophage lipid metabolism that play key roles in atherosclerosis development. We identified, for the first time, specific amino acids that promote or reduce macrophage lipid accumulation. Among all amino acids, glycine had the most prominent lipid-lowering effects in a murine macrophage model system, mediated by inhibition of VLDL uptake and triglyceride biosynthesis. Administration of glycine to apolipoprotein E-deficient (apoE^{−/−}) mice, significantly increased its serum levels, enhanced the activity of the HDL-associated enzyme paraoxonase 1 and decreased serum triglycerides. Accordingly, the lipid content in peritoneal macrophages isolated from glycine-treated apoE^{−/−} mice was lower and a trend towards reduced aortic cholesterol was noted. These findings are in line with epidemiological evidence demonstrating that glycine levels are associated with a favourable lipid profile and inversely correlated with AMI risk, suggesting an antiatherogenic potential for glycine.

- 13 ■ Mardinoglu A, Bjornson E, Zhang C, et al. Personal model-assisted identification of NAD⁺ and glutathione metabolism as intervention target in NAFLD. *Mol Syst Biol* 2017; 13:916. [PubMed: 28254760] Aimed to elucidate the molecular and metabolic mechanisms of NAFLD, this study used hepatocyte genome-scale metabolic modelling in 86 subjects with varying degrees of hepatic steatosis. Using nontargeted metabolomics profiling (~520 metabolites), plasma levels of glycine and its main precursor serine negatively correlated with the level of hepatic steatosis. Systems level analysis in human and mice demonstrated that glycine is the limiting substrate for de novo glutathione synthesis, which was found to be in higher demand in subjects with high hepatic steatosis. In a proof-of-concept human study, supplementation with the glycine precursor serine improved liver function and decreased hepatic steatosis. This study provides a strong indication for glycine potential as a biomarker and a therapeutic strategy for NAFLD.
14. Takashima S, Ikejima K, Arai K, et al. Glycine prevents metabolic steatohepatitis in diabetic KK-A^y mice through modulation of hepatic innate immunity. *Am J Physiol Gastrointest Liver Physiol* 2016; 311:G1105–G1113. [PubMed: 27659424]
15. Zhou X, Han D, Xu R, et al. Glycine protects against high sucrose and high fat-induced nonalcoholic steatohepatitis in rats. *Oncotarget* 2016; 7: 80223–80237. [PubMed: 27784003]
16. Demirkan A, Henneman P, Verhoeven A, et al. Insight in genome-wide association of metabolite quantitative traits by exome sequence analyses. *PLoS Genet* 2015; 11:e1004835. [PubMed: 25569235]
17. Mirmiran P, Bahadoran Z, Ghasemi A, Azizi F. Contribution of dietary amino acids composition to incidence of cardiovascular outcomes: a prospective population-based study. *Nutr Metab Cardiovasc Dis* 2017; 27:633–641. [PubMed: 28684082]
18. Adeva-Andany M, Souto-Adeva G, Ameneiros-Rodríguez E, et al. Insulin resistance and glycine metabolism in humans. *Amino Acids* 2018; 50:11–27. [PubMed: 29094215]
19. Grajeda-Iglesias C, Rom O, Hamoud S, et al. Leucine supplementation attenuates macrophage foam-cell formation: studies in humans, mice, and cultured macrophages. *Biofactors* 2018; 44:245–262. [PubMed: 29399895]
20. Nimri L, Grajeda-Iglesias C, Volkova N, Aviram M. Pro-atherogenic and prooxidant crosstalk between adipocytes and macrophages. *Eur J Nutr* 2018 [Epub ahead of print]
21. Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: trends, predictions, riskfactors and prevention. *Nat Rev Gastroenterol Hepatol* 2018; 15:11–20. [PubMed: 28930295]

FURTHER RECOMMENDED READING

- Cruz M, Maldonado-Bernal C, Mondragón-Gonzalez R, et al. Glycine treatment decreases proinflammatory cytokines and increases interferon-gamma in patients with type 2 diabetes. *J Endocrinol Invest* 2008; 31:694–699. [PubMed: 18852529] ■ The study demonstrates that chronic supplementation with glycine (5g/day, 3 months) to 74 patients with T2D significantly reduced the levels of glycated haemoglobin and TNFR1 and increased IFN- γ levels, indicating a role of glycine in glycaemic control and inflammatory response in T2D patients.

- Mardinoglu A, Agren R, Kampf C, et al. Genome-scale metabolic modelling of hepatocytes reveals serine deficiency in patients with nonalcoholic fatty liver disease. *Nat Commun* 2014; 5:3083. [PubMed: 24419221] ■ The study describes the reconstruction of a genome-scale metabolic model for hepatocytes (iHepatocytes2322) allowing the study of metabolic reprogramming in response to the development of NAFLD. The iHepatocytes2322 model was used to analyse transcriptomics data from NAFLD patients, demonstrating a significant decrease in the expression of genes encoding for enzymes catalysing glycine and serine biosynthesis in patients with nonalcoholic steatohepatitis.
- Ruiz-Ramirez A, Ortiz-Balderas E, Cardozo-Saldaña G, et al. Glycine restores glutathione and protects against oxidative stress in vascular tissue from sucrose-fed rats. *Clin Sci (Lond)* 2014; 126:19–29. [PubMed: 23742196] ■ The study demonstrates the protective effects of glycine supplementation to sucrose-fed rats on their isolated aortas. Glycine is shown to reduce the release of superoxide anion radical ($O_2^{\bullet -}$) and to decrease lipid peroxidation in relation with increased biosynthesis of glutathione.
- Chen J, Ma X, Yang Y, et al. Glycine enhances expression of adiponectin and IL-10 in 3T3-L1 adipocytes without affecting adipogenesis and lipolysis. *Amino Acids* 2018; 50:629–640. [PubMed: 29356901] ■ This in-vitro study in 3T3-L1 adipocytes aimed to determine the effects of glycine on adipocyte differentiation, adipogenesis and lipolysis. Although glycine had no significant effects of adipogenesis and lipolysis in the adipocytes, it markedly increased the expression of adiponectin and IL-10, two adipose-derived adipokines with anti-inflammatory properties. These findings provide a possible explanation for the observed antiobesogenic and anti-inflammatory properties of glycine.
- Rom O, Aviram M. It is not just lipids: proatherogenic vs. antiatherogenic roles for amino acids in macrophage foam cell formation. *Curr Opin Lipidol* 2017; 28:85–87. [PubMed: 28030455] ■ An overview of clinical and mechanistic evidence supporting a role of amino acids in macrophage foam cell formation, atherosclerosis development and cardiovascular diseases risk.
- Grajeda-Iglesias C, Rom O, Aviram M. Branched-chain amino acids and atherosclerosis: friends or foes? *Curr Opin Lipidol* 2018; 29:166–169. [PubMed: 29517610] ■ A concise overview discussing the role of BCAA in lipid metabolism and atherosclerosis. Emphasis is given to the discrepancy between association studies that consistently report a positive correlation between plasma levels of BCAA and the risk of cardiometabolic diseases vs. interventional studies that demonstrate beneficial effects of BCAA supplementation (mainly, leucine) in lipid metabolism-related diseases such as atherosclerosis and NAFLD.

FIGURE 1.

Emerging therapeutic potential of glycine in cardiometabolic diseases. (a) Glycine has been proposed as a biomarker for diabetes onset and progression. Association studies consistently show that plasma glycine levels are low in patients with type 2 diabetes and correlate negatively with insulin resistance. Mechanistically, human islet β-cells express glycine receptor (mainly glycine receptor α1), which mediates a depolarizing Cl⁻ current, enhances action potential firing, Ca²⁺ entry and insulin secretion from β-cells. In β-cells from donors with type 2 diabetes, glycine receptor α1 expression and glycine-activated currents are

impaired. (b) Plasma glycine levels negatively correlate with acute myocardial infarction risk and are associated with a favourable lipid and inflammatory plasma profile. Mechanistically, glycine acts via glycine receptor α_2 to protect against transverse aortic constriction-mediated or angiotensin II-mediated cardiac hypertrophy and fibrosis in mice, by preventing extracellular signal-regulated kinase 1/2 phosphorylation, overexpression of transforming growth factor β , endothelin-1 and collagen I and III. In ageing mice, glycine + *N*-acetylcysteine (but not *N*-acetylcysteine alone) reduce leucocyte infiltration and improve cardiac and mitochondrial function. Glycine decreases lipid accumulation in murine macrophages via attenuation of VLDL uptake and triglycerides biosynthesis. (c) Plasma glycine levels are low in patients with nonalcoholic fatty liver disease and obesity and negatively correlate with the severity of hepatic steatosis and obesity. Mechanistic evidence indicates that glycine is the limiting substrate for hepatic biosynthesis of glutathione, which is in higher demand in patients with hepatic steatosis. In genetic and dietary rodent nonalcoholic fatty liver disease models, glycine protects from steatohepatitis via downregulation of lipogenic genes (sterol regulatory element-binding protein-1c, stearoyl-CoA desaturase-1, acetyl-CoA carboxylase, fatty acid synthase), attenuation of lipid peroxidation (reactive oxygen species, 4-hydroxy-2-nonenal), and improvement of hepatic innate immune response (increased M2 transformation of Kupffer cells and natural killer T cells). In 3T3-L1 adipocytes, glycine increases the expression of adiponectin and IL-10. T2D, type 2 diabetes; GlyR, glycine receptor; CVD, cardiovascular diseases; AMI, acute myocardial infarction; HDL-C, HDL cholesterol; apoA1, apolipoprotein A1; TG, triglycerides; apoB, apolipoprotein B; CRP, C-reactive protein; ERK, extracellular signal-regulated kinase; TGF- β , transforming growth factor β ; ET-1, endothelin-1; NAFLD, nonalcoholic fatty liver disease; SREBP1c, sterol regulatory element-binding protein-1c; SCD1, stearoyl-CoA desaturase-1; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; ROS, reactive oxygen species; 4-HNE, 4-hydroxy-2-nonenal; NK, natural killer; GSH; glutathione.