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REVIEW

Sodium-glucose cotransporter 2 inhibitors' mechanisms of action in heart failure

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Abstract

Three major cardiovascular outcome trials (CVOTs) with a new class of antidiabetic drugs - sodium-glucose cotransporter 2 (SGLT2) inhibitors (EMPA-REG OUTCOME trial with empagliflozin, CANVAS Program with canagliflozin, DECLARE-TIMI 58 with dapagliflozin) unexpectedly showed that cardiovascular outcomes could be improved possibly due to a reduction in heart failure risk, which seems to be the most sensitive outcome of SGLT2 inhibition. No other CVOT to date has shown any significant benefit on heart failure events. Even more impressive findings came recently from the DAPA-HF trial in patients with confirmed and well-treated heart failure: Dapagliflozin was shown to reduce heart failure risk for patients with heart failure with reduced ejection fraction regardless of diabetes status. Nevertheless, despite their possible wide clinical implications, there is much doubt about the mechanisms of action and a lot of questions to unravel, especially now when their benefits translated to non-diabetic patients, rising doubts about the validity of some current mechanistic assumptions.The time frame of their cardiovascular benefits excludes glucose-lowering and antiatherosclerotic-mediated effects and multiple other mechanisms, direct cardiac as well as systemic, are suggested to explain their early cardiorenal benefits. These are: Anti-inflammatory, antifibrotic, antioxidative, antiapoptotic

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properties, then renoprotective and hemodynamic effects, attenuation of glucotoxicity, reduction of uric acid levels and epicardial adipose tissue, modification of neurohumoral system and cardiac fuel energetics, sodium-hydrogen exchange inhibition. The most logic explanation seems that SGLT2 inhibitors timely target various mechanisms underpinning heart failure pathogenesis. All the proposed mechanisms of their action could interfere with evolution of heart failure and are discussed separately within the main text.

Key words: Sodium-glucose cotransporter 2 inhibitors; Heart failure; Cardiovascular outcomes; Diabetes mellitus; Physiological mechanisms; Pleiotropic effects

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Core tip: Three major cardiovascular outcome trials with a new class of antidiabetic drugs-sodium-glucose cotransporter 2 inhibitors unexpectedly showed that cardiovascular outcomes could be improved due to a reduction in heart failure events. Moreover, recently dapagliflozin was shown to reduce heart failure risk for patients with heart failure with reduced ejection fraction regardless of diabetic status. Currently, there is much doubt regarding the mechanisms of action of these drugs. The most logic explanation is that they are timely targeting various mechanisms underpinning heart failure pathogenesis due to pleiotropic effects which are discussed in the main text.

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INTRODUCTION

Atherosclerotic cardiovascular disease is the leading cause of morbidity and mortality among diabetic patients. Due to US Food and Drug Administration requirements, since 2008 a series of large clinical trials with new hypoglycemic drugs have been designed to rule out cardiovascular harm and to show noninferiority on the cardiovascular outcomes while improving glucose control[1]. Three major cardiovascular outcome trials (CVOTs) with a new class of drugs - sodium-glucose cotransporter 2 (SGLT2) inhibitors (EMPA-REG OUTCOME trial with empagliflozin, CANVAS Program with canagliflozin, DECLARE-TIMI 58 with dapagliflozin; Table 1 unexpectedly showed that cardiovascular outcomes could be improved possibly due to a reduction in heart failure risk, which seems to be the most sensitive outcome of SGLT2 inhibition[2-4]. It is worthwhile to mention that no other CVOT to date has shown any significant benefit on heart failure events[5].These observations set the stage for the new game changers in cardiometabolic pharmacotherapy and opened up new possibilities in heart failure strategies, along with its standard medical therapy with neurohormonal antagonists [mineralocorticoid receptor antagonists, β-blockers, angiotensin-converting enzyme (ACE) inhibitors/angiotensin receptor blockers(ARBs), neprilysin inhibitors]. American Diabetes Association has already recommended new strategies in the treatment of diabetic patients with established atherosclerotic cardiovascular disease and patients with heart failure, and that is the preferential use of SGLT2 inhibitors (empa-, dapa- and canagliflozin) in these patients[1]. Furthermore, even more impressive findings came recently from the DAPA-HF trial in patients with confirmed and well-treated heart failure: dapagliflozin was shown to reduce heart failure risk in patients with heart failure with reduced ejection fraction (HFrEF) regardless of their diabetic status[6]. So, at present, there are evidences that these new drugs work both for diabetic and nondiabetic patients, regardless of existing atherosclerotic cardiovascular disease or heart failure, and probably in both HFrEF and heart failure with preserved ejection fraction (HFpEF), making them unique and important for clinical practice[7,8]. Nevertheless, despite their possible wide clinical implications, there is much doubt regarding the mechanisms of action and a lot of questions still left to unravel. While scientific and professional community is

Table 1 Major cardiovascular outcome trials with sodium-glucose cotransporter 2 inhibitors

Parameters	EMPA-REG OUTCOME	CANVAS program	DECLARE-TIMI 58
Intervention	Empagliflozin/placebo	Canagliflozin/placebo	Dapagliflozin/placebo
Median follow-up (yr)	3.1	3.6	4.2
Number of patients	7020	10142	17160
Prior cardiovascular disease/heart failure (%)	99/10	65.6/14.4	40/10
Primary outcome (3-point MACE)	0.86 (95%CI: 0.74-0.99) Noninferiority, P < 0.001; Superiority, P = 0.04	0.86 (95%CI: 0.75-0.97) Noninferiority, P < 0.001; Superiority, P = 0.02	0.93 (95%CI: 0.84-1.03) Noninferiority, P < 0.001; Superiority, P = 0.17
Cardiovascular death	0.62 (0.49-0.77)[1]	0.87 (0.72-1.06)	0.98 (0.81-1.17)
Myocardial infarction	0.87 (0.70-1.09)	0.89 (0.73-1.09)	0.89 (0.77-1.01)
Stroke	1.18 (0.89-1.56)	0.87 (0.69-1.09)	1.01 (0.84-1.21)
Heart failure hospitalization	0.65 (0.50-0.85)[1]	0.67 (0.52-0.87)[1]	0.73 (0.61-0.88)[1]
All cause mortality	0.68 (0.57-0.82)[1]	0.87 (0.74-1.01)	0.93 (0.82-1.04)

MACE: Major adverse cardiac event.

[1]Significant.

struggling in pursuing those answers, at the same time, we are expecting the results of several ongoing studies of SGLT2 inhibitors in heart failure to fully evaluate their therapeutic potential. In this review we will try to give some mechanistic insights of SGLT2 inhibitors' mode of action regarding heart failure, from current hypotheses of possible mechanisms of action to explain cardiac protection to the controversies and gaps in evidence, as well as potential future developments in the field.

SGLT2 INHIBITORS AND HEART FAILURE

Sodium-dependent glucose cotransporters are a family of active glucose transporter proteins. SGLT1 is widely expressed in numerous organs (heart, liver, small intestine, lung, kidney), while SGLT2 is mainly expressed in the renal proximal tubule. Under normal conditions, glucose is filtered into the urine at the glomerulus and reabsorbed in the proximal tubuli by SGLT2 (90%) and SGLT1 (the remaining 10%). In hyperglycemic conditions SGLT2 expression is increased, paradoxically augmenting the threshold for urinary glucose excretion in diabetic patients. SGLT2 inhibitors are a novel class of antidiabetic agents that promote urinary glucose excretion by inhibiting glucose and sodium reabsorption from the renal proximal tubules and have recently been investigated in several large randomized controlled trials for cardiovascular safety and efficacy in patients with type 2 diabetes[9-11]. Enhancing urinary glucose excretion by targeting SGLT2 represents an alternative strategy to the traditional antihyperglycemic interventions that have been focused on restoring β-cell activity, insulin sensitivity or tissue glucose uptake to normalize plasma glucose levels in patients with diabetes. SGLT2 inhibitors are generally well tolerated, and the risk of hypoglycemia is low because the efficacy of SGLT2 inhibitors to increase glucose excretion attenuates at lower plasma glucose levels[12,13]. Since regulatory agencies have issued safety warnings for several adverse events (urinary tract infections, diabetic ketoacidosis, acute kidney injury, bone fractures, lower limb amputations) based primarily on case report data, a meta-analysis of randomized controlled trials with SGLT2 inhibitors was performed and concluded that current evidences do not suggest an increased risk of harm with SGLT2 inhibitors as a class over placebo or active comparators with respect to acute kidney injury, diabetic ketoacidosis, urinary tract infections and bone fractures[14]. Further research is required to ascertain whether there is an increased risk of amputations associated with SGLT2 inhibitors. Evidence on the risk of lower limb amputations is limited to the results from CANVAS trial. There was an increased risk of amputations, although the overall incidence of these events was low and the study was not powerful enough to detect significant differences among the studied population[13,14].

According to a recent meta-analysis of the three major CVOTs with these drugs (the EMPA-REG OUTCOME trial, the CANVAS Program and the DECLARE-TIMI 58 trial), even though the exact inclusion criteria and definitions of endpoints varied among them, the presence of established atherosclerotic disease and heart failure was investigator-reported and no heart failure phenotyping was performed, SGLT2 inhibitors, as a class, have moderate benefits on atherosclerotic major adverse cardiovascular events in patients with established atherosclerotic cardiovascular disease but also have robust benefits on reducing hospitalization for heart failure and progression of renal disease regardless of existing atherosclerotic disease or a history of heart failure (they reduced the risk of heart failure hospitalization by 31% and progression of renal disease by 45%)[6,15]. This efficacy in the prevention (primary as well as secondary) of heart failure has already translated to efficacy in the treatment of heart failure as shown in the above-mentioned DAPA-HF trial including patients with HFrEF. But SGLT2 inhibitors may also be valuable in the treatment of HFpEF according to the subanalyses of CVOTs and accumulating mechanistic insights[8,16,17]. Till now, in trials of HFpEF, several established treatments for HFrEF have shown no efficacy. So, if results of ongoing studies with SGLT2 inhibitors in HFpEF would show effectiveness, this will represent a true breakthrough in heart failure treatment (Table 2).

Heart failure comprises an array of patients categorized by their symptoms and ejection fraction (HFrEF with EF < 40%, midrange EF between 40% and 49%, and HFpEF with EF > 50%). It is a growing public health problem, with an estimated 63 million people affected worldwide[16]. Heart failure has a progressive nature and preventive strategies have to be adopted early because starting treatment at the preclinical stage may improve its outcomes[18]. Hospitalization for heart failure carries a 10% mortality rate at 30 days postdischarge, 20% at 1 year, the readmission rate at 6 months is 50%, and the risk of mortality is greater with each hospitalization[16]. Despite established treatment options for HFrEF that are associated with reduced mortality, the prognosis of heart failure is still very poor. In the recent US study of Shah *et al*[19] patients across the ejection fraction spectrum have a similarly 5-year mortality around 75% with an elevated risk for heart failure and cardiovascular hospitalizations. These data warn us that treatment strategies of patients with heart failure need to be improved.

Heart failure is a particularly common complication of diabetes, with poor 5-year survival rates, but it seems to have been neglected because more attention was given to atherothrombotic complications of disease. Furthermore, patients with diabetes are predisposed to a distinct cardiomyopathy - diabetic cardiomyopathy which is independent of concomitant diabetic macro- and microvascular complications. It is still poorly understood, but of great clinical importance, given the robust association of diabetes mellitus with heart failure and increased cardiovascular mortality[20,21]. Could SGLT2 inhibitors be the new heart savers in diabetes and beyond diabetes? How is their effect so ubiquitous across the spectrum of heart failure? Are we missing something in the pathophysiology of heart failure or there is just a lot of work to do with the pleiotropic mechanisms of SGLT2 inhibitors? There are still a lot of questions regarding SGLT2 inhibitors that are awaiting answers.

SGLT2 INHIBITORS' MECHANISMS OF ACTION

Physiological mechanisms responsible for SGLT2 inhibitors' benefits are not yet well defined and the situation has become even more complicated since their benefits have recently translated to non-diabetic patients which raised doubts about the validity of some current mechanistic assumptions. The time frame of their effects excludes glucose-lowering and antiatherosclerotic-mediated mechanism of action[22]. Namely, separation of the cardiovascular event curves for SGLT2 inhibitors occurred early in the studies and persisted for the entire duration of the treatment than would be expected from effects on atherosclerosis. Furthermore, it is known that hyperglycemia is a weak risk for cardiovascular disease[23,24]. So, multiple different mechanisms, direct cardiac as well as systemic, are suggested to explain the early cardiorenal benefits of SGLT2 inhibitors seen in CVOTs and they are presented in Figure 1 and discussed below: (1) Lowering elevated blood glucose levels with SGLT2 inhibitors which promote glucose excretion and not uptake could reduce glucose toxicity, improve β-cell function and insulin sensitivity as it was shown in the metabolic study of Ferrannini *et al*[25]. Reduced effects of glucotoxicity on the heart, could also reduce the risk of heart failure in diabetic patients[23]. But as it was shown in DAPA-HF, the

Table 2 Ongoing larger clinical trials of sodium-glucose cotransporter 2 inhibitors in heart failure

Trial	Drug	Ejection fraction	Diabetic/nondiabetic
Emperor-reduced	empagliflozin	HFrEF	Both
Emperor-preserved	empagliflozin	HFpEF	Both
Deliver	dapagliflozin	HFpEF	Both

HFrEF: Heart failure with reduced ejection fraction; HFpEF: Heart failure with preserved ejection fraction.

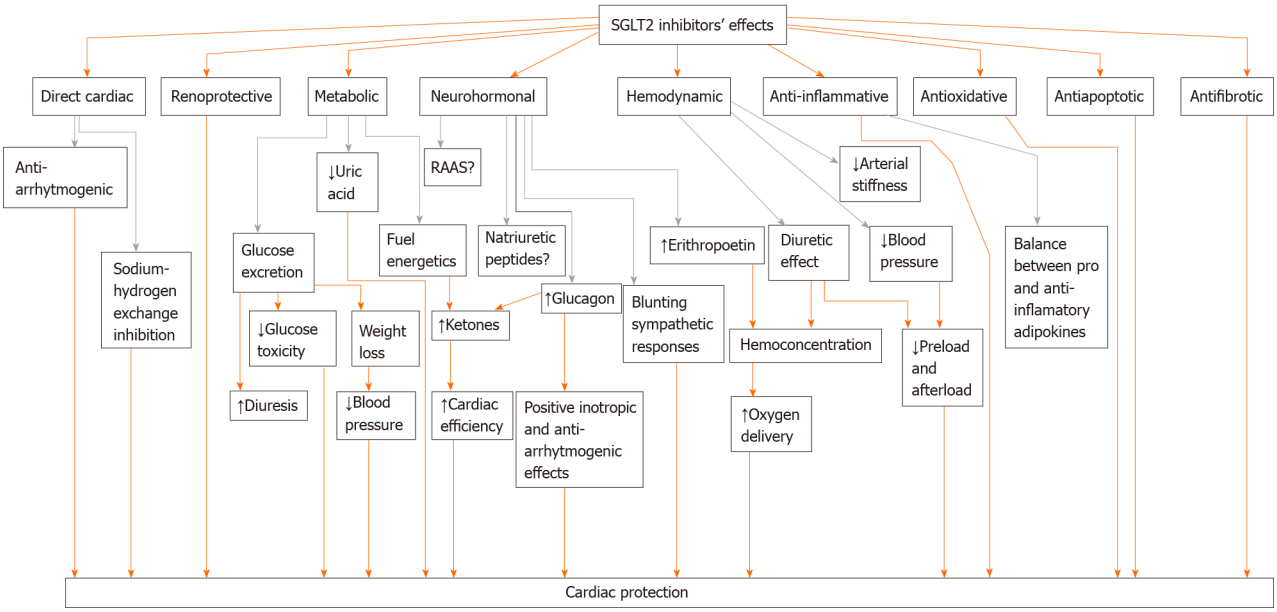

Figure 1 Summary of sodium-glucose cotransporter 2 inhibitors' proposed cardiac protection mechanisms. SGLT2: Sodium-glucose cotransporter 2; RAAS: Renin-angiotensin-aldosterone system.

benefits of dapaglifozin on the progression of heart failure occurred regardless of diabetes, which somehow undermine the hypothesis of glucotoxicity[24]. Nevertheless, it could be that in the group of patients with diabetic cardiomyopathy this particular mechanism played a role; (2) Reduced body fat and fluid loss are observed with SGLT2 inhibition due to glucose excretion. This could partially account for the blood pressure reduction, weight loss and, even though the magnitude of this effects is modest, could contribute to cardiovascular risk reduction and heart unloading[26-28]; (3) SGLT2 inhibitors produce natriuresis and osmotic diuresis that in turn cause a reduction in blood pressure and intravascular volume and in this way simultaneously reduce both preload and afterload of the heart which could give rapid results observed with SGLT2 inhibitors. Reduced arterial stiffness observed with SGLT2 inhibition and, as already mentioned, weight loss also contribute to blood pressure lowering[29]. However, how could this affect primary heart failure prevention and to which extent it can play a role in non- diabetic patients when it is known that SGLT2 inhibitors induce a greater level of glycosuria (consequently osmotic diuresis) in patients with diabetes compared to normal individuals, needs to be clarified[30]. Furthermore, previous investigations did not show that commonly used diuretics were associated with reduction in cardiovascular death, while in CVOTs and DAPA-HF, SGLT2 inhibitors reduced cardiovascular death as well as sudden death[24]; (4) Therapy with SGLT2 inhibitors is associated with small plasma uric acid reduction but this potential benefit requires further investigation[31]. It is known that uric acid may be associated with an adverse prognosis in heart failure and may play a causative role in metabolic syndrome, hypertension, renal damage or endothelial dysfunction[32,33]. In rats, hypertension associated with hyperuricemia is linked to reduced expression of macula densa neuronal nitric oxide synthase. This synthase affects cardiac function facilitating sarcoplasmic reticulum calcium release and thus modulating cardiac excitation-contraction coupling which could be a potential mechanism of SGLT2 inhibitors in

cardiovascular protection and heart failure[34]; (5) SGLT2 inhibition induce volume contraction which is accompanied by an increase in circulating renin-angiotensin-aldosterone system (RAAS) mediators even though this effect do not raise the blood pressure during treatment with SGLT2 inhibitors[35]. Combined use of RAAS and SGLT2 inhibitors may lead to synergistic beneficial cardiovascular effects. Although most of the patients in CVOTs and DAPA HF had already been taking ACE inhibitors or ARBs, this combined treatment strategy needs further investigation[36]. Furthermore, SGLT2 inhibitor treatment is associated with afferent vasoconstriction rather than efferent vasodilatation associated with RAAS inhibitors which attenuate renal hyperfiltration and contribute to renal protection in diabetes[35]; (6) Renoprotection of SGLT2 inhibitors could play a role in the observed cardiac benefits since the heart and kidney are inextricably linked (cardiorenal syndrome) and renal disease adversely impacts heart failure outcomes[16]. Recently, the CREDENCE trial (Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation) was prematurely stopped because of the achievement of the prespecified criteria for the primary composite endpoint (time to first occurrence of end-stage kidney disease, cardiovascular/renal death, doubling of serum creatinine) when investigating canagliflozin versus placebo. The trial also confirmed significant reductions in secondary endpoints of cardiovascular death or hospitalization for heart failure[24]. These results emphasize that cardiovascular benefit induced by SGLT2 inhibitors and renal protection may be connected. Sano proposed that SGLT2 inhibitors rest the exhausted kidney proximal tubular epithelial cells and restore functional and structural manifestation of diabetic kidney disease[37,38]; (7) Increased cardiac efficiency may be linked to increased oxygen delivery due to hemoconcentration and raised erythropoietin associated with SGLT2 inhibition[23,39]. On the other hand, in the study with erythropoietin-mimetic agents in patients with heart failure of Swedberg *et al*[40] the correction of anemia did not reduce the rate of death or hospitalization among patients with systolic heart failure and there was even a significant increase in the thromboembolic risk. This increase in hematocrit value during SGLT2 inhibition could be alternatively explained as being a surrogate marker of renal recovery from tubulointerstitial injury[37]. Nevertheless, the issue needs to be further clarified; (8) Increased heart rate was not observed during SGLT2 inhibitor treatment even though they affect blood pressure and induce volume contraction. It seems that the diuretic effects of SGLT2 inhibitors do not activate neurohumoral factors which is beneficial in heart failure[37]. Maybe the pharmacological implications of SGLT expression found in the brain could be manifested through this mechanism[41,42]; (9) Raised glucagon levels have been linked to SGLT2 inhibitor therapy and, considering glucagon inotropic effect independent of the catecholamine release it induces, this could lead to a better cardiac performance[25,43,44]. Glucagon inotropic effect declines with the failing heart which means that it could contribute to the maintaining of the heart function when heart failure is in its commencing stage. It has also anti-arrhythmogenic property which may be linked to the reduction of sudden death[45]. Moreover, glucagon is known for hepatic glucose production that could contribute to low risk of hypoglycemia with SGLT2 inhibition and it enhances ketogenesis, so the consequences of glucagon effects could account for SGLT2 inhibitors' benefits even in non-diabetic patients[9,46,47]. On the other hand, since this hormone has been traditionally considered harmful in diabetes, its activity regarding SGLT2 inhibition therapy needs to be further clarified; (10) SGLT2 inhibitors can redirect metabolism from glucose to fatty acid oxidation. This augments the synthesis of ketones which release energy more efficiently than glucose or fatty acids and can be used as alternate fuel source in the failing heart. These findings could contribute to increased heart function observed with SGLT2 inhibitors even in non-diabetic patients but they need further investigation[47-51]. Ketone bodies participate in epigenetic and cellular signaling and have antioxidative and anti-inflammatory properties. Oxidative stress and inflammation are key contributors to the development of diabetic cardiomyopathy but also play a role in the pathophysiology of heart failure irrespective of diabetes[52-57]; (11) From experimental animal models it is known that SGLT2 inhibitors exert systemic and cardiac anti-inflammatory effects[9]. For example, inflammatory M1 macrophages preferentially utilize glucose, so SGLT2 inhibitors could dampen inflammatory processes decreasing glucose flux and thus promoting polarization of macrophage phenotype to non-inflammatory[23]. Treatment with SGLT2 inhibitors also showed reduction in pro-inflammatory cytokines profile such as TNFα and IL-6[9]. As it was stated before, inflammation is one of the mechanisms involved in diabetic cardiomyopathy pathogenesis but also plays a role in the failing heart irrespective of diabetes. Furthermore, heart failure and inflammation are strongly connected and mutually enhance each other creating a vicious circle[58]. No doubt that SGLT2

inhibitors' anti-inflammatory capacities could be beneficial in the failing heart, but more studies in humans are needed; (12) SGLT2 inhibition is associated to antifibrotic effects in animal models. According to the study of Lin *et al*[59] empagliflozin significantly ameliorated pericoronary arterial fibrosis, cardiac interstitial fibrosis, coronary arterial thickening, cardiac interstitial macrophage infiltration and cardiac superoxide levels in db/db mice. The authors also stated that the observations might be attributable to the attenuation of oxidative stress. Recently, in a randomized trial with empagliflozin (EMPA HEART CardioLink-6 trial) it has been shown that empagliflozin caused a reduction in left ventricular mass index assessed by cardiac magnetic resonance imaging over a 6-month period in patients with type 2 diabetes mellitus and coronary disease[60]. The issue of antifibrotic effects needs further investigation in humans since it could be of great importance in heart failure and cardiac reverse remodeling; (13) Antioxidative features are attributable to SGLT2 inhibitors as it was shown in animal experiments[9]. Oxidative stress plays an important role in the pathogenesis of cardiac remodeling, and substantial evidence indicate that oxidative stress is increased both in the myocardium and systemically in patients with heart failure[56]; (14) By current evidence from experimental animal models SGLT2 inhibitors attenuate apoptosis of myocardial cells in models of myocardial ischaemia-reperfusion injury and diabetic cardiomyopathy which could be very important since apoptosis is likely to play an important role in heart failure[9,61]. Nevertheless, further clarification is required; (15) SGLT2 inhibitors could reestablish the balance between pro- and anti-inflammatory adipokines that can influence atherosclerosis, insulin resistance, inflammation, coagulation and fibrinolysis[15,62]. Additionally, perivascular and epicardial fat, through altered paracrine regulation of adipokines, is implicated in pathogenesis of heart failure[63]. According to the study of Sato *et al*[64] dapagliflozin might reduce epicardial adipose tissue volume and in this way could contribute to heart failure reduction risk while canagliflozin, in comparison with glimepiride, reduces serum leptin levels and increases the levels of the anti-inflammatory adipokine adiponectin[65]; (16) SGLT2 inhibitors could exert direct cardiac effect through sodium-hydrogen exchange inhibition. This may lead to a reduction in cardiac remodeling, injury, hypertrophy, fibrosis, and systolic dysfunction, as well as reduce cytoplasmic sodium and calcium levels, while increasing mitochondrial calcium levels[66,67]. Since heart failure is associated with intracellular cardiomyocyte sodium and calcium loading, this could affect the origin of heart failure[68,69]; (17) SGLT2 inihibitors could modulate electrophysiology in the heart. Besides the already mentioned glucagon anti-arrhythmogenic effect, one retrospective study showed that treatment with SGLT2 inhibitors reverses ventricular repolarization heterogeneity in people with type 2 diabetes, independently of their effect on glycemic control[70]. The findings may be linked to the reduction of fatal arrhythmias and thus reduced cardiovascular death seen with the SGLT2 inhibition so more studies on mechanisms of arrhythmias and SGLT2 inhibition are encouraged; and (18) The effects of SGLT2 inhibitors on plasma biomarker N-terminal pro-brain natriuretic peptide (NT-proBNP) have been inconsistent in studies on humans and in experimental studies[39,71-73]. To better elucidate SGLT2 inhibitors' action, it would be appropriate to determine their effects on NT-proBNP and other natriuretic peptides in patients with developed heart failure as well as in asymptomatic individuals. There are interesting findings of Majowicz *et al*[74] with the atrial natriuretic peptide (ANP) and endothelin-3. In their study, it has been shown that these vasoactive agents inhibit SGLT2 activity in the kidney. If SGLT2 transporters would be inhibited *via* SGLT2 inhibitors, ANP could exert and enhance other functions besides its natriuretic and diuretic actions, for example inhibition of RAAS and aldosterone production, protection against angiotensin II induced cardiac remodeling by minimizing macrophage infiltration and expression of pro-inflammatory factors, and modulation of arterial and cardiac baroreflex mechanism, *e.g.*, blunting sympathetic response. This could also give synergic effect with neprilysin inhibitors. The hypothesis still needs to be tested but theoretically it could partially contribute to SGLT2 inhibitors' cardioprotection, in asymptomatic left ventricle dysfunction and especially in the failing heart where the natriuretic peptides are significantly elevated[75,76].

CONCLUSION

In conclusion, based on current evidence, SGLT2 inhibitors are agents with pleiotropic effects that are valuable in treating diabetes and preventing its complications. They reduce the burden of cardiovascular adverse events especially through decreasing

heart failure risk. In addition, these drugs represent a new add-on strategy in the treatment of normoglycemic patients with HFrEF and carry the potential to be useful even in patients with HFpEF, but the dedicated studies are still ongoing. The benefits seen on heart failure appear to be mediated *via* glucose-independent mechanisms. Translational clues to the heart failure benefits recorded in clinical trials so far, should be sought in mechanisms of their action, which are not completely explained and are yet to be revealed. Particularly missing are human studies designed with enough power to elucidate some potential mechanisms essential for their mode of action. These data are important since SGLT2 inhibitors have great clinical potential through wide indications across the spectrum of heart failure and could lessen polypragmasy since they can target various mechanisms underpinning heart failure pathogenesis. The most logical explanation of their benefits is the timely targeting of various mechanisms implicated in the evolution of heart failure.

REFERENCES

1 **American Diabetes Association**. 10. Cardiovascular Disease and Risk Management: *Standards of Medical Care in Diabetes-2020*. *Diabetes Care* 2020; **43**: S111-S134 [PMID: 31862753 DOI: 10.2337/dc20-S010]

2 **Zinman B**, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE; EMPA-REG OUTCOME Investigators. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *N Engl J Med* 2015; **373**: 2117-2128 [PMID: 26378978 DOI: 10.1056/NEJMoa1504720]

3 **Neal B**, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, Shaw W, Law G, Desai M, Matthews DR; CANVAS Program Collaborative Group. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. *N Engl J Med* 2017; **377**: 644-657 [PMID: 28605608 DOI: 10.1056/NEJMoa1611925]

4 **Wiviott SD**, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, Silverman MG, Zelniker TA, Kuder JF, Murphy SA, Bhatt DL, Leiter LA, McGuire DK, Wilding JPH, Ruff CT, Gause-Nilsson IAM, Fredriksson M, Johansson PA, Langkilde AM, Sabatine MS; DECLARE–TIMI 58 Investigators. Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2019; **380**: 347-357 [PMID: 30415602 DOI: 10.1056/NEJMoa1812389]

5 **Giugliano D**, Meier JJ, Esposito K. Heart failure and type 2 diabetes: From cardiovascular outcome trials, with hope. *Diabetes Obes Metab* 2019; **21**: 1081-1087 [PMID: 30609236 DOI: 10.1111/dom.13629]

6 **McMurray JJV**, Solomon SD, Inzucchi SE, Køber L, Kosiborod MN, Martinez FA, Ponikowski P, Sabatine MS, Anand IS, Bělohlávek J, Böhm M, Chiang CE, Chopra VK, de Boer RA, Desai AS, Diez M, Drozdz J, Dukát A, Ge J, Howlett JG, Katova T, Kitakaze M, Ljungman CEA, Merkely B, Nicolau JC, O'Meara E, Petrie MC, Vinh PN, Schou M, Tereshchenko S, Verma S, Held C, DeMets DL, Docherty KF, Jhund PS, Bengtsson O, Sjöstrand M, Langkilde AM; DAPA-HF Trial Committees and Investigators. Dapagliflozin in Patients with Heart Failure and Reduced Ejection Fraction. *N Engl J Med* 2019; **381**: 1995-2008 [PMID: 31535829 DOI: 10.1056/NEJMoa1911303]

7 **Zelniker TA**, Wiviott SD, Raz I, Im K, Goodrich EL, Bonaca MP, Mosenzon O, Kato ET, Cahn A, Furtado RHM, Bhatt DL, Leiter LA, McGuire DK, Wilding JPH, Sabatine MS. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet* 2019; **393**: 31-39 [PMID: 30424892 DOI: 10.1016/S0140-6736(18)32590-X]

8 **Figtree GA**, Rådholm K, Barrett TD, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Matthews DR, Shaw W, Neal B. Effects of Canagliflozin on Heart Failure Outcomes Associated With Preserved and Reduced Ejection Fraction in Type 2 Diabetes Mellitus. *Circulation* 2019; **139**: 2591-2593 [PMID: 30882240 DOI: 10.1161/CIRCULATIONAHA.119.040057]

9 **Lahnwong S**, Chattipakorn SC, Chattipakorn N. Potential mechanisms responsible for cardioprotective effects of sodium-glucose co-transporter 2 inhibitors. *Cardiovasc Diabetol* 2018; **17**: 101 [PMID: 29991346 DOI: 10.1186/s12933-018-0745-5]

10 **Abdul-Ghani MA**, Norton L, DeFronzo RA. Renal sodium-glucose cotransporter inhibition in the management of type 2 diabetes mellitus. *Am J Physiol Renal Physiol* 2015; **309**: F889-F900 [PMID: 26354881 DOI: 10.1152/ajprenal.00267.2015]

11 **Rahmoune H**, Thompson PW, Ward JM, Smith CD, Hong G, Brown J. Glucose transporters in human renal proximal tubular cells isolated from the urine of patients with non-insulin-dependent diabetes. *Diabetes* 2005; **54**: 3427-3434 [PMID: 16306358 DOI: 10.2337/diabetes.54.12.3427]

12 **Heerspink HJ**, Perkins BA, Fitchett DH, Husain M, Cherney DZ. Sodium Glucose Cotransporter 2 Inhibitors in the Treatment of Diabetes Mellitus: Cardiovascular and Kidney Effects, Potential Mechanisms, and Clinical Applications. *Circulation* 2016; **134**: 752-772 [PMID: 27470878 DOI: 10.1161/CIRCULATIONAHA.116.021887]

13 **Tamargo J**. Sodium-glucose Cotransporter 2 Inhibitors in Heart Failure: Potential Mechanisms of Action, Adverse Effects and Future Developments. *Eur Cardiol* 2019; **14**: 23-32 [PMID: 31131034 DOI: 10.15420/ecr.2018.34.2]

14 **Donnan JR**, Grandy CA, Chibrikov E, Marra CA, Aubrey-Bassler K, Johnston K, Swab M, Hache J, Curnew D, Nguyen H, Gamble JM. Comparative safety of the sodium glucose co-transporter 2 (SGLT2) inhibitors: a systematic review and meta-analysis. *BMJ Open* 2019; **9**: e022577 [PMID: 30813108 DOI: 10.1136/bmjopen-2018-022577]

15 **Verma S**, McMurray JJV. SGLT2 inhibitors and mechanisms of cardiovascular benefit: a state-of-the-art review. *Diabetologia* 2018;

61: 2108-2117 [PMID: 30132036 DOI: 10.1007/s00125-018-4670-7]
16 **Lam CSP**, Chandramouli C, Ahooja V, Verma S. SGLT-2 Inhibitors in Heart Failure: Current Management, Unmet Needs, and Therapeutic Prospects. *J Am Heart Assoc* 2019; **8**: e013389 [PMID: 31607208 DOI: 10.1161/JAHA.119.013389]
17 **Kato ET**, Silverman MG, Mosenzon O, Zelniker TA, Cahn A, Furtado RHM, Kuder J, Murphy SA, Bhatt DL, Leiter LA, McGuire DK, Wilding JPH, Bonaca MP, Ruff CT, Desai AS, Goto S, Johansson PA, Gause-Nilsson I, Johanson P, Langkilde AM, Raz I, Sabatine MS, Wiviott SD. Effect of Dapagliflozin on Heart Failure and Mortality in Type 2 Diabetes Mellitus. *Circulation* 2019; **139**: 2528-2536 [PMID: 30882238 DOI: 10.1161/CIRCULATIONAHA.119.040130]
18 **Ponikowski P**, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, Falk V, González-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GM, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P; Authors/Task Force Members; Document Reviewers. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail* 2016; **18**: 891-975 [PMID: 27207191 DOI: 10.1002/ejhf.592]
19 **Shah KS**, Xu H, Matsouaka RA, Bhatt DL, Heidenreich PA, Hernandez AF, Devore AD, Yancy CW, Fonarow GC. Heart Failure With Preserved, Borderline, and Reduced Ejection Fraction: 5-Year Outcomes. *J Am Coll Cardiol* 2017; **70**: 2476-2486 [PMID: 29141781 DOI: 10.1016/j.jacc.2017.08.074]
20 **Huynh K**, Bernardo BC, McMullen JR, Ritchie RH. Diabetic cardiomyopathy: mechanisms and new treatment strategies targeting antioxidant signaling pathways. *Pharmacol Ther* 2014; **142**: 375-415 [PMID: 24462787 DOI: 10.1016/j.pharmthera.2014.01.003]
21 **Levelt E**, Gulsin G, Neubauer S, McCann GP. MECHANISMS IN ENDOCRINOLOGY: Diabetic cardiomyopathy: pathophysiology and potential metabolic interventions state of the art review. *Eur J Endocrinol* 2018; **178**: R127-R139 [PMID: 29440374 DOI: 10.1530/EJE-17-0724]
22 **Verma S**, McMurray JJV, Cherney DZI. The Metabolodiuretic Promise of Sodium-Dependent Glucose Cotransporter 2 Inhibition: The Search for the Sweet Spot in Heart Failure. *JAMA Cardiol* 2017; **2**: 939-940 [PMID: 28636701 DOI: 10.1001/jamacardio.2017.1891]
23 **Staels B**. Cardiovascular Protection by Sodium Glucose Cotransporter 2 Inhibitors: Potential Mechanisms. *Am J Med* 2017; **130**: S30-S39 [PMID: 28526184 DOI: 10.1016/j.amjmed.2017.04.009]
24 **Packer M**. Lessons learned from the DAPA-HF trial concerning the mechanisms of benefit of SGLT2 inhibitors on heart failure events in the context of other large-scale trials nearing completion. *Cardiovasc Diabetol* 2019; **18**: 129 [PMID: 31585532 DOI: 10.1186/s12933-019-0938-6]
25 **Ferrannini E**, Muscelli E, Frascerra S, Baldi S, Mari A, Heise T, Broedl UC, Woerle HJ. Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. *J Clin Invest* 2014; **124**: 499-508 [PMID: 24463454 DOI: 10.1172/JCI72227]
26 **Bolinder J**, Ljunggren Ö, Johansson L, Wilding J, Langkilde AM, Sjöström CD, Sugg J, Parikh S. Dapagliflozin maintains glycaemic control while reducing weight and body fat mass over 2 years in patients with type 2 diabetes mellitus inadequately controlled on metformin. *Diabetes Obes Metab* 2014; **16**: 159-169 [PMID: 23906445 DOI: 10.1111/dom.12189]
27 **DeFronzo RA**. The EMPA-REG study: What has it told us? A diabetologist's perspective. *J Diabetes Complications* 2016; **30**: 1-2 [PMID: 26541076 DOI: 10.1016/j.jdiacomp.2015.10.013]
28 **Packer M**. Reconceptualization of the Molecular Mechanism by Which Sodium-Glucose Cotransporter 2 Inhibitors Reduce the Risk of Heart Failure Events. *Circulation* 2019; **140**: 443-445 [PMID: 31381418 DOI: 10.1161/CIRCULATIONAHA.119.040909]
29 **Abdul-Ghani M**, Del Prato S, Chilton R, DeFronzo RA. SGLT2 Inhibitors and Cardiovascular Risk: Lessons Learned From the EMPA-REG OUTCOME Study. *Diabetes Care* 2016; **39**: 717-725 [PMID: 27208375 DOI: 10.2337/dc16-0041]
30 **List JF**, Whaley JM. Glucose dynamics and mechanistic implications of SGLT2 inhibitors in animals and humans. *Kidney Int Suppl* 2011; S20-S27 [PMID: 21358698 DOI: 10.1038/ki.2010.512]
31 **Ptaszynska A**, Hardy E, Johnsson E, Parikh S, List J. Effects of dapagliflozin on cardiovascular risk factors. *Postgrad Med* 2013; **125**: 181-189 [PMID: 23748519 DOI: 10.3810/pgm.2013.05.2667]
32 **Kittleson MM**, St John ME, Bead V, Champion HC, Kasper EK, Russell SD, Wittstein IS, Hare JM. Increased levels of uric acid predict haemodynamic compromise in patients with heart failure independently of B-type natriuretic peptide levels. *Heart* 2007; **93**: 365-367 [PMID: 17322514 DOI: 10.1136/hrt.2006.090845]
33 **Feig DI**, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. *N Engl J Med* 2008; **359**: 1811-1821 [PMID: 18946066 DOI: 10.1056/NEJMra0800885]
34 **Hare JM**, Johnson RJ. Uric acid predicts clinical outcomes in heart failure: insights regarding the role of xanthine oxidase and uric acid in disease pathophysiology. *Circulation* 2003; **107**: 1951-1953 [PMID: 12707249 DOI: 10.1161/01.CIR.0000066420.36123.35]
35 **Cherney DZ**, Perkins BA, Soleymanlou N, Maione M, Lai V, Lee A, Fagan NM, Woerle HJ, Johansen OE, Broedl UC, von Eynatten M. Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. *Circulation* 2014; **129**: 587-597 [PMID: 24334175 DOI: 10.1161/CIRCULATIONAHA.113.005081]
36 **Lo KB**, Gul F, Ram P, Kluger AY, Tecson KM, McCullough PA, Rangaswami J. The Effects of SGLT2 Inhibitors on Cardiovascular and Renal Outcomes in Diabetic Patients: A Systematic Review and Meta-Analysis. *Cardiorenal Med* 2020; **10**: 1-10 [PMID: 31743918 DOI: 10.1159/000503919]
37 **Sano M**. A new class of drugs for heart failure: SGLT2 inhibitors reduce sympathetic overactivity. *J Cardiol* 2018; **71**: 471-476 [PMID: 29415819 DOI: 10.1016/j.jjcc.2017.12.004]
38 **Perkovic V**, Jardine MJ, Neal B, Bompoint S, Heerspink HJL, Charytan DM, Edwards R, Agarwal R, Bakris G, Bull S, Cannon CP, Capuano G, Chu PL, de Zeeuw D, Greene T, Levin A, Pollock C, Wheeler DC, Yavin Y, Zhang H, Zinman B, Meininger G, Brenner BM, Mahaffey KW; CREDENCE Trial Investigators. Canagliflozin and Renal Outcomes in Type 2 Diabetes and Nephropathy. *N Engl J Med* 2019;

380: 2295-2306 [PMID: 30990260 DOI: 10.1056/NEJMoa1811744]
39 **Lambers Heerspink HJ**, de Zeeuw D, Wie L, Leslie B, List J. Dapagliflozin a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes. *Diabetes Obes Metab* 2013; **15**: 853-862 [PMID: 23668478 DOI: 10.1111/dom.12127]
40 **Swedberg K**, Young JB, Anand IS, Cheng S, Desai AS, Diaz R, Maggioni AP, McMurray JJ, O'Connor C, Pfeffer MA, Solomon SD, Sun Y, Tendera M, van Veldhuisen DJ; RED-HF Committees; RED-HF Investigators. Treatment of anemia with darbepoetin alfa in systolic heart failure. *N Engl J Med* 2013; **368**: 1210-1219 [PMID: 23473338 DOI: 10.1056/NEJMoa1214865]
41 **Yu AS**, Hirayama BA, Timbol G, Liu J, Diez-Sampedro A, Kepe V, Satyamurthy N, Huang SC, Wright EM, Barrio JR. Regional distribution of SGLT activity in rat brain in vivo. *Am J Physiol Cell Physiol* 2013; **304**: C240-C247 [PMID: 23151803 DOI: 10.1152/ajpcell.00317.2012]
42 **Poppe R**, Karbach U, Gambaryan S, Wiesinger H, Lutzenburg M, Kraemer M, Witte OW, Koepsell H. Expression of the Na+-D-glucose cotransporter SGLT1 in neurons. *J Neurochem* 1997; **69**: 84-94 [PMID: 9202297 DOI: 10.1046/j.1471-4159.1997.69010084.x]
43 **Jones BJ**, Tan T, Bloom SR. Minireview: Glucagon in stress and energy homeostasis. *Endocrinology* 2012; **153**: 1049-1054 [PMID: 22294753 DOI: 10.1210/en.2011-1979]
44 **Regan TJ**, Lehan PH, Henneman DH, Behar A, Hellems HK. Myocardial metabolic and contractile response to glucagon and epinephrine. *J Lab Clin Med* 1964; **63**: 638-647 [PMID: 14155451]
45 **Ceriello A**, Genovese S, Mannucci E, Gronda E. Glucagon and heart in type 2 diabetes: new perspectives. *Cardiovasc Diabetol* 2016; **15**: 123 [PMID: 27568179 DOI: 10.1186/s12933-016-0440-3]
46 **Schade DS**, Eaton RP. Glucagon regulation of plasma ketone body concentration in human diabetes. *J Clin Invest* 1975; **56**: 1340-1344 [PMID: 1184755 DOI: 10.1172/JCI108212]
47 **Ferrannini E**, Baldi S, Frascerra S, Astiarraga B, Heise T, Bizzotto R, Mari A, Pieber TR, Muscelli E. Shift to Fatty Substrate Utilization in Response to Sodium-Glucose Cotransporter 2 Inhibition in Subjects Without Diabetes and Patients With Type 2 Diabetes. *Diabetes* 2016; **65**: 1190-1195 [PMID: 26861783 DOI: 10.2337/db15-1356]
48 **Mudaliar S**, Alloju S, Henry RR. Can a Shift in Fuel Energetics Explain the Beneficial Cardiorenal Outcomes in the EMPA-REG OUTCOME Study? A Unifying Hypothesis. *Diabetes Care* 2016; **39**: 1115-1122 [PMID: 27289124 DOI: 10.2337/dc16-0542]
49 **Daniele G**, Xiong J, Solis-Herrera C, Merovci A, Eldor R, Tripathy D, DeFronzo RA, Norton L, Abdul-Ghani M. Dapagliflozin Enhances Fat Oxidation and Ketone Production in Patients With Type 2 Diabetes. *Diabetes Care* 2016; **39**: 2036-2041 [PMID: 27561923 DOI: 10.2337/dc15-2688]
50 **Kutoh E**, Hayashi J. Effect of Canagliflozin on Heart Function Involving Ketone Bodies in Patients with Type 2 Diabetes. *Drug Res (Stuttg)* 2019; **69**: 297-300 [PMID: 30296805 DOI: 10.1055/a-0748-5745]
51 **Bedi KC Jr**, Snyder NW, Brandimarto J, Aziz M, Mesaros C, Worth AJ, Wang LL, Javaheri A, Blair IA, Margulies KB, Rame JE. Evidence for Intramyocardial Disruption of Lipid Metabolism and Increased Myocardial Ketone Utilization in Advanced Human Heart Failure. *Circulation* 2016; **133**: 706-716 [PMID: 26819374 DOI: 10.1161/CIRCULATIONAHA.115.017545]
52 **Prattichizzo F**, De Nigris V, Micheloni S, La Sala L, Ceriello A. Increases in circulating levels of ketone bodies and cardiovascular protection with SGLT2 inhibitors: Is low-grade inflammation the neglected component? *Diabetes Obes Metab* 2018; **20**: 2515-2522 [PMID: 30073768 DOI: 10.1111/dom.13488]
53 **Haces ML**, Hernández-Fonseca K, Medina-Campos ON, Montiel T, Pedraza-Chaverri J, Massieu L. Antioxidant capacity contributes to protection of ketone bodies against oxidative damage induced during hypoglycemic conditions. *Exp Neurol* 2008; **211**: 85-96 [PMID: 18339375 DOI: 10.1016/j.expneurol.2007.12.029]
54 **Newman JC**, Verdin E. β-hydroxybutyrate: much more than a metabolite. *Diabetes Res Clin Pract* 2014; **106**: 173-181 [PMID: 25193333 DOI: 10.1016/j.diabres.2014.08.009]
55 **Newman JC**, Verdin E. Ketone bodies as signaling metabolites. *Trends Endocrinol Metab* 2014; **25**: 42-52 [PMID: 24140022 DOI: 10.1016/j.tem.2013.09.002]
56 **Burgoyne JR**, Mongue-Din H, Eaton P, Shah AM. Redox signaling in cardiac physiology and pathology. *Circ Res* 2012; **111**: 1091-1106 [PMID: 23023511 DOI: 10.1161/CIRCRESAHA.111.255216]
57 **Riehle C**, Bauersachs J. Key inflammatory mechanisms underlying heart failure. *Herz* 2019; **44**: 96-106 [PMID: 30715565 DOI: 10.1007/s00059-019-4785-8]
58 **Van Linthout S**, Tschöpe C. Inflammation - Cause or Consequence of Heart Failure or Both? *Curr Heart Fail Rep* 2017; **14**: 251-265 [PMID: 28667492 DOI: 10.1007/s11897-017-0337-9]
59 **Lin B**, Koibuchi N, Hasegawa Y, Sueta D, Toyama K, Uekawa K, Ma M, Nakagawa T, Kusaka H, Kim-Mitsuyama S. Glycemic control with empagliflozin, a novel selective SGLT2 inhibitor, ameliorates cardiovascular injury and cognitive dysfunction in obese and type 2 diabetic mice. *Cardiovasc Diabetol* 2014; **13**: 148 [PMID: 25344694 DOI: 10.1186/s12933-014-0148-1]
60 **Verma S**, Mazer CD, Yan AT, Mason T, Garg V, Teoh H, Zuo F, Quan A, Farkouh ME, Fitchett DH, Goodman SG, Goldenberg RM, Al-Omran M, Gilbert RE, Bhatt DL, Leiter LA, Jüni P, Zinman B, Connelly KA. Effect of Empagliflozin on Left Ventricular Mass in Patients With Type 2 Diabetes Mellitus and Coronary Artery Disease: The EMPA-HEART CardioLink-6 Randomized Clinical Trial. *Circulation* 2019; **140**: 1693-1702 [PMID: 31434508 DOI: 10.1161/CIRCULATIONAHA.119.042375]
61 **Abbate A**, Narula J. Role of apoptosis in adverse ventricular remodeling. *Heart Fail Clin* 2012; **8**: 79-86 [PMID: 22108728 DOI: 10.1016/j.hfc.2011.08.010]
62 **Lau DC**, Dhillon B, Yan H, Szmitko PE, Verma S. Adipokines: molecular links between obesity and atheroslcerosis. *Am J Physiol Heart Circ Physiol* 2005; **288**: H2031-H2041 [PMID: 15653761 DOI: 10.1152/ajpheart.01058.2004]
63 **Patel VB**, Shah S, Verma S, Oudit GY. Epicardial adipose tissue as a metabolic transducer: role in heart failure and coronary artery disease. *Heart Fail Rev* 2017; **22**: 889-902 [PMID: 28762019 DOI: 10.1007/s10741-017-9644-1]
64 **Sato T**, Aizawa Y, Yuasa S, Kishi S, Fuse K, Fujita S, Ikeda Y, Kitazawa H, Takahashi M, Sato M, Okabe M. The effect of dapagliflozin treatment on epicardial adipose tissue volume. *Cardiovasc Diabetol* 2018; **17**:

6 [PMID: 29301516 DOI: 10.1186/s12933-017-0658-8]

65 **Garvey WT**, Van Gaal L, Leiter LA, Vijapurkar U, List J, Cuddihy R, Ren J, Davies MJ. Effects of canagliflozin versus glimepiride on adipokines and inflammatory biomarkers in type 2 diabetes. *Metabolism* 2018; **85**: 32-37 [PMID: 29452178 DOI: 10.1016/j.metabol.2018.02.002]

66 **Packer M**, Anker SD, Butler J, Filippatos G, Zannad F. Effects of Sodium-Glucose Cotransporter 2 Inhibitors for the Treatment of Patients With Heart Failure: Proposal of a Novel Mechanism of Action. *JAMA Cardiol* 2017; **2**: 1025-1029 [PMID: 28768320 DOI: 10.1001/jamacardio.2017.2275]

67 **Baartscheer A**, Schumacher CA, Wüst RC, Fiolet JW, Stienen GJ, Coronel R, Zuurbier CJ. Empagliflozin decreases myocardial cytoplasmic Na^+ through inhibition of the cardiac Na^+/H^+ exchanger in rats and rabbits. *Diabetologia* 2017; **60**: 568-573 [PMID: 27752710 DOI: 10.1007/s00125-016-4134-x]

68 **Kohlhaas M**, Liu T, Knopp A, Zeller T, Ong MF, Böhm M, O'Rourke B, Maack C. Elevated cytosolic Na+ increases mitochondrial formation of reactive oxygen species in failing cardiac myocytes. *Circulation* 2010; **121**: 1606-1613 [PMID: 20351235 DOI: 10.1161/CIRCULATIONAHA.109.914911]

69 **Baartscheer A**, Schumacher CA, van Borren MM, Belterman CN, Coronel R, Fiolet JW. Increased Na+/H+-exchange activity is the cause of increased [Na+]i and underlies disturbed calcium handling in the rabbit pressure and volume overload heart failure model. *Cardiovasc Res* 2003; **57**: 1015-1024 [PMID: 12650879 DOI: 10.1016/s0008-6363(02)00809-x]

70 **Sato T**, Miki T, Ohnishi H, Yamashita T, Takada A, Yano T, Tanno M, Tsuchida A, Miura T. Effect of sodium-glucose co-transporter-2 inhibitors on impaired ventricular repolarization in people with Type 2 diabetes. *Diabet Med* 2017; **34**: 1367-1371 [PMID: 28703863 DOI: 10.1111/dme.13424]

71 **Tanaka H**, Takano K, Iijima H, Kubo H, Maruyama N, Hashimoto T, Arakawa K, Togo M, Inagaki N, Kaku K. Factors Affecting Canagliflozin-Induced Transient Urine Volume Increase in Patients with Type 2 Diabetes Mellitus. *Adv Ther* 2017; **34**: 436-451 [PMID: 27981497 DOI: 10.1007/s12325-016-0457-8]

72 **Eickhoff MK**, Dekkers CCJ, Kramers BJ, Laverman GD, Frimodt-Møller M, Jørgensen NR, Faber J, Danser AHJ, Gansevoort RT, Rossing P, Persson F, Heerspink HJL. Effects of Dapagliflozin on Volume Status When Added to Renin-Angiotensin System Inhibitors. *J Clin Med* 2019; **8**: 779 [PMID: 31159350 DOI: 10.3390/jcm8060779]

73 **Januzzi JL Jr**, Butler J, Jarolim P, Sattar N, Vijapurkar U, Desai M, Davies MJ. Effects of Canagliflozin on Cardiovascular Biomarkers in Older Adults With Type 2 Diabetes. *J Am Coll Cardiol* 2017; **70**: 704-712 [PMID: 28619659 DOI: 10.1016/j.jacc.2017.06.016]

74 **Majowicz MP**, Gonzalez Bosc LV, Albertoni Borghese MF, Delgado MF, Ortiz MC, Sterin Speziale N, Vidal NA. Atrial natriuretic peptide and endothelin-3 target renal sodium-glucose cotransporter. *Peptides* 2003; **24**: 1971-1976 [PMID: 15127950 DOI: 10.1016/j.peptides.2003.07.030]

75 **Volpe M**, Carnovali M, Mastromarino V. The natriuretic peptides system in the pathophysiology of heart failure: from molecular basis to treatment. *Clin Sci (Lond)* 2016; **130**: 57-77 [PMID: 26637405 DOI: 10.1042/CS20150469]

76 **Grubić Rotkvić P**, Cigrovski Berković M, Bulj N, Rotkvić L. Minireview: are SGLT2 inhibitors heart savers in diabetes? *Heart Fail Rev* 2019 [PMID: 31410757 DOI: 10.1007/s10741-019-09849-3]

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REVIEW

Islet cell dysfunction in patients with chronic pancreatitis

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Abstract

Chronic pancreatitis (CP) is characterized by progressive inflammation and fibrosis of the pancreas that eventually leads to pancreatic exocrine and endocrine insufficiency. Diabetes in the background of CP is very difficult to manage due to high glycemic variability and concomitant malabsorption. Progressive beta cell loss leading to insulin deficiency is the cardinal mechanism underlying diabetes development in CP. Alpha cell dysfunction leading to deranged glucagon secretion has been described in different studies using a variety of stimuli in CP. However, the emerging evidence is varied probably because of dependence on the study procedure, the study population as well as on the stage of the disease. The mechanism behind islet cell dysfunction in CP is multifactorial. The intra-islet alpha and beta cell regulation of each other is often lost. Moreover, secretion of the incretin hormones such as glucagon like peptide-1 and glucose-dependent insulinotropic polypeptide is dysregulated. This significantly contributes to islet cell disturbances. Persistent and progressive inflammation with changes in the function of other cells such as islet delta cells and pancreatic polypeptide cells are also implicated in CP. In addition, the different surgical procedures performed in patients with CP and antihyperglycemic drugs used to treat diabetes associated with CP also affect islet cell function. Hence, different factors such as chronic inflammation, dysregulated incretin axis, surgical interventions and anti-diabetic drugs all affect islet cell function in patients with CP. Newer therapies targeting alpha cell function and beta cell regeneration would be useful in the management of pancreatic diabetes in the near future.

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Core tip: Chronic pancreatitis (CP) is a progressive inflammatory disorder leading to islet cell dysfunction and subsequent development of diabetes. The disease pathology is complex and is characterized by dysregulation of both the islet cells and the incretin axes. The different surgical procedures performed in patients with CP and antihyperglycemic drugs used to treat diabetes associated with CP also affect islet cell function. Diabetes secondary to CP is difficult to treat and contributes to disease morbidity. Newer therapies targeting alpha cell function and beta cell regeneration would be useful in the management of pancreatic diabetes in the near future.

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INTRODUCTION

Chronic pancreatitis (CP) is a slow progressive inflammatory condition of the pancreas, resulting in pancreatic exocrine and endocrine insufficiency. Diabetes secondary to pancreatic pathology (acute pancreatitis, CP, and pancreatic adenocarcinoma) is termed type 3c diabetes[1]. Diabetes mellitus (DM) due to CP is characterized by predominant post-prandial hyperglycemia and fasting hyperglycemia usually occurs later. Hence, fasting glucose measurements alone can miss the diagnosis at an early stage of CP. It is often difficult to control blood glucose levels due to unpredictable swings between hyper and hypoglycemia, the so-called "brittle diabetes". CP patients are at increased risk of hypoglycemia, partly because of poor glycogen reserve due to malabsorption resulting from severe exocrine insufficiency.

Pancreatic endocrine insufficiency leading to diabetes usually occurs late in the natural history of CP. The prevalence of diabetes-related to the pancreatopathies is usually underestimated because it is often misclassified as either type 1 or type 2 diabetes. A study from Europe has shown the prevalence of type 3c diabetes to be around 9% among different diabetes populations[2]. About 80% of long-standing CP patients develop diabetes[3]. The prevalence of diabetes is much higher in fibro-calculous pancreatic diabetes (FCPD) typically found in the Indian subcontinent. Pancreatic islets are constituted by various cells: alpha cells secrete glucagon; beta-cells secrete insulin; delta cells secrete somatostatin and pancreatic polypeptide (PP) cells secrete PP. Progressive inflammation and fibrosis lead to atrophy of the pancreas and acinar cell death, which together culminate in pancreatic exocrine insufficiency. Nonetheless, endocrine insufficiency soon follows. However, the mechanism behind this progressive islet cell damage has remained an active area of research. Hence, we searched the evidence available on islet cell dysfunction in CP and summarized the evidence in this review.

LITERATURE SEARCH AND STUDY SELECTION

We searched the literature using the following key search terms: "islet cell" [AND] "chronic pancreatitis"; "alpha cell" [AND] "chronic pancreatitis"; "beta cell" [AND] "chronic pancreatitis"; "insulin" [AND] "chronic pancreatitis"; "glucagon" [AND] "chronic pancreatitis"; "incretin" [AND] "chronic pancreatitis"; "GLP-1" [AND] "chronic pancreatitis"; "GIP" [AND] "chronic pancreatitis"; "beta cell" [AND] "alpha cell" [AND] "chronic pancreatitis"; "islet transplantation" [AND] "chronic pancreatitis" [AND] "beta cell". Two authors [AR, JPS] independently searched the literature in PubMed. The search was restricted to English language articles and was performed from inception up to January 2020. We also looked for references in the

individual articles for their suitability and included them in this review if found to be appropriate. Studies which evaluated islet cell function in patients with CP in a comprehensive way were selected by the authors to be included in this review [JPS, SKK, DBN].

ALPHA CELL DYSFUNCTION IN CP

There has been a renewed interest in alpha cell dysfunction in both type 1 and type 2 diabetes. Alpha cell dysfunction has also been implicated in the pathogenesis of CP related diabetes (Figure 1). The destruction of beta-cell mass is higher when compared to alpha cells in patients with CP[4], which may result in higher glucagon levels in these patients. This destruction of islet cell mass occurs relatively later in the disease process. Studies have shown that alpha cell numbers are not significantly decreased in CP patients as compared to control populations[4]. One report has shown that alpha cell mass can be increased in patients with CP[5].

Glucagon secretion in CP has been assessed in several studies using different dynamic methods such as oral glucose, intravenous (IV) arginine, and IV alanine (Table 1). Baseline plasma glucagon levels were shown to be either similar[6], reduced[7] or elevated[8] compared to healthy controls in different studies. Similarly, stimulated glucagon levels are also varied among different studies[8-10]. In the study by Lundberg *et al*[11], both basal and post-meal stimulated glucagon levels were shown to be higher in CP patients compared to a normal healthy control population. Interestingly, their patient cohort had a relatively early stage of pancreatitis and none of them had diabetes.

Glucose mediated glucagon suppression was also assessed by Mumme *et al*[12]. Similar to diabetes patients without pancreatitis, glucose-induced glucagon suppression was found to be impaired in CP patients with diabetes after an oral glucose tolerance test (OGTT). Moreover, glucagon levels were lower in response to hypoglycemia in CP patients with diabetes in a stepwise hypoglycemic clamp. This finding establishes the poor alpha cell function in CP patients, particularly those developing diabetes.

Since elevated glucagon is part of the first-line defense against hypoglycemia, the diminished glucagon response exposes a patient with CP and DM to the risk of severe hypoglycemia, particularly when the disease is quite advanced. Absence of the glucagon response following hypoglycemia was also shown by Larsen *et al*[13]. We previously demonstrated that glucagon was not suppressed following an oral glucose load in patients with chronic calcific pancreatitis (CCP) irrespective of their diabetes status[14]. This suggests that the ability of alpha cells to suppress glucagon secretion in response to glucose is significantly impaired in CCP patients. In an another study by Schrader *et al*[15], it was found that the glucose-induced glucagon suppression was decreased after partial pancreatectomy. They found a trend of lower baseline glucagon after surgery. However, glucagon suppression was 22% after surgery as compared to 39% before surgery as shown by the OGTT. Interestingly, this impaired glucagon response was correlated with a reduction in insulin secretion but not with the elevated glucose level. They postulated that this alpha cell dysfunction is due to decreased beta cell mass. However, some studies have reported normal arginine stimulated glucagon response in CP patients with or without diabetes[16].

Another important consideration is whether this elevated glucagon is from outside the pancreas. Lund and colleagues[17] have shown evidence of extra-pancreatic glucagon in pancreatectomized patients as compared to normal healthy controls. They showed that hyperglucagonemia was seen after oral glucose but not during an IV isoglycemic glucose infusion in pancreatectomized patients. This suggests that CP patients with pancreatic atrophy may have elevated plasma glucagon level, which is gut derived. The possible mechanism for this may be due to a shift in the L-cells of the intestine towards secretion of more glucagon (mediated by prohormone convertase 2 enzyme) in the absence of pancreatic alpha cells. The stimulus for L-cells may be an altered delivery of nutrients including glucose secondary to the distorted anatomy of the small intestine, particularly after surgery. This area needs to be further clarified in future studies involving CP patients.

Table 1 Summary of the studies that assessed glucagon response in chronic pancreatitis

Ref.	Population characteristics	Method used	Key findings
Mumme *et al*[12], 2017	(1) Patients with diabetes due to CP; (2) Patients with type 2 diabetes without CP; and (3) NGT patients without CP	(1) OGTT; (2) Hyperinsulinemic, stepwise hypoglycemic clamp	(1) Higher fasting glucagon levels and an initial rise of glucagon after the OGTT in both groups with diabetes compared to the healthy group; and (2) Lower glucagon levels in both groups with diabetes after hypoglycemia in the clamp study
Kumar *et al*[14], 2018	Chronic calcific pancreatitis: pre- and post-Frey's procedure	OGTT	Before surgery: Elevated glucagon level at 60- and 120-min post OGTT compared to the fasting state
Lundberg *et al*[11], 2016	Non-diabetic patients with CP and healthy controls	(1) MMTT; and (2) FSIVGTT	(1) Elevated glucagon levels (total area under curve) in CP patients at both basal and post-stimulation state; and (2) No difference in basal to peak increment glucagon levels between the groups
Knop *et al*[38], 2010	(1) CP patients with NGT; (2) CP patients with IGT; (3) CP patients with DM; and (4) Normal healthy controls	(1) OGTT; and (2) IVGTT	(1) Similar fasting mean glucagon in all the groups; (2) During OGTT: No increase in glucagon level in the CP + NGT group; increased glucagon up to 60 min in the CP + IGT and CP + DM groups; a small early rise in glucagon which was suppressed later in the healthy group; and (3) During FSIVGTT, AUC for glucagon was higher in the CP + DM group compared to normal controls
Larsen *et al*[6], 1988	(1) Type 1 diabetes; (2) Insulin dependent diabetes secondary to CP; and (3) Normal healthy controls	(1) IV Glucagon; (2) IV Arginine infusion; and (3) Mixed meal test	No difference in baseline glucagon or stimulated glucagon levels between the groups with diabetes
Kannan *et al*[8], 1979	CP patients and healthy control group	(1) 50 g OGTT; and (2) L-arginine stimulation	(1) Elevated basal fasting glucagon and higher glucagon levels during the OGTT in CP patients compared to controls; and (2) An early rise in glucagon after L-arginine stimulation in CP patients compared to normal patients
Donowitz *et al*[10], 1975	CP group and healthy control group	IV L-alanine infusion	(1) Lower basal glucagon levels in CP patients; and (2) No rise in glucagon level after alanine stimulation in CP patients compared to normal controls

AUC: Area under curve; CP: Chronic pancreatitis; DM: Diabetes mellitus; FSIVGTT: Frequently sampled intravenous glucose tolerance test; IGT: Impaired glucose tolerance; IV: Intravenous; IVGTT: Intravenous glucose tolerance test; MMTT: Mixed meal tolerance test; NGT: Normal glucose tolerance; OGTT: Oral glucose tolerance test.

BETA-CELL DYSFUNCTION IN CP

Beta-cell destruction and consequently, insulin deficiency, has been viewed as the most important mechanism for the development of DM in CP (Figure 1). Meier *et al*[18] showed that the destruction of 65% of beta cells was associated with diabetes in CP patients. Postprandial hyperglycemia is more directly related to the reduction in beta-cell mass. Fasting hyperglycemia, which is more related to insulin resistance usually develops when beta-cell mass is significantly reduced. Schrader *et al*[4] reported a 29% reduction in beta-cell area in CP patients and in their study, one-third of patients had diabetes as compared to controls. A significant reduction in beta-cell mass has also been reported in patients with advanced CP without diabetes[19].

The presence of beta-cell dysfunction, in addition to reduced beta-cell mass is also very important in the development of DM in CP. The residual beta cells cannot function effectively in an environment of significant inflammation and fibrosis seen in CP. An *in vitro* analysis has shown that beta-cells retain only 53% of glucose-stimulated insulin secretive function in advanced CP patients without diabetes[19]. Lundberg *et al*[11], showed significantly lower mean disposition index (a composite

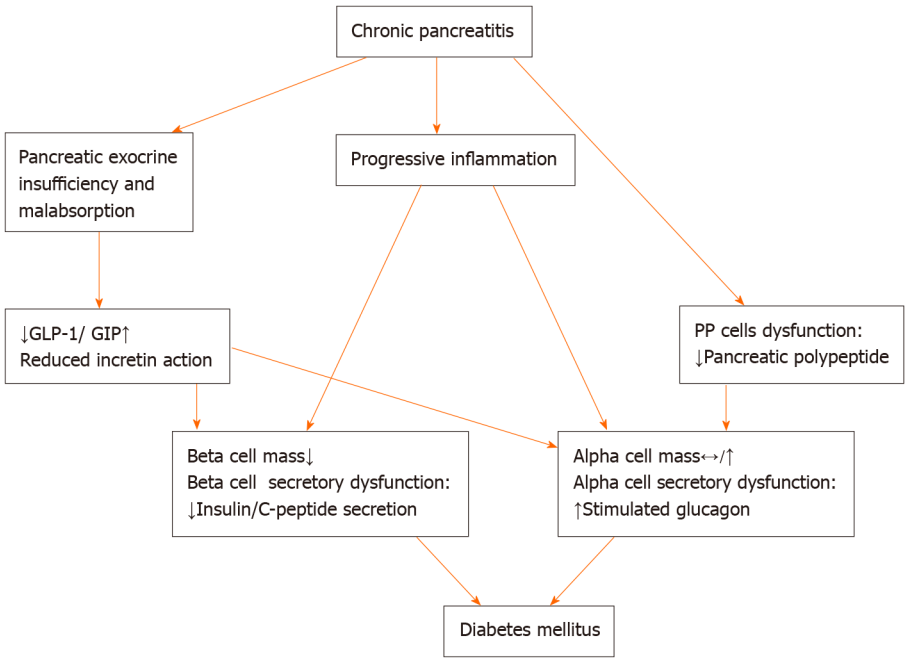

Figure 1 Mechanisms of islet cell dysfunction in patients with chronic pancreatitis. GLP-1: Glucagon-like peptide 1; GIP: Glucose-dependent insulinotropic polypeptide; PP: Pancreatic polypeptide.

marker of insulin secretion) after a frequently sampled intravenous glucose tolerance test in CP patients as compared to controls. However, following a mixed meal tolerance test (MMTT), a low C-peptide at 30 min was the only significant difference between CP and control patients. Interestingly, they also showed that calcific pancreatitis patients have a greater reduction in insulin secretion from beta cells when compared to non-calcific pancreatitis patients. This may imply that pancreatic calcification occurs in relatively advanced stages of pancreatitis.

FCPD is a distinct entity particularly prevalent in South India[20]. FCPD occurs in the 3rd or 4th decade of life and is often difficult to treat because of its brittleness. The mechanism of development of diabetes in FCPD is different when compared to other causes of CP[21]. Arginine stimulated C-peptide was found to be significantly lower in FCPD patients, but tropical calcific pancreatitis (TCP) patients without diabetes showed a normal response[16]. However, in North Indian TCP patients with mild dysglycemia, decreased beta-cell function was noted to be the major factor[22]. In our study[14], beta-cell function, as measured by insulin secretion sensitivity index-2 (another composite measure of insulin secretion) was lower in a group of CCP patients with DM compared to CCP patients with prediabetes or normal glucose tolerance (NGT).

PP CELL DYSFUNCTION IN CP

PP deficiency in CP has been observed in several studies. The most compelling evidence comes from glucose clamp studies, which showed the effect of PP deficiency in CP[23]. Seymour *et al*[24] showed that PP deficiency causes a reduction in the number of insulin receptors in the liver without altering insulin affinity. PP deficiency has been observed mostly in the postprandial state. A recent study[25] did not find any difference in fasting PP between CP patients with pancreatic adenocarcinoma and normal healthy controls irrespective of their glycemic status.

Interestingly, PP administration in CP patients reversed hepatic insulin resistance, confirming its role in CP related DM[23,24]. Subsequently, a randomized controlled trial of 72-h PP infusion showed significant improvement in insulin sensitivity in CP patients with diabetes[26]. Considering this evidence, an absent response of PP following a mixed meal was considered to be pathognomonic of CP related diabetes by a group of experts[27]. However, further studies are required to identify the effect of PP in CP patients. PP also suppresses glucagon secretion from alpha cells. This action is mediated by the PPYR1 receptor present in the islet alpha cells of both humans and

mice[28]. Since PP deficiency in CP has been demonstrated in different studies, it is possible that this suppressive effect on alpha cell glucagon secretion is lost in CP patients thereby resulting in hyperglucagonemia (Figure 1).

DELTA CELL DYSFUNCTION IN CP

Studies on somatostatin secreting delta cells are scarce and their exact role in CP has not been established. The study by Larsen *et al*[6] showed higher somatostatin levels following a mixed meal and arginine stimulation in diabetes secondary to pancreatitis as compared to type 1 diabetes and healthy controls. It was postulated that higher somatostatin levels may help to lower blood glucose level in patients with CP. The mechanism suggested was an inhibitory effect of somatostatin on both insulin and glucagon secretion. Somatostatin may also delay glucose absorption from the gut.

MECHANISMS OF ISLET CELL DYSFUNCTION IN CP

Disruption in the interaction between alpha and beta cells

Alpha cell secretion of glucagon is indirectly controlled by several mechanisms and these are perturbed in CP. One such mechanism is a beta cell defect leading to less insulin secretion in CP. Insulin suppresses glucagon during hyperglycemia and reciprocally, glucagon levels rise when insulin levels decline during hypoglycemia[29]. This switching mechanism may be lost in CP as progressive beta-cell failure is an established feature of CP. However, recent studies in animal models have shown that glucagon has potentiating action on beta cells to secrete more insulin, particularly in the postprandial state[30]. It is possible that hyperglucagonemia observed in CP is a compensatory response to falling insulin levels due to beta-cell loss.

Altered incretin axis in CP

Incretin hormones such as glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are secreted from L- and K-cells of the small intestine, respectively. Both GLP-1 and GIP physiologically regulate glucose metabolism by increasing insulin secretion *via* G-protein coupled receptors in beta-cells. However, GLP-1 suppresses glucagon secretion from alpha cells, whereas GIP augments glucagon secretion in the presence of hyperglycemia[31,32]. The reduced incretin hormone effect is a well-documented pathophysiological abnormality in type 2 diabetes[32].

The reduced incretin effect has been demonstrated in different studies in CP patients (Figure 1). However, whether this is primarily due to the effect of CP per se or due to the development of diabetes in CP patients is unclear. Vilsbøll *et al*[33] showed that the late phase insulin response (30-120 min) to GIP is particularly impaired in CP patients, whereas the response to GLP-1 was preserved in both the first and second phases. This difference was attributed to different post-receptor signaling between GIP and GLP-1, although they act through the same G-protein coupled receptor. The late phase insulin response (30-120 min) to GIP and not to GLP-1 infusion was shown to be impaired in CP patients with diabetes compared to CP patients with NGT[34].

Knop *et al*[35] compared CP patients with DM, CP patients with NGT, type 2 diabetics and healthy controls. They found a significantly lower incretin effect [measured by 100% × (β-cell secretory response to OGTT - intravenous β-cell secretory response)/β-cell secretory response to OGTT] in CP patients with diabetes, whereas CP patients without hyperglycemia had similar incretin hormones to the healthy population. However, basal GLP-1 was higher in CP patients with diabetes and type-2 diabetes patients. Importantly, beta-cell function was found to be similar in all four groups implying that the reduced incretin effect may be a consequence of the development of diabetes itself.

However, Lundberg *et al*[11] found no significant difference in either GLP-1 or GIP levels during MMTT in CP patients. Hornum *et al*[36] also did not find any difference in GIP response following a mixed meal in CP patients with pancreatic exocrine insufficiency compared to healthy subjects. Nevertheless, they showed an increased GLP-2 response in CP patients. This correlated with a higher superior mesenteric arterial flow. The incremental GLP-2 was possibly due to the easy access of more nutrients to the distal intestine stimulating GLP-2 secretion as a result of quick transit in CP patients. At present, the clinical role of GLP-2 is yet to be established in the

pathogenesis of diabetes, and in CP patients.

The role of incretin hormones is also implicated in the pathogenesis of alpha cell abnormality in CP. GLP-1 suppresses glucagon, whereas GIP has the opposite effect on glucagon[37]. Interestingly, Knop *et al*[38] demonstrated that glucagon levels were elevated in the first 60 min following the OGTT, but glucagon was suppressed during the IVGTT in CP patients. Importantly, glucagon suppression deteriorated throughout the spectrum of diabetes in CP patients. Glucagon suppression was low in CP patients with NGT, absent in CP with impaired glucose tolerance and glucagon levels were paradoxically elevated in CP with frank DM. The authors found that both basal and stimulated GIP response was higher in CP patients with DM, but GLP-1 levels were similar to the controls. This suggests that a change in the delicate balance between stimulatory and inhibitory incretin hormones that regulate glucagon secretion from alpha cells is an important contributor to hyperglucagonemia. However, Lund *et al*[17] found a significantly higher GLP-1 response during the OGTT in pancreatectomized patients compared to normal controls, but no difference in GIP response was seen. This area requires further studies to analyze the contribution of the extent of incretin hormone defect in alpha cell dysfunction.

CP is a state of malabsorption leading to steatorrhea as a result of pancreatic exocrine insufficiency. Both nutrient absorption and assimilation are important drivers of adequate incretin secretion (Figure 1). In an elegant study by Knop *et al*[39], it was found that CP patients with pancreatic exocrine insufficiency had higher GLP-1 and GIP responses to a mixed meal when the patients were supplemented with pancreatic enzymes. This higher incretin response was also associated with higher insulin secretion. A comparable result of increased GIP was shown previously by Ebert *et al*[40]. Similarly, higher GLP-2 levels after ingestion of a mixed meal were noted in CP patients when supplemented with pancreatic enzymes[41]. This is an active area of research. Pancreatic enzyme replacement therapy in CP and its effect on glucose homeostasis needs to be clarified in future studies. Studies are lacking in this area and whether enzyme supplementation itself improves glycemic control *via* the increased incretin effect or improved glycemic control per se leads to improved incretin response is still debated. Moreover, studies are also needed to identify how altered gut motility affects incretin hormones and glucose homeostasis in CP patients.

Dysregulation of amino acid metabolism

Another important consideration is the altered amino acid metabolism in CP. Amino acids are important mediators of the "liver-alpha cell axis". Increased hepatic resistance to glucagon increases the levels of certain amino acids and that, in turn, stimulates glucagon secretion from alpha cells[42]. It was recently shown that glucagon resistance in the liver increases the production of alanine, causing hyperglucagonemia further resulting in elevated glucose levels[43]. Indeed, the "glucagon-alanine index" is suggested to be a marker of glucagon's biological effect.

Abnormalities in amino acid metabolism have already been described in CP. It was found that the concentration of citrulline, gamma-aminobutyric acid, taurine, and aspartic acid were significantly decreased in CP patients[44]. On the other hand, hepatic steatosis has been described in pancreatectomized patients which may also cause elevated amino acids[45]. Thus, the contribution of disturbed amino acid metabolism on elevated glucagon level is yet to be established. Therefore, this area requires further active research to establish the relationship between hepatic steatosis, alpha cell dysfunction and disturbed amino acid metabolism in CP patients.

Chronic inflammation

CP is currently viewed as a progressive fibro-inflammatory disorder. Persistent inflammation is found in early CP and is more pronounced in established CP. This finally results in fibrosis of the pancreas with exocrine insufficiency, islet cell dysfunction, development of diabetes, and pancreatic carcinoma at the advanced stage[46] (Figure 1). It has also been suggested by Lundberg *et al*[11], that alpha cells present in the islet are under stress due to chronic inflammation and secrete higher amounts of glucagon until the disease reaches an advanced stage, when the alpha cell mass/secretory function starts to decline resulting in hypoglucagonemia. The pancreatic inflammation in CP also leads to altered sensitivity of alpha cells to oral glucose resulting in decreased glucagon suppression as suggested by Knop *et al*[38].

Beta-cell dysfunction usually occurs late in this process possibly due to a lack of TNF-related apoptosis-inducing ligand and apoptotic receptor (CD95)[47]. However, persistent inflammation and subsequent changes in the intra-islet cytokine milieu may adversely affect the intrinsic signaling machinery of beta cells, thus resulting in beta cell dysfunction and less insulin secretion[48]. In CP with autoimmune etiology, $CD8^+$ T

cell-mediated beta cell dysfunction was described earlier[49]. Talukdar *et al*[50] showed that pancreatic islet cells in CP patients with DM are infiltrated with Th1 cells and this correlated with the increased rate of beta-cell apoptosis in this group of patients. The same study reported increased signal transducer and activator of transcription 1 or decreased pancreas and duodenal homeobox gene 1 (PDX-1) expression in CP patients with diabetes. The intra-islet increase in the interferon-gamma results in the reduction of PDX-1 expression and finally, beta-cell dysfunction.

Another important concept is beta cell dedifferentiation, wherein the mature beta-cells regress to more like precursor cells, which are less glucose-sensitive. A recent study by Sun *et al*[51] showed that CP patients without diabetes had a higher percentage of dedifferentiated cells in their islets (10.4% *vs* 3.6%) as compared to normal controls. Importantly, the beta-cell apoptosis rate in CP patients with DM was similar to the normal population. Interestingly, this finding is strongly correlated with islet inflammation and fibrosis associated with atrophy. This shows that the direct effect of inflammation plays an important role in beta cell dysfunction even in the early stages of CP.

Role of pancreatic stellate cells

Stellate cells have been identified in pancreatic islets and their role in chronic progressive fibrosis of the pancreas in type 2 diabetes has been described recently[52]. However, its effect on islet cells remains uncertain. Activated stellate cell-induced dysfunction in pancreatic beta-cells has been described recently in a few animal studies[53,54] although human studies are lacking.

DIFFERENCE IN ISLET CELL FUNCTION BETWEEN CP WITH DM *VS* CP WITH NGT

The development of type 2 DM is seen as a continuum of different stages from NGT to prediabetes to frankly elevated blood glucose to satisfy the criteria for diabetes. It is intriguing to look at the changes in islet cell function in CP patients as they gradually progress to diabetes from normal glucose levels. As already described, early dysfunction in insulin secretion and glucagon suppression is seen in CP patients without diabetes[11]. Indeed, it has been shown that beta cell function and insulin secretion is lower in CP patients with DM compared to CP patients with NGT[14,38]. CP patients with prediabetes are intermediate in terms of beta cell secretary function[14,38]. Although our study[14] showed no difference in glucagon suppression between CP subjects with NGT, prediabetes and diabetes groups at baseline, Knop *et al*[38] clearly demonstrated a rise in glucagon level in a continuous manner across NGT to diabetes in CP. Moreover, CP patients with DM have significantly low GIP stimulated late insulin secretion (20-120 min) compared to CP with NGT who had a significantly greater insulin response to GIP. Taken together these findings show that the development of diabetes in CP is related to alterations in both alpha and beta cell function, whereas islet cell function is maintained in CP patients with NGT.

EFFECT OF DIFFERENT INTERVENTIONS ON ISLET CELL FUNCTION IN CP

It is likely that patients with CP will undergo interventional procedures such as pancreatectomy (including Frey's procedure) for various reasons including intractable pain. Indeed, recent studies have shown that pancreatic surgery is a strong and independent risk factor for the development of diabetes in CP patients[55,56]. It is of great interest to determine the changes in islet cell function following an intervention and how these changes translate into the development of diabetes.

Menge *et al*[57] showed a 50% decline in insulin secretion after a 75-g OGTT in CP patients undergoing hemi-pancreatectomy. Interestingly, follow-up of these participants (n = 10) for 3 years (range 2.2-3.8 years) showed a considerable improvement in the level of plasma insulin and C-peptide, although preoperative basal values were not reached[58]. The authors attributed this limited functional recovery of beta cells to improvement in the inflammatory milieu and gastric motility in the long term as compared to the immediate postoperative period. Importantly, in the immediate postoperative period, it was found that resection of the pancreatic tail was associated with significant worsening of glycemia after glucose challenge,

whereas pancreatic head resection resulted in lower glycemic excursion. This finding suggests that different procedures have a different impact on postoperative beta cell function. Similar findings of initial deterioration followed by relatively stable beta cell function up to 36 months were also demonstrated in an earlier study[59]. On the contrary, a recent study[60] showed that CP patients who underwent a surgical drainage procedure (Puestow, Frey, or similar procedure) had a rapid decline in C-peptide level during a mixed meal test. However, studies have also reported no change in beta cell function in subjects with CP following surgical interventions such as resection and drainage[14,61].

Alpha cell function is also affected following surgery in patients with CP. Altered glucagon suppression following surgery has been described in a few studies[14,15]. Fasting lower glucagon after surgery probably reflected a loss of alpha cell mass[15] during the procedure, but there was a significant increase in glucagon secretion post-glucose challenge after surgery[14,15]. This could reflect the possible effect of impaired beta cell function on alpha cells as insulin secretion is also simultaneously impaired. Taken together, it is evident that beta cell function declines after surgery and it may stabilize in the long-term, but at a lower level compared to the presurgical state, and there is definite evidence of alpha cell dysfunction following surgical procedures in CP.

Another issue that needs to be considered in patients with CP is assessment of the functional preservation of islet cell function following total pancreatectomy and autologous islet transplantation (TP-AIT). A detailed discussion on this topic can be found elsewhere[62,63]. Roughly one third of patients maintain insulin independence following TP-AIT at around two years post-transplantation and a significant proportion of patients require a reduced insulin dose[64,65]. However, the percentage of patients achieving insulin independence declines as time progresses. In some studies, almost 50% of patients achieved insulin independence at a median follow- up of approximately two years[66].

The formal assessment of beta cell functionality is by fasting insulin, C-peptide, glycosylated hemoglobin (HbA1c) and importantly by the requirement of exogenous insulin for glycemic control. Recently Ali and colleagues showed that there is a gradual decrease in post-meal stimulated insulin and C-peptide following islet transplant and more so after 6 months of follow-up[67]. They also demonstrated a gradual decline in the beta 2 score (a composite score to assess islet viability based on fasting glucose, fasting C-peptide, insulin dose and HbA1C) after transplantation. However, the study by Robertson *et al*[68] demonstrated no difference in insulin and C-peptide response to glucose potentiated acute arginine stimulation in CP patients after TP-AIT (follow up of 1-8 years). The beta cell response strongly correlated with the number of transplanted islets. These authors also observed that transplanted alpha cells showed a normal response to arginine stimulation and glucagon was appropriately suppressed after glucose infusion. This result suggests that alpha cells function normally after islet transplant. However, in an earlier study, it was shown that the glucagon response to hypoglycemia was impaired during a 3-h hypoglycemic hyperinsulinemic clamp study in recipients of autologous islet transplant in CP after pancreatectomy[69].

EFFECTS OF ANTI-HYPERGLYCEMIC DRUGS ON ISLET CELL FUNCTION IN PATIENTS WITH CP

There are very few studies in the literature which have evaluated the effect of antidiabetic drugs on islet cell function in CP patients. Of the drugs used to treat diabetes in CP, the effect of thiazolidinediones (TZDs) has been noted, particularly in animal studies. TZDs have been shown to limit the progression of pancreatitis by inhibiting the fibrogenic action of pancreatic stellate cells in rat models[70]. However, human studies on the effect of TZDs on islet cells are lacking. Side effects such as fluid retention, bone loss[71] in the face of malnutrition in CP patients are important and TZDs should be used cautiously in CP.

A randomized controlled trial did not show any difference in insulin and C-peptide response in sitagliptin treated patients with CP who underwent TP-AIT in comparison to placebo after up to 18 months of treatment[72]. There was no difference in insulin dependence or insulin dose reduction either. There is good evidence that GLP-1 analogues increase beta cell mass and prevent apoptosis in rodent models[73,74]. However, the effect of incretin therapy on human pancreatic islet cell morphology was not marked in diabetes patients who received incretin therapies as compared to those

who did not[75]. Importantly, the use of these agents is not suitable in patients with CP due to the risk of precipitating pancreatitis itself[76] and some histological evidence of cellular changes that may increase the risk of neoplasia[77].

CONCLUSION

CP is a progressive inflammatory disorder leading to islet cell disturbances and the subsequent development of diabetes. The disease pathology is complex and is characterized by dysregulation of islet cells. An ongoing inflammatory milieu affects different aspects of the functionality of islet cells. A critical decrease in beta cell mass as well as a decline in insulin and C-peptide secretion are the classic defects found in CP. At baseline, patients with CP but without diabetes have adequate beta cell function which is progressively lost along with the development of prediabetes to diabetes. There is also evidence of inappropriate glucagon secretion in CP patients more so after surgical procedures; however, their impact on glycemic control remains to be determined. The source of this elevated glucagon needs to be confirmed in future studies. PP cells and delta cell functional abnormalities as well as altered secretion of incretin hormones (GLP-1/GIP) are evident in CP and significantly contribute to alpha and beta cell dysregulation.

The different surgical procedures performed in patients with CP and the antihyperglycemic drugs used to treat diabetes associated with CP also affect islet cell function. Surgical intervention in CP can lead to stabilization of beta cell decline albeit at a lower level than the presurgical state. Islet cell transplantation is promising in the management of diabetes in CP following total pancreatectomy. It seems to be an effective measure to curtail the risk of diabetes development by maintaining adequate beta cell function. Newer therapies targeting alpha cell function and beta cell regeneration would be useful in the management of pancreatic diabetes in the near future.

REFERENCES

1 **Hart PA**, Bellin MD, Andersen DK, Bradley D, Cruz-Monserrate Z, Forsmark CE, Goodarzi MO, Habtezion A, Korc M, Kudva YC, Pandol SJ, Yadav D, Chari ST; Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer(CPDPC). Type 3c (pancreatogenic) diabetes mellitus secondary to chronic pancreatitis and pancreatic cancer. *Lancet Gastroenterol Hepatol* 2016; **1**: 226-237 [PMID: 28404095 DOI: 10.1016/S2468-1253(16)30106-6]

2 **Ewald N**, Kaufmann C, Raspe A, Kloer HU, Bretzel RG, Hardt PD. Prevalence of diabetes mellitus secondary to pancreatic diseases (type 3c). *Diabetes Metab Res Rev* 2012; **28**: 338-342 [PMID: 22121010 DOI: 10.1002/dmrr.2260]

3 **Ewald N**, Hardt PD. Diagnosis and treatment of diabetes mellitus in chronic pancreatitis. *World J Gastroenterol* 2013; **19**: 7276-7281 [PMID: 24259958 DOI: 10.3748/wjg.v19.i42.7276]

4 **Schrader H**, Menge BA, Schneider S, Belyaev O, Tannapfel A, Uhl W, Schmidt WE, Meier JJ. Reduced pancreatic volume and beta-cell area in patients with chronic pancreatitis. *Gastroenterology* 2009; **136**: 513-522 [PMID: 19041312 DOI: 10.1053/j.gastro.2008.10.083]

5 **Webb MA**, Chen JJ, James RFL, Davies MJ, Dennison AR. Elevated Levels of Alpha Cells Emanating from the Pancreatic Ducts of a Patient with a Low BMI and Chronic Pancreatitis. *Cell Transplant* 2018; **27**: 902-906 [PMID: 29852747 DOI: 10.1177/0963689718755707]

6 **Larsen S**, Hilsted J, Tronier B, Worning H. Pancreatic hormone secretion in chronic pancreatitis without residual beta-cell function. *Acta Endocrinol (Copenh)* 1988; **118**: 357-364 [PMID: 2899369 DOI: 10.1530/acta.0.1180357]

7 **Linde J**, Nilsson LH, Barany FR. Diabetes and hypoglycemia in chronic pancreatitis. *Scand J Gastroenterol* 1977; **12**: 369-373 [PMID: 867001 DOI: 10.3109/00365527709180943]

8 **Kannan V**, Nabarro JD, Cotton PB. Glucagon secretion in chronic pancreatitis. *Horm Res* 1979; **11**: 203-212 [PMID: 387567 DOI: 10.1159/000179055]

9 **Kalk WJ**, Vinik AI, Bank S, Buchanan KD, Keller P, Jackson WP. Glucagon responses to arginine in chronic pancreatitis. Possible pathogenic significance in diabetes. *Diabetes* 1974; **23**: 257-263 [PMID: 4596075 DOI: 10.2337/diab.23.4.257]

10 **Donowitz M**, Hendler R, Spiro HM, Binder HJ, Felig P. Glucagon secretion in acute and chronic pancreatitis. *Ann Intern Med* 1975; **83**: 778-781 [PMID: 1200523 DOI: 10.7326/0003-4819-83-6-778]

11 **Lundberg R**, Beilman GJ, Dunn TB, Pruett TL, Freeman ML, Ptacek PE, Berry KL, Robertson RP, Moran A, Bellin MD. Early Alterations in Glycemic Control and Pancreatic Endocrine Function in Nondiabetic Patients With Chronic Pancreatitis. *Pancreas* 2016; **45**: 565-571 [PMID: 26918872 DOI: 10.1097/MPA.0000000000000491]

12 **Mumme L**, Breuer TGK, Rohrer S, Schenker N, Menge BA, Holst JJ, Nauck MA, Meier JJ. Defects in α-Cell Function in Patients With Diabetes Due to Chronic Pancreatitis Compared With Patients With Type 2 Diabetes and Healthy Individuals. *Diabetes Care* 2017; **40**: 1314-1322 [PMID: 28751547 DOI:

10.2337/dc17-0792]
13 **Larsen S**, Hilsted J, Philipsen EK, Tronier B, Christensen NJ, Damkjaer Nielsen M, Worning H. Glucose counterregulation in diabetes secondary to chronic pancreatitis. *Metabolism* 1990; **39**: 138-143 [PMID: 1967815 DOI: 10.1016/0026-0495(90)90066-l]
14 **Kumar R**, Sahoo JP, Pottakat B, Kamalanathan S, Mohan P, Kate V, Kar SS, Selviambigapathy J. Effect of Frey's procedure on islet cell function in patients with chronic calcific pancreatitis. *Hepatobiliary Pancreat Dis Int* 2018; **17**: 358-362 [PMID: 30029953 DOI: 10.1016/j.hbpd.2018.06.001]
15 **Schrader H**, Menge BA, Breuer TG, Ritter PR, Uhl W, Schmidt WE, Holst JJ, Meier JJ. Impaired glucose-induced glucagon suppression after partial pancreatectomy. *J Clin Endocrinol Metab* 2009; **94**: 2857-2863 [PMID: 19491219 DOI: 10.1210/jc.2009-0826]
16 **Rossi L**, Parvin S, Hassan Z, Hildebrand P, Keller U, Ali L, Beglinger C, Azad Khan AK, Whitcomb DC, Gyr N. Diabetes mellitus in Tropical Chronic Pancreatitis Is Not Just a Secondary Type of Diabetes. *Pancreatology* 2004; **4**: 461-467 [PMID: 15258409 DOI: 10.1159/000079849]
17 **Lund A**, Bagger JI, Wewer Albrechtsen NJ, Christensen M, Grøndahl M, Hartmann B, Mathiesen ER, Hansen CP, Storkholm JH, van Hall G, Rehfeld JF, Hornburg D, Meissner F, Mann M, Larsen S, Holst JJ, Vilsbøll T, Knop FK. Evidence of Extrapancreatic Glucagon Secretion in Man. *Diabetes* 2016; **65**: 585-597 [PMID: 26672094 DOI: 10.2337/db15-1541]
18 **Meier JJ**, Breuer TG, Bonadonna RC, Tannapfel A, Uhl W, Schmidt WE, Schrader H, Menge BA. Pancreatic diabetes manifests when beta cell area declines by approximately 65% in humans. *Diabetologia* 2012; **55**: 1346-1354 [PMID: 22286529 DOI: 10.1007/s00125-012-2466-8]
19 **Mitnala S**, Pondugala PK, Guduru VR, Rabella P, Thiyyari J, Chivukula S, Boddupalli S, Hardikar AA, Reddy DN. Reduced expression of PDX-1 is associated with decreased beta cell function in chronic pancreatitis. *Pancreas* 2010; **39**: 856-862 [PMID: 20467340 DOI: 10.1097/MPA.0b013e3181d6bc69]
20 **Unnikrishnan R**, Mohan V. Fibrocalculous pancreatic diabetes (FCPD). *Acta Diabetol* 2015; **52**: 1-9 [PMID: 25395047 DOI: 10.1007/s00592-014-0685-9]
21 **Dasgupta R**, Naik D, Thomas N. Emerging concepts in the pathogenesis of diabetes in fibrocalculous pancreatic diabetes. *J Diabetes* 2015; **7**: 754-761 [PMID: 25707547 DOI: 10.1111/1753-0407.12280]
22 **Mehrotra RN**, Bhatia E, Choudhuri G. Beta-cell function and insulin sensitivity in tropical calcific pancreatitis from north India. *Metabolism* 1997; **46**: 441-444 [PMID: 9109851 DOI: 10.1016/s0026-0495(97)90063-9]
23 **Brunicardi FC**, Chaiken RL, Ryan AS, Seymour NE, Hoffmann JA, Lebovitz HE, Chance RE, Gingerich RL, Andersen DK, Elahi D. Pancreatic polypeptide administration improves abnormal glucose metabolism in patients with chronic pancreatitis. *J Clin Endocrinol Metab* 1996; **81**: 3566-3572 [PMID: 8855802 DOI: 10.1210/jcem.81.10.8855802]
24 **Seymour NE**, Volpert AR, Lee EL, Andersen DK, Hernandez C. Alterations in hepatocyte insulin binding in chronic pancreatitis: effects of pancreatic polypeptide. *Am J Surg* 1995; **169**: 105-9; discussion 110 [PMID: 7817978 DOI: 10.1016/s0002-9610(99)80117-2]
25 **Nagpal SJS**, Bamlet WR, Kudva YC, Chari ST. Comparison of Fasting Human Pancreatic Polypeptide Levels Among Patients With Pancreatic Ductal Adenocarcinoma, Chronic Pancreatitis, and Type 2 Diabetes Mellitus. *Pancreas* 2018; **47**: 738-741 [PMID: 29771765 DOI: 10.1097/MPA.0000000000001077]
26 **Rabiee A**, Galiatsatos P, Salas-Carrillo R, Thompson MJ, Andersen DK, Elahi D. Pancreatic polypeptide administration enhances insulin sensitivity and reduces the insulin requirement of patients on insulin pump therapy. *J Diabetes Sci Technol* 2011; **5**: 1521-1528 [PMID: 22226275 DOI: 10.1177/193229681100500629]
27 **Rickels MR**, Bellin M, Toledo FG, Robertson RP, Andersen DK, Chari ST, Brand R, Frulloni L, Anderson MA, Whitcomb DC; PancreasFest Recommendation Conference Participants. Detection, evaluation and treatment of diabetes mellitus in chronic pancreatitis: recommendations from PancreasFest 2012. *Pancreatology* 2013; **13**: 336-342 [PMID: 23890130 DOI: 10.1016/j.pan.2013.05.002]
28 **Aragón F**, Karaca M, Novials A, Maldonado R, Maechler P, Rubí B. Pancreatic polypeptide regulates glucagon release through PPYR1 receptors expressed in mouse and human alpha-cells. *Biochim Biophys Acta* 2015; **1850**: 343-351 [PMID: 25445712 DOI: 10.1016/j.bbagen.2014.11.005]
29 **Cooperberg BA**, Cryer PE. Insulin reciprocally regulates glucagon secretion in humans. *Diabetes* 2010; **59**: 2936-2940 [PMID: 20811038 DOI: 10.2337/db10-0728]
30 **Capozzi ME**, Wait JB, Koech J, Gordon AN, Coch RW, Svendsen B, Finan B, D'Alessio DA, Campbell JE. Glucagon lowers glycemia when β-cells are active. *JCI Insight* 2019; 5 [PMID: 31335319 DOI: 10.1172/jci.insight.129954]
31 **Lund A**, Bagger JI, Christensen M, Knop FK, Vilsbøll T. Glucagon and type 2 diabetes: the return of the alpha cell. *Curr Diab Rep* 2014; **14**: 555 [PMID: 25344790 DOI: 10.1007/s11892-014-0555-4]
32 **Nauck MA**, Meier JJ. The incretin effect in healthy individuals and those with type 2 diabetes: physiology, pathophysiology, and response to therapeutic interventions. *Lancet Diabetes Endocrinol* 2016; **4**: 525-536 [PMID: 26876794 DOI: 10.1016/S2213-8587(15)00482-9]
33 **Vilsbøll T**, Knop FK, Krarup T, Johansen A, Madsbad S, Larsen S, Hansen T, Pedersen O, Holst JJ. The pathophysiology of diabetes involves a defective amplification of the late-phase insulin response to glucose by glucose-dependent insulinotropic polypeptide-regardless of etiology and phenotype. *J Clin Endocrinol Metab* 2003; **88**: 4897-4903 [PMID: 14557471 DOI: 10.1210/jc.2003-030738]
34 **Knop FK**, Vilsbøll T, Højberg PV, Larsen S, Madsbad S, Holst JJ, Krarup T. The insulinotropic effect of GIP is impaired in patients with chronic pancreatitis and secondary diabetes mellitus as compared to patients with chronic pancreatitis and normal glucose tolerance. *Regul Pept* 2007; **144**: 123-130 [PMID: 17692937 DOI: 10.1016/j.regpep.2007.07.002]
35 **Knop FK**, Vilsbøll T, Højberg PV, Larsen S, Madsbad S, Vølund A, Holst JJ, Krarup T. Reduced incretin effect in type 2 diabetes: cause or consequence of the diabetic state? *Diabetes* 2007; **56**: 1951-1959 [PMID: 17513701 DOI: 10.2337/db07-0100]
36 **Hornum M**, Pedersen JF, Larsen S, Olsen O, Holst JJ, Knop FK. Increased postprandial response of glucagon-like peptide-2 in patients with chronic pancreatitis and pancreatic exocrine insufficiency.

Pancreatology 2010; **10**: 201-207 [PMID: 20460948 DOI: 10.1159/000243729]
37 **Seino Y**, Fukushima M, Yabe D. GIP and GLP-1, the two incretin hormones: Similarities and differences. *J Diabetes Investig* 2010; **1**: 8-23 [PMID: 24843404 DOI: 10.1111/j.2040-1124.2010.00022.x]
38 **Knop FK**, Vilsbøll T, Larsen S, Madsbad S, Holst JJ, Krarup T. Glucagon suppression during OGTT worsens while suppression during IVGTT sustains alongside development of glucose intolerance in patients with chronic pancreatitis. *Regul Pept* 2010; **164**: 144-150 [PMID: 20573586 DOI: 10.1016/j.regpep.2010.05.011]
39 **Knop FK**, Vilsbøll T, Larsen S, Højberg PV, Vølund A, Madsbad S, Holst JJ, Krarup T. Increased postprandial responses of GLP-1 and GIP in patients with chronic pancreatitis and steatorrhea following pancreatic enzyme substitution. *Am J Physiol Endocrinol Metab* 2007; **292**: E324-E330 [PMID: 16954337 DOI: 10.1152/ajpendo.00059.2006]
40 **Ebert R**, Creutzfeldt W. Reversal of impaired GIP and insulin secretion in patients with pancreatogenic steatorrhea following enzyme substitution. *Diabetologia* 1980; **19**: 198-204 [PMID: 6997121 DOI: 10.1007/bf00275269]
41 **Knop FK**, Vilsbøll T, Lund A, Krarup T, Holst JJ, Hornum M. The impact of pancreatic enzyme supplementation on postprandial responses of glucagon-like Peptide-2 in patients with chronic pancreatitis and pancreatic exocrine insufficiency. *JOP* 2010; **11**: 489-491 [PMID: 20818126 DOI: 10.6092/1590-8577/3445]
42 **Wewer Albrechtsen NJ**, Pedersen J, Galsgaard KD, Winther-Sørensen M, Suppli MP, Janah L, Gromada J, Vilstrup H, Knop FK, Holst JJ. The Liver-α-Cell Axis and Type 2 Diabetes. *Endocr Rev* 2019; **40**: 1353-1366 [PMID: 30920583 DOI: 10.1210/er.2018-00251]
43 **Wewer Albrechtsen NJ**, Færch K, Jensen TM, Witte DR, Pedersen J, Mahendran Y, Jonsson AE, Galsgaard KD, Winther-Sørensen M, Torekov SS, Lauritzen T, Pedersen O, Knop FK, Hansen T, Jørgensen ME, Vistisen D, Holst JJ. Evidence of a liver-alpha cell axis in humans: hepatic insulin resistance attenuates relationship between fasting plasma glucagon and glucagonotropic amino acids. *Diabetologia* 2018; **61**: 671-680 [PMID: 29305624 DOI: 10.1007/s00125-017-4535-5]
44 **Schrader H**, Menge BA, Belyaev O, Uhl W, Schmidt WE, Meier JJ. Amino acid malnutrition in patients with chronic pancreatitis and pancreatic carcinoma. *Pancreas* 2009; **38**: 416-421 [PMID: 19169171 DOI: 10.1097/MPA.0b013e318194fc7a]
45 **Dresler CM**, Fortner JG, McDermott K, Bajorunas DR. Metabolic consequences of (regional) total pancreatectomy. *Ann Surg* 1991; **214**: 131-140 [PMID: 1867520 DOI: 10.1097/00000658-199108000-00007]
46 **Whitcomb DC**, Frulloni L, Garg P, Greer JB, Schneider A, Yadav D, Shimosegawa T. Chronic pancreatitis: An international draft consensus proposal for a new mechanistic definition. *Pancreatology* 2016; **16**: 218-224 [PMID: 26924663 DOI: 10.1016/j.pan.2016.02.001]
47 **Hasel C**, Dürr S, Rau B, Sträter J, Schmid RM, Walczak H, Bachem MG, Möller P. In chronic pancreatitis, widespread emergence of TRAIL receptors in epithelia coincides with neoexpression of TRAIL by pancreatic stellate cells of early fibrotic areas. *Lab Invest* 2003; **83**: 825-836 [PMID: 12808117 DOI: 10.1097/01.lab.0000073126.56932.46]
48 **Sasikala M**, Talukdar R, Pavan kumar P, Radhika G, Rao GV, Pradeep R, Subramanyam C, Nageshwar Reddy D. β-Cell dysfunction in chronic pancreatitis. *Dig Dis Sci* 2012; **57**: 1764-1772 [PMID: 22383081 DOI: 10.1007/s10620-012-2086-7]
49 **Tanaka S**, Kobayashi T, Nakanishi K, Okubo M, Murase T, Hashimoto M, Watanabe G, Matsushita H, Endo Y, Yoshizaki H, Kosuge T, Sakamoto M, Takeuchi K. Evidence of primary beta-cell destruction by T-cells and beta-cell differentiation from pancreatic ductal cells in diabetes associated with active autoimmune chronic pancreatitis. *Diabetes Care* 2001; **24**: 1661-1667 [PMID: 11522716 DOI: 10.2337/diacare.24.9.1661]
50 **Talukdar R**, Sasikala M, Pavan Kumar P, Rao GV, Pradeep R, Reddy DN. T-Helper Cell-Mediated Islet Inflammation Contributes to β-Cell Dysfunction in Chronic Pancreatitis. *Pancreas* 2016; **45**: 434-442 [PMID: 26474432 DOI: 10.1097/MPA.0000000000000479]
51 **Sun J**, Ni Q, Xie J, Xu M, Zhang J, Kuang J, Wang Y, Ning G, Wang Q. β-Cell Dedifferentiation in Patients With T2D With Adequate Glucose Control and Nondiabetic Chronic Pancreatitis. *J Clin Endocrinol Metab* 2019; **104**: 83-94 [PMID: 30085195 DOI: 10.1210/jc.2018-00968]
52 **Bynigeri RR**, Jakkampudi A, Jangala R, Subramanyam C, Sasikala M, Rao GV, Reddy DN, Talukdar R. Pancreatic stellate cell: Pandora's box for pancreatic disease biology. *World J Gastroenterol* 2017; **23**: 382-405 [PMID: 28210075 DOI: 10.3748/wjg.v23.i3.382]
53 **Lee E**, Ryu GR, Ko SH, Ahn YB, Song KH. A role of pancreatic stellate cells in islet fibrosis and β-cell dysfunction in type 2 diabetes mellitus. *Biochem Biophys Res Commun* 2017; **485**: 328-334 [PMID: 28232184 DOI: 10.1016/j.bbrc.2017.02.082]
54 **Zang G**, Sandberg M, Carlsson PO, Welsh N, Jansson L, Barbu A. Activated pancreatic stellate cells can impair pancreatic islet function in mice. *Ups J Med Sci* 2015; **120**: 169-180 [PMID: 25854824 DOI: 10.3109/03009734.2015.1032453]
55 **Bellin MD**, Whitcomb DC, Abberbock J, Sherman S, Sandhu BS, Gardner TB, Anderson MA, Lewis MD, Alkaade S, Singh VK, Baillie J, Banks PA, Conwell D, Cote GA, Guda NM, Muniraj T, Tang G, Brand RE, Gelrud A, Amann ST, Forsmark CE, Wilcox CM, Slivka A, Yadav D. Patient and Disease Characteristics Associated With the Presence of Diabetes Mellitus in Adults With Chronic Pancreatitis in the United States. *Am J Gastroenterol* 2017; **112**: 1457-1465 [PMID: 28741615 DOI: 10.1038/ajg.2017.181]
56 **Olesen SS**, Poulsen JL, Novovic S, Nøjgaard C, Kalaitzakis E, Jensen NM, Engjom T, Tjora E, Waage A, Hauge T, Haas SL, Vujasinovic M, Barauskas G, Pukitis A, Ozola-Zālīte I, Okhlobystin A, Parhiala M, Laukkarinen J, Drewes AM. Multiple risk factors for diabetes mellitus in patients with chronic pancreatitis: A multicentre study of 1117 cases. *United European Gastroenterol J* 2020; **8**: 453-461 [PMID: 32213024 DOI: 10.1177/2050640620901973]
57 **Menge BA**, Schrader H, Breuer TG, Dabrowski Y, Uhl W, Schmidt WE, Meier JJ. Metabolic consequences of a 50% partial pancreatectomy in humans. *Diabetologia* 2009; **52**: 306-317 [PMID: 19037627 DOI:

10.1007/s00125-008-1219-1]
58 **Menge BA**, Breuer TG, Ritter PR, Uhl W, Schmidt WE, Meier JJ. Long-term recovery of β-cell function after partial pancreatectomy in humans. *Metabolism* 2012; **61**: 620-624 [PMID: 22079939 DOI: 10.1016/j.metabol.2011.09.019]
59 **Schneider MU**, Meister R, Domschke S, Zirngibl H, Strebl H, Heptner G, Gebhardt C, Gall FP, Domschke W. Whipple's procedure plus intraoperative pancreatic duct occlusion for severe chronic pancreatitis: clinical, exocrine, and endocrine consequences during a 3-year follow-up. *Pancreas* 1987; **2**: 715-726 [PMID: 3438310 DOI: 10.1097/00006676-198711000-00016]
60 **Gutama BW**, Yang Y, Beilman GJ, Freeman ML, Kirchner VA, Pruett TL, Chinnakotla S, Downs EM, Trikudanathan G, Schwarzenberg SJ, Hodges JS, Bellin MD. Risk Factors Associated With Progression Toward Endocrine Insufficiency in Chronic Pancreatitis. *Pancreas* 2019; **48**: 1160-1166 [PMID: 31593013 DOI: 10.1097/MPA.0000000000001394]
61 **Heise JW**, Becker H, Niederau C, Röher HD. [Effect of resection or duct drainage on glucose stimulated beta cell function in chronic pancreatitis]. *Langenbecks Arch Chir* 1994; **379**: 44-49 [PMID: 7908397 DOI: 10.1007/bf00206561]
62 **Chang CA**, Haque WZ, Yoshimatsu G, Balajii PS, Lawrence MC, Naziruddin B. Monitoring of beta cell replacement outcomes. *Panminerva Med* 2016; **58**: 59-71 [PMID: 26763742]
63 **Rickels MR**, Robertson RP. Pancreatic Islet Transplantation in Humans: Recent Progress and Future Directions. *Endocr Rev* 2019; **40**: 631-668 [PMID: 30541144 DOI: 10.1210/er.2018-00154]
64 **Johnston PC**, Lin YK, Walsh RM, Bottino R, Stevens TK, Trucco M, Bena J, Faiman C, Hatipoglu BA. Factors associated with islet yield and insulin independence after total pancreatectomy and islet cell autotransplantation in patients with chronic pancreatitis utilizing off-site islet isolation: Cleveland Clinic experience. *J Clin Endocrinol Metab* 2015; **100**: 1765-1770 [PMID: 25781357 DOI: 10.1210/jc.2014-4298]
65 **Garcea G**, Weaver J, Phillips J, Pollard CA, Ilouz SC, Webb MA, Berry DP, Dennison AR. Total pancreatectomy with and without islet cell transplantation for chronic pancreatitis: a series of 85 consecutive patients. *Pancreas* 2009; **38**: 1-7 [PMID: 18665009 DOI: 10.1097/MPA.0b013e3181825c00]
66 **Savari O**, Golab K, Wang LJ, Schenck L, Grose R, Tibudan M, Ramachandran S, Chon WJ, Posner MC, Millis JM, Matthews JB, Gelrud A, Witkowski P. Preservation of beta cell function after pancreatic islet autotransplantation: University of Chicago experience. *Am Surg* 2015; **81**: 421-427 [PMID: 25831191]
67 **Ali KF**, San Martin VT, Walsh RM, Bottino R, Stevens T, Trucco M, Hatipoglu B. Change in Functional Beta Cell Capacity With Time Following Autologous Islet Transplantation. *Pancreas* 2019; **48**: 656-661 [PMID: 31091212 DOI: 10.1097/MPA.0000000000001315]
68 **Robertson RP**, Bogachus LD, Oseid E, Parazzoli S, Patti ME, Rickels MR, Schuetz C, Dunn T, Pruett T, Balamurugan AN, Sutherland DE, Beilman G, Bellin MD. Assessment of β-cell mass and α- and β-cell survival and function by arginine stimulation in human autologous islet recipients. *Diabetes* 2015; **64**: 565-572 [PMID: 25187365 DOI: 10.2337/db14-0690]
69 **Kendall DM**, Teuscher AU, Robertson RP. Defective glucagon secretion during sustained hypoglycemia following successful islet allo- and autotransplantation in humans. *Diabetes* 1997; **46**: 23-27 [PMID: 8971076 DOI: 10.2337/diab.46.1.23]
70 **Shimizu K**, Shiratori K, Kobayashi M, Kawamata H. Troglitazone inhibits the progression of chronic pancreatitis and the profibrogenic activity of pancreatic stellate cells via a PPARgamma-independent mechanism. *Pancreas* 2004; **29**: 67-74 [PMID: 15211114 DOI: 10.1097/00006676-200407000-00058]
71 **Kahn SE**, Zinman B, Lachin JM, Haffner SM, Herman WH, Holman RR, Kravitz BG, Yu D, Heise MA, Aftring RP, Viberti G; Diabetes Outcome Progression Trial (ADOPT) Study Group. Rosiglitazone-associated fractures in type 2 diabetes: an Analysis from A Diabetes Outcome Progression Trial (ADOPT). *Diabetes Care* 2008; **31**: 845-851 [PMID: 18223031 DOI: 10.2337/dc07-2270]
72 **Bellin MD**, Beilman GJ, Dunn TB, Pruett TL, Sutherland DE, Chinnakotla S, Hodges JS, Lane A, Ptacek P, Berry KL, Hering BJ, Moran A. Sitagliptin Treatment After Total Pancreatectomy With Islet Autotransplantation: A Randomized, Placebo-Controlled Study. *Am J Transplant* 2017; **17**: 443-450 [PMID: 27459721 DOI: 10.1111/ajt.13979]
73 **Xu G**, Stoffers DA, Habener JF, Bonner-Weir S. Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes* 1999; **48**: 2270-2276 [PMID: 10580413 DOI: 10.2337/diabetes.48.12.2270]
74 **Kapodistria K**, Tsilibary EP, Kotsopoulou E, Moustardas P, Kitsiou P. Liraglutide, a human glucagon-like peptide-1 analogue, stimulates AKT-dependent survival signalling and inhibits pancreatic β-cell apoptosis. *J Cell Mol Med* 2018; **22**: 2970-2980 [PMID: 29524296 DOI: 10.1111/jcmm.13259]
75 **Ueberberg S**, Jütte H, Uhl W, Schmidt W, Nauck M, Montanya E, Tannapfel A, Meier J. Histological changes in endocrine and exocrine pancreatic tissue from patients exposed to incretin-based therapies. *Diabetes Obes Metab* 2016; **18**: 1253-1262 [PMID: 27545110 DOI: 10.1111/dom.12766]
76 **Knapen LM**, de Jong RG, Driessen JH, Keulemans YC, van Erp NP, De Bruin ML, Leufkens HG, Croes S, de Vries F. Use of incretin agents and risk of acute and chronic pancreatitis: A population-based cohort study. *Diabetes Obes Metab* 2017; **19**: 401-411 [PMID: 27883260 DOI: 10.1111/dom.12833]
77 **Gier B**, Matveyenko AV, Kirakossian D, Dawson D, Dry SM, Butler PC. Chronic GLP-1 receptor activation by exendin-4 induces expansion of pancreatic duct glands in rats and accelerates formation of dysplastic lesions and chronic pancreatitis in the Kras(G12D) mouse model. *Diabetes* 2012; **61**: 1250-1262 [PMID: 22266668 DOI: 10.2337/db11-1109]

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REVIEW

Gut microbiota and diabetes: From correlation to causality and mechanism

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Abstract

In this review, we summarize the recent microbiome studies related to diabetes disease and discuss the key findings that show the early emerging potential causal roles for diabetes. On a global scale, diabetes causes a significant negative impact to the health status of human populations. This review covers type 1 diabetes and type 2 diabetes. We examine promising studies which lead to a better understanding of the potential mechanism of microbiota in diabetes diseases. It appears that the human oral and gut microbiota are deeply interdigitated with diabetes. It is that simple. Recent studies of the human microbiome are capturing the attention of scientists and healthcare practitioners worldwide by focusing on the interplay of gut microbiome and diabetes. These studies focus on the role and the potential impact of intestinal microflora in diabetes. We paint a clear picture of how strongly microbes are linked and associated, both positively and negatively, with the fundamental and essential parts of diabetes in humans. The microflora seems to have an endless capacity to impact and transform diabetes. We conclude that there is clear and growing evidence of a close relationship between the microbiota and diabetes and this is worthy of future investments and research efforts.

Key words: Diabetes; Microbiota; Causality; Mechanism; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Inflammation; Metabolites

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Core tip: Current research continues to uncover associations between microbiota and diabetes [type 1 diabetes (T1D) and type 2 diabetes (T2D)], and these appear to involve metabolic effects and immune response processes. Understanding the consequences of balance in human gut microbiota and diabetes may prove very useful in developing future therapeutic interventions. This review summarizes recent studies in both mouse models and human cases that support a potential cause-effect relationship, and discusses the role of gut microbial metabolites on T1D and T2D.

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INTRODUCTION

Recently, studies of the human microbiome are capturing the attention of scientists and healthcare practitioners worldwide by focusing on the interplay of gut microbiome and diabetes. Understanding the consequences of balance in human gut microbiota and diabetes should prove very useful in developing future promising therapeutic interventions. Diabetes is a common chronic endocrine and metabolic disease, which impacts humans globally. Type 1 diabetes (T1D) is prevalent among children and adolescents, although the disease can occur at any age. The pathogenesis of T1D occurs when the endocrine system cannot produce insulin due to an autoimmune-mediated response leading to both inflammation and destruction of pancreatic β-islet cells. Type 2 diabetes (T2D) is a more prevalent form of diabetes most commonly occurring among adults and is usually caused by a combination of insulin resistance and an insulin deficiency.

Among the risk factors associated with diabetes are often things like a family history

of diabetes, unhealthy eating habits, and obesity. The increasing prevalence of diabetes is a worldwide phenomenon following the continuous growth in urbanization, changes in diet, and the emergence of more sedentary lifestyles. According to a 2019 report, about 463 million adults worldwide currently have diabetes and future projections indicate the number of diabetic patients will reach 700 million by 2045[1]. According to epidemiological observations, specific changes in the diversity of intestinal microflora are one of the characteristics of diabetic patients[2]. At the same time, there is also growing evidence of a close association between gut microbiota and diabetes[3].

The human gut is a complex ecosystem consisting of microbiome, host cells and nutrients[4]. There are about 100 trillion bacteria in the intestinal tract and they form the gut microbiota. Gut microbiota are composed of many diverse species of bacteria. These are taxonomically classified by genus, family, order and phylum. The intestinal microflora of healthy adults principally consists of six phyla: Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, Fusobacteria and Verrucomicrobia. Bacteroidetes and Firmicutes occupy the dominant position in the human intestinal tract and play a pivotal role in the nutritional absorption system and support intestinal barrier enhancement. Genomic analysis of lean mice and healthy humans also confirmed the dominance of Firmicutes and Bacteroidetes, and most research indicates that Bacteroidetes outnumber Firmicutes[5].

Current research continues to find associations between microbiota and diabetes (T1D and T2D), and these appear to involve many metabolic effects and immune response processes, and most of these associate with more specific mechanisms. Some of the future research activities exploring gut microbiota balance variations and diabetes will lead to new interventional experiments, and potential evaluation of a causal hypothesis. This review provides an overview of studies that focuses on gut microbiota balance in humans with diabetes. So far, we know there is a range of recent evidence leading to some support for the potential causal role of gut microbiota in aspects of diabetic disease. It is now clear that future research will examine the potential for and discovery of the microbiota-related underlying mechanisms of diabetes[5,6]. It is only a matter of time and effort to follow the increasing evidence supporting these linkages.

DIET IS A CRUCIAL REGULATOR OF INTESTINAL MICROFLORA

The composition of the microbial community ecosystem is dynamic and its composition is dependent upon many factors[7]. Recent experiments using animal models indicate that intestinal microflora is regulated by factors including genes, medication, and diet. The gut microflora is easily altered by dietary changes. Experiments have shown that dietary changes can induce temporary shifts in a large number of microorganisms as rapidly as within 24 h[8]. Since diet is the main source of energy for individuals and a crucial method for humans to maintain health and growth, the diet composition has a big impact on gut microbiota[9]. It therefore follows that diet is also a vital regulator of gut microbiota. Gut microbiota composition also varies with an individual's age, and studies have shown these age-related gut microflora changes could possibly occur due to changes in diet at different ages and changes in inflammation due to some age-related diseases and changes leading to decreased immune system function[10]. At the same time, the varying composition of gut microorganisms has been identified in disparate geographical regions and this may also be related to different regional eating habits[11]. The gut microflora plays a pivotal role in the body's metabolism and immunity responses can also become a regulator of the effect of diet on the host's metabolic state[12]. On the other hand, these factors may also provide a potential impact on the onset of metabolic diseases like diabetes. The type, quality, components and source of human food intake will affect the composition of gut microbiome, as well as the functions and interactions in the microbiome ecosystem.

The main energy source of the gut microflora is dietary carbohydrates. The incidence of T2D is inversely associated with the total amount of dietary fiber intake. Dietary fiber is also found to impact intestinal microflora populations, and research indicates that fiber intake is associated with an increase in microbial diversity and the ratio of Firmicutes/Bacteroidetes[13]. Some studies have confirmed that an increase in dietary fiber intake also increases the abundance of the human intestinal microflora and leads to higher microflora richness. Fiber intake is also associated with higher microflora stability[14]. Dietary fiber intake promotes the fermentation of intestinal

microbes and this appears to cause an increase in short-chain fatty acids (SCFAs). As ligands of free fatty acid receptor 2 (FFAR2) and free fatty acid receptor 3 (FFAR3), SCFAs participate in the regulation mechanism of glucose homeostasis[15]. Propionic acid is reported to be produced mainly by threonine[16]; glycine, glutamic acid, lysine, ornithine and aspartic acid can be used to synthesize acetate; threonine, glutamic acid and lysine acid can be used to synthesize butyric acid, of which threonine can produce three main SCFAs[17]. Studies have reported that soluble fiber has a direct blood glucose lowering effect. Intake of soluble dietary fiber increases the viscosity of gastric juices, the more viscous fiber leads to gastric emptying times that are longer. Additionally these changes lead to small intestine transit time slowing, and increased starch digestion, which is associated with a reduced rate of glucose absorption, leading to changes in blood glucose and cholesterol concentrations[18]. Consuming more dietary fiber appears to reduce the risk of T2D, and is also associated with maintaining a healthy weight. Healthy adults and children can increase their intake of plant foods rich in fiber, while reducing total energy intake that is more often associated with high-sugar, high-fat, and low-fiber foods[19]. Nevertheless, some SCFAs appear to be involved in some of the mechanisms associated with diabetes, which also establishes the link between microbiota and diabetes[17,20].

A recent study combined measurements of intestinal microbiome diversity with diet history, and blood test parameters from volunteers. These data were evaluated using machine learning algorithms to predict how an individual's postprandial blood glucose production responded to real-life diets[21]. This study indicated that a personalized diet can successfully improve postprandial blood glucose elevation[21]. By combining these techniques and big data analysis, and the use of more specific medicinal nutrition recommendations shows the possible prevention and management of T2D with more effective personalized nutrition guidance. The widespread use of personalized nutrition also faces many challenges, such as the historic lack of reliable and repeatable results, also there are omics technology problems such as high cost, and the need for more research evidence to support actual effectiveness[22].

In addition to SCFAs, intestinal microflora appears to regulate lipopolysaccharide (LPS) levels and these levels are also thought to be involved in the development of diabetes[23]. Patients with T2D have fewer butyrate-producing bacteria than non-diabetic patients. Additionally, the ratio of Firmicutes/Bacteroidetes is also significantly lower in T2D patients than in non-diabetic patients[24]. By reviewing the results from across numerous studies, we can observe which intestinal microflora types and balances are co-occurring and possibly correlated with diabetes. In T2D patients, there is an abundance of *Bacteroides*, *Faecalibacterium*, *Akkermansia*, and there are lower concentrations of *Roseburia*, while *Ruminococcus* and *Fusobacterium* are elevated. Gut microbiota was also reported to have a relationship with T1D in previous studies[25]. Gut microbial communities appear to have an impact starting in infancy, and it is speculated that T1D is possibly related to the early effects of the gut microbiome. The interaction between the human body and the intestinal microflora appears to start at birth, and the development of the gut microbiome then evolves and goes through three fundamental stages: The first is a developmental stage (occurring during months 3-14), the second is a transition stage (occurring during months 15-30) and finally the third stage is a stable period (occurring during months 31-46)[26]. Abnormal gut microbiota is often observed in pre-diabetic patients. A controlled study was conducted to analyze 134 Danish patients with prediabetes. When these subjects were compared with normal controls, the intestinal microflora of patients with prediabetes showed abnormal characteristics, with low concentrations of *Clostridium* and mucin-degrading *Akkermansia muciniphila*[27]. In another study, vertical stool samples from 903 children aged 3-46 mo were analyzed, and the study found that early intestinal microorganism ecology is impacted by breastfeeding and childbirth[26]. The full implications of these observations, although not conclusive, appear to indicate that there is a developmental impact on microbiome development and the strength and outcome of these factors will need to be more fully explored in future research.

STUDIES USING ANIMAL MODELS

The mouse model is commonly used in the study of intestinal microflora, and the function of the intestinal microflora can model that for mammals. Studies using mice as models provide important insights and help to build an understanding of the relationship between the intestinal microflora and diabetes. Mice are generally used as the preferred model for research, because the intestinal structures of mice and human

subjects are quite similar. These models can also provide an evaluation of experiments designed to disturb the intestinal microbiota using controlled experimental apparatus. Closer observations of the microbiota composition is helpful in identifying and evaluating the potential causal relationships and possible mechanism of the interaction between host and intestinal microorganisms[28]. Although there is more work to be accomplished here, it is expected that a better understanding of how these balances in microbiota impact both health and diabetic disease processes will be forthcoming.

It is important to note that previous research indicated that when mice do not have gut microbiota (germ-free mice) they also have lower body fat and insulin resistance than conventional mice, and the tolerance of insulin and glucose in germ-free mice was higher than that observed in routinely fed mice. This study also paved the way for the examination of many potential mechanisms in the past decade[29]. This was followed by a subsequent intestinal microflora transplantation experiment, and the germ-free mice that received transplanted gut microflora from ob/ob mice showed a significant increase in obesity with associated insulin resistance[30]. In subsequent weight-loss surgery experiments, the correlation between obesity and intestinal microflora was also demonstrated, with an observed increase in fat mass in germ-free mice transplanted with altered microbiome[31].

In a recent study, the Koch hypothesis was a useful method to examine the possible causal link between gut microflora and obesity[32]. These studies all started to substantiate the potential cause-and-effect relationship. However, the results of gut microbiological studies in mouse models cannot be simply directly translated into human comparisons and these pitfalls of direct comparisons need to be avoided until more evidence from human studies can be completed to evaluate any potential causality.

TRANSLATIONAL STUDIES AND EXPLORATIONS

Studies in mouse models support the hypothesis of potential causality between gut microbiota and the development of obesity and diabetes, but so far there has been little research completed related to causality in human subjects. The reproducibility of human experimental studies is also sometimes limited, which may also be influenced by variations in differences among study settings, geographic locations of sample preparation, as well as inconsistencies in data analysis. Moreover, there are some studies which have produced contradictory observations and data in human research. It is unclear, as to the root cause of this variation; however, it may be partially attributed to different dietary habits and environmental/cultural factors around the world as well as to different experimental methods used. However, future conclusions regarding human microflora connections to diabetes will require intervention studies to determine if there is a causal relationship with microflora as a driving factor for disease development. To date, fecal microbiota transplantation (FMT), antibiotic therapy, diet, and probiotic therapy are considered effective in various intervention studies[33].

Contemporary research shows that FMT has also been considered an effective tool to gain evidence of microbiome association and the causality of many diseases[34]. In a randomized, double-blind controlled experiment of insulin-resistant men, patients received gut microbiota from lean body mass donors, and analysis of the experimental results demonstrated that FMT improved insulin sensitivity and the number of butyrate-producing bacteria also increased significantly. However, not all patients receiving FMT from lean donors experienced the same beneficial effects, and more research is required for comparative analysis[35].

Metformin

Forslund *et al*[36] proposed that changes in gut microbiome in diabetic patients are not entirely endogenous and can be explained in a large part by metformin treatment. Upregulation of glucagon-like peptide-1 (GLP-1) and peroxisome proliferator-activated receptors has been reported in healthy individuals and in T2D patients after metformin treatment. Metformin is also an insulin hormone regulator that has multiple effects in the intestine, such as increasing GLP-1 concentration in the intestine and extraction of glucose[37]. Metformin can reduce lipid absorption and inflammation caused by LPS, and can also reverse T2D-related changes because the abundance of several gut microbiota appears more similar to non-diabetic control levels when treated with metformin[36].

Recent studies have shown that metformin disrupts the microbial characteristics

associated with diabetes, including changes in the composition of the intestinal microflora[38]. A double-blind, placebo-controlled experiment of T2D patients showed that metformin altered the intestinal microflora balance in treatment-naive T2D patients, while germ-free mice had glucose tolerance after receiving metformin-modified microbiota and showed improved results[39]. Metformin was used in a controlled experiment in mice fed a high-fat diet (HFD), and the results showed that the abundance of the mucin-degrading bacteria *Akkermansia muciniphila* (*A. muciniphila*) was higher than that observed in the control group[40]. Similar conclusions have been found in other human studies[41]. A recent study analyzed the gut microbiome of Chinese T2D patients receiving different anti-diabetes drugs, and metformin recipients showed enrichment of *Turicibacter* and *Spirochaete*[42]. Another study used genomic analysis to analyze the composition of intestinal microflora in diabetic patients taking metformin. The results showed that *A. muciniphila* and several SCFAs-producing microbiota were low when compared to non-diabetic patients who had a relatively high abundance, and this study revealed some of the mechanism by which metformin changes the composition of intestinal microflora by enriching *A. muciniphila* and several SCFAs-producing microbiota[41].

Probiotics and intervention experiments

Probiotics appear to have a wide range of effects on the host, including improved regulation of insulin sensitivity, which may also be related to host metabolism mediated by the gut microbiome balance, by improving host metabolism composition, by reducing pro-inflammatory cytokines, and by reducing intestinal permeability[43]. In addition, probiotics have the potential to directly improve host metabolism and increase SCFAs production. Supplementing probiotics can also improve intestinal balance through the production of antibacterial compounds and competition with pathogens. Probiotics may also regulate the host's immune response, and activate specific gene activation and impact extra-intestine processes and disorders[44].

Numerous experiments in mouse models and human experiments have confirmed that multiple probiotics reduce insulin resistance by affecting gut microbiota and consequently, may influence health. Preliminary studies have shown that ingestion of fermented dairy products such as yogurt can transport lactic acid bacteria to the gut, alter gut microbial composition, inhibit the production of LPS, and increase the close connection of gut epithelial cells[45]. At the same time, a prospective, double-blind, randomized trial of 21 people with high glucose tolerance showed that oral administration of *Lactobacillus reuteri* also improved insulin secretion[46].

Recently, *A. muciniphila* has been frequently mentioned in current studies, and these studies show it reduces insulin resistance and reduces destruction of the intestinal barrier. *A. muciniphila* was reported to be less abundant in pre-diabetic patients, as well as among newly diagnosed T2D patients, suggesting that the low levels of *A. muciniphila* may be a biomarker for impaired glucose tolerance[47]. A recent study found that *A. muciniphila*-derived extracellular vesicles (AmEVs) can regulate gut permeability. The analysis of fecal samples revealed that AmEVs levels were low in T2D patients. Moreover, in a study of diabetic mice, the administration of AmEVs was associated with an observed decrease in fat content and an increase in glucose tolerance in diabetic mice[48]. Studies in mouse models have shown that supplementation with *A. muciniphila* can reduce low-grade inflammatory responses and metabolic disorders[49]. In another study of HFD mice, *Akkermansia* was reported to be associated with reduced LPS levels, which may be related to the ability of *Akkermansia* to maintain mucus layer thickness, which reduces intestinal permeability and LPS leakage[50]. *A. muciniphila* is a mucus-degrading bacterium, and its abundance is negatively correlated with glucose tolerance and fat accumulation in mouse models, but more evidence needs to be acquired in human studies to establish clear results[51]. The mechanism of decreasing insulin sensitivity of *A. muciniphila* may also be related to its membrane protein. Amuc_1100 is a special membrane protein isolated from *A. muciniphila*. Studies have shown that the special protein binds to Toll-like receptor 2 (TLR2) and participates in the protective mechanism of the intestinal barrier[52].

Clinical experiments are increasing in frequency and new results are encouraging. A recent randomized, double-blind placebo trial of 40 insulin-resistant adults who were orally supplemented with *A. muciniphila* showed that it played a role in reducing biomarkers associated with inflammatory responses, these biomarkers have also been linked to diabetes. Experiments have also shown that *A. muciniphila* improves insulin sensitivity in patients[53].

However, the regulatory effects of probiotics on improving insulin sensitivity have population limitations and may not work for everyone. It is worth noting, for example, that two recent studies have shown that probiotics have no effect on gestational

diabetes as this disorder appears entirely hormonal[54,55].

METABOLIC PRODUCTS AFFECT THE UNDERLYING MECHANISMS

Obesity and T2D are often characterized by changes in intestinal microflora, inflammation, and disruption of the intestinal barrier. Chronic, low-grade inflammatory response is a common characteristic of T2D and obesity, and this systemic inflammatory response is also thought to drive insulin resistance. Previous research in mouse models has confirmed that the intestinal microflora is responsible for the increased inflammatory response in obese patients[28]. Furthermore, the gut microbiome can interact with dietary components and habits to influence host insulin sensitivity, intestinal permeability, glucose and fat metabolism[56]. The gut microbiota has long been regarded as a virtual organ of human metabolic activity[57], and its metabolic activity interacts with insulin resistance and diabetes. Gut microbial metabolites can affect host physiological functions. Metagenomic analysis showed that the intestinal microflora of T2D patients and healthy individuals is often markedly different, and the decline in butyrate-producing bacteria may be the cause of impaired glucose metabolism[58]. Modification of gut microbiota caused by external interventions such as diet leads to dysregulation and secretory changes of intestinal microbial metabolites, triggering a variety of potential mechanisms leading to insulin resistance and diabetes. At the same time, intestinal microflora can also affect metabolism and the potential risk of diabetes by changing the way they respond to dietary ingredients[12]. There are many ways to interact with the host and intestinal microorganisms, and in the past decade, many studies were conducted to understand mechanisms for the analysis and hypothesis of microflora involved in regulating insulin resistance, including LPS and SCFAs. Most of the studies have focused on triggering the markers of diabetes: A low-grade inflammatory response and an immune response, in which intestinal microflora and its metabolites play a key role[5].

LPS

LPS is reported to induce inflammatory cytokines through immune cells and adipocytes, causing low-grade inflammation, while acetic acid or butyrate can regulate the function of immune cells. According to Gram staining analysis, the two most common phyla in clinical classification belong to different groups, namely Gram-positive bacteria and Gram-negative bacteria. LPS is derived from the cell wall of Gram-negative bacteria[59]. The LPS of gut microbiota binds to Toll-like receptor 4 (TLR4), then it initiates a signal cascade with good characteristics, inducing the inflammatory response and the expression and secretion of cytokines[60]. The TLR4 signaling pathway is considered to be one of the main triggers of the obesity-induced inflammatory response. Studies have shown that saturated fatty acids can cause insulin resistance and low-grade inflammation by activating the TLR4 signaling pathway[61]. At the same time, different studies have shown that TLR2 is also involved in the inflammatory response when the signaling cascade caused by LPS-LBP-TLR4 is activated[15]. The integrity of the gut barrier seems to play a crucial role in the development of obesity and T2D. The intestinal epithelium acts as a barrier, and its basic function is to limit the interaction between the intestinal microflora, the basic local immunity and other parts of the body[62]. The integrity of the gut barrier can maintain the functional balance of the mucosa, which can be maximally absorbed while maintaining an effective defense response[63]. Increased production of LPS by the intestinal microflora will also activate the endocannabinoid system. In addition, too much LPS may destroy the integrity of the intestinal barrier, and increase LPS absorption[64]. Animal studies have indicated that LPS is involved in the regulation of diabetes-related mechanisms, which can be characterized by the occurrence of increased inflammatory response[65].

SCFAs

SCFAs are composed of acetic acid, propionic acid and butyric acid. The deficiency in SCFAs is thought to be associated with T2D. Currently, studies have shown there is confirmation that SCFAs have a protective effect on the gut barrier, and result in a decrease in the number of butyrate-producing bacteria that may lead to changes in intestinal permeability. Studies have shown that butyrate can promote the expression of tight junction proteins and affect the mucosal barrier function[66], while acetate has also been reported to have a good performance in reducing mucosal permeability and enhancing the intestinal barrier function[67]. The SCFAs mechanism involves activation

of G proteins of the L-cells to promote the release of GLP-1 and peptide YY (PYY) to regulate glucose homeostasis, and at the same time, the SCFAs also effect the intestinal barrier, up-regulate 5'-AMP activated in muscle and liver tissues and the protein kinase signaling pathway, which are related to insulin resistance and inflammation, and oxidative stress may have a potential role[43].

Clinical studies have shown that dietary fiber promotes SCFAs production by gut microorganisms, while most other potential producers are relatively reduced in T2D patients[68]. In a recent study, intestinal microflora before and after dietary fiber interventions in volunteers were transplanted into germ-free mice. The study indicated the strong and significant association between gut microbiome and improved fiber glucose-induced host glycemic control. At the same time, the study proposed that when the SCFAs-producing bacteria promoted by dietary fiber have greater abundance and diversity, participants' glycated hemoglobin levels were improved[68]. On the other hand, SCFAs activate the vagal afferent neurons, which establish a connection between the intestinal information and the brain. This connection has been proved to play a role in controlling human feeding behavior, which also raises new considerations for the potential mechanism of SCFAs in increasing the risk of diabetes by controlling human feeding behavior and selection of dietary response[69].

In a recent study, genome-wide genotyping, intestinal genomic sequences, and fecal SCFAs level information from 952 normal blood glucose individuals were synthesized. A two-way Mendelian randomization (MR) analysis was used to assess causality, and the results showed that butyrate and propionate were proved to be involved in a causal relationship with diabetes, with oral glucose tolerance test showing a positive correlation between butyrate and improved insulin resistance and between malabsorption of propionic acid and the incidence of T2D, which offers evidence for the causal effect of gut microbiota on metabolic characteristics[70].

Butyrate

In a fecal bacteria transplantation experiment, insulin resistance patients received fecal microflora from insulin-sensitive donors, which resulted in a significant improvement in insulin sensitivity with increased abundance of butyrate-producing bacteria[71]. Through the analysis of human fecal samples, *Faecalibacterium prausnitzii* (*F. prausnitzii*) was found to be the main butyrate-producing bacteria. The abundance of *F. prausnitzii* and *Roseburia* in intestinal microflora of T2D patients is lower than that of healthy individuals, according to large scale metagenomic association studies in different populations[72]. Other studies have also demonstrated that the enrichment of *F. prausnitzii* can reduce inflammatory symptoms and insulin resistance. *Roseburia spp*. is also a butyrate-producing bacteria, which has a pivotal part in maintaining intestinal health and immune defense. It can regulate the dynamic balance of T cells by producing butyric acid[73]. Butyrate has a protective effect on the intestinal barrier by inducing the synthesis of mucin, it reduces the intestinal permeability and prevents bacteria from passing through. Butyrate also acts on the colonic epithelium, reducing oxidative stress and inflammation. In addition, the abundance of butyrate-producing bacteria is lower in prediabetic patients than in healthy people[27], which may indicate that the absence of butyrate-producing bacteria is one of the precursors of diabetes.

Bile acids and branched-chain amino acids

Bile acids are synthesized in the liver, and are transformed into secondary bile acids through the enzyme metabolism of gut microbiota[74]. In an experiment on rats, the intestinal microflora of oral bile acid treated rats was analyzed and showed there were significant changes in phylum levels and an increased ratio of Firmicutes/Bacteroidetes[75]. Secondary bile acids are associated with the regulation of insulin sensitivity through activation of Farnesoid X receptor (FXR) and the Takeda G protein-coupled receptor 5 (TGR5) receptors[76]. A study reported reduced genetic and diet-induced insulin resistance in FXR knockout mice[77]. FXR activation induces increased secretion of fibroblast growth factor 19 (FGF19 in humans, FGF15 in rodents), which improves glucose tolerance and insulin resistance[78]. Activation of the TGR receptor stimulates intestinal L cells to secrete GLP-1, thereby improving insulin sensitivity[79].

Branched-chain amino acids (BCAA) are thought to be related to the risk of developing T2D and are considered to be predictive markers for T2D[80,81]. Several studies have reported decreasing plasma BCAA levels in T2D patients[82,83].

A large cohort study also demonstrated the strong association between BCAA and diabetes, as well as the potential role of amino acid metabolism in the early stage of diabetes[80]. Studies in rats have demonstrated that high-fat dietary supplementation with BCAA also leads to insulin resistance[84]. Human studies have confirmed the

conclusion that supplementing BCAA in diet increases the risk of T2D and insulin resistance[85]. In a recent study, patients with T2D received a short-term dietary supplement of BCAA, which showed reduced insulin secretion after a meal and changes in the composition of the intestinal microflora. The synthesis pathway of BCAA has been shown to be related to *Prevotella copri* (*P. copri*) and *Bacteroides vulgatus* in intestinal microflora[86]. Subsequent experiments showed increased BCAA levels and increased insulin resistance in germ-free mice transplanted with *P. copri*[86]. The mechanism of BCAA inducing insulin resistance has been proposed to be attributed to the increased oxidation of free fatty acids and the activation of phosphatidylinositol 3-kinase (PI3K)[87]. However, the exact mechanism is still unclear and needs further study.

GUT MICROBIOTA AND T1D

Both T1D and T2D are associated with complex immune system and gut microbiome interactions. Gut microbiota disorders are associated with the pathogenesis of T1D, and the incidence of T1D is related to the interaction of gut microbiota and the innate immunity. Non-obese diabetic (NOD) mice have developed into the prototype model of T1D. The occurrence of T1D in NOD mice depends on the composition of gut microflora and LPS-mediated gut signals involving TLR4 and MyD88[88]. MyD88 is a key signal transduction factor in interleukin (IL)-1 and TLR signal transduction pathway. Its defect alters the composition of distal intestinal microflora. Studies have reported that NOD mice lacking MyD88 protein will not develop T1D[89]. In the follow-up study, the gut microflora of MyD88-deficient NOD mice protected by diabetes was transferred to wild-type NOD female mice, which reduced the intensity of pancreatitis and significantly delayed the occurrence of autoimmune glycosuria[90].

The gut microflora of preclinical T1D patients is characterized by the dominance of Bacteroidetes, the lack of butyric acid-producing bacteria, and the decrease of bacterial and functional diversity. A study in which colonic bacteria released large amounts of acetic acid or butyrate by feeding NOD mice with specific foods found that the key characteristics of the disease were negatively correlated with the concentrations of butyrate and acetate in blood and feces[91]. The mechanism is believed to be that the acetate diet reduces the frequency of autoimmune T cells in lymphoid tissues, while butyrate diet increases the number and function of regulatory T cells[91]. Human studies have also shown that SCFAs are involved in the prevention mechanism of early-onset human T1D. A recent prospective study demonstrated the protective effect of SCFAs on early-onset human T1D. This study analyzed 10913 metagenomes from 783 stool samples, and increased several bacterial pathways that promote SCFAs biosynthesis was found in healthy controls[92].

However, unlike T2D, transfer of the whole microbiota may not reduce the incidence of T1D. Recently, a study investigated the incidence of T1D in two NOD groups with different gut microbiota. Afterwards, 16S rRNA gene sequencing was used to analyze the gut microbiota with high or low incidence of T1D in the two groups of NOD mice, and the high incidence population was colonized with the microflora of the low incidence population. The results showed that the gut microbiota changed but the incidence of diabetes did not[88]. In another study, germ-free mice received fecal microflora from children with loss of β-cells, the result of which indicated that loss of β-cells after human T1D onset cannot be converted in germ-free NOD mice by FMT[93]. However, it is interesting that single symbiotic bacteria, such as *A. muciniphila*, can be used as probiotics to reduce the incidence of diabetes[88]. LPS also participates in the regulation of autoimmunity, most of which are *Escherichia coli* LPS involved in suppressing innate immune signals, but *Bacteroides dorei* LPS does not show significant improvement in T1D incidence[94]. In a recent study, intraperitoneal injection of *Escherichia coli* LPS in T1D mice showed a decrease in the incidence of T1D and an improved autoimmune response[94], while another study of NOD mice that received oral injection of *Escherichia coli* LPS also demonstrated an improvement in local immunity[95]. The concept that the pathogenesis of T1D is affected by gut microbiota has been well established in mouse models, but human studies on the microbiome in T1D are still few and far between to provide convincing evidence.

Gut microbial colonization in fetuses and infants can lead to dynamic changes in diversity, which may further affect disease susceptibility. A study of 33 infants with T1D genetic predisposition observed a significant decrease in alpha diversity among T1D progenitors, along with peaks in inflammatory organisms, gene function, serum and fecal metabolites, and this diversity difference occurred after serum conversion

and was determined to be specific to T1D[96].

The pre-clinical T1D patients' intestinal microflora is characterized by a dominant Bacteroidetes, with low stability and diversity of intestinal microflora. Studies have shown that these changes were found after the body produced auto-antibodies, which could indicate the role of gut microbiota in the autoimmune process, while the triggering mechanism of T1D disease was not determined[97]. There is growing evidence that islet autoimmunity is the first stage of T1D. Islet autoimmunity refers to the continuous existence of islet antigen autoantibodies, which usually begin in early childhood[98]. The role of gut microbiota in activating T1D is still a very vague concept, current studies have few observations or evidence to support the explanation that gut microbiota activates T1D, and most studies focus on the involvement of gut microbiota in the β-cell autoimmunity process. The causal relationship between intestinal microflora and T1D is still unclear, because most studies are only observational studies, and lack specific mechanical and intervention.

ORAL MICROBIOTA: ANOTHER FACTOR OF GUT MICROBIOME AND DIABETES

As the starting point of the digestive tract, the importance of oral microbiota and its association with the intestinal microbiota are received increasing attention. The oral cavity serves as an endogenous reservoir for gut microbial strains, and oral-fecal transmission is an important process that shapes the gastrointestinal microbiome in both health and disease[99]. Oral bacteria can translocate to the gut and lead to changes in its microbiota and possibly immune defense. It has been recognized that oral microorganisms may cause diseases mainly by a synergistic or cooperative way, and oral diseases (*e.g.*, caries, periodontal disease) and T2D appear to be mutually correlated[100]. Studies have reported significant differences in oral microbiota between patients with T2D and non-diabetic patients. Oral microbial biomarkers have been identified for T2D screening, diagnosis and prognosis[101-103]. Recently, researchers provided a possible mechanism for the improved understanding of how diabetes increases the risk and severity of tooth loss. Diabetes may cause changes in oral bacterial composition, and the oral microbiota of diabetic mice was found to be more pathogenic in studies transplanting to germ-free mice[104]. These studies suggested that oral microbiota is an important factor in the development of diabetes, and on the other hand, oral microbiota is also an important avenue for diabetes to cause other oral or systemic complications. This new area of investigation may represent another pathway for the oral-gut axis to potentially cause an increase in diabetic disease and deserves more in-depth research moving forward.

CONCLUSION

The current research into gut microbiome in the field of diabetes has gradually moved step by step from the initial correlation studies, which proved a strong association, to exploring the causality and potential mechanisms (Figure 1). It is very clear that as science looks to the future this will be a very promising frontier. It can be foreseen that the gut microbiota will be used not only as a biomarker for diabetes, but also as a target for potential therapeutic treatments. Through the intervention of gut microflora, it will eventually be possible to achieve a more precise and personalized diagnosis as well as treatment of diabetes (Table 1). This is only going to be possible with a significant investment in extensive multicenter, longitudinal, interventional and double-blind randomized clinical trials. Additionally, these will yield an extensive knowledge base upon which data science and exploration can occur. The scientific research community must proceed with a sense of urgency, if these data are to be used to their fullest advantage, as many new discoveries are waiting just ahead.

Table 1 A summary of products of gut microbiota and their mechanism of action

Gut microbiota products		Source	Mechanism	Function		Ref.
LPS		The cell wall of Gram-negative bacteria	Activates the receptor TLR4	Increase the occurrence of inflammatory response		[59,60]
SCFAs	Acetate	Carbohydrate fermentation	Activates the receptor FFAR2	Reduce the frequency of autoimmune T cells in lymphoid tissues	FFAR2 and FFAR3 stimulate the release of GLP-1 and PYY, which improve insulin secretion.	[15,91, 105]
	Propionate		Activates the receptor FFAR2 and FFAR3	Promote intestinal gluconeogenesis		
	Butyrate		Activates the receptor FFAR3	Increase the number and function of regulatory T cells		
Bile acids		The microbiota from host cholesterol	Bind to the receptor TGR5 and FXR	Improve insulin sensitivity		[74,76]
BCAA		*Prevotella copri* and *Bacteroides vulgatus*	Activate PI3K and increase the oxidation of free fatty acids	Increase the risk of insulin resistance		[86,87]

LPS: Lipopolysaccharide; TLR4: Toll-like receptor 4; SCFAs: Short-chain fatty acids; FFAR 2: Free fatty acid receptor 2; FFAR 3: Free fatty acid receptor 3; GLP-1: Glucagon-like peptide-1; PYY: Peptide YY; TGR5: Takeda G protein-coupled receptor 5; FXR: Farnesoid X receptor; BCAA: Branched-Chain Amino Acids; PI3K: Phosphatidylinositol 3-kinase.

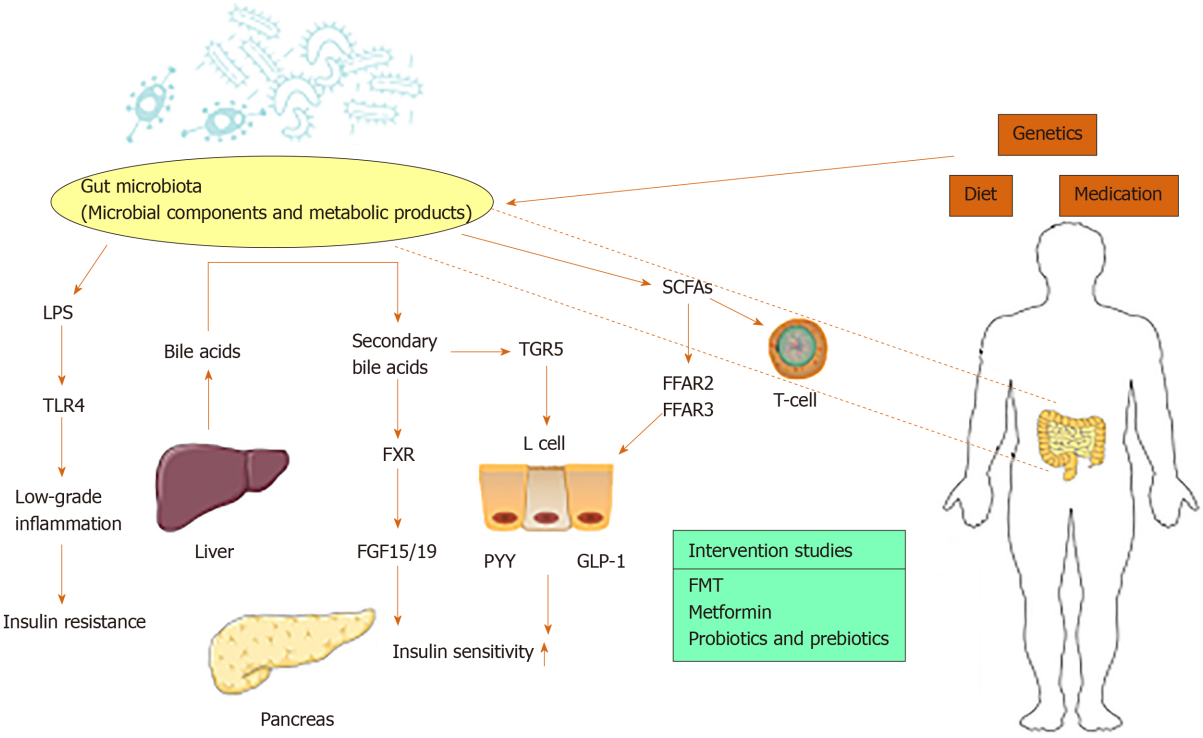

Figure 1 The main mechanism of gut microbiota affecting insulin resistance and diabetes. Gut microbes are influenced by diet, genetics and medication, and common types of interventions in humans include fecal microbiota transplantation, metformin and probiotics. Lipopolysaccharide (LPS), short-chain fatty acids (SCFAs) and bile acids are major regulators of diabetes. LPS binds to the Toll-like receptor 4 to induce low-grade inflammation and insulin resistance. Bile acids are synthesized by the liver and transformed into secondary bile acids through the metabolism of gut microbiota. Secondary bile acids activate Farnesoid X receptor to induce increased secretion of fibroblast growth factor 15/19. Secondary bile acids activate Takeda G protein-coupled receptor to stimulate intestinal L cells to secrete glucagon-like peptide-1 (GLP-1). SCFAs activate L cells to promote the release of GLP-1 and peptide YY to increase insulin sensitivity. SCFAs also have a regulatory effect on T cells. LPS: Lipopolysaccharide; TLR4: Toll-like receptor 4; FXR: Farnesoid X receptor; FGF15/19: Fibroblast growth factor 15/19; TGR5: Takeda G protein-coupled receptor 5; PYY: Peptide YY; GLP-1: Glucagon-like peptide-1; SCFAs: Short-chain fatty acids; FFAR 2: Free fatty acid receptor 2; FFAR 3: Free fatty acid receptor 3; FMT: Fecal microbiota transplantation.

REFERENCES

1 **Saeedi P**, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA,

Ogurtsova K, Shaw JE, Bright D, Williams R; IDF Diabetes Atlas Committee. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 2019; **157**: 107843 [PMID: 31518657 DOI: 10.1016/j.diabres.2019.107843]

2 **Gowd V**, Xie L, Zheng X, Chen W. Dietary fibers as emerging nutritional factors against diabetes: focus on the involvement of gut microbiota. *Crit Rev Biotechnol* 2019; **39**: 524-540 [PMID: 30810398 DOI: 10.1080/07388551.2019.1576025]

3 **Brunkwall L**, Orho-Melander M. The gut microbiome as a target for prevention and treatment of hyperglycaemia in type 2 diabetes: from current human evidence to future possibilities. *Diabetologia* 2017; **60**: 943-951 [PMID: 28434033 DOI: 10.1007/s00125-017-4278-3]

4 **Azad MAK**, Sarker M, Li T, Yin J. Probiotic Species in the Modulation of Gut Microbiota: An Overview. *Biomed Res Int* 2018; **2018**: 9478630 [PMID: 29854813 DOI: 10.1155/2018/9478630]

5 **Saad MJ**, Santos A, Prada PO. Linking Gut Microbiota and Inflammation to Obesity and Insulin Resistance. *Physiology (Bethesda)* 2016; **31**: 283-293 [PMID: 27252163 DOI: 10.1152/physiol.00041.2015]

6 **Han JL**, Lin HL. Intestinal microbiota and type 2 diabetes: from mechanism insights to therapeutic perspective. *World J Gastroenterol* 2014; **20**: 17737-17745 [PMID: 25548472 DOI: 10.3748/wjg.v20.i47.17737]

7 **Cox LM**, Blaser MJ. Antibiotics in early life and obesity. *Nat Rev Endocrinol* 2015; **11**: 182-190 [PMID: 25488483 DOI: 10.1038/nrendo.2014.210]

8 **Singh RK**, Chang HW, Yan D, Lee KM, Ucmak D, Wong K, Abrouk M, Farahnik B, Nakamura M, Zhu TH, Bhutani T, Liao W. Influence of diet on the gut microbiome and implications for human health. *J Transl Med* 2017; **15**: 73 [PMID: 28388917 DOI: 10.1186/s12967-017-1175-y]

9 **Makki K**, Deehan EC, Walter J, Bäckhed F. The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. *Cell Host Microbe* 2018; **23**: 705-715 [PMID: 29902436 DOI: 10.1016/j.chom.2018.05.012]

10 **Vaiserman AM**, Koliada AK, Marotta F. Gut microbiota: A player in aging and a target for anti-aging intervention. *Ageing Res Rev* 2017; **35**: 36-45 [PMID: 28109835 DOI: 10.1016/j.arr.2017.01.001]

11 **Heath RD**, Cockerell C, Mankoo R, Ibdah JA, Tahan V. Fecal microbiota transplantation and its potential therapeutic uses in gastrointestinal disorders. *North Clin Istanb* 2018; **5**: 79-88 [PMID: 29607440 DOI: 10.14744/nci.2017.10692]

12 **Sonnenburg JL**, Bäckhed F. Diet-microbiota interactions as moderators of human metabolism. *Nature* 2016; **535**: 56-64 [PMID: 27383980 DOI: 10.1038/nature18846]

13 **Martínez I**, Lattimer JM, Hubach KL, Case JA, Yang J, Weber CG, Louk JA, Rose DJ, Kyureghian G, Peterson DA, Haub MD, Walter J. Gut microbiome composition is linked to whole grain-induced immunological improvements. *ISME J* 2013; **7**: 269-280 [PMID: 23038174 DOI: 10.1038/ismej.2012.104]

14 **Tap J**, Furet JP, Bensaada M, Philippe C, Roth H, Rabot S, Lakhdari O, Lombard V, Henrissat B, Corthier G, Fontaine E, Doré J, Leclerc M. Gut microbiota richness promotes its stability upon increased dietary fibre intake in healthy adults. *Environ Microbiol* 2015; **17**: 4954-4964 [PMID: 26235304 DOI: 10.1111/1462-2920.13006]

15 **Gholizadeh P**, Mahallei M, Pormohammad A, Varshochi M, Ganbarov K, Zeinalzadeh E, Yousefi B, Bastami M, Tanomand A, Mahmood SS, Yousefi M, Asgharzadeh M, Kafil HS. Microbial balance in the intestinal microbiota and its association with diabetes, obesity and allergic disease. *Microb Pathog* 2019; **127**: 48-55 [PMID: 30503960 DOI: 10.1016/j.micpath.2018.11.031]

16 **Davila AM**, Blachier F, Gotteland M, Andriamihaja M, Benetti PH, Sanz Y, Tomé D. Intestinal luminal nitrogen metabolism: role of the gut microbiota and consequences for the host. *Pharmacol Res* 2013; **68**: 95-107 [PMID: 23183532 DOI: 10.1016/j.phrs.2012.11.005]

17 **Neis EP**, Dejong CH, Rensen SS. The role of microbial amino acid metabolism in host metabolism. *Nutrients* 2015; **7**: 2930-2946 [PMID: 25894657 DOI: 10.3390/nu7042930]

18 **Fuller S**, Beck E, Salman H, Tapsell L. New Horizons for the Study of Dietary Fiber and Health: A Review. *Plant Foods Hum Nutr* 2016; **71**: 1-12 [PMID: 26847187 DOI: 10.1007/s11130-016-0529-6]

19 **Dahl WJ**, Stewart ML. Position of the Academy of Nutrition and Dietetics: Health Implications of Dietary Fiber. *J Acad Nutr Diet* 2015; **115**: 1861-1870 [PMID: 26514720 DOI: 10.1016/j.jand.2015.09.003]

20 **Kasubuchi M**, Hasegawa S, Hiramatsu T, Ichimura A, Kimura I. Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. *Nutrients* 2015; **7**: 2839-2849 [PMID: 25875123 DOI: 10.3390/nu7042839]

21 **Zeevi D**, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, Ben-Yacov O, Lador D, Avnit-Sagi T, Lotan-Pompan M, Suez J, Mahdi JA, Matot E, Malka G, Kosower N, Rein M, Zilberman-Schapira G, Dohnalová L, Pevsner-Fischer M, Bikovsky R, Halpern Z, Elinav E, Segal E. Personalized Nutrition by Prediction of Glycemic Responses. *Cell* 2015; **163**: 1079-1094 [PMID: 26590418 DOI: 10.1016/j.cell.2015.11.001]

22 **Wang DD**, Hu FB. Precision nutrition for prevention and management of type 2 diabetes. *Lancet Diabetes Endocrinol* 2018; **6**: 416-426 [PMID: 29433995 DOI: 10.1016/S2213-8587(18)30037-8]

23 **Cani PD**, Delzenne NM. The role of the gut microbiota in energy metabolism and metabolic disease. *Curr Pharm Des* 2009; **15**: 1546-1558 [PMID: 19442172 DOI: 10.2174/138161209788168164]

24 **Yoo JY**, Kim SS. Probiotics and Prebiotics: Present Status and Future Perspectives on Metabolic Disorders. *Nutrients* 2016; **8**: 173 [PMID: 26999199 DOI: 10.3390/nu8030173]

25 **Gavin PG**, Hamilton-Williams EE. The gut microbiota in type 1 diabetes: friend or foe? *Curr Opin Endocrinol Diabetes Obes* 2019; **26**: 207-212 [PMID: 31145129 DOI: 10.1097/MED.0000000000000483]

26 **Stewart CJ**, Ajami NJ, O'Brien JL, Hutchinson DS, Smith DP, Wong MC, Ross MC, Lloyd RE, Doddapaneni H, Metcalf GA, Muzny D, Gibbs RA, Vatanen T, Huttenhower C, Xavier RJ, Rewers M, Hagopian W, Toppari J, Ziegler AG, She JX, Akolkar B, Lernmark A, Hyoty H, Vehik K, Krischer JP, Petrosino JF. Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature* 2018; **562**: 583-588 [PMID: 30356187 DOI: 10.1038/s41586-018-0617-x]

27 **Allin KH**, Tremaroli V, Caesar R, Jensen BAH, Damgaard MTF, Bahl MI, Licht TR, Hansen TH, Nielsen T, Dantoft TM, Linneberg A, Jørgensen T, Vestergaard H, Kristiansen K, Franks PW; IMI-DIRECT

consortium, Hansen T, Bäckhed F, Pedersen O. Aberrant intestinal microbiota in individuals with prediabetes. *Diabetologia* 2018; **61**: 810-820 [PMID: 29379988 DOI: 10.1007/s00125-018-4550-1]
28 **Nguyen TL**, Vieira-Silva S, Liston A, Raes J. How informative is the mouse for human gut microbiota research? *Dis Model Mech* 2015; **8**: 1-16 [PMID: 25561744 DOI: 10.1242/dmm.017400]
29 **Bäckhed F**, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 2004; **101**: 15718-15723 [PMID: 15505215 DOI: 10.1073/pnas.0407076101]
30 **Turnbaugh PJ**, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; **444**: 1027-1031 [PMID: 17183312 DOI: 10.1038/nature05414]
31 **Tremaroli V**, Karlsson F, Werling M, Ståhlman M, Kovatcheva-Datchary P, Olbers T, Fändriks L, le Roux CW, Nielsen J, Bäckhed F. Roux-en-Y Gastric Bypass and Vertical Banded Gastroplasty Induce Long-Term Changes on the Human Gut Microbiome Contributing to Fat Mass Regulation. *Cell Metab* 2015; **22**: 228-238 [PMID: 26244932 DOI: 10.1016/j.cmet.2015.07.009]
32 **Zhao L**. The gut microbiota and obesity: from correlation to causality. *Nat Rev Microbiol* 2013; **11**: 639-647 [PMID: 23912213 DOI: 10.1038/nrmicro3089]
33 **Meijnikman AS**, Gerdes VE, Nieuwdorp M, Herrema H. Evaluating Causality of Gut Microbiota in Obesity and Diabetes in Humans. *Endocr Rev* 2018; **39**: 133-153 [PMID: 29309555 DOI: 10.1210/er.2017-00192]
34 **Smits LP**, Bouter KE, de Vos WM, Borody TJ, Nieuwdorp M. Therapeutic potential of fecal microbiota transplantation. *Gastroenterology* 2013; **145**: 946-953 [PMID: 24018052 DOI: 10.1053/j.gastro.2013.08.058]
35 **Hartstra AV**, Bouter KE, Bäckhed F, Nieuwdorp M. Insights into the role of the microbiome in obesity and type 2 diabetes. *Diabetes Care* 2015; **38**: 159-165 [PMID: 25538312 DOI: 10.2337/dc14-0769]
36 **Forslund K**, Hildebrand F, Nielsen T, Falony G, Le Chatelier E, Sunagawa S, Prifti E, Vieira-Silva S, Gudmundsdottir V, Pedersen HK, Arumugam M, Kristiansen K, Voigt AY, Vestergaard H, Hercog R, Costea PI, Kultima JR, Li J, Jørgensen T, Levenez F, Dore J; MetaHIT consortium, Nielsen HB, Brunak S, Raes J, Hansen T, Wang J, Ehrlich SD, Bork P, Pedersen O. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature* 2015; **528**: 262-266 [PMID: 26633628 DOI: 10.1038/nature15766]
37 **McCreight LJ**, Bailey CJ, Pearson ER. Metformin and the gastrointestinal tract. *Diabetologia* 2016; **59**: 426-435 [PMID: 26780750 DOI: 10.1007/s00125-015-3844-9]
38 **Vallianou NG**, Stratigou T, Tsagarakis S. Metformin and gut microbiota: their interactions and their impact on diabetes. *Hormones (Athens)* 2019; **18**: 141-144 [PMID: 30719628 DOI: 10.1007/s42000-019-00093-w]
39 **Wu H**, Esteve E, Tremaroli V, Khan MT, Caesar R, Mannerås-Holm L, Ståhlman M, Olsson LM, Serino M, Planas-Fèlix M, Xifra G, Mercader JM, Torrents D, Burcelin R, Ricart W, Perkins R, Fernàndez-Real JM, Bäckhed F. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nat Med* 2017; **23**: 850-858 [PMID: 28530702 DOI: 10.1038/nm.4345]
40 **Shin NR**, Lee JC, Lee HY, Kim MS, Whon TW, Lee MS, Bae JW. An increase in the Akkermansia spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut* 2014; **63**: 727-735 [PMID: 23804561 DOI: 10.1136/gutjnl-2012-303839]
41 **de la Cuesta-Zuluaga J**, Mueller NT, Corrales-Agudelo V, Velásquez-Mejía EP, Carmona JA, Abad JM, Escobar JS. Metformin Is Associated With Higher Relative Abundance of Mucin-Degrading Akkermansia muciniphila and Several Short-Chain Fatty Acid-Producing Microbiota in the Gut. *Diabetes Care* 2017; **40**: 54-62 [PMID: 27999002 DOI: 10.2337/dc16-1324]
42 **Zhang F**, Wang M, Yang J, Xu Q, Liang C, Chen B, Zhang J, Yang Y, Wang H, Shang Y, Wang Y, Mu X, Zhu D, Zhang C, Yao M, Zhang L. Response of gut microbiota in type 2 diabetes to hypoglycemic agents. *Endocrine* 2019; **66**: 485-493 [PMID: 31410749 DOI: 10.1007/s12020-019-02041-5]
43 **Kim YA**, Keogh JB, Clifton PM. Probiotics, prebiotics, synbiotics and insulin sensitivity. *Nutr Res Rev* 2018; **31**: 35-51 [PMID: 29037268 DOI: 10.1017/S095442241700018X]
44 **George Kerry R**, Patra JK, Gouda S, Park Y, Shin HS, Das G. Benefaction of probiotics for human health: A review. *J Food Drug Anal* 2018; **26**: 927-939 [PMID: 29976412 DOI: 10.1016/j.jfda.2018.01.002]
45 **Wen L**, Duffy A. Factors Influencing the Gut Microbiota, Inflammation, and Type 2 Diabetes. *J Nutr* 2017; **147**: 1468S-1475S [PMID: 28615382 DOI: 10.3945/jn.116.240754]
46 **Simon MC**, Strassburger K, Nowotny B, Kolb H, Nowotny P, Burkart V, Zivehe F, Hwang JH, Stehle P, Pacini G, Hartmann B, Holst JJ, MacKenzie C, Bindels LB, Martinez I, Walter J, Henrich B, Schloot NC, Roden M. Intake of Lactobacillus reuteri improves incretin and insulin secretion in glucose-tolerant humans: a proof of concept. *Diabetes Care* 2015; **38**: 1827-1834 [PMID: 26084343 DOI: 10.2337/dc14-2690]
47 **Zhang X**, Shen D, Fang Z, Jie Z, Qiu X, Zhang C, Chen Y, Ji L. Human gut microbiota changes reveal the progression of glucose intolerance. *PLoS One* 2013; **8**: e71108 [PMID: 24013136 DOI: 10.1371/journal.pone.0071108]
48 **Chelakkot C**, Choi Y, Kim DK, Park HT, Ghim J, Kwon Y, Jeon J, Kim MS, Jee YK, Gho YS, Park HS, Kim YK, Ryu SH. Akkermansia muciniphila-derived extracellular vesicles influence gut permeability through the regulation of tight junctions. *Exp Mol Med* 2018; **50**: e450 [PMID: 29472701 DOI: 10.1038/emm.2017.282]
49 **Schneeberger M**, Everard A, Gómez-Valadés AG, Matamoros S, Ramírez S, Delzenne NM, Gomis R, Claret M, Cani PD. Akkermansia muciniphila inversely correlates with the onset of inflammation, altered adipose tissue metabolism and metabolic disorders during obesity in mice. *Sci Rep* 2015; **5**: 16643 [PMID: 26563823 DOI: 10.1038/srep16643]
50 **Anhê FF**, Roy D, Pilon G, Dudonné S, Matamoros S, Varin TV, Garofalo C, Moine Q, Desjardins Y, Levy E, Marette A. A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice. *Gut* 2015; **64**: 872-883 [PMID: 25080446 DOI: 10.1136/gutjnl-2014-307142]

51 **Dao MC**, Everard A, Aron-Wisnewsky J, Sokolovska N, Prifti E, Verger EO, Kayser BD, Levenez F, Chilloux J, Hoyles L; MICRO-Obes Consortium, Dumas ME, Rizkalla SW, Doré J, Cani PD, Clément K. Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. *Gut* 2016; **65**: 426-436 [PMID: 26100928 DOI: 10.1136/gutjnl-2014-308778]

52 **Plovier H**, Everard A, Druart C, Depommier C, Van Hul M, Geurts L, Chilloux J, Ottman N, Duparc T, Lichtenstein L, Myridakis A, Delzenne NM, Klievink J, Bhattacharjee A, van der Ark KC, Aalvink S, Martinez LO, Dumas ME, Maiter D, Loumaye A, Hermans MP, Thissen JP, Belzer C, de Vos WM, Cani PD. A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat Med* 2017; **23**: 107-113 [PMID: 27892954 DOI: 10.1038/nm.4236]

53 **Depommier C**, Everard A, Druart C, Plovier H, Van Hul M, Vieira-Silva S, Falony G, Raes J, Maiter D, Delzenne NM, de Barsy M, Loumaye A, Hermans MP, Thissen JP, de Vos WM, Cani PD. Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. *Nat Med* 2019; **25**: 1096-1103 [PMID: 31263284 DOI: 10.1038/s41591-019-0495-2]

54 **Badehnoosh B**, Karamali M, Zarrati M, Jamilian M, Bahmani F, Tajabadi-Ebrahimi M, Jafari P, Rahmani E, Asemi Z. The effects of probiotic supplementation on biomarkers of inflammation, oxidative stress and pregnancy outcomes in gestational diabetes. *J Matern Fetal Neonatal Med* 2018; **31**: 1128-1136 [PMID: 28326881 DOI: 10.1080/14767058.2017.1310193]

55 **Callaway LK**, McIntyre HD, Barrett HL, Foxcroft K, Tremellen A, Lingwood BE, Tobin JM, Wilkinson S, Kothari A, Morrison M, O'Rourke P, Pelecanos A, Dekker Nitert M. Probiotics for the Prevention of Gestational Diabetes Mellitus in Overweight and Obese Women: Findings From the SPRING Double-Blind Randomized Controlled Trial. *Diabetes Care* 2019; **42**: 364-371 [PMID: 30659070 DOI: 10.2337/dc18-2248]

56 **Gurung M**, Li Z, You H, Rodrigues R, Jump DB, Morgun A, Shulzhenko N. Role of gut microbiota in type 2 diabetes pathophysiology. *EBioMedicine* 2020; **51**: 102590 [PMID: 31901868 DOI: 10.1016/j.ebiom.2019.11.051]

57 **O'Hara AM**, Shanahan F. The gut flora as a forgotten organ. *EMBO Rep* 2006; **7**: 688-693 [PMID: 16819463 DOI: 10.1038/sj.embor.7400731]

58 **Tang WH**, Kitai T, Hazen SL. Gut Microbiota in Cardiovascular Health and Disease. *Circ Res* 2017; **120**: 1183-1196 [PMID: 28360349 DOI: 10.1161/CIRCRESAHA.117.309715]

59 **Blaut M**, Klaus S. Intestinal microbiota and obesity. *Handb Exp Pharmacol* 2012; 251-273 [PMID: 22249818 DOI: 10.1007/978-3-642-24716-3_11]

60 **Boutagy NE**, McMillan RP, Frisard MI, Hulver MW. Metabolic endotoxemia with obesity: Is it real and is it relevant? *Biochimie* 2016; **124**: 11-20 [PMID: 26133659 DOI: 10.1016/j.biochi.2015.06.020]

61 **Rogero MM**, Calder PC. Obesity, Inflammation, Toll-Like Receptor 4 and Fatty Acids. *Nutrients* 2018; **10** [PMID: 29601492 DOI: 10.3390/nu10040432]

62 **Odenwald MA**, Turner JR. The intestinal epithelial barrier: a therapeutic target? *Nat Rev Gastroenterol Hepatol* 2017; **14**: 9-21 [PMID: 27848962 DOI: 10.1038/nrgastro.2016.169]

63 **Bron PA**, Kleerebezem M, Brummer RJ, Cani PD, Mercenier A, MacDonald TT, Garcia-Ródenas CL, Wells JM. Can probiotics modulate human disease by impacting intestinal barrier function? *Br J Nutr* 2017; **117**: 93-107 [PMID: 28102115 DOI: 10.1017/S0007114516004037]

64 **Woting A**, Blaut M. The Intestinal Microbiota in Metabolic Disease. *Nutrients* 2016; **8**: 202 [PMID: 27058556 DOI: 10.3390/nu8040202]

65 **Delzenne NM**, Cani PD. Gut microbiota and the pathogenesis of insulin resistance. *Curr Diab Rep* 2011; **11**: 154-159 [PMID: 21431853 DOI: 10.1007/s11892-011-0191-1]

66 **Peng L**, Li ZR, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *J Nutr* 2009; **139**: 1619-1625 [PMID: 19625695 DOI: 10.3945/jn.109.104638]

67 **Suzuki T**, Yoshida S, Hara H. Physiological concentrations of short-chain fatty acids immediately suppress colonic epithelial permeability. *Br J Nutr* 2008; **100**: 297-305 [PMID: 18346306 DOI: 10.1017/S0007114508888733]

68 **Zhao L**, Zhang F, Ding X, Wu G, Lam YY, Wang X, Fu H, Xue X, Lu C, Ma J, Yu L, Xu C, Ren Z, Xu Y, Xu S, Shen H, Zhu X, Shi Y, Shen Q, Dong W, Liu R, Ling Y, Zeng Y, Wang X, Zhang Q, Wang J, Wang L, Wu Y, Zeng B, Wei H, Zhang M, Peng Y, Zhang C. Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science* 2018; **359**: 1151-1156 [PMID: 29590046 DOI: 10.1126/science.aao5774]

69 **Goswami C**, Iwasaki Y, Yada T. Short-chain fatty acids suppress food intake by activating vagal afferent neurons. *J Nutr Biochem* 2018; **57**: 130-135 [PMID: 29702431 DOI: 10.1016/j.jnutbio.2018.03.009]

70 **Sanna S**, van Zuydam NR, Mahajan A, Kurilshikov A, Vich Vila A, Võsa U, Mujagic Z, Masclee AAM, Jonkers DMAE, Oosting M, Joosten LAB, Netea MG, Franke L, Zhernakova A, Fu J, Wijmenga C, McCarthy MI. Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. *Nat Genet* 2019; **51**: 600-605 [PMID: 30778224 DOI: 10.1038/s41588-019-0350-x]

71 **Vrieze A**, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JF, Dallinga-Thie GM, Ackermans MT, Serlie MJ, Oozeer R, Derrien M, Druesne A, Van Hylckama Vlieg JE, Bloks VW, Groen AK, Heilig HG, Zoetendal EG, Stroes ES, de Vos WM, Hoekstra JB, Nieuwdorp M. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012; **143**: 913-6.e7 [PMID: 22728514 DOI: 10.1053/j.gastro.2012.06.031]

72 **Jumpertz R**, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, Krakoff J. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. *Am J Clin Nutr* 2011; **94**: 58-65 [PMID: 21543530 DOI: 10.3945/ajcn.110.010132]

73 **Tamanai-Shacoori Z**, Smida I, Bousarghin L, Loreal O, Meuric V, Fong SB, Bonnaure-Mallet M, Jolivet-Gougeon A. Roseburia spp.: a marker of health? *Future Microbiol* 2017; **12**: 157-170 [PMID: 28139139 DOI: 10.2217/fmb-2016-0130]

74 **Fiorucci S**, Distrutti E. Bile Acid-Activated Receptors, Intestinal Microbiota, and the Treatment of

Metabolic Disorders. *Trends Mol Med* 2015; **21**: 702-714 [PMID: 26481828 DOI: 10.1016/j.molmed.2015.09.001]

75 **Islam KB**, Fukiya S, Hagio M, Fujii N, Ishizuka S, Ooka T, Ogura Y, Hayashi T, Yokota A. Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. *Gastroenterology* 2011; **141**: 1773-1781 [PMID: 21839040 DOI: 10.1053/j.gastro.2011.07.046]

76 **Pathak P**, Xie C, Nichols RG, Ferrell JM, Boehme S, Krausz KW, Patterson AD, Gonzalez FJ, Chiang JYL. Intestine farnesoid X receptor agonist and the gut microbiota activate G-protein bile acid receptor-1 signaling to improve metabolism. *Hepatology* 2018; **68**: 1574-1588 [PMID: 29486523 DOI: 10.1002/hep.29857]

77 **Prawitt J**, Abdelkarim M, Stroeve JH, Popescu I, Duez H, Velagapudi VR, Dumont J, Bouchaert E, van Dijk TH, Lucas A, Dorchies E, Daoudi M, Lestavel S, Gonzalez FJ, Oresic M, Cariou B, Kuipers F, Caron S, Staels B. Farnesoid X receptor deficiency improves glucose homeostasis in mouse models of obesity. *Diabetes* 2011; **60**: 1861-1871 [PMID: 21593203 DOI: 10.2337/db11-0030]

78 **Massafra V**, van Mil SWC. Farnesoid X receptor: A "homeostat" for hepatic nutrient metabolism. *Biochim Biophys Acta Mol Basis Dis* 2018; **1864**: 45-59 [PMID: 28986309 DOI: 10.1016/j.bbadis.2017.10.003]

79 **Thomas C**, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, Macchiarulo A, Yamamoto H, Mataki C, Pruzanski M, Pellicciari R, Auwerx J, Schoonjans K. TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell Metab* 2009; **10**: 167-177 [PMID: 19723493 DOI: 10.1016/j.cmet.2009.08.001]

80 **Wang TJ**, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, O'Donnell CJ, Carr SA, Mootha VK, Florez JC, Souza A, Melander O, Clish CB, Gerszten RE. Metabolite profiles and the risk of developing diabetes. *Nat Med* 2011; **17**: 448-453 [PMID: 21423183 DOI: 10.1038/nm.2307]

81 **Giesbertz P**, Daniel H. Branched-chain amino acids as biomarkers in diabetes. *Curr Opin Clin Nutr Metab Care* 2016; **19**: 48-54 [PMID: 26485337 DOI: 10.1097/MCO.0000000000000235]

82 **Niewczas MA**, Sirich TL, Mathew AV, Skupien J, Mohney RP, Warram JH, Smiles A, Huang X, Walker W, Byun J, Karoly ED, Kensicki EM, Berry GT, Bonventre JV, Pennathur S, Meyer TW, Krolewski AS. Uremic solutes and risk of end-stage renal disease in type 2 diabetes: metabolomic study. *Kidney Int* 2014; **85**: 1214-1224 [PMID: 24429397 DOI: 10.1038/ki.2013.497]

83 **Huang M**, Liang Q, Li P, Xia J, Wang Y, Hu P, Jiang Z, He Y, Pang L, Han L, Wang Y, Luo G. Biomarkers for early diagnosis of type 2 diabetic nephropathy: a study based on an integrated biomarker system. *Mol Biosyst* 2013; **9**: 2134-2141 [PMID: 23719966 DOI: 10.1039/c3mb25543c]

84 **Newgard CB**, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, Haqq AM, Shah SH, Arlotto M, Slentz CA, Rochon J, Gallup D, Ilkayeva O, Wenner BR, Yancy WS Jr, Eisenson H, Musante G, Surwit RS, Millington DS, Butler MD, Svetkey LP. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab* 2009; **9**: 311-326 [PMID: 19356713 DOI: 10.1016/j.cmet.2009.02.002]

85 **Asghari G**, Farhadnejad H, Teymoori F, Mirmiran P, Tohidi M, Azizi F. High dietary intake of branched-chain amino acids is associated with an increased risk of insulin resistance in adults. *J Diabetes* 2018; **10**: 357-364 [PMID: 29281182 DOI: 10.1111/1753-0407.12639]

86 **Pedersen HK**, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BA, Forslund K, Hildebrand F, Prifti E, Falony G, Le Chatelier E, Levenez F, Doré J, Mattila I, Plichta DR, Pöhö P, Hellgren LI, Arumugam M, Sunagawa S, Vieira-Silva S, Jørgensen T, Holm JB, Trošt K; MetaHIT Consortium, Kristiansen K, Brix S, Raes J, Wang J, Hansen T, Bork P, Brunak S, Oresic M, Ehrlich SD, Pedersen O. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature* 2016; **535**: 376-381 [PMID: 27409811 DOI: 10.1038/nature18646]

87 **Kawaguchi T**, Izumi N, Charlton MR, Sata M. Branched-chain amino acids as pharmacological nutrients in chronic liver disease. *Hepatology* 2011; **54**: 1063-1070 [PMID: 21563202 DOI: 10.1002/hep.24412]

88 **Hänninen A**, Toivonen R, Pöysti S, Belzer C, Plovier H, Ouwerkerk JP, Emani R, Cani PD, De Vos WM. *Akkermansia muciniphila* induces gut microbiota remodelling and controls islet autoimmunity in NOD mice. *Gut* 2018; **67**: 1445-1453 [PMID: 29269438 DOI: 10.1136/gutjnl-2017-314508]

89 **Wen L**, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, Hu C, Wong FS, Szot GL, Bluestone JA, Gordon JI, Chervonsky AV. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature* 2008; **455**: 1109-1113 [PMID: 18806780 DOI: 10.1038/nature07336]

90 **Peng J**, Narasimhan S, Marchesi JR, Benson A, Wong FS, Wen L. Long term effect of gut microbiota transfer on diabetes development. *J Autoimmun* 2014; **53**: 85-94 [PMID: 24767831 DOI: 10.1016/j.jaut.2014.03.005]

91 **Mariño E**, Richards JL, McLeod KH, Stanley D, Yap YA, Knight J, McKenzie C, Kranich J, Oliveira AC, Rossello FJ, Krishnamurthy B, Nefzger CM, Macia L, Thorburn A, Baxter AG, Morahan G, Wong LH, Polo JM, Moore RJ, Lockett TJ, Clarke JM, Topping DL, Harrison LC, Mackay CR. Gut microbial metabolites limit the frequency of autoimmune T cells and protect against type 1 diabetes. *Nat Immunol* 2017; **18**: 552-562 [PMID: 28346408 DOI: 10.1038/ni.3713]

92 **Vatanen T**, Franzosa EA, Schwager R, Tripathi S, Arthur TD, Vehik K, Lernmark Å, Hagopian WA, Rewers MJ, She JX, Toppari J, Ziegler AG, Akolkar B, Krischer JP, Stewart CJ, Ajami NJ, Petrosino JF, Gevers D, Lähdesmäki H, Vlamakis H, Huttenhower C, Xavier RJ. The human gut microbiome in early-onset type 1 diabetes from the TEDDY study. *Nature* 2018; **562**: 589-594 [PMID: 30356183 DOI: 10.1038/s41586-018-0620-2]

93 **Neuman V**, Cinek O, Funda DP, Hudcovic T, Golias J, Kramna L, Petruzelkova L, Pruhova S, Sumnik Z. Human gut microbiota transferred to germ-free NOD mice modulate the progression towards type 1 diabetes regardless of the pace of beta cell function loss in the donor. *Diabetologia* 2019; **62**: 1291-1296 [PMID: 31025045 DOI: 10.1007/s00125-019-4869-2]

94 **Vatanen T**, Kostic AD, d'Hennezel E, Siljander H, Franzosa EA, Yassour M, Kolde R, Vlamakis H, Arthur TD, Hämäläinen AM, Peet A, Tillmann V, Uibo R, Mokurov S, Dorshakova N, Ilonen J, Virtanen SM, Szabo SJ, Porter JA, Lähdesmäki H, Huttenhower C, Gevers D, Cullen TW, Knip M; DIABIMMUNE Study Group, Xavier RJ. Variation in Microbiome LPS Immunogenicity Contributes to Autoimmunity in Humans.

Cell 2016; **165**: 842-853 [PMID: 27133167 DOI: 10.1016/j.cell.2016.04.007]
95 **Kihl P**, Krych L, Deng L, Kildemoes AO, Laigaard A, Hansen LH, Hansen CHF, Buschard K, Nielsen DS, Hansen AK. Oral LPS Dosing Induces Local Immunological Changes in the Pancreatic Lymph Nodes in Mice. *J Diabetes Res* 2019; **2019**: 1649279 [PMID: 30956991 DOI: 10.1155/2019/1649279]
96 **Kostic AD**, Gevers D, Siljander H, Vatanen T, Hyötyläinen T, Hämäläinen AM, Peet A, Tillmann V, Pöhö P, Mattila I, Lähdesmäki H, Franzosa EA, Vaarala O, de Goffau M, Harmsen H, Ilonen J, Virtanen SM, Clish CB, Orešič M, Huttenhower C, Knip M; DIABIMMUNE Study Group, Xavier RJ. The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell Host Microbe* 2015; **17**: 260-273 [PMID: 25662751 DOI: 10.1016/j.chom.2015.01.001]
97 **Knip M**, Siljander H. The role of the intestinal microbiota in type 1 diabetes mellitus. *Nat Rev Endocrinol* 2016; **12**: 154-167 [PMID: 26729037 DOI: 10.1038/nrendo.2015.218]
98 **Rewers M**, Ludvigsson J. Environmental risk factors for type 1 diabetes. *Lancet* 2016; **387**: 2340-2348 [PMID: 27302273 DOI: 10.1016/S0140-6736(16)30507-4]
99 **Schmidt TS**, Hayward MR, Coelho LP, Li SS, Costea PI, Voigt AY, Wirbel J, Maistrenko OM, Alves RJ, Bergsten E, de Beaufort C, Sobhani I, Heintz-Buschart A, Sunagawa S, Zeller G, Wilmes P, Bork P. Extensive transmission of microbes along the gastrointestinal tract. *Elife* 2019; **8** [PMID: 30747106 DOI: 10.7554/eLife.42693]
100 **He J**, Li Y, Cao Y, Xue J, Zhou X. The oral microbiome diversity and its relation to human diseases. *Folia Microbiol (Praha)* 2015; **60**: 69-80 [PMID: 25147055 DOI: 10.1007/s12223-014-0342-2]
101 **Chen B**, Wang Z, Wang J, Su X, Yang J, Zhang Q, Zhang L. The oral microbiome profile and biomarker in Chinese type 2 diabetes mellitus patients. *Endocrine* 2020 [PMID: 32246318 DOI: 10.1007/s12020-020-02269-6]
102 **Long J**, Cai Q, Steinwandel M, Hargreaves MK, Bordenstein SR, Blot WJ, Zheng W, Shu XO. Association of oral microbiome with type 2 diabetes risk. *J Periodontal Res* 2017; **52**: 636-643 [PMID: 28177125 DOI: 10.1111/jre.12432]
103 **Matsha TE**, Prince Y, Davids S, Chikte U, Erasmus RT, Kengne AP, Davison GM. Oral Microbiome Signatures in Diabetes Mellitus and Periodontal Disease. *J Dent Res* 2020; **99**: 658-665 [PMID: 32298191 DOI: 10.1177/0022034520913818]
104 **Xiao E**, Mattos M, Vieira GHA, Chen S, Corrêa JD, Wu Y, Albiero ML, Bittinger K, Graves DT. Diabetes Enhances IL-17 Expression and Alters the Oral Microbiome to Increase Its Pathogenicity. *Cell Host Microbe* 2017; **22**: 120-128.e124 [PMID: 28704648 DOI: 10.1016/j.chom.2017.06.014]
105 **Hurst NR**, Kendig DM, Murthy KS, Grider JR. The short chain fatty acids, butyrate and propionate, have differential effects on the motility of the guinea pig colon. *Neurogastroenterol Motil* 2014; **26**: 1586-1596 [PMID: 25223619 DOI: 10.1111/nmo.12425]

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ORIGINAL ARTICLE

Observational Study

Relationship between diabetic polyneuropathy, serum visfatin, and oxidative stress biomarkers

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Abstract

BACKGROUND

Diabetic polyneuropathy is a very common complication of diabetes. Numerous studies are available in terms of pathogenesis. But examination methods with low reliability are still not standardized and generally time consuming. High-sensitive, easy-to-access methods are expected. Biochemical markers are one of the subjects of research. We aimed to discover a potential biomarker that can be used for this purpose in patients with diabetes who have not yet developed symptoms of neuropathy.

AIM

To determine the place and availability of visfatin and thiol-disulfide homeostasis in this disorder.

METHODS

A total of 392 patients with type 2 diabetes mellitus were included in the study. The polyneuropathy clinical signs were evaluated with the Subjective Peripheral Neuropathy Screen Questionnaire and Michigan Neuropathy Screening Instrument questionnaire and examination. The biochemical parameters, oxidative stress markers, visfatin, and thiol-disulfide homeostasis were analyzed and correlated with each other and clinical signs.

RESULTS

additional data are available.

STROBE statement: The authors have read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

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Subjective Peripheral Neuropathy Screen Questionnaire and Michigan Neuropathy Screening Instrument questionnaire with examination scores were correlated with each other and diabetes duration ($P < 0.005$). Neuropathy related symptoms were present in 20.7% of the patients, but neuropathy related findings were observed in 43.9% of the patients. Serum glucose, glycated hemoglobin, and visfatin were positively correlated with each other. Also, these parameters were positively correlated with the total oxidative stress index. Total and native thiol was positively correlated with total antioxidant status and negatively with oxidant status. Inversely thiol-disulfide positively correlated with higher glucose and oxidant status and negatively with total antioxidant status ($P < 0.005$). There was no correlation between visfatin and thiol-disulphide ($P = 0.092$, $r = 0.086$). However, a significant negative correlation was observed between visfatin and total with native thiol ($P < 0.005$, $r = -0.338$), ($P < 0.005$, $r = -0.448$).

CONCLUSION

Diagnosis of neuropathy is one of the issues studied in patients with diabetes. Visfatin and thiol-disulfide balance were analyzed for the first time in this study with inspiring results.

Key words: Diabetic neuropathy; Diabetic foot; Early detection; Oxidative stress; Thiol-disulfide; Visfatin protein

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Core tip: Early diagnosis and management of micro and macrovascular complications are vital in patients with diabetes. Many algorithms and early diagnostic tools have been developed for this purpose. Yet it is still difficult to identify neuropathy because of the prolonged preclinical phase. This patient group has uncontrolled blood sugar and hypertension, accelerated renal replacement need, and life-threatening cardiac or cerebral macrovascular complications. With this study, we wanted to emphasize that screening of neuropathy should not be ignored in the follow-up of these cases. As an early diagnostic tool, many parameters that are responsible for pathogenesis should be investigated.

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INTRODUCTION

Type 2 diabetes mellitus (DM) is a global epidemic and a highly complex disease. The incidence is estimated to be 366 million between the years 2000 and 2030[1]. Diabetic polyneuropathy (DN) is the most common complication along with a 50% lifetime prevalence[2]. DM leads to various peripheral neuronal damages, but the most common type is the bilaterally symmetric, distal to the proximal severity of nerve damage known as stocking-glove neuropathy. Although 50% of the patients are asymptomatic, the progressive nerve damage results in instability, falls, and numb, insensate feet. DN negatively affects the quality of life and increases health expenses. The yearly medical expense for diabetes is $6632 per patient. However, the presence of DN doubles this amount, and the presence of severe neuropathy quadruples the amount[3].

For the early diagnosis, practical and reliable methods are essential[4]. Monofilament tests for superficial and vibratory stimulus for deep sensation may provide early data for neuropathy. Nerve conduction studies and skin biopsy are more detailed and useful but not practical methods[5,6]. The Toronto Consensus Panel defined diagnostic guidelines based on these methods[7]. Subjective Peripheral Neuropathy Screen (SPNS) and Michigan Neuropathy Screening Instrument (MNSI) are other reliable procedures that are simple and easily self-completed methods applied by the patient or the physician[8,9].

In the pathogenesis of neuropathy, the metabolic redox state, extreme production of

mitochondrial, and cytosolic reactive oxygen species of dorsal root ganglia and Schwann cells are the major determinants[10]. Reactive oxygen species are one of the major determinants in the pathophysiology of many diseases[11]. Increased reactive oxygen species are one of the factors leading to programmed cell death of neurons. Oxidative stress is an oxidant/antioxidant imbalance, and oxidative stress index (OSI) is the ratio of total oxidant status (TOS) to total antioxidant status (TAS)[12]. Thiols, functional SH groups, have been identified as one of the main antioxidants. The plasma thiols are albumin, cysteine, cysteinylglycine, glutathione, homocysteine, and γ-glutamylcysteine. Under oxidative status, thiols transform into their reversible oxidized disulfide forms. These disulfide bonds are not covalent. Thiols may return the reduced form, and thiol-disulfide balance is maintained. Thiol-disulfide provides homeostatic redox status, and it has been associated with many clinical disorders including DM[13,14]. Visfatin, a 52 kDa protein, is produced by visceral adipose tissue. It induces cytokine production and is accepted as a proinflammatory adipokine. In the clinical studies, the relationship between visfatin and intracerebral hemorrhage, acute ischemic stroke, acute pancreatitis, and myocardial infarction have been identified[15,16]. Although it was generally associated with long-term unfavorable outcomes, it has been shown to have a regulatory effect in myocardium, neurons, and even mitochondria[17,18]. Further studies are necessary to define the precise effect of this adipocytokine in critically ill patients.

In this study, patients with diabetes were evaluated using the Subjective Peripheral Neuropathy Screen and MNSI questionnaires and MNSI examination. For all patients, oxidative status, visfatin, and thiol-disulfide balance were determined. The measured parameters were compared to each other and differences of parameters between patients with and without DN clinical signs were compared.

MATERIALS AND METHODS

Study population

This study was performed at the Bezmialem Vakif University Internal Medicine and Endocrinology Department policlinics. In these clinics, our patients with diabetes come for routine control every 3 mo with an appointment. Between October 2018 and April 2019, we randomly included this group of patients in the study with informed consent. A total of 392 patients with neuropathy examinations were included in the study. Exclusion criteria were as follows: (1) Patients with acute infection or other lymphoproliferative and chronic infection like human immunodeficiency virus; (2) Patients with monoclonal gammopathy, vasculitis, alcoholism, chronic renal failure, sarcoidosis, Sjogren disease, amyloidosis, neoplasms, and paraneoplastic syndromes; and (3) Patients with a certain diagnosis for hereditary, demyelinating or multifocal neuropathies, radiculopathy, mononeuritis, cerebrovascular diseases, and chronic renal or hepatic failure. For the other possible macro and microvascular complications, we did not apply any exclusion. The age of the patients and diabetes duration was registered. Although the diabetic medications and other related disorders were registered, we were not able to group and compare the possible effect of related disorders and using agent drugs including vitamin B_{12}.

The study was performed based on the Helsinki Declaration, and ethical consent was obtained from Bezmialem Clinical Research Ethics Committee (Number 2016/14823). Informed voluntary consent form was received from all patients.

Diagnosis for DN

The patients were examined on a stretcher in policlinic conditions. The researchers were educated and used the same instructions. Complaints of the patients were evaluated using the Subjective Peripheral Neuropathy Screen Questionnaire (SPNSQ) and MNSI[8,9]. SPNSQ contains 15 questions about the symptoms of neuropathy. The total score is obtained by counting the yes answers. The sum of the scores range from 0 to 15 and determine the cases from no neuropathic symptoms to the severe neuropathic symptoms. MNSI questionnaire contains 15 "yes/no" questions regarding neuropathy. For the questions 1-3, 5-6, 8-9, 11-12, 14-15, "yes" answers and for the questions 7 and 13 "no" answers were scored as one point. Questions 4 and 10 were not included in the published scoring algorithm. The sum of the questionnaire score of 7 ≥ was accepted as abnormal[9]. MNSI examination was performed by the physicians participating in the study. Each foot was examined for dryness, fissures, ulcers, and infections. The detected abnormality was scored with 1 point.

The feet were also inspected for ulcers and each foot with an ulcer received 1 point.

The Achilles reflex was evaluated. If it was absent, Jendrassic maneuver was performed. If it is present, the reflex was scored as 0.5 points for each foot. If the reflex was absent with the maneuver, it was scored as 1 point. Vibration sensation was evaluated using a tuning fork placed on the dorsal face of the big toe. With covered vision, the vibration was scored according to the duration of the sensation. Below 10 s, the sensation was accepted as reduced and scored as 0.5 points. The patient with no perception was scored as 1 point for each foot. For monofilament evaluation, the feet were rested on a flat, warm surface. The filament was applied perpendicularly and briefly (< 1 s) with 10 gr pressure to ten designated spots for each foot. Eight correct answers were considered normal. One to seven correct answers were considered decreased. No correct answers were considered a loss of sensation. The total MNSI score was over 10 points, and the score ≥ 2.5 was accepted as abnormal[19].

Sample collection

Fasting venous blood samples were provided from the antecubital vein. The samples were centrifuged at 3000 × *g* for 10 min to dissociate the serum. The serum samples were aliquoted and kept at -80 °C until further analysis.

Biochemical parameters

The biochemical parameters serum glucose, glycated hemoglobin (HbA1c), serum triglycerides, low density lipoprotein cholesterol, serum creatinine, alanine aminotransferase, aspartate aminotransferase, and vitamin B_{12} were analyzed using commercial assay kits.

Measurement of visfatin

Serum visfatin levels were analyzed using an enzyme-linked immunosorbent assay kit (Elabscience, Houston, TX, United States). Results were obtained by spectrophotometric method according to the manufacturer's directions and specified in pg/mL.

Analyses of oxidative stress

Serum TAS was evaluated with the method based by Erel *et al*[20]. The method encompasses the formation of hydroxyl radicals, which is a potent reactive substance. A ferrous ion solution (reagent 1) is stirred with hydrogen peroxide (reagent 2). The antioxidative capacity of a sample can be measured in terms of the inhibition of free radical reactions initiated by the generation of the hydroxyl radical. The change in assay data was very low (< 3%), and results were in mmol Trolox Eq./L.

For TOS analysis, the oxidants formed in the serum oxidize the ferrous ion of an o-dianisidine compound to the ferric ion. For the calibration of the analysis, hydrogen peroxide was used, and the results were presented with micromole hydrogen peroxide equivalents per liter (μmol H_2O_2 Eq./L).

OSI was calculated as OSI (arbitrary unit) = (TOS, μmol H_2O_2 Eq./L) / (TAS, mmol Trolox Eq./L)[21].

Determination of thiol-disulfide homeostasis

For the evaluation, dynamic disulfide bonds (–S–S–) in the serum sample were reduced to native thiol groups (–SH) by NaBH4. The total thiol ingredient was measured using a derivative of Ellman reagent. Native thiol was subtracted from the total thiol, and half of the obtained difference gave the disulfide bond amount. Biomarkers were measured using a spectrophotometer (Varioskan Flash Multimode Reader; Thermo, Waltham, MA, United States)[13]. After the measurement of native thiol and disulfide concentrations in the samples, the disulfide/native thiol ratio (-S-S-/-SH) was calculated as dynamic thiol-disulfide homeostasis (TDH).

Statistical methods

All of the statistical analyses were performed using the IBM SPSS 22.0. Mean standard deviation and percentages are presented as descriptive statistics. When comparing the groups for categorical data MNSI neuropathy score categories, the chi-square test was used. For the cuts of averages, the independent sample *t*-test was used. The Pearson correlation coefficient was calculated for the relationship between variables. For all data, $P < 0.05$ was accepted as significant.

RESULTS

A total of 392 patients were evaluated (271 female, 121 male). The mean age of the patients was 57.5 ± 9.0 years. The mean diabetes period was 12.00 ± 7.29 years. For all patients the mean SPNSQ score was 5.6 ± 3.6, the mean MNSI questionnaire score was 4.5 ± 2.3, and the mean MNSI exam score was 2.4 ± 2.0 points. SPNSQ, MNSI questionnaire, and MNSI exam scores were correlated with each other ($P < 0.005$). Between the disease duration and SPNSQ, MNSI questionnaire, and MNSI examination, significant positive correlation was observed ($P < 0.005$, $r = 0.275$, $P < 0.005$, $r = 0.242$, $P = 0.027$, $r = 0.119$). However, there was no correlation between the disease duration and all other measured parameters.

MNSI questionnaire score was less than seven points in 311 patients (79.3%). In 81 patients it was more than seven points (20.7%). MNSI examination score of less than 2.5 points was observed in 220 patients (56.1%), and it was more than 2.5 points in 172 patients (43.9%). In females, the disease duration was much longer ($P = 0.003$), and both questionnaire scores were much higher than males ($P = 0.001$ and $P = 0.044$). But in terms of MNSI examination, there was no difference between males and females ($P = 0.059$).

There was no correlation between questionnaire results and the biochemical parameters, visfatin, oxidative stress biomarkers, and TDH. There was a positive linear relationship between MNSI examination scores and HbA1c, visfatin, TOS, and OSI. The same correlation was observed in the divided MNSI score analysis. These correlations with detailed information are presented in Tables 1 and 2.

Serum HbA1c was correlated with TOS and OSI in the same direction and oppositely with TAS ($P < 0.005$, $r = 0.503$, $r = 0.702$, $r = -0.593$). Visfatin was positively correlated with higher glucose, HbA1c, TOS, and OSI ($P < 0.005$, $r = 0.537$, $r = 0.753$, $r = 0.407$, $r = 0.587$), and it was negatively correlated with TAS ($r = -0.499$).

Total and native thiol were negatively correlated with glucose, HbA1c, TOS, and OSI, but it was positively correlated with TAS. There were oppositely directed correlations between thiol-disulfide and the same parameters. Detailed results and correlations of all these parameters are presented in Table 3.

There was no significant correlation between visfatin and thiol-disulfide ($P = 0.092$, $r = 0.086$). However, a statistically significant negative correlation was detected between visfatin and total with native thiol ($P < 0.005$, $r = - 0.338$), ($P < 0.005$, $r = -0.448$). All correlations between visfatin and oxidative stress biomarkers and TDH were presented in Figures 1-6.

DISCUSSION

Type 2 DM is a major health problem worldwide. Along with increased cardiovascular risk, severe chronic complications, retinopathy, nephropathy, and neuropathy are associated with morbidity and mortality. DN is a common complication and affects more than one-third of patients. Although there are routine protocols for early diagnosis of retinopathy and nephropathy, there is no practical method with proven reliability for DN.

Microalbuminuria is controlled quarterly, and retinopathy is examined yearly if there are no additional problems. However, there is no standardized follow-up protocol and reliable methods for DN. The sensitivity of the surveys used is weak. Also in our patients, SPNSQ average score was 5.6 ± 3.6 over fifteen questions. SPNSQ is simple and easy to apply method. This survey was used to screen neuropathy in patients with human immunodeficiency virus and presented with 70% positive predictivity and 67% diagnostic efficacy[9]. This survey was compared with the Neuropathy Symptoms Score and DN 4 in patients with diabetes and reported as a proposed method for neuropathy screening. But sensitivity and reliability are insufficient because of subjective property as in our patient group[22].

The sensitivity of electrophysiological studies was observed in the preclinical period of DN[23]. Among the surveys, MNSI has been reported to have a linear relationship with the electrophysiological studies[24]. In our study, along with the MNSI questionnaire, 20.7% of the patients had neuropathy related symptoms. But examination findings were detected in 43.9% of the patients. Screening only symptoms does not seem like an effective and sensitive method. Neuropathy examination appears to be part of the clinical evaluation in these patients. We detected higher positive examination findings than similar studies[25,26]. Mean diabetes duration of 12.00 ± 7.29 years was significantly correlated with questionnaires and examination scores,

Table 1 The median of the parameters and the correlation between neuropathy clinical scores and biochemical parameters, visfatin, and oxidative stress biomarkers

	SPNSQ score, 5.66 ± 3.64	MNSI q, 4.5 ± 2.28	MNSI exam, 2.42 ± 1.99
Glucose, mg/dL, 154.0 ± 57.5	P = 0.099, r = 0.084	P = 0.110, r = 0.081	P = 0.110, r = 0.081
HbA1c, 7.68 ± 1.52	P = 0.099, r = 0.084	P = 0.062, r = 0.094	[b]P < 0.01, r = 0.170
Creatinine, mg/dL, 0.81 ± 0.13	P = 0.017, r = -0.121	P = 0.053, r = -0.098	P = 0.143, r = 0.074
ALT, U/L, 22.3 ± 12.8	P = 0.177, r = 0.000	P = 0.518, r = 0.033	P = 0.585, r = 0.028
AST, U/L, 18.6 ± 7.8	P = 0.587, r = 0.028	P = 0.676, r = 0.021	P = 0.810, r = -0.012
LDL cholesterol, mg/dL, 118.12 ± 28.00	P = 0.077, r = 0.090	P = 0.151, r = 0.073	P = 0.868, r = 0.008
Triglycerides, mg/dL, 160.3 ± 85.0	P =0.123, r = 0.078	P = 0.355, r = 0.047	P = 0.994, r = 0.000
Vitamin B12, pg/mL, 425.5 ± 32.0	[a]P < 0.05, r = 0.132	P = 0.062, r = 0.095	P = 0.049, r = 0.100
Visfatin, pg/mL, 19.34 ± 8.07	P = 0.579, r = 0.028	P = 0.330, r = 0.049	[a]P < 0.05, r = 0.122
TOS, μmol H_2O_2/L, 12.91 ± 1.92	P = 0.417, r = 0.041	P = 0.340, r = 0.048	[a]P < 0.05, r = 0.155
TAS, mmol Trolox Eq./L, 0.87 ± 0.14	P = 0.385, r = 0.044	P = 0.160, r = -0.071	[a]P < 0.05, r = -0.127
OSI (Arbitrary units), r = 0.20, 15.29 ± 4.12	P = 0.283, r = 0.054	P = 0.170, r = 0,069	[b]P < 0.01, P = 0.000
Total thiol, mmol/L, 0.51 ± 0.05	P = 0.070, r = -0.092	P = 0.053, r = -0.098	P = 0.251, r = -0.05
Native thiol, mmol/L, 0.35 ± 0.05	P = 0.822, r = 0.011	P = 0.903, r = -0.006	P = 0.864, r = -0.009
Thiol-disulfide, mmol/L, 0.08 ± 0.03	P = 0.059, r = -0.096	P = 0.069, r = -0.092	P = 0.270, r = -0.056

[a]P < 0.05,

[b]P < 0.01. SPNSQ: Subjective Peripheral Neuropathy Screen Questionnaire; MNSI q: Michigan Neuropathy Screening Instrument questionnaire; MNSI exam: Michigan Neuropathy Screening Instrument examination; HbA1c: Glycosylated hemoglobin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TOS: Total oxidant status; TAS: Total antioxidant status; OSI: Oxidative stress index; LDL: Low density lipoprotein.

Table 2 The correlation between the Michigan Neuropathy Screening Instrument neuropathy scores and visfatin with oxidative stress biomarkers

	MNSI q < 7	MNSI q ≥ 7	*P* value	MNSI exam < 2.5	MNSI exam ≥ 2.5	*P* value
Visfatin	19.15 ± 7.93	20.08 ± 8.62	0.357	18.32 ± 6.97	20.64 ± 9.16	< 0.01
TOS	12.88 ± 1.90	13.04 ± 2.01	0.486	12.64 ± 1.63	13.26 ± 2.20	< 0.05
TAS	0.88 ± 0.14	0.85 ± 0.13	0.102	0.88 ± 0.12	0.85 ± 0.15	< 0.05
OSI	15.14 ± 4.09	15.84 ± 4.23	0.178	14.62 ± 3.45	16.64 ± 4.72	< 0.01
Total thiol	0.51 ± 0.00	0.51 ± 0.05	0.385	0.52 ± 0.05	0.51 ± 0.05	0.228
Native thiol	0.35 ± 0.05	0.34 ± 0.06	0.570	0.35 ± 0.05	0.35 ± 0.06	0.638
Thiol-disulfide	0.08 ± 0.03	0.08 ± 0.03	0.850	0.08 ± 0.03	0.08 ± 0.03	0.399

MNSI q: Michigan Neuropathy Screening Instrument questionnaire; MNSI exam: Michigan Neuropathy Screening Instrument examination; TOS: Total oxidant status; TAS: Total antioxidant status; OSI: Oxidative stress index.

and we thought that long term disease duration may be partly responsible for this result.

In patients with diabetes, microvascular complications are associated with glycemic regulation. For DN, this relationship is more evident in patients with type 1 diabetes[27]. A series of systemic and cellular imbalances were responsible in this process: Increased oxidative/nitrosative stress, activation of poly-ADP ribosylation, activation of the polyol and protein kinase C pathways, endothelial dysfunction, altered Na^+/K^+-ATPase pump function, cyclooxygenase-2 activation, endoplasmic reticulum stress, and impaired C-peptide-related pathways. These factors lead to cytokine and chemokine generation that induce cellular oxidative/nitrosative stress and finally neuronal damage[28,29]. In a large cohort with over one thousand

Table 3 The median of the parameters and the correlation between glucose, serum oxidative stress biomarkers, and visfatin with thiol groups

	Visfatin, 19.34 ± 8.07	Total thiol, 0.51 ± 0.05	Native thiol, 0.35 ± 0.05	Thiol-disulfide, 0.08 ± 0.03
Glucose, 154.0 ± 57.5	$P < 0.05$, $r = 0.537$	$P < 0.01$, $r = -0.204$[1]	$P < 0.05$[1], $r = -0.452$[1]	$P < 0.01$, $r = 0.206$
HbA1c, 7.68 ± 1.52	$P < 0.05$, $r = 0.753$	$P < 0.01$, $r = -0.351$[1]	$P < 0.05$[1], $r = -0.597$[1]	$P < 0.01$, $r = 0.194$
TOS, 12.91 ± 1.92	$P < 0.05$, $r = 0.407$	$P < 0.05$, $r = -0.236$[1]	$P < 0.05$[1], $r = -0.352$[1]	$P < 0.01$, $r = 0.094$
TAS, 0.87 ± 0.14	$P < 0.05$, $r = -0.499$[1]	$P < 0.01$, $r = 0.243$	$P < 0.05$, $r = 0.408$	$P < 0.01$, $r = -0.134$[1]
OSI, 15.29 ± 4.12	$P < 0.05$, $r = 0.587$	$P < 0.05$, $r = -0.322$[1]	$P < 0.05$[1], $r = -0.485$[1]	$P < 0.01$, $r = 0.132$
Visfatin		$P < 0.01$, $r = -0.338$[1]	$P < 0.01$[1], $r = -0.448$[1]	$P = 0.092$

[1]Correlations with negative directions are presented. HbA1c: Glycosylated hemoglobin; TOS: Total oxidant status; TAS: Total antioxidant status; OSI: Oxidative stress index.

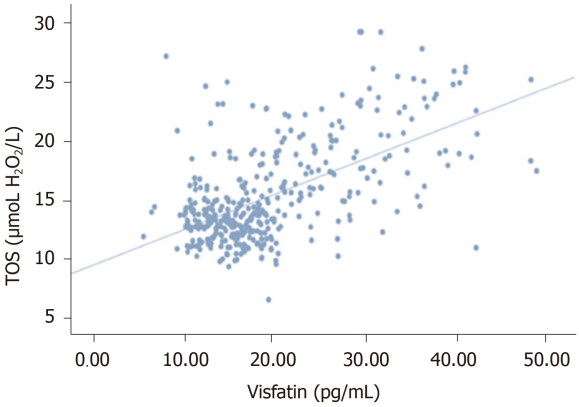

Figure 1 Positive correlation between visfatin and total oxidant status ($P < 0.05$, $r = 0.407$). TOS: Total oxidant status.

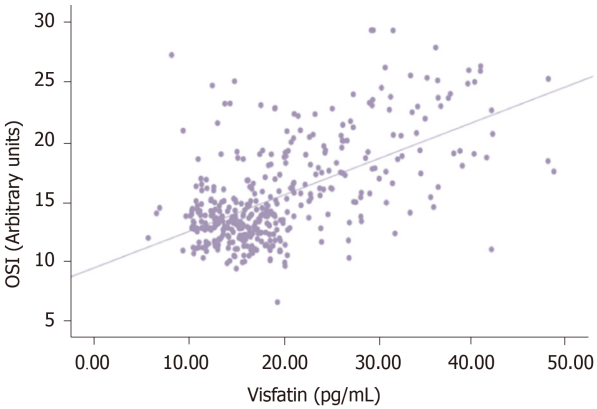

Figure 2 Positive correlation between visfatin and oxidative stress index ($P < 0.05$, $r = 0.587$). OSI: Oxidative stress index.

participants, the relationship between IL-1β, IL-6, and measures of DN was demonstrated[30]. In another study with a small number of patients, it was shown that antioxidant activity evaluated by superoxide dismutase, catalase, and glutathione peroxidase decreased in patients with DN[31]. In our patients, the examination findings associated with neuropathy were more pronounced in patients with poor glycemic control. Also, a statistically significant higher level of TOS, OSI, visfatin and lower TAS levels were observed in this patient group. These results show that oxidative balance is one of the main determinants for the development of DN, and visfatin is likely to be effective in the oxidative direction.

With this result, the place of adipose tissue dysfunction in the pathogenesis of

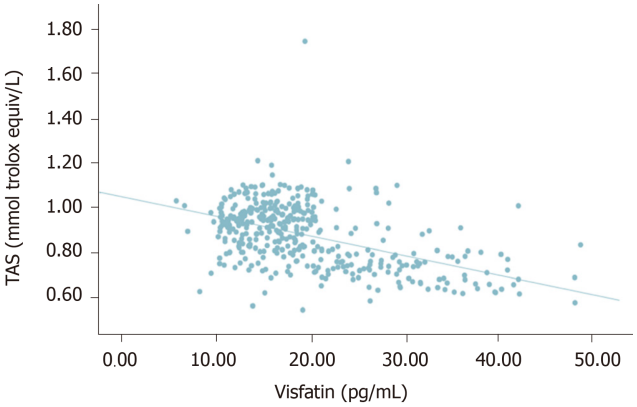

Figure 3 Negative correlation between visfatin and total antioxidant status ($P < 0.05$, $r = -0.499$). TAS: Total antioxidant status.

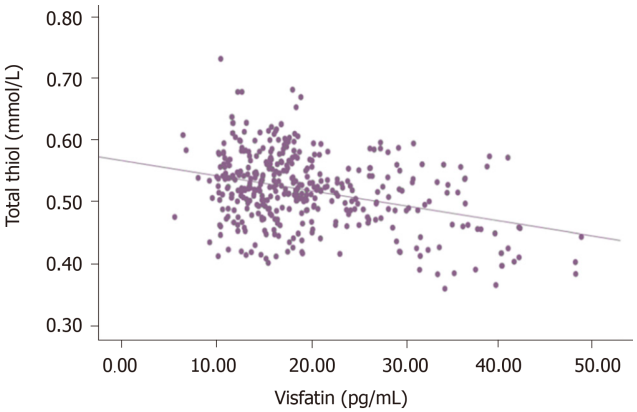

Figure 4 Negative correlation between visfatin and total thiol ($P < 0.05$, $r = -0.338$).

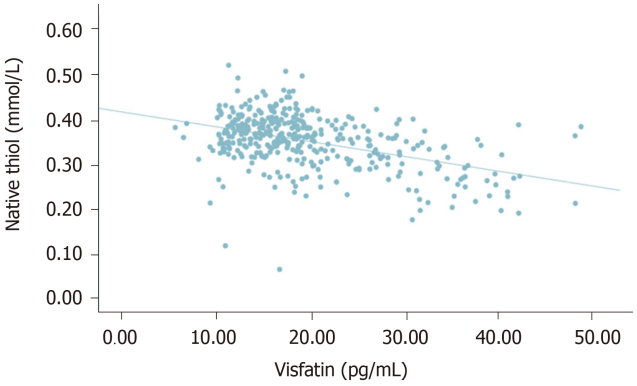

Figure 5 Negative correlation between visfatin and native thiol ($P < 0.05$, $r = -0.448$).

neuropathy once again became evident. Adipokines have endocrine, autocrine, and paracrine effects and are responsible for appetite regulation, insulin resistance, lipid metabolism, immunity, inflammation, vascular homeostasis, angiogenesis, and endothelial function. Visfatin is one of these adipokines, and it induces immune cell activation by β cell maturation and leukocyte, TNF/IL-6/IL-1b, and NFkB activation. At the same time, it leads to immune cell support on endothelial cells and vascular smooth muscle[32]. Although visfatin has been studied in many chronic disorders and has been associated with long-term unfavorable clinical outcomes and disease severity, its pathogenic or protective role has not been well established, especially in cardiovascular and cerebrovascular events[15,16].

Visfatin has been shown as a potential marker of inflammation and endothelial

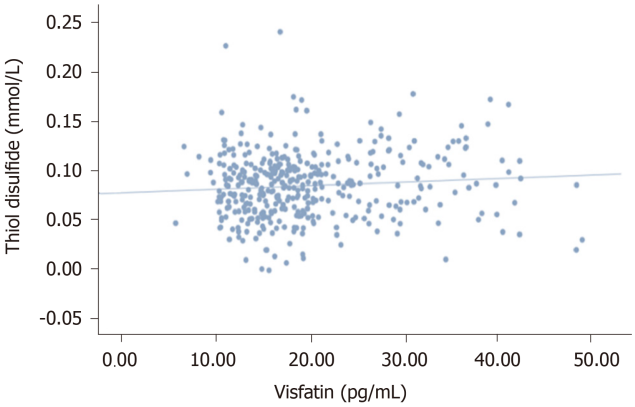

Figure 6 There is no statistical relationship between visfatin and thiol-disulfide (P = 0.092, r = 0.086).

dysfunction in patients with myocardial infarction in a clinical trial[33]. In another case-controlled study in overweight patients with diabetes, visfatin, leptin, resistin, monocyte chemoattractive protein-1, and retinol-binding protein 4 were evaluated. Visfatin was associated with higher HbA1c and HOMA-β levels[34]. Visfatin has been evaluated in the pathogenesis of diabetic nephropathy. Patients with renal failure in different stages were compared with each other, and a major difference was not observed between the patient groups[35]. However, in another study, high visfatin was associated with a decrease in kidney function in patients with diabetic nephropathy[36]. Visfatin is possibly upregulated in a dose dependent manner for the stability of pro- and anti-inflammatory cytokines. The possible place of visfatin on the pathogenesis of DN was first investigated in our study. Our patients with neuropathy findings had higher levels of visfatin. Also, it had a positive linear relationship with the oxidative status of the patients (TOS and OSI). There are studies in the literature confirming our results. Increased visfatin along with resistin was associated with increased diabetes and cardiovascular risk in obese patients[37]. In another study, higher visfatin and TOS and lower TAS levels were observed in infants born to mothers who smoked[38]. Patients with psoriasis were evaluated for the same relationship. Increased oxidative stress associated with visfatin has been associated with chronic inflammation in patients with nonsevere psoriasis[39].

One of the parameters studied on oxidative stress in recent years is TDH. TDH has been associated with several pathophysiologies[40,41]. In our study, TAS had a positive correlation with total and native thiol and a negative correlation with thiol-disulfide. We also found a negative correlation between total and native thiol and HbA1c, TOS, and OSI. TDH appears to have antioxidant properties. In the literature, there are a few studies in which TDH is studied in patients with diabetes. In a study where prediabetic patients were compared with the control, lower native and total thiol and higher disulfide ratios were observed in the prediabetic group[14]. In another study, high levels of TDH were associated with poor glycemic control in patients with diabetes[42]. We also obtained similar results in terms of TDH and glycemic control. TDH was evaluated in patients with diabetic retinopathy, and it was found that disulfide ratios were higher in the advanced stage of retinopathy[43].

There is only one study that investigated TDH in DN pathogenesis. Patients with and without diabetes who were predominantly diagnosed with axonal polyneuropathy were evaluated in terms of TDH with the control group. In that study, total and native thiol levels were lower in patients with neuropathy than the control group, but there was no significant difference between patients with and without diabetes. However, patients with DN had higher disulfide levels than patients with nondiabetic polyneuropathy. In that study, it was emphasized that, regardless of etiology, TDH may be the last common pathway in patients with axonal damage polyneuropathy[44]. Our study is the first in the literature in which TDH was evaluated with visfatin. We did not find a significant correlation between visfatin and thiol-disulfide. However, we observed a significant negative correlation between visfatin and total native thiol. When the relationship between visfatin and TDH is evaluated together with other results of our study, it becomes evident that the effect of this adipokine on oxidative stress warrants further studies.

In conclusion, DN is one of the common complications of diabetes. Although there are many studies in terms of pathogenesis, there is currently no evidence-based and

practical method for early diagnosis. Surveys and clinical examinations are insufficient and time-consuming methods. Oxidative stress has an important place in the pathogenesis of DN, and our results are consistent with the literature. In this study, oxidative stress was evaluated with visfatin and TDH in patients with DN, and significant correlations were found between these markers. We believe that more comprehensive studies involving TDH and visfatin are needed in the clinical management of DN.

ARTICLE HIGHLIGHTS

Research background

Diabetic polyneuropathy is the most common complication of type 2 diabetes. However, there is no standard method for clinical follow-up and early diagnosis.

Research motivation

Diagnosis of neuropathy is possible only with special examination methods before clinical signs and symptoms. Studies on pathogenesis continue to be conducted on the basis of evidence. However, there is a need for practical methods for early diagnosis.

Research objectives

With this study, we aimed to investigate the frequency of neuropathy in our patients, to test the sensitivity of the interrogation methods used, and to investigate the location of visfatin and thiol balance, which have not yet been studied in pathogenesis.

Research methods

Neuropathy examinations were completed with two defined questionnaires and examination methods: Subjective Peripheral Neuropathy Screen and Michigan Neuropathy Screening Instrument (MNSI). At the same time, venous samples were taken and stored under appropriate conditions until analysis. The analysis included biochemistry panels, oxidative stress parameters, visfatin, and thiol disulfide balance. The last two parameters were evaluated for the first time specifically for this patient group.

Research results

A total of 392 patients were evaluated (271 female, 121 male). The mean age of the patients was 57.5 ± 9.0 years. The mean diabetes period was 12.00 ± 7.29 years. The mean Subjective Peripheral Neuropathy Screen Questionnaire score was 5.6 ± 3.6, the mean MNSI questionnaire score was 4.5 ± 2.3, and the mean MNSI exam score was 2.4 ± 2.0 points. Subjective Peripheral Neuropathy Screen Questionnaire, MNSI questionnaire, and MNSI exam scores were correlated with each other ($P < 0.005$). There was a positive linear relationship between MNSI examination scores and glycated hemoglobin, visfatin, total oxidant status, and oxidative stress index. Visfatin was positively correlated with higher glucose, glycated hemoglobin, total oxidant status and oxidative stress index ($P < 0.005$, $r = 0.537$, $r = 0.753$, $r = 0.407$, $r = 0.587$), and it was negatively correlated with total antioxidant status ($r = -0.499$). Total and native thiol was negatively correlated with glucose, glycated hemoglobin, total oxidant status, and oxidative stress index, but it was positively correlated with total antioxidant status. A statistically significant negative correlation was detected between visfatin and total with native thiol ($P < 0.005$, $r = -0.338$), ($P < 0.005$, $r = -0.448$).

Research conclusions

The sensitivity of the survey methods is low in the diagnosis of neuropathy. The place of oxidative stress in pathogenesis is indisputable. Neuropathy complaints must be included in the clinical examination of the patient, but its reliability is low. The sensitivity of the neuropathy examination is partially higher. However, its applicability is time consuming and difficult in the internal medicine clinic. Increased oxidative stress starts nerve damage in these patients without any clinical symptoms. Visfatin and thiol disulfide balance are being investigated in the pathogenesis of many diseases. It has been shown with this study that they may have a role in the development of polyneuropathy in pathogenesis. Routine monitoring of these parameters in patients with diabetes may be a practical approach for early diagnosis. However, the sensitivity levels of these techniques should be tested together with standard methods. In addition, comparisons for these parameters between patients

with different levels of neuropathy, comorbidities, glycemic regulation, and using drugs are promising studies.

Research perspectives

With this study, we observed how often neuropathy was in patients admitted to internal medicine clinics. We found that there is a need for a practical method for early diagnosis within the clinic. The pathogenesis of neuropathy is one of the issues illuminated in many aspects. These markers, which are thought to be involved in the pathogenesis, should continue to be studied, and their practical use should be evaluated.

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REFERENCES

1 **Hossain P**, Kawar B, El Nahas M. Obesity and diabetes in the developing world--a growing challenge. *N Engl J Med* 2007; **356**: 213-215 [PMID: 17229948 DOI: 10.1056/NEJMp068177]
2 **Tesfaye S**, Vileikyte L, Rayman G, Sindrup SH, Perkins BA, Baconja M, Vinik AI, Boulton AJ; Toronto Expert Panel on Diabetic Neuropathy. Painful diabetic peripheral neuropathy: consensus recommendations on diagnosis, assessment and management. *Diabetes Metab Res Rev* 2011; **27**: 629-638 [PMID: 21695762 DOI: 10.1002/dmrr.1225]
3 **Sadosky A**, Mardekian J, Parsons B, Hopps M, Bienen EJ, Markman J. Healthcare utilization and costs in diabetes relative to the clinical spectrum of painful diabetic peripheral neuropathy. *J Diabetes Complications* 2015; **29**: 212-217 [PMID: 25498300 DOI: 10.1016/j.jdiacomp.2014.10.013]
4 **Vas PRJ**, Edmonds ME. Early recognition of diabetic peripheral neuropathy and the need for one-stop microvascular assessment. *Lancet Diabetes Endocrinol* 2016; **4**: 723-725 [PMID: 27180137 DOI: 10.1016/S2213-8587(16)30063-8]
5 **Bourcier ME**, Ullal J, Parson HK, Dublin CB, Witherspoon CA, Ward SA, Vinik AI. Diabetic peripheral neuropathy: how reliable is a homemade 1-g monofilament for screening? *J Fam Pract* 2006; **55**: 505-508 [PMID: 16750065]
6 **McArthur JC**, Stocks EA, Hauer P, Cornblath DR, Griffin JW. Epidermal nerve fiber density: normative reference range and diagnostic efficiency. *Arch Neurol* 1998; **55**: 1513-1520 [PMID: 9865794 DOI: 10.1001/archneur.55.12.1513]
7 **Perkins BA**, Olaleye D, Zinman B, Bril V. Simple screening tests for peripheral neuropathy in the diabetes clinic. *Diabetes Care* 2001; **24**: 250-256 [PMID: 11213874 DOI: 10.2337/diacare.24.2.250]
8 **Venkataramana AB**, Skolasky RL, Creighton JA, McArthur JC. Diagnostic utility of the subjective peripheral neuropathy screen in HIV-infected persons with peripheral sensory polyneuropathy. *AIDS Read* 2005; **15**: 341-344, 348-349, 354 [PMID: 16044575]
9 **Feldman EL**, Stevens MJ, Thomas PK, Brown MB, Canal N, Greene DA. A practical two-step quantitative clinical and electrophysiological assessment for the diagnosis and staging of diabetic neuropathy. *Diabetes Care* 1994; **17**: 1281-1289 [PMID: 7821168 DOI: 10.2337/diacare.17.11.1281]
10 **Feldman EL**, Nave KA, Jensen TS, Bennett DLH. New Horizons in Diabetic Neuropathy: Mechanisms, Bioenergetics, and Pain. *Neuron* 2017; **93**: 1296-1313 [PMID: 28334605 DOI: 10.1016/j.neuron.2017.02.005]
11 **Uzar E**, Tamam Y, Evliyaoglu O, Tuzcu A, Beyaz C, Acar A, Aydın B, Tasdemir N. Serum prolidase activity and oxidative status in patients with diabetic neuropathy. *Neurol Sci* 2012; **33**: 875-880 [PMID: 22120188 DOI: 10.1007/s10072-011-0857-0]
12 **Aslan M**, Sabuncu T, Kocyigit A, Celik H, Selek S. Relationship between total oxidant status and severity of diabetic nephropathy in type 2 diabetic patients. *Nutr Metab Cardiovasc Dis* 2007; **17**: 734-740 [PMID: 17321120 DOI: 10.1016/j.numecd.2006.08.005]
13 **Erel O**, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem* 2014; **47**: 326-332 [PMID: 25304913 DOI: 10.1016/j.clinbiochem.2014.09.026]
14 **Ates I**, Kaplan M, Inan B, Alisik M, Erel O, Yilmaz N, Guler S. How does thiol/disulfide homeostasis change in prediabetic patients? *Diabetes Res Clin Pract* 2015; **110**: 166-171 [PMID: 26421363 DOI: 10.1016/j.diabres.2015.09.011]
15 **Huang Q**, Dai WM, Jie YQ, Yu GF, Fan XF, Wu A. High concentrations of visfatin in the peripheral blood of patients with acute basal ganglia hemorrhage are associated with poor outcome. *Peptides* 2013; **39**: 55-58 [PMID: 23174347 DOI: 10.1016/j.peptides.2012.11.006]
16 **Schäffler A**, Hamer OW, Dickopf J, Goetz A, Landfried K, Voelk M, Herfarth H, Kopp A, Buechler C, Schölmerich J, Brünnler T. Admission visfatin levels predict pancreatic and peripancreatic necrosis in acute pancreatitis and correlate with clinical severity. *Am J Gastroenterol* 2011; **106**: 957-967 [PMID: 21245835 DOI: 10.1038/ajg.2010.503]
17 **Hajri T**, Gharib M, Kaul S, Karpeh MS Jr. Association between adipokines and critical illness outcomes. *J Trauma Acute Care Surg* 2017; **83**: 507-519 [PMID: 28697011 DOI: 10.1097/TA.0000000000001610]
18 **Kang YS**, Bae MK, Kim JY, Jeong JW, Yun I, Jang HO, Bae SK. Visfatin induces neurite outgrowth in PC12 cells via ERK1/2 signaling pathway. *Neurosci Lett* 2011; **504**: 121-126 [PMID: 21945543 DOI:

10.1016/j.neulet.2011.09.014]
19 **Herman WH**, Pop-Busui R, Braffett BH, Martin CL, Cleary PA, Albers JW, Feldman EL; DCCT/EDIC Research Group. Use of the Michigan Neuropathy Screening Instrument as a measure of distal symmetrical peripheral neuropathy in Type 1 diabetes: results from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications. *Diabet Med* 2012; **29**: 937-944 [PMID: 22417277 DOI: 10.1111/j.1464-5491.2012.03644.x]
20 **Erel O**. A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin Biochem* 2004; **37**: 112-119 [PMID: 14725941 DOI: 10.1016/j.clinbiochem.2003.10.014]
21 **Erel O**. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* 2005; **38**: 1103-1111 [PMID: 16214125 DOI: 10.1016/j.clinbiochem.2005.08.008]
22 **Agathos E**, Tentolouris A, Eleftheriadou I, Katsaouni P, Nemtzas I, Petrou A, Papanikolaou C, Tentolouris N. Effect of α-lipoic acid on symptoms and quality of life in patients with painful diabetic neuropathy. *J Int Med Res* 2018; **46**: 1779-1790 [PMID: 29517942 DOI: 10.1177/0300060518756540]
23 **Hyllienmark L**, Alstrand N, Jonsson B, Ludvigsson J, Cooray G, Wahlberg-Topp J. Early electrophysiological abnormalities and clinical neuropathy: a prospective study in patients with type 1 diabetes. *Diabetes Care* 2013; **36**: 3187-3194 [PMID: 23723354 DOI: 10.2337/dc12-2226]
24 **Fateh HR**, Madani SP, Heshmat R, Larijani B. Correlation of Michigan neuropathy screening instrument, United Kingdom screening test and electrodiagnosis for early detection of diabetic peripheral neuropathy. *J Diabetes Metab Disord* 2015; **15**: 8 [PMID: 27019831 DOI: 10.1186/s40200-016-0229-7]
25 **D'Souza M**, Kulkarni V, Bhaskaran U, Ahmed H, Naimish H, Prakash A, S T, Dahiya B, Thapar R, Mithra P, Kumar N, Holla R, Bb D, Kumar A. Diabetic Peripheral Neuropathy and its Determinants among Patients Attending a Tertiary Health Care Centre in Mangalore, India. *J Public Health Res* 2015; **4**: 450 [PMID: 26425491 DOI: 10.4081/jphr.2015.450]
26 **Khawaja N**, Abu-Shennar J, Saleh M, Dahbour SS, Khader YS, Ajlouni KM. The prevalence and risk factors of peripheral neuropathy among patients with type 2 diabetes mellitus; the case of Jordan. *Diabetol Metab Syndr* 2018; **10**: 8 [PMID: 29483946 DOI: 10.1186/s13098-018-0309-6]
27 **Dziemidok P**, Szcześniak G, Kostrzewa-Zabłocka E, Paprzycki P, Korzon-Burakowska A. Current glycaemic control has no impact on the advancement of diabetic neuropathy. *Ann Agric Environ Med* 2012; **19**: 742-745 [PMID: 23311800]
28 **Callaghan BC**, Cheng HT, Stables CL, Smith AL, Feldman EL. Diabetic neuropathy: clinical manifestations and current treatments. *Lancet Neurol* 2012; **11**: 521-534 [PMID: 22608666 DOI: 10.1016/S1474-4422(12)70065-0]
29 **Doupis J**, Lyons TE, Wu S, Gnardellis C, Dinh T, Veves A. Microvascular reactivity and inflammatory cytokines in painful and painless peripheral diabetic neuropathy. *J Clin Endocrinol Metab* 2009; **94**: 2157-2163 [PMID: 19276232 DOI: 10.1210/jc.2008-2385]
30 **Herder C**, Bongaerts BW, Rathmann W, Heier M, Kowall B, Koenig W, Thorand B, Roden M, Meisinger C, Ziegler D. Association of subclinical inflammation with polyneuropathy in the older population: KORA F4 study. *Diabetes Care* 2013; **36**: 3663-3670 [PMID: 24009302 DOI: 10.2337/dc13-0382]
31 **Kasznicki J**, Kosmalski M, Sliwinska A, Mrowicka M, Stanczyk M, Majsterek I, Drzewoski J. Evaluation of oxidative stress markers in pathogenesis of diabetic neuropathy. *Mol Biol Rep* 2012; **39**: 8669-8678 [PMID: 22718504 DOI: 10.1007/s11033-012-1722-9]
32 **Guzik TJ**, Skiba DS, Touyz RM, Harrison DG. The role of infiltrating immune cells in dysfunctional adipose tissue. *Cardiovasc Res* 2017; **113**: 1009-1023 [PMID: 28838042 DOI: 10.1093/cvr/cvx108]
33 **Smekal A**, Vaclavik J. Adipokines and cardiovascular disease: A comprehensive review. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2017; **161**: 31-40 [PMID: 28228651 DOI: 10.5507/bp.2017.002]
34 **Toan NL**, Van Hoan N, Cuong DV, Dung NV, Dung PT, Hang NT, Dieu DTH, Chung DT, Son HA, Phong PX, Lenon GB, Van De D, Van Tong H. Adipose tissue-derived cytokines and their correlations with clinical characteristics in Vietnamese patients with type 2 diabetes mellitus. *Diabetol Metab Syndr* 2018; **10**: 41 [PMID: 29785210 DOI: 10.1186/s13098-018-0343-4]
35 **Cha JJ**, Min HS, Kim K, Lee MJ, Lee MH, Kim JE, Song HK, Cha DR, Kang YS. Long-term study of the association of adipokines and glucose variability with diabetic complications. *Korean J Intern Med* 2018; **33**: 367-382 [PMID: 27809453 DOI: 10.3904/kjim.2016.114]
36 **Akbarian N**, Zarghami N, Mota A, Abediazar S, Abroon S, Mihanfar A, Amanzadeh M, Darbin A, Bannazadeh Baghi H, Rahmati-Yamchi M. Correlation Between Circulating Visfatin and Nitric Oxide Metabolites Levels in Patients With Diabetic Nephropathy. *Iran J Kidney Dis* 2018; **12**: 163-168 [PMID: 29891746]
37 **Indulekha K**, Surendar J, Anjana RM, Geetha L, Gokulakrishnan K, Pradeepa R, Mohan V. Metabolic obesity, adipocytokines, and inflammatory markers in Asian Indians--CURES-124. *Diabetes Technol Ther* 2015; **17**: 134-141 [PMID: 25478993 DOI: 10.1089/dia.2014.0202]
38 **Chełchowska M**, Ambroszkiewicz J, Gajewska J, Rowicka G, Maciejewski TM, Mazur J. Cord Blood Adiponectin and Visfatin Concentrations in relation to Oxidative Stress Markers in Neonates Exposed and Nonexposed In Utero to Tobacco Smoke. *Oxid Med Cell Longev* 2016; **2016**: 4569108 [PMID: 27525051 DOI: 10.1155/2016/4569108]
39 **Haberka M**, Bańska-Kisiel K, Bergler-Czop B, Biedroń M, Brzezińska-Wcisło L, Okopień B, Gąsior Z. Mild to moderate psoriasis is associated with oxidative stress, subclinical atherosclerosis, and endothelial dysfunction. *Pol Arch Intern Med* 2018; **128**: 434-439 [PMID: 30057390 DOI: 10.20452/pamw.4301]
40 **Kolgelier S**, Ergin M, Demir LS, Inkaya AC, Aktug Demir N, Alisik M, Erel O. Impaired Thiol-Disulfide Balance in Acute Brucellosis. *Jpn J Infect Dis* 2017; **70**: 258-262 [PMID: 27795469 DOI: 10.7883/yoken.JJID.2016.196]
41 **Ozler S**, Oztas E, Caglar AT, Uygur D, Ergin M, Erel O, Danisman N. Thiol/disulfide homeostasis in predicting adverse perinatal outcomes at 24-28 weeks of pregnancy in gestational diabetes. *J Matern Fetal Neonatal Med* 2016; **29**: 3699-3704 [PMID: 26769102 DOI: 10.3109/14767058.2016.1141885]
42 **Ergin M**, Aydin C, Yurt EF, Cakir B, Erel O. The Variation of Disulfides in the Progression of Type 2 Diabetes Mellitus. *Exp Clin Endocrinol Diabetes* 2020; **128**: 77-81 [PMID: 29378378 DOI:

10.1055/s-0044-100376]

43 **Gulpamuk B**, Tekin K, Sonmez K, Inanc M, Neselioglu S, Erel O, Yilmazbas P. The significance of thiol/disulfide homeostasis and ischemia-modified albumin levels to assess the oxidative stress in patients with different stages of diabetes mellitus. *Scand J Clin Lab Invest* 2018; **78**: 136-142 [PMID: 29298534 DOI: 10.1080/00365513.2017.1422540]

44 **Vural G**, Bektas H, Gumusyayla S, Deniz O, Alışık M, Erel O. Impaired thiol-disulphide homeostasis in patients with axonal polyneuropathy. *Neurol Res* 2018; **40**: 166-172 [PMID: 29359643 DOI: 10.1080/01616412.2018.1428522]

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