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ABOUT COVER

Associate Editor of *World Journal of Diabetes*, Chin-Hsiao Tseng, MD, PhD, Professor, Department of Internal Medicine, National Taiwan University College of Medicine/National Taiwan University Hospital, Taipei 10015, Taiwan. ccktsh@ms6.hinet.net

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MINIREVIEWS

Metabolically healthy obesity: Is it really healthy for type 2 diabetes mellitus?

Qi Wu, Ming-Feng Xia, Xin Gao

ORCID number: Qi Wu 0000-0002-0859-702X; Ming-Feng Xia 0000-0002-5650-0165; Xin Gao 0000-0003-1864-7796.

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Qi Wu, Ming-Feng Xia, Xin Gao, Department of Endocrinology and Metabolism, Zhongshan Hospital, Fudan University, Shanghai 200032, China

Qi Wu, Ming-Feng Xia, Xin Gao, Fudan Institute for Metabolic Disease, Fudan University, Shanghai 200032, China

Corresponding author: Ming-Feng Xia, MD, Associate Professor, Department of Endocrinology and Metabolism, Zhongshan Hospital, Fudan University, No. 180 Fenglin Road, Shanghai 200032, China. dr_xiamingfeng@163.com

Abstract

Metabolically healthy obese (MHO) individuals are reported to have a lower risk of developing cardiovascular diseases in comparison with individuals with metabolic syndrome. However, the association between MHO and type 2 diabetes (T2DM) is still controversial. Some studies indicated that MHO is a favorable phenotype for T2DM, but more studies showed that MHO individuals have an increased risk of developing T2DM compared with metabolically healthy normal-weight individuals, especially among those who would acquire metabolically unhealthy obesity. This has been supported by finding insulin resistance and low-grade inflammatory responses in MHO individuals with a tendency for impaired beta-cell dysfunction. Studies also showed that liver fat accumulation increased the risk of incidence of T2DM in MHO. Here, we reviewed current literature on the relationship between MHO and T2DM, discussed the determinants for the development of diabetes in MHO, and summarized the measures for the prevention of T2DM in MHO.

Key Words: Metabolically healthy obesity; Type 2 diabetes; Non-alcoholic fatty liver diseases; Insulin resistance; Low-grade inflammatory status; Beta-cell dysfunction

Core Tip: Metabolically healthy obese individuals have already developed impaired insulin sensitivity with dysfunction of insulin action on subcutaneous tissue, as well as a tendency for beta-cell dysfunction and a chronic low-grade inflammatory status compared with metabolically healthy normal-weight individuals. Thus, it is an

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unfavorable phenotype for type 2 diabetes, with metabolic changes preceding the incidence of diabetes. Liver fat content might be an important contributor to the development of diabetes in metabolically healthy obesity among all risk factors. More attention should be paid to the weight management and metabolic status of these individuals.

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INTRODUCTION

Obesity and diabetes have been growing public health problems for decades. The prevalence of obesity had doubled worldwide in 2015 compared with that in 1980[1]. Individuals with obesity are generally likely to develop type 2 diabetes mellitus (T2DM), since obesity is linked to increased risk of insulin resistance, beta-cell dysfunction, and imbalanced fat tissue metabolism[2]. However, there is a subset of obese individuals who are at low risk of cardiovascular disease with a relatively normal metabolic profile compared with metabolic unhealthy obesity (MUO) individuals, a condition known as metabolically healthy obesity (MHO)[3]. Some studies showed that MHO individuals were not at increased risk for diabetes compared with those who are classified as metabolically healthy normal weight (MHNW)[4,5], but others indicated that MHO was associated with an increased risk of developing T2DM over a lifetime than MHNW[6,7]. Whether MHO is a real health status, or more specifically, whether it predisposes individuals to T2DM, is still controversial.

In this review, we address the above questions by discussing controversies related to metabolically healthy obesity, including the causal relationship between MHO and T2DM and its related diseases as well as the underlying mechanisms.

PREVALENCE OF METABOLICALLY HEALTHY OBESITY

MHO was described by Sims in 2001 as obesity with the absence of metabolic syndrome and metabolic complications[8]. Most definitions of MHO are based on the criteria for metabolic syndrome based on the definition provided by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP-III)[9], which include: (1) The presence of central obesity, waist circumference ≥ 102 cm (90 cm for Asians) in men and ≥ 88 cm (80 cm for Asians) in women; (2) Systolic blood pressure ≥ 17.3 kPa (130 mmHg) and/or diastolic blood pressure ≥ 11.3 kPa (85 mmHg); (3) Triglycerides ≥ 1.7 mmol/L (150 mg/dL); (4) Fasting blood glucose ≥ 5.6 mmol/L (100 mg/dL); and (5) High-density lipoprotein cholesterol (HDL-C) less than 1.03 mmol/L (40 mg/dL) in men or less than 1.30 mmol/L (50 mg/dL) in women. Most definitions of MHO require fewer than two or the absence of any metabolic abnormalities except for waist circumference[7,10-13]. However, the details of the MHO definitions are slightly different. One study defined MHO as individuals who possess no more than two of four metabolic abnormalities except waist circumference[14]. Some researchers believe that those who use anti-hypertension drugs, lipid-lowering agents, or glucose-lowering medicines are also metabolically abnormal even though their metabolic levels are good[15,16]. The level of low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) were also included in the definition of MHO by Karelis *et al*[17]. Insulin resistance evaluated by the homeostasis model assessment for insulin resistance (HOMA-IR) and inflammatory status expressed by C-reactive protein (CRP) has been added to the criteria for MHO by Wildman *et al*[18]. Lwow *et al*[19] proposed using a combined lipid accumulation product with the criteria mentioned above as new criteria for MHO. Smith *et al*[20] decreased the cut point of triglyceride to a level of 95 mg/dL and includes the criteria for the evaluation of intrahepatic lipid content. MHO was also defined as the absence of metabolic diseases such as hypertension, T2DM, and dyslipidemia[15]. The detailed information of common definitions of

MHO was showed in Table 1.

The prevalence of MHO differs from 2.2% to 11.9% in the general population according to the different definitions of MHO[21]. The prevalence of MHO in Americans from the National Health and Nutrition Examination Survey was 19.9% when metabolic health was defined as the absence of components of NCEP ATP-III; the prevalence decreased to 16.0% when the threshold of glucose was reduced to 100 mg/dL, and it decreased to 14.8% when HbA1c was included in the definition of MHO. The prevalence further decreased to 12.2% when the cut-off point of blood pressure was reduced from 17.3/11.3 kPa (130/85 mmHg) to 16.0/10.6 kPa (120/80 mmHg)[22]. Using the criteria of less than three components of NCEP ATP-III, the prevalence of MHO was 8.6% in Spanish[23] and 10.3% in China[24]. There was an age-related reduction in the proportion of MHO regardless of different definitions[24]. Besides, obese patients with higher body mass index (BMI) levels had a lower proportion of MHO, which accounted for 53.7% of participants with BMI at 30-34.9 kg/m^2 and 4.9% of participants with BMI at 35-39.9 kg/m^2[25]. When a more stringent criterion of having no components of NECP ATP-III was applied to the definition of MHO, there was no metabolic healthy individual with BMI ≥ 35 kg/m^2[23]. It means that there might be a cut-off point in individuals with MHO, beyond which their metabolic status would no longer be healthy.

Metabolically healthy individuals will develop metabolic disorders over time. Feng *et al*[7] discovered that only 42.84% of individuals in a group of MHO remained metabolic healthy after a 4-year follow-up. Gilardini *et al*[26] reported that 44% of MHO became metabolically unhealthy after 6-year follow-up, and the proportion increased to 62% after 12-year follow-up. The proportion of transition from MHO to MUO might differ because of different definitions of MHO and various lengths of follow-up[27]. Generally speaking, MHO is not a health status according to the current definitions of having one or two abnormal conditions but rather a transient state that can transition to an unhealthy state over time. Thus, it is fundamentally inaccurate to define those groups of people as "healthy" and worthwhile to investigate the relationship between MHO and T2DM.

RISK OF T2DM IN MHO SUBJECTS

The association between T2DM and MHO has been studied with diverse results, as shown in Table 2. Although MHO is believed to be a healthier phenotype for T2DM when compared with metabolically unhealthy normal weight and MUO individuals, most of the current studies supported that MHO phenotype relates to an increased incidence of T2DM in cohort studies compared to MHNW individuals, independent of the length of follow-up[7,16,28-31]. Wei *et al*[30] examined 17801 individuals in the Dongfeng-Tongji cohort study and showed that the hazard ratio [95% confidence interval (CI)] of diabetes for MHO was 1.74 (1.16-2.59). The multivariate-adjusted hazard ratio (95%CI) of diabetes for MHO without non-alcoholic fatty liver diseases (NAFLD) was 1.57 (1.14-2.16) after an average 4.1-year follow-up in The Kangbuk Samsung Health Study[16]. However, studies have also found that different sub-groups of MHO individuals have different risks of developing diabetes at follow-up [14,30,32,33]. For example, Wang *et al*[33] found that an MHO phenotype that is stable over time is not significantly related to an increased risk of incident diabetes in a 6-year follow-up cohort study when compared with the MHNW phenotype, while the majority of MHO participants had an increased risk of developing diabetes over their lifetimes. Consistently, our human data from Shanghai Changfeng Study showed a similar result that MHO individuals who transition into MUO had a higher risk of developing T2DM while there was no significant association between MHO and incidence of diabetes in the whole population (unpublished data). Thus, it will be of great importance to investigate the determinants related to incident diabetes in MHO individuals.

Several factors might contribute to the development of T2DM in the MHO participants. Baseline body weight is an important factor associated with the high risk of incidence of diabetes. It is universally known that obesity can increase the risk of T2DM. One study found that obese individuals (BMI ≥ 30 kg/m^2) with a healthy metabolic status were at greater risk of developing diabetes than either overweight or normal-weight subjects, and the risk was in proportion to the degree of obesity[14,34]. The previous study has also shown that all metabolically unhealthy individuals, regardless of their body weight, have a higher risk of diabetes[14]. Unstable MHO individuals who progress into unhealthy metabolic statuses also have an elevated risk

Table 1 Definitions of metabolic health in previous publications

Ref.	BP, kPa (mmHg)	Plasma glucose, mmol/L	TG, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	TC, mmol/L	WC, cm	Insulin sensitivity	CRP, mg/L	Intrahepatic lipid content	Others	Metabolic health
NECP ATP III [9]	SBP ≥ 17.3 (130) and/or DBP ≥ 11.3 (85) and/or treatment	FPG ≥ 5.60	≥ 1.70	< 1.29 in women, < 1.03 in men	-	-	> 88 in women, >102 in men	-	-	-	-	< 3 of above
Karelis *et al*[17]	-	-	≤ 1.70	≥ 1.30 and no treatment	≤ 2.60 and no treatment	≤ 5.20	-	HOMA-IR ≤ 1.95	-	-	-	> 3 of above
Meigs *et al*[4]	SBP ≥ 17.3 (130) or DBP ≥ 11.3(85) or treatment	5.6 < FPG ≤ 6.9	≥ 1.70	< 1.30 in women, < 1.00 in men	-	-	> 88 in women, > 102 in men	-	-	-	-	< 2 of above
Meigs *et al*[4]	-	-	-	-	-	-	-	HOMA-IR ≥ 75th percentile	-	-	-	None of above
Aguilar-Salinas *et al*[89]	SBP > 18.6 (140) and/or DBP > 12.0 (90) and/or treatment	FPG ≥ 7.0, or 2-h OGTT ≥ 11.1, or RBG ≥ 11.11 or treatment	-	< 1.04	-	-	-	-	-	-	-	None of above
Wildman *et al* [18]	SBP ≥ 17.3 (130) or DBP ≥ 11.3 (85) or treatment	FPG ≥ 5.56 or treatment	≥ 1.70	< 1.30 in women, < 1.04 in men or treatment	-	-	-	HOMA-IR > 90th percentile	> 90th percentile	-	-	< 2 of above
van Vliet-Ostaptchouk *et al*[90]	SBP ≥ 17.3 (130) or DBP ≥ 11.3 (85) or treatment	FPG ≥ 6.10 or treatment or history/diagnosis of type 2 diabetes	≥ 1.70 or treatment	< 1.03 in men or < 1.30 in women or treatment	-	-	-	-	-	-	-	<2 of above
Jana V van Vliet-Ostaptchouk *et al*[90]	SBP ≥ 18.6 (140) or DBP ≥ 12.0(90) or treatment	FPG ≥ 7.0 or treatment or history/diagnosis of type 2 diabetes	≥ 1.70 or treatment	< 1.03 in men or < 1.30 in women or treatment	-	-	-	-	-	-	-	< 2 of above
Smith *et al*[20]	SBP < 17.3 (130) and/or DBP < 11.3 (85)	FPG < 5.60, or 2-h OGTT glucose < 7.80	< 1.07	≥ 1.29 in women, ≥ 1.04 in men	-	-	-	GIR > 8 mg/kg FFM/min during an HECP (insulin infusion rate: 40 mU/m^2/min)	-	< 5% of liver volume by imaging or < 5% of hepatocytes with intracellular TG by histology	Basic criteria: Absence of diagnosis or therapy of cardiometabolic diseases	all of above

BP: Blood pressure; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FPG: Fasting plasma glucose; OGTT: Oral glucose tolerance test; RBG: Random blood glucose; HbA1c: Glycosylated hemoglobin A1c; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TC: Total cholesterol; WC: Waist circumference; HOMA-IR: Homeostasis model assessment of insulin resistance; GIR: Glucose infusion rate; HECP: Hyperinsulinemic-euglycemic clamp procedure; hs-CRP: High-sensitivity C-reactive protein.

Table 2 Cohort studies of the association of metabolically healthy obesity and type 2 diabetes in the last 5 years

Ref.	Definition of "metabolic health"	MHO, *n*	Main findings
Wei *et al*[30], 2020	Having < 2 of the following criteria: (1) TG ≥ 1.7 mmol/L or lipid-lowering drugs; (2) SBP ≥ 17.3 kPa (130 mmHg) or DBP ≥ 11.3 kPa (85 mmHg) or anti-hypertensive drugs; (3) FPG ≥ 5.6 mmol/L; and (4) HDL-C < 1.04 mmol/L for men and < 1.29 mmol/L for women.	693	MHO was associated with an increased incidence of diabetes, and the association did not differ by the presence or absence of NAFLD.
Feng *et al*[7], 2020	Having < 2 of the following criteria: (1) Hyperglycemia, defined as FPG ≥ 5.6 mmol/L (100 mg/dL); (2) Elevated blood pressure, defined as SBP ≥ 17.3 kPa (130 mmHg) and/or DBP ≥11.3 kPa (85 mmHg) or antihypertensive drug treatment; (3) Hypertriglyceridemia, defined as TG ≥ 1.7 mmol/L (150 mg/dL); and (4) Reduced HDL-C levels, defined as drug treatment to increase HDL-C levels.	3728	The MHO phenotype was associated with an increased incidence of diabetes in older adults. The presence of metabolic disorders in the group with MHO was associated with increased diabetes risk and was predicted by the waist circumference at baseline.
Kim *et al*[32], 2019	Having two or fewer metabolic abnormalities as follows: (1) WC ≥ 90 cm in men and ≥ 85 cm in women; (2) SBP ≥ 17.3 kPa (130 mmHg) or DBP ≥ 11.3 kPa (85 mmHg) or medication use; (3) FPG ≥ 5.6 mmol/L (100 mg/dL) or claim for T2DM or on anti-diabetic medications; (4) Hypertriglyceridemia ≥ 1.7 mmol/L (150 mg/dL) or on lipid medications; and (5) HDL-C < 1.04 mmol/L (40 mg/dL) in men and < 1.29 mmol/L (50 mg/dL) in women, or medication use.	796371	MHO and MHNW phenotypes were transient phenotypes, and their change into metabolic unhealthy status was an important risk factor for the development of T2DM both in obese and normal-weight subjects. Transition into a metabolically unhealthy phenotype was a more significant risk factor of developing T2DM than obesity itself.
Wang *et al*[33], 2018	Having < 2 of the following criteria: (1) SBP ≥ 17.3 kPa (130 mmHg) or DBP ≥ 11.3 kPa (85 mmHg) or current treatment for hypertension; (2) Fasting TG level ≥ 1.7 mmol/L; (3) HDL-C level < 1.03 mmol/l for males or < 1.29 mmol/L for females; and (4) FPG ≥ 5.60 mmol/L.	2153	Stable metabolically healthy overweight/obesity Individuals and those who transitioned to the metabolically healthy status from MUNW did not have an increased risk of incident T2DM. Participants who transitioned from the metabolically healthy overweight/obesity to metabolically unhealthy overweight/obesity phenotype and stable MUNW phenotype showed an increased risk of incident T2DM.
Fingeret *et al* [31], 2018	Having two or fewer metabolic abnormalities as follows: (1) FPG ≥ 5.6 mmol/L or drug treatment; (2) Fasting TG ≥ 1.7 mmol/L or drug treatment; (3) Fasting HDL-C < 1.30 mmol/L in women and < 1.00 mmol/L in men or drug treatment; (4) SBP ≥ 17.3 kPa (130 mmHg), DBP ≥ 11.3 kPa (85 mmHg), or drug treatment; and (5) WC ≥ 102 cm for men and ≥ 88 cm for women.	170	MHO leads to a higher risk of developing cardiovascular risk factors such as hypertension, diabetes, dyslipidemia as compared with MHNW. MHO is transient and should be regarded by clinicians as a warning sign.
Liu *et al*[91], 2018	Having < 2 of metabolic abnormalities as follows: (1) TG ≥ 1.7 mmol/L; (2) HDL-C < 1.0 mmol/L; (3) SBP ≥ 17.3 kPa (130 mmHg) and/or DBP ≥ 11.3 kPa (85 mmHg); and (4) FPG ≥ 5.6 mmol/ L (≥ 100 mg/dL).	1184	MHO and MUNW phenotypes had an increased risk for diabetes. Both baseline metabolic status and follow-up changes played more important roles than obesity for diabetes incidence after adjusted for potential confounding factors. MHO is a transient condition.
Janghorbani *et al*[29], 2017	Having none of metabolic abnormalities as follows: (1) TG ≥ 1.7 mmol/L (150 mg/dL); (2) HDL < 1.04 mmol/L(40 mg/dL) in men and < 1.29 mmol/L(50 mg/dL) in women; (3)BP ≥ 17.3/11.3 kPa (130/85 mmHg) or on antihypertensive medication; and (4) FPG ≥ 5.6 mmol/L (100 mg/dL).	75	Metabolic abnormalities increased risk for incident T2D at any BMI status. Also, obesity is a risk factor for the incidence of T2DM, even in the absence of any metabolic abnormalities.
Latifi *et al*[25], 2017	Having none of metabolic abnormalities as follows: (1) WC ≥ 102 cm in men and ≥ 88 cm in women; (2) TG ≥ 1.7 mmol/L (150 mg/dL) or drug use; (3) HDL < 1.04 mmol/L (40 mg/dL) in men and 1.29 mmol/L (50 mg/dL) in women or drug consumption for hyperlipidemia; (4) BP ≥ 17.3/10.6 kPa (130/80 mmHg) or a history of anti-hypertensive drug consumption; and (5) FPG ≥ 5.6 mmol/L (100 mg/dL), or a history of diabetes mellitus or consumption of anti-diabetes drugs.	NA	There was a specific higher risk of developing metabolic syndrome and diabetes in MHO.
Navarro-Gonzalez *et al* [14], 2016	Having < 3 of the following criteria: (1) TG ≥ 1.7 mmol/L (150 mg/dL); (2) HDL-C > 1.04 mmol/L (40 mg/dL) for men and > 1.29 mmol/L (50 mg/dL) for women; (3) BP ≥ 17.3/11.3 kPa (130/85 mmHg); or (4) FPG ≥ 5.6 mmol/L (100 mg/dL). All individuals currently taking a pharmacological treatment for hypertension were assumed to have raised BP.	389	MHO individuals had an increased risk of incident type 2 diabetes but mainly among those who progressed MUO. MHO individuals who remained with one or no metabolic health risk factors or lost weight overtime did not have a significant risk of diabetes. Metabolically unhealthy individuals had a greater risk of diabetes compared with subjects with MHO.
Guo *et al*[3], 2016	Having all three components as follows: (1) Untreated SBP < 17.3 kPa (130 mmHg) and DBP < 11.3 kPa (85 mmHg); (2) Untreated FPG < 5.6 mmol/L (100 mg/dl) or HbA1c < 5.7%; and (3) Untreated TC < 6.2 mmol/L (240 mg/dL) and HDL ≥ 1.04 mmol/L (40 mg/dL) in men and ≥ 1.29 mmol/L (50 mg/dL) in women.	260	People with healthy obesity have lower risks for diabetes, coronary heart disease, stroke, and mortality compared with unhealthy subjects regardless of their BMI status. Obesity did not affect the risks of coronary heart disease, stroke, and mortality, but did increase diabetes risk.

Jung *et al*[40], 2016	Having < 2 of the following criteria: (1) SBP ≥ 17.3 kPa (130 mmHg) and/or a DBP ≥ 11.3 kPa (85 mmHg), or on antihypertensive treatment; (2) TG ≥ 1.7 mmol/L; (3) FPG ≥ 5.6 mmol/L (impaired fasting glucose, IFG); (4) HDL-C < 1.0 mmol/L in men and < 1.3 mmol/L in women; (5) HOMA-IR ≥ 90th percentile (≥ 2.91); and (6) Hs-CRP ≥ 90th percentile (≥ 2.0 mg/L).	4635	MHO subjects have a substantially increased risk of incident type 2 diabetes compared with MHNO subjects in an Asian population. The presence of FLD assessed by FLI partially explains this increased risk.
Chang *et al*[16], 2016	Having none of the following criteria: (1) BP ≥ 17.3/11.3 kPa (130/85 mmHg) or current use of blood pressure-lowering agents; (2) FPG ≥ 5.6 mmol/L (100 mg/dL) or current use of blood glucose-lowering agents; (3) TG ≥ 1.7 mmol/L (150 mg/dL) or current use of lipid-lowering agents (15); (4) HDL-C < 1.04 mmol/L (40 mg/dL) in men or < 1.29 mmol/L (50 mg/dL) in women; or (5) Insulin resistance, defined as HOMA-IR score ≥ 2.5.	8140	Metabolically healthy overweight and obese individuals were both associated with an increased incidence of diabetes, even in the absence of NAFLD. Obese phenotype itself can drive the development of diabetes, even in the absence of metabolic abnormalities and NAFLD.
Ryoo *et al*[34], 2015	Having < 2 of the following criteria: (1) SBP ≥ 17.3 kPa (130 mmHg) and/or DBP ≥ 11.3 kPa (85 mmHg); (2) TG ≥ 1.7 mmol/L; (3) FPG ≥ 5.6 mmol/L; (4) HDL-C < 1.0 mmol/L; and (5) HOMA-IR ≥ 90th percentile.	240	The risk for diabetes was in proportion to both metabolic health status and degree of obesity in Korean men. Additionally, metabolically healthy status was a more significant determinant for the development of diabetes than obesity itself.

TG: Triglycerides; BP: Blood pressure; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FPG: Fasting plasma glucose; HOMA-IR: Homeostatic model assessment of insulin resistance; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; hs-CRP: High sensitivity C reactive protein; WC: Waist circumference; T2DM: Type 2 diabetes mellitus; MHO: Metabolically healthy obesity; MUO: Metabolically unhealthy obesity; MHNW: Metabolically healthy normal weight; MUNW: Metabolically unhealthy normal weight; NAFLD: Non-alcoholic fatty liver disease; FLI: Fatty liver index.

of developing diabetes. Weight gain was a risk factor for the progression from a healthy condition to an unhealthy one, which further develops into T2DM. In one study, MHO individuals who developed cardiometabolic risk complications gained 6% ± 14% of their body weight (4.9 ± 11.8 kg) compared to 5% ± 14% (3.9 ± 11.3 kg) for those that retained a healthy status[35]. Besides, MHO participants with larger waist circumference at baseline are more likely to transition into an unhealthy phenotype[7]. This has been supported by studies showing that visceral abdominal fat accumulation and fatty liver in MHO contribute to this transition[12,36,37]. Thus, MHO individuals with high liver fat content or large waist circumference are possibly associated with a high risk of diabetes as they have a trend to transferring into MUO phenotype. Our previous study found that visceral adipose area measured by visceral adiposity index in Chinese adults has a more favorable function to predict the development of diabetes than BMI and waist circumference in MHO individuals[38]. Some researchers found that MHO individuals with a high fatty liver index[39] have an increased risk of incident T2DM[40].

LIVER FAT ACCUMULATION IS CRUCIAL FOR DETERMINING THE DEVELOPMENT OF T2DM IN MHO

NAFLD is believed to be significantly associated with the long-term risk of T2DM, and increased liver fat can predict the incidence of T2DM independent of obesity[41,42]. Bian *et al*[43] found that elevated liver fat content (LFC) showed a positive association with insulin resistance and a higher level of nocturnal mean blood concentration before the onset of diabetes. The presence of NAFLD will promote the transition from MHO to a metabolic unhealthy state, and further increases the long-term risk of

incidence of T2DM and even aggravates the deterioration of liver diseases in MHO. Hwang *et al*[12] found that the presence of NAFLD in MHO could predict the conversion from a metabolic health status into a metabolic unhealthy status independent of age, sex, BMI, lifestyle factors, components of metabolic syndrome, and insulin resistance evaluated by HOMA-IR. This result was supported by Hashimoto *et al*[37] with findings that fatty liver index was a predictor for the transition from MHO to MUO phenotype even adjusted for body weight change. However, Hwang *et al*[12] also found that the association between the NAFLD and future transition of MHO into MUO weakened as BMI increased, and the relationship was more prominent in lower BMI individuals. Studies also found that the risk of NAFLD, non-alcoholic steatohepatitis, and liver fibrosis increased as BMI elevated in MHO[16,44]. The unstable MHO status predicted by NAFLD would increase the risk for the development of T2DM, as mentioned above, and therefore the presence of NAFLD in MHO might increase the risk of incident T2DM. Chang *et al*[16] supported this with the result that the risk of incidence of T2DM in MHO subjects with NAFLD increased compared to those free of NAFLD. Ampuero *et al*[45] also found that MHO individuals with biopsy-proven NAFLD or with an intermediate-to-high risk of significant fibrosis evaluated by Hepanet Fibrosis Score (> 0.12) were at risk of developing T2DM.

However, despite the presence of elevated LFC in MHO increasing the risk for the transition of MHO and the incidence of T2DM, few studies regarded intrahepatic lipids content as one of the criteria for the definition of metabolic health. Our previous study found that LFC was positively associated with metabolic disorders independent of related anthropometric and metabolic parameters, and the risk for metabolic diseases increased in an LFC-dependent manner when LFC ≥ 5%[46]. Besides, part of normal individuals without metabolic disorders had a higher LFC[46]. Hence, we agree with Smith *et al*[20] that the evaluation of LFC should be regarded as another crucial criterion for defining "metabolic health".

ASSOCIATION BETWEEN MHO AND METABOLIC DISEASES RELATED TO T2DM

Cardiovascular disease

Studies have found that subjects with MHO have a lower risk of cardiovascular disease (CVD) than MUO individuals over their lifetimes but still have a higher risk than MHNW subjects[47-50]. The transition to an unhealthier metabolic status and the longer duration of unhealthy metabolic conditions contribute to the increased risk of developing CVD among MHO subjects[50-52]. Furthermore, the risk of developing CVD for MHO subjects who initially develop diabetes, hypertension, or hypercholesterolemia tends to be higher than in MHNW subjects[53]. Obesity might increase the risk of CVD independently. A meta-analysis concluded that CVD risk is increased in metabolically healthy overweight or obese participants than in MHNW individuals even when there are no metabolic risk factors[54]. Similarly, obese individuals have been reported to be at higher risk of coronary heart disease irrespective of metabolic health, which challenges the concept of "metabolically healthy obesity"[55].

Chronic kidney disease

Previous studies have shown an increased risk of developing chronic kidney disease (CKD), defined as an estimated glomerular filtration rate of less than 60 mL/min/1.73 m^2 in metabolically healthy overweight/obese subjects compared to MHNW individuals at follow-up, with metabolic health judged as having less than two metabolic abnormalities[56]. Another study showed a similar result in which MHO individuals with no metabolic abnormalities had a higher risk of developing CKD, and this risk was greater in those 40 years or older than in the young[57]. Systemic inflammation measured by high sensitivity-CRP (hs-CRP) might partially contribute to the association between MHO and CKD[11]. Furthermore, individuals who progress to MUO at follow-up show a higher risk of CKD compared with remaining MHO subjects[58,59]. However, Chen *et al*[60] found that the risk difference was not significant in MHO subjects compared to MHNW individuals in the early stage of CKD. This discrepancy might come from the different definitions of CKD, as Chen *et al*[60] combined proteinuria and structural changes in the kidney as indicators.

POSSIBLE MECHANISMS OF THE FUTURE INCIDENCE OF T2DM IN MHO

The possible mechanisms underlying the pathophysiology of incident T2DM in MHO include beta-cell dysfunction, insulin resistance, leptin and adiponectin imbalance, as well as a chronic low-grade inflammatory status (Figure 1). The presence of NAFLD in MHO is also an important factor for the development of T2DM.

Impaired insulin action and insulin resistance

Mature insulin and C-peptide are produced from the precursor proinsulin, and increased proinsulin is observed in insulin-resistant and/or glucose-intolerant individuals[61]. Significantly increased levels of plasma proinsulin, split proinsulin, and C-peptide are observed in MHO subjects compared to MHNW subjects[62]. A similar result has been found in a Chinese population, in which the serum insulin of MHO subjects is significantly elevated[63]. Studies have confirmed the above results showing that HOMA-IR evaluations are significantly different between MHO and MHNW subjects, with a higher value of HOMA-IR in MHO individuals[62,63].

The action of insulin on subcutaneous adipocytes is impaired as well. Rydén *et al*[10] compared the inhibitory action on lipolysis and the stimulatory effect on lipogenesis of insulin in metabolically healthy subjects who were lean, overweight, or obese and found that insulin resistance was already observed in metabolically healthy overweight and obese subjects. In the classical agonist-receptor interaction model, the half-maximum effects for insulin to inhibit adipocyte lipolysis and lipogenesis in overweight/obese people were 10 times and 100 times higher than that in lean people, respectively. The above model suggested that alterations in intracellular events downstream of the insulin receptor and their initial signaling steps have already happened in those individuals. The decreased expression of the insulin signaling mediator *AKT2* might partially explain the increased maximum concentration of insulin hormones needed for an antilipolytic effect and lipogenesis, as AKT2 is an early signaling factor common to the two pathways[64]. Furthermore, the impaired lipogenic function might in part result from a decrease in *SLC2A4* (glucose transporter type 4) mRNA expression, which is essential for insulin-induced glucose uptake by fat cells and stimulating lipogenesis[64]. When testing the maximum insulin action on subcutaneous adipocytes, Rydén *et al*[10] found that the lipogenic effect of insulin hormone was reduced by more than 50% in healthy overweight/obese subjects comparing to lean individuals, and the effect was further impaired in the unhealthy obese groups. Thus, there are reasons to believe that insulin resistance is already present in MHO individuals.

Beta-cell dysfunction

There is no apparent evidence that beta cells in MHO subjects are severely impaired, but they may be partially impaired according to previous studies. Hjelmgren *et al*[62] found that MHO individuals are at increased risk for having β-cell dysfunction, as evaluated by proinsulin levels > 11 pmol/L compared to MHNW subjects, with a relative risk of 18.2 (95%CI: 2.1-159.3). However, Zhao *et al*[63] failed to find a significant difference in HOMA-β between MHNW and obese subjects, though the value of HOMA-β in MHO tended to be higher than that in MHNW individuals among middle-aged subjects. The discrepancy between the two studies might come from their different definitions of metabolic health, differences in race and age, and the relatively small sample size in Zhao's study for evaluating statistical differences. Overall, studies on beta-cell dysfunction are too few to confirm their impaired function in MHO individuals.

Immune and inflammatory responses

Obesity has always been believed to be a chronic low-grade inflammatory status[65], referred to as meta-inflammation. This chronic low-grade inflammation is believed to be a central link between obesity and T2DM[66,67]. A previous study showed that meta-inflammation is presented in MHO subjects as well[68].

Macrophage infiltration in adipose tissue causes increased proinflammatory cytokines and contributes to the development of insulin resistance and T2DM[69]. Christou *et al*[70] found that circulating inflammatory intermediate monocytes [Mon2 ($CD14^{++}CD16^{+}$)] are upregulated in MHO individuals, and nonclassical monocytes [Mon3 ($CD14^{+}CD16^{++}$)] tended to be higher in comparison to metabolically healthy lean individuals when metabolic health was defined as fewer than two metabolic disabilities. The absolute counts of nonclassical Mon3 showed a positive association with HOMA-IR in that study. However, that result differed from previous studies, as

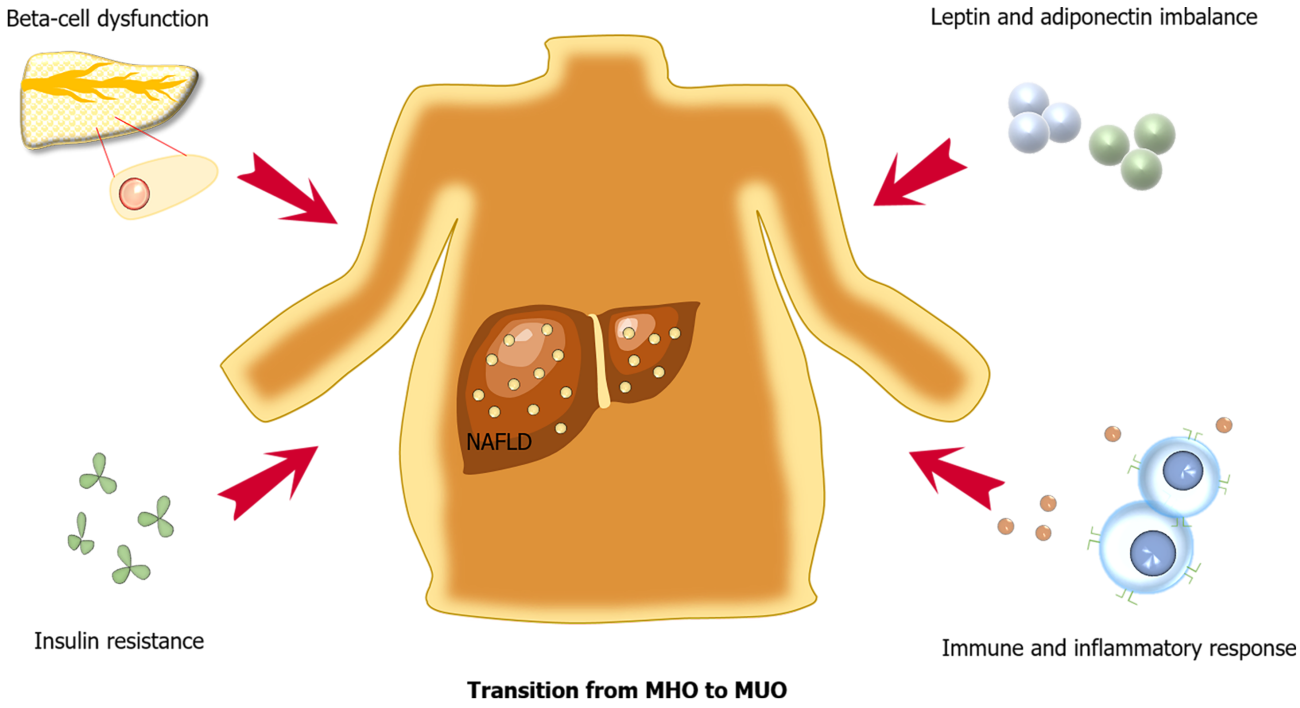

Figure 1 Possible mechanisms that contribute to the future incidence of type 2 diabetes mellitus in the transition from metabolically healthy obesity to metabolically unhealthy obesity. The presence of non-alcoholic fatty liver disease in metabolically healthy obesity is crucial to the incidence of type 2 diabetes mellitus. The possible mechanisms underlying the future development of type 2 diabetes mellitus in metabolically healthy obesity include beta-cell dysfunction, insulin resistance with impaired insulin action, adiponectin concentration reduction, as well as a chronic low-grade inflammatory status. MHO: Metabolically healthy obesity; MUO: Metabolically unhealthy obesity; NAFLD: Non-alcoholic fatty liver disease.

the participants they recruited were taking antidiabetic medications, which might have disturbed the relationship between Mon3 and the level of insulin resistance[71,72].

A previous study found that an imbalance of T cell subsets is responsible for the pathogenesis of obesity and T2DM[68]. Th22 subsets might play a role in obesity and T2DM progression, with MHO and T2DM individuals having significantly elevated peripheral blood Th22 frequencies[73]. This might partially result from the significantly increased transcription of aryl hydrocarbon receptor (AHR), a transcription factor responsible for the differentiation of Th22, on peripheral blood mononuclear cells in both obese and T2DM individuals compared with metabolically healthy normal BMI subjects. AHR is significantly positively associated with elevated hs-CRP and HOMA-IR levels in MHO individuals. Although it was tested in peripheral blood mononuclear cells and not T cells in that study, AHR expression in peripheral blood mononuclear cells is more likely to be a causative factor in Th polarization with a currently unknown mechanism[63].

Leptin and adiponectin

Adipose tissue is not only an energy storage depot, it also has endocrine functions and produces some cytokines that influence metabolism throughout the human body. White fat tissue can participate in regulating insulin sensitivity, lipid metabolism, and low-grade inflammation[74,75]. Leptin and adiponectin are important factors in these conditions. Leptin is responsible for food intake and metabolism regulation, while adiponectin release contributes to energy metabolism, insulin action, lipid metabolism regulation, and oxidative stress. Increased adiponectin is associated with better insulin sensitivity in the human body[76]. A previous study found that adiponectin is significantly decreased in MHO Han Chinese adolescents compared with a normal-weight control group, and a similar result was also found in middle-aged Norwegians [77,78]. Thus, insulin sensitivity might be disturbed in MHO individuals with elevated adiponectin. However, Carvalho *et al*[79] found an inconsistent result that the serum adiponectin concentration in MHO subjects had no significant difference with MHNW individuals. The small sample size of the latter study might have contributed to the inability to find statistically significant differences in adiponectin. Taken together, most studies indicated an increased leptin/adiponectin ratio in MHO compared to MHNW individuals[77-79], which was already regarded as a sensitive indicator of metabolic syndrome and insulin sensitivity[80]. Thus, no matter whether adiponectin

is decreased in MHO individuals, it can be deduced that insulin sensitivity has been already impaired in MHO subjects with an elevated leptin/adiponectin ratio.

WEIGHT CONTROL MIGHT IMPROVE T2DM RISK IN MHO

There are few clinical procedures for MHO individuals to prevent the high risk of incidence of T2DM, but studies have shown evidence of benefits of weight loss for MHO with the improvement of metabolic parameters and inflammatory biomarkers.

A cohort study has found that bariatric surgery could significantly achieve a great deal of total weight loss in MHO patients at follow-up[81]. Some studies have shown that MHO could achieve more weight loss than that in MUO participants after bariatric surgery[82-84], suggesting that the MHO phenotype is an independent predictor for greater body weight loss and more effective bariatric surgery in obese individuals before metabolic abnormalities appear[83]. Furthermore, cardiovascular risk factors such as blood pressure, lipid levels, and plasma glucose are improved after bariatric surgery, even when some of these levels are "normal" preoperatively[81]. Otherwise, these indexes show more improvement in metabolically unhealthy individuals[81,84]. However, Pelascini *et al*[82] failed to find significant improvements in HDL-C and plasma glucose in MHO participants, which might have resulted from their relatively small sample size and strict definition of "metabolic health" plus HOMA-IR and hs-CRP. In summary, the benefits of bariatric surgery for the MHO phenotype are considerable, potentially comparable in benefit to the unhealthier phenotype with much better weight loss[84]. However, this has only been tested and observed in MHO subjects whose BMI was ≥ 40 kg/m^2. For the majority of MHO individuals, the application of bariatric surgery is not recommended in the current clinical environment with no more solid testimonies.

For the majority of those individuals with MHO, cultivating a favorable lifestyle might be a more feasible method to achieve weight loss. Studies have demonstrated that a healthier diet with a higher proportion of fruit, vegetables, and fish and longer mealtimes (more than 10 min) in women and higher degrees of physical activity is associated with the MHO phenotype compared with the MUO phenotype[85,86]. Gomez-Huelgas *et al*[87] found that intensive lifestyle modification could induce clinically significant weight loss in MHO phenotype women, leading to the reduction of serum adipokines and inflammatory biomarkers such as hs-CRP, interleukin-6, and tumor necrosis factor-α, which play important roles in the pathological mechanism of obesity and insulin resistance.

In a prospective cohort study of the MHO population, it was found that air pollution had a significantly positive correlation with adiponectin and hs-CRP, which suggests that air pollution plays an important role in the occurrence and development of diabetes in MHO individuals[88]. It will be interesting to compare the risk of the incident in MHO with and without exposure to polluted air.

CONCLUSION

Current MHO diagnostic criteria are insufficient to exclude all obese people with the potential to develop future metabolic disorders. How to define MHO is an issue worth discussing. MHO is not absolutely "metabolically healthy" compared to MHNW with potential risks for T2DM and its related metabolic disorders. This might be explained by mechanisms such as the expansion and hypoxia of adipose tissue, increased inflammation, and decreased adiponectin concentrations in the MHO population. Liver fat accumulation is also a crucial risk factor for the incidence of T2DM in MHO. Thus, we recommend adding the intrahepatic fat content into the criteria for "metabolic health". Weight control might effectively protect the MHO individuals from the development of diabetes and its related metabolic diseases. In addition, MHO is a transitional phenotype between MHNW and MUO. It will be worthwhile to investigate the crucial factors that are responsible for the transition from MHO to MUO. The advance of multi-omics technology might help us to identify better MHO with a higher risk of developing diabetes and multiple metabolic disorders.

REFERENCES

1 **GBD 2015 Obesity Collaborators**, Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Lee A, Marczak L, Mokdad AH, Moradi-Lakeh M, Naghavi M, Salama JS, Vos T, Abate KH, Abbafati C, Ahmed MB, Al-Aly Z, Alkerwi A, Al-Raddadi R, Amare AT, Amberbir A, Amegah AK, Amini E, Amrock SM, Anjana RM, Ärnlöv J, Asayesh H, Banerjee A, Barac A, Baye E, Bennett DA, Beyene AS, Biadgilign S, Biryukov S, Bjertness E, Boneya DJ, Campos-Nonato I, Carrero JJ, Cecilio P, Cercy K, Ciobanu LG, Cornaby L, Damtew SA, Dandona L, Dandona R, Dharmaratne SD, Duncan BB, Eshrati B, Esteghamati A, Feigin VL, Fernandes JC, Fürst T, Gebrehiwot TT, Gold A, Gona PN, Goto A, Habtewold TD, Hadush KT, Hafezi-Nejad N, Hay SI, Horino M, Islami F, Kamal R, Kasaeian A, Katikireddi SV, Kengne AP, Kesavachandran CN, Khader YS, Khang YH, Khubchandani J, Kim D, Kim YJ, Kinfu Y, Kosen S, Ku T, Defo BK, Kumar GA, Larson HJ, Leinsalu M, Liang X, Lim SS, Liu P, Lopez AD, Lozano R, Majeed A, Malekzadeh R, Malta DC, Mazidi M, McAlinden C, McGarvey ST, Mengistu DT, Mensah GA, Mensink GBM, Mezgebe HB, Mirrakhimov EM, Mueller UO, Noubiap JJ, Obermeyer CM, Ogbo FA, Owolabi MO, Patton GC, Pourmalek F, Qorbani M, Rafay A, Rai RK, Ranabhat CL, Reinig N, Safiri S, Salomon JA, Sanabria JR, Santos IS, Sartorius B, Sawhney M, Schmidhuber J, Schutte AE, Schmidt MI, Sepanlou SG, Shamsizadeh M, Sheikhbahaei S, Shin MJ, Shiri R, Shiue I, Roba HS, Silva DAS, Silverberg JI, Singh JA, Stranges S, Swaminathan S, Tabarés-Seisdedos R, Tadese F, Tedla BA, Tegegne BS, Terkawi AS, Thakur JS, Tonelli M, Topor-Madry R, Tyrovolas S, Ukwaja KN, Uthman OA, Vaezghasemi M, Vasankari T, Vlassov VV, Vollset SE, Weiderpass E, Werdecker A, Wesana J, Westerman R, Yano Y, Yonemoto N, Yonga G, Zaidi Z, Zenebe ZM, Zipkin B, Murray CJL. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *N Engl J Med* 2017; **377**: 13-27 [PMID: 28604169 DOI: 10.1056/NEJMoa1614362]

2 **Mokdad AH**, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS, Marks JS. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA* 2003; **289**: 76-79 [PMID: 12503980 DOI: 10.1001/jama.289.1.76]

3 **Guo F**, Garvey WT. Cardiometabolic disease risk in metabolically healthy and unhealthy obesity: Stability of metabolic health status in adults. *Obesity (Silver Spring)* 2016; **24**: 516-525 [PMID: 26719125 DOI: 10.1002/oby.21344]

4 **Meigs JB**, Wilson PW, Fox CS, Vasan RS, Nathan DM, Sullivan LM, D'Agostino RB. Body mass index, metabolic syndrome, and risk of type 2 diabetes or cardiovascular disease. *J Clin Endocrinol Metab* 2006; **91**: 2906-2912 [PMID: 16735483 DOI: 10.1210/jc.2006-0594]

5 **Appleton SL**, Seaborn CJ, Visvanathan R, Hill CL, Gill TK, Taylor AW, Adams RJ; North West Adelaide Health Study Team. Diabetes and cardiovascular disease outcomes in the metabolically healthy obese phenotype: a cohort study. *Diabetes Care* 2013; **36**: 2388-2394 [PMID: 23491523 DOI: 10.2337/dc12-1971]

6 **Bell JA**, Kivimaki M, Hamer M. Metabolically healthy obesity and risk of incident type 2 diabetes: a meta-analysis of prospective cohort studies. *Obes Rev* 2014; **15**: 504-515 [PMID: 24661566 DOI: 10.1111/obr.12157]

7 **Feng S**, Gong X, Liu H, Lu R, Duan T, Wang M, Wang J, Wang H, Chen J, Liu Y, Li C, Ma J, Wu L, Lin Y, Hou F, Zhang Y, Lu C, Yu P, Cui Z. The Diabetes Risk and Determinants of Transition from Metabolically Healthy to Unhealthy Phenotypes in 49,702 Older Adults: 4-Year Cohort Study. *Obesity (Silver Spring)* 2020; **28**: 1141-1148 [PMID: 32374520 DOI: 10.1002/oby.22800]

8 **Sims EA**. Are there persons who are obese, but metabolically healthy? *Metabolism* 2001; **50**: 1499-1504 [PMID: 11735101 DOI: 10.1053/meta.2001.27213]

9 **Grundy SM**, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F; American Heart Association; National Heart, Lung, and Blood Institute. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005; **112**: 2735-2752 [PMID: 16157765 DOI: 10.1161/CIRCULATIONAHA.105.169404]

10 **Rydén M**, Petrus P, Andersson DP, Medina-Gómez G, Escasany E, Corrales Cordón P, Dahlman I, Kulyté A, Arner P. Insulin action is severely impaired in adipocytes of apparently healthy overweight and obese subjects. *J Intern Med* 2019; **285**: 578-588 [PMID: 30758089 DOI: 10.1111/joim.12887]

11 **Lin L**, Peng K, Du R, Huang X, Lu J, Xu Y, Xu M, Chen Y, Bi Y, Wang W. Metabolically healthy obesity and incident chronic kidney disease: The role of systemic inflammation in a prospective study. *Obesity (Silver Spring)* 2017; **25**: 634-641 [PMID: 28160438 DOI: 10.1002/oby.21768]

12 **Hwang YC**, Ahn HY, Park CY. Association Between Nonalcoholic Fatty Liver Disease and Future Deterioration of Metabolic Health: A Cohort Study. *Obesity (Silver Spring)* 2019; **27**: 1360-1366 [PMID: 31245932 DOI: 10.1002/oby.22536]

13 **Chang Y**, Jung HS, Cho J, Zhang Y, Yun KE, Lazo M, Pastor-Barriuso R, Ahn J, Kim CW, Rampal S, Cainzos-Achirica M, Zhao D, Chung EC, Shin H, Guallar E, Ryu S. Metabolically Healthy Obesity and the Development of Nonalcoholic Fatty Liver Disease. *Am J Gastroenterol* 2016; **111**: 1133-1140 [PMID: 27185080 DOI: 10.1038/ajg.2016.178]

14 **Navarro-González D**, Sánchez-Íñigo L, Fernández-Montero A, Pastrana-Delgado J, Alfredo Martínez J. Are all metabolically healthy individuals with obesity at the same risk of diabetes onset? *Obesity (Silver Spring)* 2016; **24**: 2615-2623 [PMID: 27804254 DOI: 10.1002/oby.21667]

15 **Ahl S**, Guenther M, Zhao S, James R, Marks J, Szabo A, Kidambi S. Adiponectin Levels Differentiate Metabolically Healthy *vs* Unhealthy Among Obese and Nonobese White Individuals. *J*

Clin Endocrinol Metab 2015; **100**: 4172-4180 [PMID: 26401592 DOI: 10.1210/jc.2015-2765]
16 **Chang Y**, Jung HS, Yun KE, Cho J, Ahn J, Chung EC, Shin H, Ryu S. Metabolically healthy obesity is associated with an increased risk of diabetes independently of nonalcoholic fatty liver disease. *Obesity (Silver Spring)* 2016; **24**: 1996-2003 [PMID: 27474900 DOI: 10.1002/oby.21580]
17 **Karelis AD**, Brochu M, Rabasa-Lhoret R. Can we identify metabolically healthy but obese individuals (MHO)? *Diabetes Metab* 2004; **30**: 569-572 [PMID: 15671927 DOI: 10.1016/s1262-3636(07)70156-8]
18 **Wildman RP**, Muntner P, Reynolds K, McGinn AP, Rajpathak S, Wylie-Rosett J, Sowers MR. The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). *Arch Intern Med* 2008; **168**: 1617-1624 [PMID: 18695075 DOI: 10.1001/archinte.168.15.1617]
19 **Lwow F**, Jedrzejuk D, Milewicz A, Szmigiero L. Lipid accumulation product (LAP) as a criterion for the identification of the healthy obesity phenotype in postmenopausal women. *Exp Gerontol* 2016; **82**: 81-87 [PMID: 27329927 DOI: 10.1016/j.exger.2016.06.007]
20 **Smith GI**, Mittendorfer B, Klein S. Metabolically healthy obesity: facts and fantasies. *J Clin Invest* 2019; **129**: 3978-3989 [PMID: 31524630 DOI: 10.1172/JCI129186]
21 **Phillips CM**. Metabolically healthy obesity: definitions, determinants and clinical implications. *Rev Endocr Metab Disord* 2013; **14**: 219-227 [PMID: 23928851 DOI: 10.1007/s11154-013-9252-x]
22 **Araújo J**, Cai J, Stevens J. Prevalence of Optimal Metabolic Health in American Adults: National Health and Nutrition Examination Survey 2009-2016. *Metab Syndr Relat Disord* 2019; **17**: 46-52 [PMID: 30484738 DOI: 10.1089/met.2018.0105]
23 **Goday A**, Calvo E, Vázquez LA, Caveda E, Margallo T, Catalina-Romero C, Reviriego J. Prevalence and clinical characteristics of metabolically healthy obese individuals and other obese/non-obese metabolic phenotypes in a working population: results from the Icaria study. *BMC Public Health* 2016; **16**: 248 [PMID: 27036105 DOI: 10.1186/s12889-016-2921-4]
24 **Liu C**, Wang C, Guan S, Liu H, Wu X, Zhang Z, Gu X, Zhang Y, Zhao Y, Tse LA, Fang X. The Prevalence of Metabolically Healthy and Unhealthy Obesity according to Different Criteria. *Obes Facts* 2019; **12**: 78-90 [PMID: 30814477 DOI: 10.1159/000495852]
25 **Latifi SM**, Karandish M, Shahbazian H, Taha JM, Cheraghian B, Moradi M. Prevalence of Metabolically Healthy Obesity (MHO) and its relation with incidence of metabolic syndrome, hypertension and type 2 Diabetes amongst individuals aged over 20 years in Ahvaz: A 5 Year cohort Study (2009-2014). *Diabetes Metab Syndr* 2017; **11** Suppl 2: S1037-S1040 [PMID: 28781161 DOI: 10.1016/j.dsx.2017.07.036]
26 **Gilardini L**, Zambon A, Soranna D, Croci M, Invitti C. Predictors of the transition from metabolically healthy obesity to unhealthy obesity. *Eat Weight Disord* 2018; **23**: 739-744 [PMID: 30374903 DOI: 10.1007/s40519-018-0600-4]
27 **Mongraw-Chaffin M**, Foster MC, Kalyani RR, Vaidya D, Burke GL, Woodward M, Anderson CA. Obesity Severity and Duration Are Associated With Incident Metabolic Syndrome: Evidence Against Metabolically Healthy Obesity From the Multi-Ethnic Study of Atherosclerosis. *J Clin Endocrinol Metab* 2016; **101**: 4117-4124 [PMID: 27552544 DOI: 10.1210/jc.2016-2460]
28 **Hashimoto Y**, Hamaguchi M, Tanaka M, Obora A, Kojima T, Fukui M. Metabolically healthy obesity without fatty liver and risk of incident type 2 diabetes: A meta-analysis of prospective cohort studies. *Obes Res Clin Pract* 2018; **12**: 4-15 [PMID: 29307656 DOI: 10.1016/j.orcp.2017.12.003]
29 **Janghorbani M**, Salamat MR, Amini M, Aminorroaya A. Risk of diabetes according to the metabolic health status and degree of obesity. *Diabetes Metab Syndr* 2017; **11** Suppl 1: S439-S444 [PMID: 28404516 DOI: 10.1016/j.dsx.2017.03.032]
30 **Wei Y**, Wang J, Han X, Yu C, Wang F, Yuan J, Miao X, Yao P, Wei S, Wang Y, Liang Y, Zhang X, Guo H, Zheng D, Tang Y, Yang H, He M. Metabolically healthy obesity increased diabetes incidence in a middle-aged and elderly Chinese population. *Diabetes Metab Res Rev* 2020; **36**: e3202 [PMID: 31291052 DOI: 10.1002/dmrr.3202]
31 **Fingeret M**, Marques-Vidal P, Vollenweider P. Incidence of type 2 diabetes, hypertension, and dyslipidemia in metabolically healthy obese and non-obese. *Nutr Metab Cardiovasc Dis* 2018; **28**: 1036-1044 [PMID: 30139688 DOI: 10.1016/j.numecd.2018.06.011]
32 **Kim JA**, Kim DH, Kim SM, Park YG, Kim NH, Baik SH, Choi KM, Han K, Yoo HJ. Impact of the Dynamic Change of Metabolic Health Status on the Incident Type 2 Diabetes: A Nationwide Population-Based Cohort Study. *Endocrinol Metab (Seoul)* 2019; **34**: 406-414 [PMID: 31884741 DOI: 10.3803/EnM.2019.34.4.406]
33 **Wang B**, Zhang M, Wang S, Wang C, Wang J, Li L, Zhang L, Ren Y, Han C, Zhao Y, Zhou J, Wang G, Shen Y, Wu D, Pang C, Yin L, Feng T, Zhao J, Luo X, Hu D. Dynamic status of metabolically healthy overweight/obesity and metabolically unhealthy and normal weight and the risk of type 2 diabetes mellitus: A cohort study of a rural adult Chinese population. *Obes Res Clin Pract* 2018; **12**: 61-71 [PMID: 29100915 DOI: 10.1016/j.orcp.2017.10.005]
34 **Ryoo JH**, Park SK, Ye S, Choi JM, Oh CM, Kim SY, Shin JY, Park JH, Hong HP, Ko TS. Estimation of risk for diabetes according to the metabolically healthy status stratified by degree of obesity in Korean men. *Endocrine* 2015; **50**: 650-658 [PMID: 26022652 DOI: 10.1007/s12020-015-0635-5]
35 **Espinosa De Ycaza AE**, Donegan D, Jensen MD. Long-term metabolic risk for the metabolically healthy overweight/obese phenotype. *Int J Obes (Lond)* 2018; **42**: 302-309 [PMID: 29064474 DOI: 10.1038/ijo.2017.233]

36 **Hwang YC**, Hayashi T, Fujimoto WY, Kahn SE, Leonetti DL, McNeely MJ, Boyko EJ. Visceral abdominal fat accumulation predicts the conversion of metabolically healthy obese subjects to an unhealthy phenotype. *Int J Obes (Lond)* 2015; **39**: 1365-1370 [PMID: 25920773 DOI: 10.1038/ijo.2015.75]

37 **Hashimoto Y**, Hamaguchi M, Fukuda T, Ohbora A, Kojima T, Fukui M. Fatty liver as a risk factor for progression from metabolically healthy to metabolically abnormal in non-overweight individuals. *Endocrine* 2017; **57**: 89-97 [PMID: 28508194 DOI: 10.1007/s12020-017-1313-6]

38 **Xia MF**, Chen Y, Lin HD, Ma H, Li XM, Aleteng Q, Li Q, Wang D, Hu Y, Pan BS, Li XJ, Li XY, Gao X. A indicator of visceral adipose dysfunction to evaluate metabolic health in adult Chinese. *Sci Rep* 2016; **6**: 38214 [PMID: 27905531 DOI: 10.1038/srep38214]

39 **Bedogni G**, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, Tiribelli C. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006; **6**: 33 [PMID: 17081293 DOI: 10.1186/1471-230X-6-33]

40 **Jung CH**, Kang YM, Jang JE, Hwang JY, Kim EH, Park JY, Kim HK, Lee WJ. Fatty liver index is a risk determinant of incident type 2 diabetes in a metabolically healthy population with obesity. *Obesity (Silver Spring)* 2016; **24**: 1373-1379 [PMID: 27112320 DOI: 10.1002/oby.21483]

41 **Mantovani A**, Byrne CD, Bonora E, Targher G. Nonalcoholic Fatty Liver Disease and Risk of Incident Type 2 Diabetes: A Meta-analysis. *Diabetes Care* 2018; **41**: 372-382 [PMID: 29358469 DOI: 10.2337/dc17-1902]

42 **Sung KC**, Wild SH, Kwag HJ, Byrne CD. Fatty liver, insulin resistance, and features of metabolic syndrome: relationships with coronary artery calcium in 10,153 people. *Diabetes Care* 2012; **35**: 2359-2364 [PMID: 22829522 DOI: 10.2337/dc12-0515]

43 **Bian H**, Yan H, Zeng M, Rao S, Yao X, Zhou J, Jia W, Gao X. Increased liver fat content and unfavorable glucose profiles in subjects without diabetes. *Diabetes Technol Ther* 2011; **13**: 149-155 [PMID: 21284482 DOI: 10.1089/dia.2010.0101]

44 **Kim Y**, Chang Y, Cho YK, Ahn J, Shin H, Ryu S. Metabolically healthy versus unhealthy obesity and risk of fibrosis progression in non-alcoholic fatty liver disease. *Liver Int* 2019; **39**: 1884-1894 [PMID: 31226232 DOI: 10.1111/liv.14184]

45 **Ampuero J**, Aller R, Gallego-Durán R, Crespo J, Calleja JL, García-Monzón C, Gómez-Camarero J, Caballería J, Lo Iacono O, Ibañez L, García-Samaniego J, Albillos A, Francés R, Fernández-Rodríguez C, Diago M, Soriano G, Andrade RJ, Latorre R, Jorquera F, Morillas RM, Escudero D, Estévez P, Guerra MH, Augustín S, Banales JM, Aspichueta P, Benlloch S, Rosales JM, Salmerón J, Turnes J, Romero Gómez M; HEPAmet Registry. Significant fibrosis predicts new-onset diabetes mellitus and arterial hypertension in patients with NASH. *J Hepatol* 2020; **73**: 17-25 [PMID: 32147361 DOI: 10.1016/j.jhep.2020.02.028]

46 **Xia MF**, Bian H, Yan HM, Lin HD, Chang XX, Li XM, Ma H, He WY, Zhao NQ, Xia P, Gao X. Assessment of liver fat content using quantitative ultrasonography to evaluate risks for metabolic diseases. *Obesity (Silver Spring)* 2015; **23**: 1929-1937 [PMID: 26239703 DOI: 10.1002/oby.21182]

47 **Caleyachetty R**, Thomas GN, Toulis KA, Mohammed N, Gokhale KM, Balachandran K, Nirantharakumar K. Metabolically Healthy Obese and Incident Cardiovascular Disease Events Among 3.5 Million Men and Women. *J Am Coll Cardiol* 2017; **70**: 1429-1437 [PMID: 28911506 DOI: 10.1016/j.jacc.2017.07.763]

48 **Yeh TL**, Chen HH, Tsai SY, Lin CY, Liu SJ, Chien KL. The Relationship between Metabolically Healthy Obesity and the Risk of Cardiovascular Disease: A Systematic Review and Meta-Analysis. *J Clin Med* 2019; **8** [PMID: 31443279 DOI: 10.3390/jcm8081228]

49 **Gao M**, Lv J, Yu C, Guo Y, Bian Z, Yang R, Du H, Yang L, Chen Y, Li Z, Zhang X, Chen J, Qi L, Chen Z, Huang T, Li L; China Kadoorie Biobank (CKB) Collaborative Group. Metabolically healthy obesity, transition to unhealthy metabolic status, and vascular disease in Chinese adults: A cohort study. *PLoS Med* 2020; **17**: e1003351 [PMID: 33125374 DOI: 10.1371/journal.pmed.1003351]

50 **Kouvari M**, Panagiotakos DB, Yannakoulia M, Georgousopoulou E, Critselis E, Chrysohoou C, Tousoulis D, Pitsavos C; ATTICA Study Investigators. Transition from metabolically benign to metabolically unhealthy obesity and 10-year cardiovascular disease incidence: The ATTICA cohort study. *Metabolism* 2019; **93**: 18-24 [PMID: 30639450 DOI: 10.1016/j.metabol.2019.01.003]

51 **Mongraw-Chaffin M**, Foster MC, Anderson CAM, Burke GL, Haq N, Kalyani RR, Ouyang P, Sibley CT, Tracy R, Woodward M, Vaidya D. Metabolically Healthy Obesity, Transition to Metabolic Syndrome, and Cardiovascular Risk. *J Am Coll Cardiol* 2018; **71**: 1857-1865 [PMID: 29699611 DOI: 10.1016/j.jacc.2018.02.055]

52 **Guo X**, Li Z, Zhou Y, Yu S, Yang H, Sun G, Zheng L, Afzal J, Liu Y, Sun Y. The effects of transitions in metabolic health and obesity status on incident cardiovascular disease: Insights from a general Chinese population. *Eur J Prev Cardiol* 2020; 2047487320935550 [PMID: 32611251 DOI: 10.1177/2047487320935550]

53 **Eckel N**, Li Y, Kuxhaus O, Stefan N, Hu FB, Schulze MB. Transition from metabolic healthy to unhealthy phenotypes and association with cardiovascular disease risk across BMI categories in 90 257 women (the Nurses' Health Study): 30 year follow-up from a prospective cohort study. *Lancet Diabetes Endocrinol* 2018; **6**: 714-724 [PMID: 29859908 DOI: 10.1016/S2213-8587(18)30137-2]

54 **Opio J**, Croker E, Odongo GS, Attia J, Wynne K, McEvoy M. Metabolically healthy overweight/obesity are associated with increased risk of cardiovascular disease in adults, even in the absence of metabolic risk factors: A systematic review and meta-analysis of prospective cohort studies. *Obes Rev* 2020; **21**: e13127 [PMID: 32869512 DOI: 10.1111/obr.13127]

55 **Lassale C**, Tzoulaki I, Moons KGM, Sweeting M, Boer J, Johnson L, Huerta JM, Agnoli C, Freisling H, Weiderpass E, Wennberg P, van der A DL, Arriola L, Benetou V, Boeing H, Bonnet F, Colorado-Yohar SM, Engström G, Eriksen AK, Ferrari P, Grioni S, Johansson M, Kaaks R, Katsoulis M, Katzke V, Key TJ, Matullo G, Melander O, Molina-Portillo E, Moreno-Iribas C, Norberg M, Overvad K, Panico S, Quirós JR, Saieva C, Skeie G, Steffen A, Stepien M, Tjønneland A, Trichopoulou A, Tumino R, van der Schouw YT, Verschuren WMM, Langenberg C, Di Angelantonio E, Riboli E, Wareham NJ, Danesh J, Butterworth AS. Separate and combined associations of obesity and metabolic health with coronary heart disease: a pan-European case-cohort analysis. *Eur Heart J* 2018; **39**: 397-406 [PMID: 29020414 DOI: 10.1093/eurheartj/ehx448]

56 **Wang Y**, Sun B, Sheng LT, Pan XF, Zhou Y, Zhu J, Li X, Yang K, Guo K, Zhang X, He M, Yang H, Wu T, Pan A. Association between weight status, metabolic syndrome, and chronic kidney disease among middle-aged and elderly Chinese. *Nutr Metab Cardiovasc Dis* 2020; **30**: 2017-2026 [PMID: 32826134 DOI: 10.1016/j.numecd.2020.06.025]

57 **Chang Y**, Ryu S, Choi Y, Zhang Y, Cho J, Kwon MJ, Hyun YY, Lee KB, Kim H, Jung HS, Yun KE, Ahn J, Rampal S, Zhao D, Suh BS, Chung EC, Shin H, Pastor-Barriuso R, Guallar E. Metabolically Healthy Obesity and Development of Chronic Kidney Disease: A Cohort Study. *Ann Intern Med* 2016; **164**: 305-312 [PMID: 26857595 DOI: 10.7326/M15-1323]

58 **Nam KH**, Yun HR, Joo YS, Kim J, Lee S, Lee C, Park KS, Park JT, Chang TI, Kang EW, Yoo TH, Kang SW, Han SH. Changes in obese metabolic phenotypes over time and risk of incident chronic kidney disease. *Diabetes Obes Metab* 2018; **20**: 2778-2791 [PMID: 29971899 DOI: 10.1111/dom.13458]

59 **Cho YK**, Lee J, Kim HS, Park JY, Lee WJ, Kim YJ, Jung CH. Impact of Transition in Metabolic Health and Obesity on the Incident Chronic Kidney Disease: A Nationwide Cohort Study. *J Clin Endocrinol Metab* 2020; **105** [PMID: 31967306 DOI: 10.1210/clinem/dgaa033]

60 **Chen HY**, Lu FH, Chang CJ, Wang RS, Yang YC, Chang YF, Wu JS. Metabolic abnormalities, but not obesity per se, associated with chronic kidney disease in a Taiwanese population. *Nutr Metab Cardiovasc Dis* 2020; **30**: 418-425 [PMID: 31744713 DOI: 10.1016/j.numecd.2019.09.029]

61 **Vangipurapu J**, Stančáková A, Kuulasmaa T, Kuusisto J, Laakso M. Both fasting and glucose-stimulated proinsulin levels predict hyperglycemia and incident type 2 diabetes: a population-based study of 9,396 Finnish men. *PLoS One* 2015; **10**: e0124028 [PMID: 25853252 DOI: 10.1371/journal.pone.0124028]

62 **Hjelmgren O**, Gummesson A, Bergström G, Schmidt C. Beta-Cell Function, Self-rated Health, and Lifestyle Habits in 64-Year-Old Swedish Women with Metabolically Healthy Obesity Phenotype. *J Obes Metab Syndr* 2020; **29**: 39-46 [PMID: 32200605 DOI: 10.7570/jomes19078]

63 **Zhao RX**, He Q, Sha S, Song J, Qin J, Liu P, Sun YJ, Sun L, Hou XG, Chen L. Increased AHR Transcripts Correlate With Pro-inflammatory T-Helper Lymphocytes Polarization in Both Metabolically Healthy Obesity and Type 2 Diabetic Patients. *Front Immunol* 2020; **11**: 1644 [PMID: 32849564 DOI: 10.3389/fimmu.2020.01644]

64 **Hellmér J**, Arner P, Lundin A. Automatic luminometric kinetic assay of glycerol for lipolysis studies. *Anal Biochem* 1989; **177**: 132-137 [PMID: 2742143 DOI: 10.1016/0003-2697(89)90027-4]

65 **Prospective Studies Collaboration**. , Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, Halsey J, Qizilbash N, Collins R, Peto R. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *Lancet* 2009; **373**: 1083-1096 [PMID: 19299006 DOI: 10.1016/S0140-6736(09)60318-4]

66 **Shu CJ**, Benoist C, Mathis D. The immune system's involvement in obesity-driven type 2 diabetes. *Semin Immunol* 2012; **24**: 436-442 [PMID: 23333525 DOI: 10.1016/j.smim.2012.12.001]

67 **Ndisang JF**, Rastogi S, Vannacci A. Immune and inflammatory processes in obesity, insulin resistance, diabetes, and related cardiometabolic complications. *J Immunol Res* 2014; **2014**: 579560 [PMID: 25328894 DOI: 10.1155/2014/579560]

68 **Zhao R**, Tang D, Yi S, Li W, Wu C, Lu Y, Hou X, Song J, Lin P, Chen L, Sun L. Elevated peripheral frequencies of Th22 cells: a novel potent participant in obesity and type 2 diabetes. *PLoS One* 2014; **9**: e85770 [PMID: 24465695 DOI: 10.1371/journal.pone.0085770]

69 **Weisberg SP**, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003; **112**: 1796-1808 [PMID: 14679176 DOI: 10.1172/jci200319246]

70 **Christou KA**, Christou GA, Karamoutsios A, Vartholomatos G, Gartzonika K, Tsatsoulis A, Tigas S. Metabolically Healthy Obesity Is Characterized by a Proinflammatory Phenotype of Circulating Monocyte Subsets. *Metab Syndr Relat Disord* 2019; **17**: 259-265 [PMID: 30864887 DOI: 10.1089/met.2018.0132]

71 **Patiño R**, Ibarra J, Rodriguez A, Yagüe MR, Pintor E, Fernandez-Cruz A, Figueredo A. Circulating monocytes in patients with diabetes mellitus, arterial disease, and increased CD14 expression. *Am J Cardiol* 2000; **85**: 1288-1291 [PMID: 10831941 DOI: 10.1016/s0002-9149(00)00757-8]

72 **Terasawa T**, Aso Y, Omori K, Fukushima M, Momobayashi A, Inukai T. Bezafibrate, a peroxisome proliferator-activated receptor α agonist, decreases circulating CD14(+)CD16(+) monocytes in patients with type 2 diabetes. *Transl Res* 2015; **165**: 336-345 [PMID: 25134759 DOI: 10.1016/j.trsl.2014.07.008]

73 **Dalmas E**, Venteclef N, Caer C, Poitou C, Cremer I, Aron-Wisnewsky J, Lacroix-Desmazes S, Bayry J, Kaveri SV, Clément K, André S, Guerre-Millo M. T cell-derived IL-22 amplifies IL-1β-driven inflammation in human adipose tissue: relevance to obesity and type 2 diabetes. *Diabetes* 2014; **63**:

1966-1977 [PMID: 24520123 DOI: 10.2337/db13-1511]
74 **Cristancho AG**, Lazar MA. Forming functional fat: a growing understanding of adipocyte differentiation. *Nat Rev Mol Cell Biol* 2011; **12**: 722-734 [PMID: 21952300 DOI: 10.1038/nrm3198]
75 **Jaganathan R**, Ravindran R, Dhanasekaran S. Emerging Role of Adipocytokines in Type 2 Diabetes as Mediators of Insulin Resistance and Cardiovascular Disease. *Can J Diabetes* 2018; **42**: 446-456.e1 [PMID: 29229313 DOI: 10.1016/j.jcjd.2017.10.040]
76 **Tschritter O**, Fritsche A, Thamer C, Haap M, Shirkavand F, Rahe S, Staiger H, Maerker E, Häring H, Stumvoll M. Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. *Diabetes* 2003; **52**: 239-243 [PMID: 12540592 DOI: 10.2337/diabetes.52.2.239]
77 **Fu J**, Li Y, Esangbedo IC, Li G, Feng D, Li L, Xu L, Han L, Li M, Li C, Gao S, Willi SM. Circulating Osteonectin and Adipokine Profiles in Relation to Metabolically Healthy Obesity in Chinese Children: Findings From BCAMS. *J Am Heart Assoc* 2018; **7**: e009169 [PMID: 30571596 DOI: 10.1161/JAHA.118.009169]
78 **Larsen MA**, Isaksen VT, Moen OS, Wilsgaard L, Remijn M, Paulssen EJ, Florholmen J, Goll R. Leptin to adiponectin ratio - A surrogate biomarker for early detection of metabolic disturbances in obesity. *Nutr Metab Cardiovasc Dis* 2018; **28**: 1114-1121 [PMID: 30145019 DOI: 10.1016/j.numecd.2018.06.020]
79 **Carvalho LP**, Basso-Vanelli RP, Di Thommazo-Luporini L, Mendes RG, Oliveira-Junior MC, Vieira RP, Bonjorno-Junior JC, Oliveira CR, Luporini R, Borghi-Silva A. Myostatin and adipokines: The role of the metabolically unhealthy obese phenotype in muscle function and aerobic capacity in young adults. *Cytokine* 2018; **107**: 118-124 [PMID: 29246653 DOI: 10.1016/j.cyto.2017.12.008]
80 **Zhuo Q**, Wang Z, Fu P, Piao J, Tian Y, Xu J, Yang X. Comparison of adiponectin, leptin and leptin to adiponectin ratio as diagnostic marker for metabolic syndrome in older adults of Chinese major cities. *Diabetes Res Clin Pract* 2009; **84**: 27-33 [PMID: 19181412 DOI: 10.1016/j.diabres.2008.12.019]
81 **Goday A**, Benaiges D, Parri A, Ramón JM, Flores-Le Roux JA, Pedro Botet J; Obemar Group. Can bariatric surgery improve cardiovascular risk factors in the metabolically healthy but morbidly obese patient? *Surg Obes Relat Dis* 2014; **10**: 871-876 [PMID: 25282193 DOI: 10.1016/j.soard.2014.04.022]
82 **Pelascini E**, Disse E, Pasquer A, Poncet G, Gouillat C, Robert M. Should we wait for metabolic complications before operating on obese patients? *Surg Obes Relat Dis* 2016; **12**: 49-56 [PMID: 26164112 DOI: 10.1016/j.soard.2015.04.024]
83 **Genua I**, Tuneu L, Ramos A, Stantonyonge N, Caimari F, Balagué C, Fernández-Ananin S, Sánchez-Quesada JL, Pérez A, Miñambres I. Effectiveness of Bariatric Surgery in Patients with the Metabolically Healthy Obese Phenotype. *Obes Surg* 2021; **31**: 517-522 [PMID: 32915359 DOI: 10.1007/s11695-020-04967-6]
84 **Barzin M**, Aryannezhad S, Khalaj A, Mahdavi M, Valizadeh M, Ghareh S, Azizi F, Hosseinpanah F. Effects of bariatric surgery in different obesity phenotypes: Tehran Obesity Treatment Study (TOTS). *Obes Surg* 2020; **30**: 461-469 [PMID: 31650407 DOI: 10.1007/s11695-019-04182-y]
85 **Slagter SN**, Corpeleijn E, van der Klauw MM, Sijtsma A, Swart-Busscher LG, Perenboom CWM, de Vries JHM, Feskens EJM, Wolffenbuttel BHR, Kromhout D, van Vliet-Ostaptchouk JV. Dietary patterns and physical activity in the metabolically (un)healthy obese: the Dutch Lifelines cohort study. *Nutr J* 2018; **17**: 18 [PMID: 29433580 DOI: 10.1186/s12937-018-0319-0]
86 **de Winter M**, Rioux BV, Boudreau JG, Bouchard DR, Sénéchal M. Physical Activity and Sedentary Patterns among Metabolically Healthy Individuals Living with Obesity. *J Diabetes Res* 2018; **2018**: 7496768 [PMID: 29707585 DOI: 10.1155/2018/7496768]
87 **Gomez-Huelgas R**, Ruiz-Nava J, Santamaria-Fernandez S, Vargas-Candela A, Alarcon-Martin AV, Tinahones FJ, Bernal-Lopez MR. Impact of Intensive Lifestyle Modification on Levels of Adipokines and Inflammatory Biomarkers in Metabolically Healthy Obese Women. *Mediators Inflamm* 2019; **2019**: 4165260 [PMID: 31093012 DOI: 10.1155/2019/4165260]
88 **Lucht S**, Hennig F, Moebus S, Führer-Sakel D, Herder C, Jöckel KH, Hoffmann B; Heinz Nixdorf Recall Study Investigative Group. Air pollution and diabetes-related biomarkers in non-diabetic adults: A pathway to impaired glucose metabolism? *Environ Int* 2019; **124**: 370-392 [PMID: 30660850 DOI: 10.1016/j.envint.2019.01.005]
89 **Aguilar-Salinas CA**, García EG, Robles L, Riaño D, Ruiz-Gomez DG, García-Ulloa AC, Melgarejo MA, Zamora M, Guillen-Pineda LE, Mehta R, Canizales-Quinteros S, Tusie Luna MT, Gómez-Pérez FJ. High adiponectin concentrations are associated with the metabolically healthy obese phenotype. *J Clin Endocrinol Metab* 2008; **93**: 4075-4079 [PMID: 18682512 DOI: 10.1210/jc.2007-2724]
90 **van Vliet-Ostaptchouk JV**, Nuotio ML, Slagter SN, Doiron D, Fischer K, Foco L, Gaye A, Gögele M, Heier M, Hiekkalinna T, Joensuu A, Newby C, Pang C, Partinen E, Reischl E, Schwienbacher C, Tammesoo ML, Swertz MA, Burton P, Ferretti V, Fortier I, Giepmans L, Harris JR, Hillege HL, Holmen J, Jula A, Kootstra-Ros JE, Kvaløy K, Holmen TL, Männistö S, Metspalu A, Midthjell K, Murtagh MJ, Peters A, Pramstaller PP, Saaristo T, Salomaa V, Stolk RP, Uusitupa M, van der Harst P, van der Klauw MM, Waldenberger M, Perola M, Wolffenbuttel BH. The prevalence of metabolic syndrome and metabolically healthy obesity in Europe: a collaborative analysis of ten large cohort studies. *BMC Endocr Disord* 2014; **14**: 9 [PMID: 24484869 DOI: 10.1186/1472-6823-14-9]
91 **Liu M**, Tang R, Wang J, He Y. Distribution of metabolic/obese phenotypes and association with diabetes: 5 years' cohort based on 22,276 elderly. *Endocrine* 2018; **62**: 107-115 [PMID: 30006803 DOI: 10.1007/s12020-018-1672-7]

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MINIREVIEWS

Role of dipeptidyl peptidase 4 inhibitors in the new era of antidiabetic treatment

Matilda Florentin, Michael S Kostapanos, Athanasia K Papazafiropoulou

ORCID number: Matilda Florentin 0000-0002-0135-9694; Michael S Kostapanos 0000-0002-7513-5319; Athanasia K Papazafiropoulou 0000-0002-7596-4942.

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Matilda Florentin, Department of Internal Medicine, School of Medicine, University of Ioannina, Ioannina 45221, Greece

Michael S Kostapanos, Lipid Clinic, Department of General Medicine, Cambridge University Hospitals NHS Foundation Trust, Addenbrooke's Hospital, Cambridge CB2 0QQ, United Kingdom

Athanasia K Papazafiropoulou, 1st Department of Internal Medicine and Diabetes Center, Tzaneio General Hospital of Piraeus, Athens 18536, Greece

Corresponding author: Athanasia K Papazafiropoulou, MD, MSc, PhD, Consultant Physician-Scientist, 1st Department of Internal Medicine and Diabetes Center, Tzaneio General Hospital of Piraeus, Leoforos Afentouli, Athens 18536, Greece. athpapazafiropoulou@gmail.com

Abstract

The last few years important changes have occurred in the field of diabetes treatment. The priority in the therapy of patients with diabetes is not glycemic control per se rather an overall management of risk factors, while individualization of glycemic target is suggested. Furthermore, regulatory authorities now require evidence of cardiovascular (CV) safety in order to approve new antidiabetic agents. The most novel drug classes, *i.e.*, sodium-glucose transporter 2 inhibitors (SGLT2-i) and some glucagon-like peptide-1 receptor agonists (GLP-1 RA), have been demonstrated to reduce major adverse CV events and, thus, have a prominent position in the therapeutic algorithm of hyperglycemia. In this context, the role of previously used hypoglycemic agents, including dipeptidyl peptidase 4 (DPP-4) inhibitors, has been modified. DPP-4 inhibitors have a favorable safety profile, do not cause hypoglycemia or weight gain and do not require dose uptitration. Furthermore, they can be administered in patients with chronic kidney disease after dose modification and elderly patients with diabetes. Still, though, they have been undermined to a third line therapeutic choice as they have not been shown to reduce CV events as is the case with SGLT2-i and GLP-1 RA. Overall, DPP-4 inhibitors appear to have a place in the management of patients with diabetes as a safe class of oral glucose lowering agents with great experience in their use.

Key Words: Cardiovascular safety; Dipeptidyl peptidase 4 inhibitors; Glucose lowering; Hypoglycemia; Therapeutic algorithm; Weight gain

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Core Tip: Dipeptidyl peptidase 4 inhibitors have a favorable safety profile, do not frequently cause hypoglycemia and weight gain, while they may be used in patients with kidney impairment and the elderly. Despite not reducing cardiovascular events, they still have a place in the diabetes treatment algorithm in several patients.

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INTRODUCTION

Diabetes mellitus (DM) is a worldwide health problem with epidemic proportions and a huge economic burden. The global prevalence of DM in 2019 was estimated to be 9.3% (463 million people) with a projection to rise to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045[1]. DM is a major cause of blindness, chronic kidney disease (CKD), stroke, lower extremity amputations and death from coronary heart disease and heart failure (HF)[2].

Until a few years ago the main focus of the management of patients with DM was the adequate or even strict glycemic control, mainly based on the fact that a glycated hemoglobin (HbA1c) of < 7% has been associated with a reduction in microvascular complications[3]. However, intensive glycemic control not only does not appear to reduce all-cause mortality and macrovascular endpoints in patients with DM type 2 (DM2), but it may increase the relative risk (RR) of severe hypoglycemia up to 30%[3,4]. Therefore, the glycemic target needs to be individualized and associated risk factors and co-morbidities be appropriately managed[5].

Another issue which emerged over a decade ago, due to concerns about agents such as rosiglitazone, is the cardiovascular (CV) safety of antidiabetic agents[6,7]. Ever since the regulatory authorities, such as the U.S. Food and Drug Administration (FDA)[8] and the European Medicines Agency (EMA)[9], require large CV outcomes trials (CVOTs) for all new treatments for DM2. Incretin-based therapies, *i.e.*, glucagon-like peptide-1 (GLP-1) receptor agonists (GLP-1 RA) and dipeptidyl peptidase 4 (DPP-4) inhibitors, and subsequent drug classes have, thus, been approved after their efficacy was established in CVOTs.

Importantly, about 6 years ago a novel class of drugs, namely sodium-glucose transporter 2 (SGLT2) inhibitors (SGLT2-i), was demonstrated to reduce major adverse CV events (MACE) and mainly hospitalizations for HF[10]. Of note, a recent meta-analysis demonstrated that SGLT2-i significantly improve CV outcomes including CV and all-cause mortality in patients with HF without excess risk of serious adverse events[11], while their capacity to slow the progression of CKD and/or albuminuria or even improve renal function has already been established[12-14].

Some GLP-1 RA were also found to decrease MACE, as well as secondary outcomes (*e.g.*, HF and progression of renal disease) in patients with established CV disease (CVD) or CKD. Furthermore, recent evidence demonstrated that these drugs reduce the risk of nonfatal stroke in patients with DM2[15].

These findings consequently changed the guidelines for the management of hyperglycemia in patients with DM2[5]. Therefore, the role of drugs which were used as second line agents (after metformin) in the therapeutic algorithm has been adjusted. DPP-4 inhibitors fall into this category. In this paper, we discuss the characteristics and CVOTs of this class of drugs as well as their current role in the therapeutic armamentarium of DM2.

MECHANISM OF ACTION AND CHARACTERISTICS OF DPP-4 INHIBITORS

In 2006 the first DPP-4 inhibitor, sitagliptin, was approved for the treatment of diabetes[16,17]. These drugs inhibit DPP-4, *i.e.*, the enzyme that degrades incretins,

subsequently prolonging their half-life[18]. Two such hormones have been identified in humans; glucose-dependent insulinotropic peptide or gastric inhibitory polypeptide (GIP) and GLP-1. The latter may achieve glucose lowering *via* various actions. Specifically, GLP-1 enhances glucose-dependent insulin secretion[19], activates insulin biosynthesis and gene transcription, thus restoring the cellular supplies of insulin for subsequent release[20], while it suppresses glucagon secretion[21,22] and food intake [23,24] and slows gastric emptying[25].

In DM2 there is a reduction in GLP-1 secretion[26], an effect which in part accounts for the impaired "incretin effect" in patients with diabetes[27]. The "incretin effect" stands for the observation that insulin response to glucose is amplified when insulin is delivered orally *vs* intravenously[28]. By inhibiting the enzyme which is responsible for the degradation of incretin hormones, *i.e.*, DPP-4, DPP-4 inhibitors prevent the proteolytic breakdown and inactivation of GLP-1 and GIP[29,30]. Typically, these drugs decrease serum DPP-4 activity by > 80%, which translates in doubling of intact, biologically active GLP-1 concentration[31] along with a significant reduction in postprandial glucose levels[31,32] and an approximately 0.8% decrease in HbA1c[33]. Importantly, DPP-4 inhibitors do not increase the risk of hypoglycemia, which is a major concern and an unfavorable prognostic factor in patients treated with antidiabetic agents. This occurs as native GLP-1, whose action is prolonged by DPP-4 inhibitors, stimulates glucose-dependent insulin secretion from pancreatic β-cells[34].

Dissimilarities in the chemical structure of the different DPP-4 inhibitors affect their pharmacokinetic properties, formulation and daily dosing (Table 1). The relatively long half-lives of sitagliptin, linagliptin and alogliptin allow for once-daily dosing. Saxagliptin, which has a short half life, may also be administered once daily due to the presence of its active metabolite, BMS-510849, which inhibits DPP-4[35-37]. In contrast, vildagliptin has a short half-life and, thus, requires twice-daily dosing[38]. As far as route of elimination is concerned, sitagliptin and alogliptin are primarily excreted renally, whereas saxagliptin undergoes both renal and hepatic clearance. In contrast, linagliptin is predominately (approximately 90%) secreted unchanged in the feces[39], while vildagliptin is metabolized *via* at least four pathways before excretion[38,40]. Regarding CKD, all DPP-4 inhibitors may be given to patients at all CKD stages in reduced doses in order to avoid increased drug exposure[38,40], with the exception of linagliptin which does not require dose modification. Furthermore, saxagliptin is contraindicated in end-stage renal disease (ESRD) and in dialysis[38] (Table 2). This agent is also prone to drug-drug interactions as it is metabolized *via* cytochrome P450 (CYP450). Hence, patients co-administered saxagliptin and CYP3A4/5 inhibitors should reduce saxagliptin dose[38,41]. Table 3 summarizes the doses which are appropriate for all stages of hepatic impairment for each DPP-4 inhibitor.

DDP-4 INHIBITORS IN CVOTS

Since over 10 years ago concerns have been raised as to the CV safety of certain antidiabetic drugs[42]. Subsequently, the FDA requires evidence of CV safety before approval of any new antidiabetic agent. In this context, no drugs that could be associated with an unacceptable level of CV risk in clinical trials would be approved for the management of DM2. Incretin-based therapies, including DDP-4 inhibitors, were the newer antidiabetic agents added to the DM2 treatment armamentarium at the time of this statement[42].

Consequently, randomized placebo-controlled clinical trials were designed to assess the CV safety of DDP-4 inhibitors. These studies mostly included high-risk patients with DM2. They had a non-inferiority design since the research question to be addressed at the time was safety rather than additional CV benefits, which were demonstrated only later with SGLT2-i and GLP-1 RA. To date, every DDP-4 inihibitor available for clinical use has been assessed in at least one of these trials (Table 4).

The trial evaluating cardiovascular outcomes with Sitagliptin (TECOS) trial included 14671 patients with DM2 with an HbA1c between 6.5 and 8.0% when treated with stable doses of one or two oral agents (*i.e.*, metformin, pioglitazone or sulfonylurea) or insulin (with or without metformin) and established CVD[43]. These patients were randomized to sitagliptin 50-100 mg/d *vs* placebo on top of standard treatment. The primary endpoint of this study was the composite of CV death, nonfatal myocardial infarction, nonfatal stroke, or hospitalization for unstable angina. This was a non-inferiority trial with upper safety boundary of 1.3 RR. During the 3 years of follow-up (median) sitagliptin was associated with mild though significant hypoglycemic effect; by lowering mean HbA1c by 0.29% points [95% confidence

Table 1 Characteristics of dipeptidyl peptidase 4 inhibitors

	Chemistry	Half-life	HbA1c reduction (%)	Metabolism	Eliminationroute
Alogliptin	Modifiedpyrimidinedione	20 h	0.6 (mean value)	Minimal	Predominantly (> 70%) renal
Linagliptin	Xanthine-based	Approxmately 12 h (effective), > 100 h (terminal)	0.5-0.7	Minimal	Predominantly biliary (< 6% renal)
Saxagliptin	Cyanopyrrolidine	2.5 h (parent), 3 h (metabolite)	0.5-1.0	Hydrolysis (cytochrome P450 3A4 or P450 3A5) to form an active metabolite	Metabolism (parent) and renal (metabolite)
Sitagliptin	β-aminoacid based	12.5 h	0.5-1.0	Minimal	Predominantly (> 80%)
Vildagliptin	Cyanopyrrolidine	Approxmately 2 h	0.9 (mean value)	Hydrolysis (cytochrome-independent) to form an inactive metabolite	Metabolism (parent) and renal (metabolite)

Table 2 Renal dosing of dipeptidyl peptidase 4 inhibitors

Renal impairment	Alogliptin	Linagliptin	Sitagliptin	Vildagliptin	Saxagliptin
Mild (eGFR > 50 mL/min)	25 mg o.d.	5 mg o.d.	100 mg o.d.	50 mg b.i.d.	5 mg o.d.
Moderate (eGFR 30-50 mL/min)	12.5 mg o.d.	5mg o.d.	50 mg o.d.	50 mg o.d.	2.5 mg o.d.
Severe (eGFR < 30 mL/min)	6.25 mg o.d.	5 mg o.d.	25 mg o.d.	50 mg o.d.	2.5 mg o.d.
ESRD	6.25 mg o.d.	5 mg o.d.	25 mg o.d.	50 mg o.d.	Contraindicated
Renal dialysis	6.25 mg o.d.	5 mg o.d.	25 mg o.d.	50 mg o.d.	Contraindicated

eGFR: Estimated glomerular filtration rate; ESRD: End-stage renal disease.

Table 3 Modification of dosing for dipeptidyl peptidase 4 inhibitors in hepatic impairment

Hepatic impairment	Alogliptin	Linagliptin	Sitagliptin	Vildagliptin	Saxagliptin
Mild	25 mg o.d.	5 mg o.d.	100 mg o.d.	Not recommended in liver disease, including AST or ALT > 3 × ULN	5 mg o.d.
Moderate	25 mg o.d.	5mg o.d.	100 mg o.d.		Can be used with caution
Severe	Not recommended	5 mg o.d.	Can be used with caution		Not recommended

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ULN: Upper limit normal.

interval (CI): -0.32 to -0.27] compared with placebo. In the intention-to-treat analysis sitagliptin was non-inferior to placebo in the primary composite endpoint [hazard ratio (HR) 0.98; 95%CI: 0.88-1.09; $P < 0.001$ for non-inferiority]. The same was relevant for all secondary CV endpoints in this trial. Interestingly, acute pancreatitis or pancreatic cancer events did not differ significantly between the sitagliptin and the placebo group. Also, sitagliptin was not associated with any excessive risk of hospitalizations for HF compared with placebo[43].

Linagliptin was evaluated in a non-inferiority multicenter randomized placebo-controlled clinical trial. The Cardiovascular And Renal Microvascular Outcome study with Linagliptin (CARMELINA) study included 6979 patients at high risk for CVD [established CVD and significant albuminuria; urine albumin creatinine ratio (UACR) > 200 mg/g] or renal disease [low estimated glomerular estimated glomerular filtration rate (eGFR) and micro- or macro-albuminuria] and suboptimal glycaemic control (baseline HbA1c 6.5%-10%)[44]. These patients were randomized to linagliptin 5 mg/d *vs* placebo. The primary composite endpoint was the time to first occurrence of CV death or nonfatal myocardial infarction or stroke. The non-inferiority margins

Table 4 Cardiovascular outcome trials with dipeptidyl peptidase 4 inhibitors

	CVOT	Comparator	Cardiovascular safety (MACE) (HR)	Risk of hospitalization for heart failure (HR)
Alogliptin	EXAMINE	Placebo	0.96	1.07
Linagliptin	CARMELINA	Placebo	1.02	0.90
	CAROLINA	Glimepiride	0.98	1.21
Saxagliptin	SAVOR-TIMI	Placebo	1.00	1.27
Sitagliptin	TECOS	Placebo	0.98	1.00

CVOT: Cardiovascular outcome trial; HR: Hazard ratio; MACE: Major adverse cardiovascular events.

were the same as in the TECOS trial.

After 2.2 years (median) follow-up the overall difference in HbA1c over the full study duration was -0.36% (95%CI: -0.42% to -0.29% based on least-square means). The primary composite outcome occurred in 5.77/100 person-years *vs* 5.63/100 person-years in the linagliptin *vs* placebo group respectively; absolute incidence rate difference was 0.13 (95%CI: -0.63 to 0.90 per 100 person-years) (HR = 1.02; 95%CI: 0.89-1.17; $P < 0.001$ for non-inferiority). Similar were the findings for the key secondary renal endpoint of composite of adjudication-confirmed ESRD, death due to renal failure, or a sustained decrease of at least 40% in eGFR from baseline. No difference in the total mortality rates was noted between groups, too. Similarly, no difference between groups was observed in the components of the key secondary renal endpoint except for progression of albuminuria which occurred less frequently in the linagliptin *vs* the placebo group: 21.4/100 person-years *vs* 24.5/100 person-years respectively; absolute incidence rate difference, -3.18; 95%CI: -5.44 to -0.92) (HR = 0.86; 95%CI: 0.78-0.95; $P = 0.003$). Regarding safety, the incidence of pancreatitis episodes and pancreatic cancer was higher in the linagliptin compared with the placebo group though the number of cases was very limited in both groups to reach safe conclusions. No statistically significant different between groups was noted in hospitalizations for HF.

The CAROLINA study was another non-inferiority study that compared linagliptin with glimepiride as an active comparator[45]. It included patients with DM2 and suboptimal glycemic control (HbA1c 6.5%-8.5%) and high CV risk. The latter was defined as the presence of established CVD or microvascular complications, the presence of multiple CV risk factors or age > 70 years. These patients were randomized to linagliptin 5 mg/d *vs* glimepiride 1-4 mg/d with investigator-led option to add other antidiabetic agents titrated to achieve sufficient glycemic control. The primary composite endpoint and the non-inferiority margins were the same as in the CARMELINA study. After 6.3 years (median) no significant difference between groups was noted in the glycemic control. Similarly, linagliptin was non-inferior to glimepiride in the primary composite endpoint which occurred in 11.8% *vs* 12.0%, respectively [HR = 0.98 (95.47% CI: 0.84-1.14); $P < 0.001$ for noninferiority; $P = 0.76$ for superiority]. The same was relevant also for the individual components of the primary endpoint[45].

Furthermore, no differences between groups were noted in overall deaths and in hospitalizations for HF. As expected, the incidence of hypoglycemic events was lower in the linagliptin than in the glimepiride group: incidence rate difference, -8.7 [95%CI: -9.4 to -8.0; HR, 0.23 (95%CI: 0.21-0.26); $P < 0.001$]. Also, more weight gain was noted in the glimepiride group, with a mean between group difference of -1.54 kg (95%CI: -1.80 to -1.28). However, no difference in fasting plasma glucose, lipids and blood pressure was noted between groups. The results of this study established the role of linagliptin as a non-inferior to sulfonylureas second-line option (after metformin) for the management of DM2[45].

Non-inferiority of alogliptin (6.25-25 mg/d adjusted according to eGFR) *vs* placebo was evaluated in 5380 high-risk participants with DM2 of the Examination of Cardiovascular Outcomes with Alogliptin *vs* Standard of Care (EXAMINE) study[46]. These patients had a recent (within 15-90 d) hospitalization for an acute coronary syndrome and suboptimal glycemic control (HbA1c 6.5%-11.0% at screening or 7.0%-11.0% if the antidiabetic regimen included insulin). The primary endpoint was the composite of CV death or nonfatal myocardial infarction or stroke and the non-inferiority margins were similar to the studies above. After 17.5 mo (median) alogliptin was associated with a mild though significant hypoglycemic effect compared with placebo; mean difference in HbA1c between groups -0.36% points (95%CI: -0.43 to -

0.28; $P < 0.001$). No significant changes between groups were noted in body weight changes or changes in lipoprotein levels. At the end of follow-up the primary endpoint occurred in similar rates in both groups: 11.3% *vs* 11.8% in the alogliptin *vs* placebo group, respectively (HR = 0.96; upper boundary of the one-sided repeated CI, 1.16; $P < 0.001$ for non-inferiority; $P = 0.32$ for superiority). No difference between groups was noted in the individual components of this endpoint or in the overall or CV mortality. No safety signal regarding the risk of acute pancreatitis or pancreatic cancer was noted in this study. Changes in eGFR throughout the study were similar between groups.

Similar was the design of the Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus-Thrombolysis in Myocardial Infarction 53 (SAVOR-TIMI53) trial[47]. This was a phase 4 randomized placebo-controlled trial including 16492 patients with DM2 with suboptimal glycemic control (6.5%-12.0%) and high CV risk (in secondary prevention or in primary prevention with multiple CV risk factors). These patients were randomized to saxagliptin 2.5-5 mg/d (adjusted based on eGFR) *vs* placebo for 2.1 years (median). The primary endpoint was the same as in the EXAMINE trial, whilst a secondary major composite endpoint of CV death, myocardial infarction, stroke, hospitalization for unstable angina, coronary revascularization, or HF was assessed too. Saxagliptin was associated with significantly reduced HbA1c compared with placebo throughout the study (difference by 0.2% points at the end of follow-up) and with more patients achieving glycemic targets. However, no significant difference between groups was noted either in the primary or in the secondary major endpoint at the end of follow-up: HR = 1.00; 95%CI: 0.89-1.12; $P = 0.99$ for superiority; $P < 0.001$ for non-inferiority for the primary endpoint and HR = 1.02; 95%CI: 0.94-1.11; $P = 0.66$ for the secondary endpoint. Interestingly, among the individual components of these endpoints saxagliptin was associated with an increased risk of hospitalization for HF compared with placebo (HR = 1.27; 95%CI: 1.07-1.51; $P = 0.007$). As mentioned above no similar signal was identified with sitagliptin and linagliptin in the TECOS and CARMELINA trial, respectively.

This matter is of particular significance since worsening of HF has been associated with excessive mortality in patients with DM2. To further assess this question the Vildagliptin in Ventricular Dysfunction Diabetes (VIVIDD) trial included 254 patients with symptomatic HF [New York Heart Association (NYHA) class II and III] with reduced left ventricular ejection fraction (LVEF < 40%) and a HbA1c of 6.5%-10%[48]. These patients were randomized to vildagliptin 50 mg twice daily *vs* placebo for 52 wk. Vildagliptin was non-inferior to placebo in mean changes of LVEF assessed after ≥ 22 wk on treatment (adjusted mean change 4.95% ± 1.25% *vs* 4.33% ± 1.23% in the vildagliptin *vs* placebo group, respectively). This was not accompanied by any differences between the 2 groups regarding other HF outcomes, including NYHA classification status and hospitalizations for HF.

However, vildagliptin was associated with significant increases in the end-diastolic LV volume as well as a non-significant trend to increased end-systolic one. The latter could be attributed to pre-treatment differences between groups in this regard. Namely, mean baseline end-diastolic volumes and brain natriuretic peptide were higher in the vildagliptin than in the placebo group. Hence, patients randomized to vildagliptin may have been more susceptible to such changes. However, the clinical relevance of this finding was uncertain and was not accompanied with any worse HF outcomes.

Overall, the large-scale randomized placebo-controlled trials with DDP-4 inhibitors established their CV and overall safety for the management of high-risk patients with DM2. However, no evidence of superiority was demonstrated in CV outcomes as compared with controls or sulfonylurea treatment. To date, there are no published head-to-head comparison CVOTs between DDP-4 inhibitors and antidiabetic drugs with established CV efficacy such as SGLT2-i or GLP1-RA. Overall, the modest hypoglycemic effects alongside the neutral effect of DDP-4 inhibitors on the lipid profile, blood pressure and body weight make DDP-4 inhibitors less promising for CVD prevention compared with the SGLT2-i and GLP-1 RA[49]. Indeed, in a network meta-analysis (236 trials; 176,310 patients) the use of SGLT2-i or GLP1-RA was associated with lower mortality compared with DPP-4 inhibitors or placebo or no treatment. Treatment with DPP-4 inhibitors was not associated with lower mortality compared with placebo or no treatment[50].

SAFETY

No safety signals were identified in the aforementioned clinical trials in the risk of

acute pancreatitis or pancreatic cancer. These two clinical entities were regarded important safety issues until up to a few years ago, as there were several relevant reports and signals from clinical studies with these drugs[51]. However, a recent meta-analysis of randomized controlled trials demonstrated that the available data do not support an association of DPP-4 inhibitors with pancreatitis or pancreatic cancer. We should note that the evidence regarding pancreatic cancer is more limited and, thus, insufficient to draw definitive conclusions[52]. The excess of hospitalizations for HF associated with saxagliptin in the SAVOR-TIMI53 trial was not observed with the other DDP-4 inhibitors in CVOTs except a non-significant rise in the EXAMINE trial with alogliptin. In this context, regulatory authorities have added a warning in the labels of saxagliptin and alogliptin for the increased risk of HF[53]. The results of the VIVIDD study were reassuring as for the drug class. However, this matter should be investigated more in future longitudinal studies as the relatively short follow-up of these CVOTs may not be sufficient to detect a relevant safety signal.

Furthermore, as previously mentioned, this drug class does not increase the risk of hypoglycemia and is neutral in terms of weight gain, two issues important for patients with DM2, while other side effects are minor and reversible (*e.g.*, gastrointestinal adverse effects, flu-like symptoms).

CURRENT USE OF DPP-4 INHIBITORS

DPP-4 inhibitors were the first therapeutic choice after metformin initiation only up to a few years ago as they improve glycemic control without producing hypoglycemia or weight gain[54]. However, the inability to show a beneficial effect in morbidity and mortality as well as the significant findings of the large-scale CVOTs of the newer antidiabetic agents (*i.e.*, SGLT2-i and GLP-1 RA) have moved DPP-4 inhibitors lower in the algorithm of hyperglycemia management[5]. The above-mentioned change in the prescription of antidiabetic agents during the last years is reflected by the results of a recent study in Greece[55]. The percentage of patients treated with a DPP-4 inhibitor, a GLP-1 RA or a SGLT2-i in 2018 was 43.4%, 18.5% and 16.5%, respectively[55].

However, previous studies reflect the large use of DPP-4 inhibitors as a second choice of antidiabetic agents almost a decade ago. A large epidemiology study in the United States in a cohort of patients aged 18 years to 100 years who were newly initiated on oral hypoglycemic monotherapy between January 1, 2006, and December 31, 2008, showed that the greatest relative change for the study period was observed for the DPP-4 inhibitors, increasing from 0.4% to 7.3% or 0.15% per month[56]. Of note, during the period that the study was conducted GLP-1 RA and SGLT2-i were not available and, therefore, were not included in the analysis. The same pattern was observed in a study in Germany in elderly patients with an initial diagnosis of DM2 between January 2011 and December 2015, where the use of DPP-4 inhibitors raised from 13.4% to 19.8% during the study period[57]. The results of the study showed that DPP-4 inhibitors might be preferred over other drugs due to the good safety profile in elderly patients with DM2. At this point we should mention that there is lack of evidence regarding the trends of prescription of DPP-4 inhibitors. Another rather important issue is that there are large differences in prescription patterns, suggesting that the screening and management of DM2 varies among different countries.

THE PLACE OF DPP-4 INHIBITORS IN THE THERAPEUTIC ALGORITHM OF HYPERGLYCEMIA

In general, DPP-4 inhibitors cause a clinically meaningful reduction in blood glucose, have a low risk of hypoglycemia and a neutral effect on body weight, while their safety profile is overall favorable. They are also easy to use, requiring no dose titration and can be taken at any time of day regardless of meal times. Furthermore, DPP-4 inhibitors exhibit non-glycemic favorable effects including reductions in systolic blood pressure, total cholesterol and triglycerides, as well as improvement in β-cell function [35]. For the above reasons, until recently, they were a safe choice for the up titration of antidiabetic therapy after metformin. However, the large CVOTs with the newest agents, namely GLP-1 RA and SGLT2-i, have changed the treatment algorithm as well as the selection of DPP-4 inhibitors as a second-line add-on therapy to metformin[5].

DPP-4 inhibitors still have a place in the treatment of certain patients, such as those who take many drugs due to longstanding DM2 and have multiple co-morbidities, as

well as in those with renal impairment, where other anti-diabetic medications might be contraindicated. The frail elderly population may also benefit due to the low risk of hypoglycemia with DPP-4 inhibitors. Post-hoc analysis of the SAVOR-TIMI 53 data established the safety and efficacy of saxagliptin in the elderly[58], an observation that has been confirmed by other studies of DPP-4 inhibitors in this patient population[59, 60]. We should stress that saxagliptin is contraindicated in patients with HF due to the increased risk of hospitalizations for HF associated with its use[47].

Patients with advanced renal failure have fewer options of glucose lowering agents and often resort to treatment with complicated insulin regimens facing their accompanying increased hypoglycemia risk. Linagliptin might be a good choice as initial therapy in a patient with CKD at risk for hypoglycemia, while other DPP-4 inhibitors might be used with proper dose adjustment in these patients[38,39]. More recently, renoprotection was suggested as another beneficial property of DPP-4 inhibitors[36], which may be of clinical importance as diabetic nephropathy is a major complication of DM. Experimental data suggest that the modulation of innate immunity and inflammation are probably involved in these kidney-protective effects. The degradation of DPP-4, which is known to be expressed on the cell membrane of many types of cells including immune cells, as well as of several chemokines and cytokines[36], the attenuation of oxidative stress, fibrosis and cellular apoptosis in the kidney[37] are plausible underlying mechanisms.

According to recent guidelines, in patients with DM2 and established atherosclerotic CVD a GLP-1 RA or an SGLT2-i with proven CV safety (*i.e.*, it has label indication of reducing CVD events) should be preferably used. In patients with HF or CKD an SGLT2-i should be used due to the beneficial effects of these drugs in CVOTs, unless they are contraindicated (according to GFR levels); then a GLP-1 RA should be used [61].

When the therapeutic goals are not achieved with the previous antidiabetic agents, a combination with a DPP-4 inhibitor is recommended as a possible third-line therapy. The triple therapy of metformin with a DPP-4 and an SGLT2-i has a very low risk of hypoglycemia, leads to a further reduction in HbA1c, followed by weight loss and a reduction of blood pressure secondary to SGLT2-i administration[62-64]. Moreover, the dual effects of DPP4-i on α-cells and β-cells of the pancreas may combine well with the pancreatic islet-independent action of SGLT2-i.

DPP-4 inhibitors still remain a reasonable second-line add-on therapy to metformin, especially in individuals at high risk for hypoglycemia (*i.e.*, elderly) or when an oral regimen is preferred. DPP-4 inhibitors can also be combined with insulin therapy. The combination of basal insulin with a DPP-4 inhibitor is a practical treatment option without the need for multiple injections and glucose self-measurements for the adjustments of insulin[61].

CONCLUSION

Despite the establishment of SGLT2-i and GLP-1 RA as a second-line therapy in current diabetes treatment algorithms, DPP-4 inhibitors still remain a useful tool for the management of patients with diabetes. Furthermore, the lack of evidence with SGLT2-i and GLP-1 RA in elderly patients with diabetes as well as the contraindication of SGLT2-i in patients with CKD grade 3A and lower, make DPP-4 inhibitors a safe choice in such populations. Concluding, DPP-4 inhibitors still appear to have a place in the management of patients with DM2 as a safe class of oral glucose lowering agents with great experience in their use.

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 **World Health Organization**. Diabetes. [cited 25 April 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/news-room/fact-sheets/detail/diabetes

3 **Cosentino F**, Grant PJ, Aboyans V, Bailey CJ, Ceriello A, Delgado V, Federici M, Filippatos G, Grobbee DE, Hansen TB, Huikuri HV, Johansson I, Jüni P, Lettino M, Marx N, Mellbin LG, Östgren CJ, Rocca B, Roffi M, Sattar N, Seferović PM, Sousa-Uva M, Valensi P, Wheeler DC; ESC Scientific

Document Group. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. *Eur Heart J* 2020; **41**: 255-323 [PMID: 31497854 DOI: 10.1093/eurheartj/ehz486]

4 **Hemmingsen B**, Lund SS, Gluud C, Vaag A, Almdal T, Hemmingsen C, Wetterslev J. Intensive glycaemic control for patients with type 2 diabetes: systematic review with meta-analysis and trial sequential analysis of randomised clinical trials. *BMJ* 2011; **343**: d6898 [PMID: 22115901 DOI: 10.1136/bmj.d6898]

5 **Davies MJ**, D'Alessio DA, Fradkin J, Kernan WN, Mathieu C, Mingrone G, Rossing P, Tsapas A, Wexler DJ, Buse JB. Management of Hyperglycemia in Type 2 Diabetes, 2018. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 2018; **41**: 2669-2701 [PMID: 30291106 DOI: 10.2337/dci18-0033]

6 **Caveney E**, Turner JR. White paper. A review of FDA guidance: understanding the FDA guidance on assessing cardiovascular risks for new antidiabetic therapies. [cited 25 April 2021]. In: IQVIA [Internet]. Available from: http://www.quintiles.com/Library/white-papers/newfda-guidance-on-antidiabetic-therapies

7 **Nissen SE**, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* 2007; **356**: 2457-2471 [PMID: 17517853 DOI: 10.1056/NEJMoa072761]

8 **U.S. Food and Drug Administration**. Guidance for industry: diabetes mellitus-evaluating cardiovascular risk in new antidiabetic therapies to treat type 2 diabetes. [cited 25 April 2021]. In: U.S. Food and Drug Administration [Internet]. Available from: www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm071627.pdf

9 **European Medicines Agency**. Guideline on clinical investigation of medicinal products in the treatment or prevention of diabetes mellitus. [cited 25 April 2021]. In: European Medicines Agency [Internet]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/06/WC500129256.pdf

10 **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]

11 **Butler J**, Usman MS, Khan MS, Greene SJ, Friede T, Vaduganathan M, Filippatos G, Coats AJS, Anker SD. Efficacy and safety of SGLT2 inhibitors in heart failure: systematic review and meta-analysis. *ESC Heart Fail* 2020; **7**: 3298-3309 [PMID: 33586910 DOI: 10.1002/ehf2.13169]

12 **Butler J**, Zannad F, Fitchett D, Zinman B, Koitka-Weber A, von Eynatten M, Zwiener I, George J, Brueckmann M, Cheung AK, Wanner C. Empagliflozin Improves Kidney Outcomes in Patients With or Without Heart Failure. *Circ Heart Fail* 2019; **12**: e005875 [PMID: 31163986 DOI: 10.1161/CIRCHEARTFAILURE.118.005875]

13 **Heerspink HJL**, Stefánsson BV, Correa-Rotter R, Chertow GM, Greene T, Hou FF, Mann JFE, McMurray JJV, Lindberg M, Rossing P, Sjöström CD, Toto RD, Langkilde AM, Wheeler DC; DAPA-CKD Trial Committees and Investigators. Dapagliflozin in Patients with Chronic Kidney Disease. *N Engl J Med* 2020; **383**: 1436-1446 [PMID: 32970396 DOI: 10.1056/NEJMoa2024816]

14 **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]

15 **Bellastella G**, Maiorino MI, Longo M, Scappaticcio L, Chiodini P, Esposito K, Giugliano D. Glucagon-Like Peptide-1 Receptor Agonists and Prevention of Stroke Systematic Review of Cardiovascular Outcome Trials With Meta-Analysis. *Stroke* 2020; **51**: 666-669 [PMID: 31813360 DOI: 10.1161/STROKEAHA.119.027557]

16 **Nathan DM**. Finding new treatments for diabetes--how many, how fast... how good? *N Engl J Med* 2007; **356**: 437-440 [PMID: 17267901 DOI: 10.1056/NEJMp068294]

17 **Nathan DM**, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, Zinman B. Management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: update regarding thiazolidinediones: a consensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2008; **31**: 173-175 [PMID: 18165348 DOI: 10.2337/dc08-9016]

18 **Drucker DJ**. Enhancing incretin action for the treatment of type 2 diabetes. *Diabetes Care* 2003; **26**: 2929-2940 [PMID: 14514604 DOI: 10.2337/diacare.26.10.2929]

19 **Nauck MA**, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W. Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 1993; **36**: 741-744 [PMID: 8405741 DOI: 10.1007/BF00401145]

20 **Drucker DJ**, Philippe J, Mojsov S, Chick WL, Habener JF. Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc Natl Acad Sci U S A* 1987; **84**: 3434-3438 [PMID: 3033647 DOI: 10.1073/pnas.84.10.3434]

21 **Komatsu R**, Matsuyama T, Namba M, Watanabe N, Itoh H, Kono N, Tarui S. Glucagonostatic and insulinotropic action of glucagonlike peptide I-(7-36)-amide. *Diabetes* 1989; **38**: 902-905 [PMID: 2661287 DOI: 10.2337/diab.38.7.902]

22 **Hare KJ**, Vilsbøll T, Asmar M, Deacon CF, Knop FK, Holst JJ. The glucagonostatic and

insulinotropic effects of glucagon-like peptide 1 contribute equally to its glucose-lowering action. *Diabetes* 2010; **59**: 1765-1770 [PMID: 20150286 DOI: 10.2337/db09-1414]

23 **Gutzwiller JP**, Drewe J, Göke B, Schmidt H, Rohrer B, Lareida J, Beglinger C. Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. *Am J Physiol* 1999; **276**: R1541-R1544 [PMID: 10233049 DOI: 10.1152/ajpregu.1999.276.5.R1541]

24 **Gutzwiller JP**, Göke B, Drewe J, Hildebrand P, Ketterer S, Handschin D, Winterhalder R, Conen D, Beglinger C. Glucagon-like peptide-1: a potent regulator of food intake in humans. *Gut* 1999; **44**: 81-86 [PMID: 9862830 DOI: 10.1136/gut.44.1.81]

25 **Meier JJ**, Gallwitz B, Salmen S, Goetze O, Holst JJ, Schmidt WE, Nauck MA. Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2003; **88**: 2719-2725 [PMID: 12788879 DOI: 10.1210/jc.2003-030049]

26 **Vilsbøll T**, Holst JJ. Incretins, insulin secretion and Type 2 diabetes mellitus. *Diabetologia* 2004; **47**: 357-366 [PMID: 14968296 DOI: 10.1007/s00125-004-1342-6]

27 **Nauck M**, Stöckmann F, Ebert R, Creutzfeldt W. Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia* 1986; **29**: 46-52 [PMID: 3514343 DOI: 10.1007/BF02427280]

28 **Perley MJ**, Kipnis DM. Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic sujbjects. *J Clin Invest* 1967; **46**: 1954-1962 [PMID: 6074000 DOI: 10.1172/JCI105685]

29 **Deacon CF**, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Holst JJ. Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH2-terminus in type II diabetic patients and in healthy subjects. *Diabetes* 1995; **44**: 1126-1131 [PMID: 7657039 DOI: 10.2337/diab.44.9.1126]

30 **Nauck MA**. Incretin-based therapies for type 2 diabetes mellitus: properties, functions, and clinical implications. *Am J Med* 2011; **124**: S3-18 [PMID: 21194578 DOI: 10.1016/j.amjmed.2010.11.002]

31 **Ahrén B**, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A. Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. *J Clin Endocrinol Metab* 2004; **89**: 2078-2084 [PMID: 15126524 DOI: 10.1210/jc.2003-031907]

32 **Herman GA**, Bergman A, Stevens C, Kotey P, Yi B, Zhao P, Dietrich B, Golor G, Schrodter A, Keymeulen B, Lasseter KC, Kipnes MS, Snyder K, Hilliard D, Tanen M, Cilissen C, De Smet M, de Lepeleire I, Van Dyck K, Wang AQ, Zeng W, Davies MJ, Tanaka W, Holst JJ, Deacon CF, Gottesdiener KM, Wagner JA. Effect of single oral doses of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on incretin and plasma glucose levels after an oral glucose tolerance test in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2006; **91**: 4612-4619 [PMID: 16912128 DOI: 10.1210/jc.2006-1009]

33 **Craddy P**, Palin HJ, Johnson KI. Comparative effectiveness of dipeptidylpeptidase-4 inhibitors in type 2 diabetes: a systematic review and mixed treatment comparison. *Diabetes Ther* 2014; **5**: 1-41 [PMID: 24664619 DOI: 10.1007/s13300-014-0061-3]

34 **Nauck MA**, Vilsbøll T, Gallwitz B, Garber A, Madsbad S. Incretin-based therapies: viewpoints on the way to consensus. *Diabetes Care* 2009; **32** Suppl 2: S223-S231 [PMID: 19875556 DOI: 10.2337/dc09-S315]

35 **Kim NH**, Yu T, Lee DH. The nonglycemic actions of dipeptidyl peptidase-4 inhibitors. *Biomed Res Int* 2014; **2014**: 368703 [PMID: 25140306 DOI: 10.1155/2014/368703]

36 **Kodera R**, Shikata K. Renoprotective effects of incretin-based drugs: A novel pleiotropic effect of dipeptidyl peptidase-4 inhibitor. *J Diabetes Investig* 2016; **7**: 29-31 [PMID: 26816598 DOI: 10.1111/jdi.12380]

37 **Yang CT**, Lin WH, Li LJ, Ou HT, Kuo S. Association of Renal and Cardiovascular Safety With DPP-4 Inhibitors vs. Sulfonylureas in Patients With Type 2 Diabetes and Advanced Chronic Kidney Disease. *Clin Pharmacol Ther* 2021; **110**: 464-472 [PMID: 33866549 DOI: 10.1002/cpt.2262]

38 **Deacon CF**. Dipeptidyl peptidase-4 inhibitors in the treatment of type 2 diabetes: a comparative review. *Diabetes Obes Metab* 2011; **13**: 7-18 [PMID: 21114598 DOI: 10.1111/j.1463-1326.2010.01306.x]

39 **Forst T**, Pfützner A. Linagliptin, a dipeptidyl peptidase-4 inhibitor with a unique pharmacological profile, and efficacy in a broad range of patients with type 2 diabetes. *Expert Opin Pharmacother* 2012; **13**: 101-110 [PMID: 22149370 DOI: 10.1517/14656566.2012.642863]

40 **Scheen AJ**. Pharmacokinetics of dipeptidylpeptidase-4 inhibitors. *Diabetes Obes Metab* 2010; **12**: 648-658 [PMID: 20590741 DOI: 10.1111/j.1463-1326.2010.01212.x]

41 **Deacon CF**, Knudsen LB, Madsen K, Wiberg FC, Jacobsen O, Holst JJ. Dipeptidyl peptidase IV resistant analogues of glucagon-like peptide-1 which have extended metabolic stability and improved biological activity. *Diabetologia* 1998; **41**: 271-278 [PMID: 9541166 DOI: 10.1007/s001250050903]

42 **Kalyani RR**. Glucose-Lowering Drugs to Reduce Cardiovascular Risk in Type 2 Diabetes. *N Engl J Med* 2021; **384**: 1248-1260 [PMID: 33789013 DOI: 10.1056/NEJMcp2000280]

43 **Green JB**, Bethel MA, Armstrong PW, Buse JB, Engel SS, Garg J, Josse R, Kaufman KD, Koglin J, Korn S, Lachin JM, McGuire DK, Pencina MJ, Standl E, Stein PP, Suryawanshi S, Van de Werf F, Peterson ED, Holman RR; TECOS Study Group. Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2015; **373**: 232-242 [PMID: 26052984 DOI: 10.1056/NEJMoa1501352]

44 **Rosenstock J**, Perkovic V, Johansen OE, Cooper ME, Kahn SE, Marx N, Alexander JH, Pencina M, Toto RD, Wanner C, Zinman B, Woerle HJ, Baanstra D, Pfarr E, Schnaidt S, Meinicke T, George JT,

von Eynatten M, McGuire DK; CARMELINA Investigators. Effect of Linagliptin *vs* Placebo on Major Cardiovascular Events in Adults With Type 2 Diabetes and High Cardiovascular and Renal Risk: The CARMELINA Randomized Clinical Trial. *JAMA* 2019; **321**: 69-79 [PMID: 30418475 DOI: 10.1001/jama.2018.18269]

45 **Rosenstock J**, Kahn SE, Johansen OE, Zinman B, Espeland MA, Woerle HJ, Pfarr E, Keller A, Mattheus M, Baanstra D, Meinicke T, George JT, von Eynatten M, McGuire DK, Marx N; CAROLINA Investigators. Effect of Linagliptin *vs* Glimepiride on Major Adverse Cardiovascular Outcomes in Patients With Type 2 Diabetes: The CAROLINA Randomized Clinical Trial. *JAMA* 2019; **322**: 1155-1166 [PMID: 31536101 DOI: 10.1001/jama.2019.13772]

46 **White WB**, Cannon CP, Heller SR, Nissen SE, Bergenstal RM, Bakris GL, Perez AT, Fleck PR, Mehta CR, Kupfer S, Wilson C, Cushman WC, Zannad F; EXAMINE Investigators. Alogliptin after acute coronary syndrome in patients with type 2 diabetes. *N Engl J Med* 2013; **369**: 1327-1335 [PMID: 23992602 DOI: 10.1056/NEJMoa1305889]

47 **Scirica BM**, Bhatt DL, Braunwald E, Steg PG, Davidson J, Hirshberg B, Ohman P, Frederich R, Wiviott SD, Hoffman EB, Cavender MA, Udell JA, Desai NR, Mosenzon O, McGuire DK, Ray KK, Leiter LA, Raz I; SAVOR-TIMI 53 Steering Committee and Investigators. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *N Engl J Med* 2013; **369**: 1317-1326 [PMID: 23992601 DOI: 10.1056/NEJMoa1307684]

48 **McMurray JJV**, Ponikowski P, Bolli GB, Lukashevich V, Kozlovski P, Kothny W, Lewsey JD, Krum H; VIVIDD Trial Committees and Investigators. Effects of Vildagliptin on Ventricular Function in Patients With Type 2 Diabetes Mellitus and Heart Failure: A Randomized Placebo-Controlled Trial. *JACC Heart Fail* 2018; **6**: 8-17 [PMID: 29032139 DOI: 10.1016/j.jchf.2017.08.004]

49 **Phrommintikul A**, Wongcharoen W, Kumfu S, Jaiwongkam T, Gunaparn S, Chattipakorn S, Chattipakorn N. Effects of dapagliflozin *vs* vildagliptin on cardiometabolic parameters in diabetic patients with coronary artery disease: a randomised study. *Br J Clin Pharmacol* 2019; **85**: 1337-1347 [PMID: 30767253 DOI: 10.1111/bcp.13903]

50 **Zheng SL**, Roddick AJ, Aghar-Jaffar R, Shun-Shin MJ, Francis D, Oliver N, Meeran K. Association Between Use of Sodium-Glucose Cotransporter 2 Inhibitors, Glucagon-like Peptide 1 Agonists, and Dipeptidyl Peptidase 4 Inhibitors With All-Cause Mortality in Patients With Type 2 Diabetes: A Systematic Review and Meta-analysis. *JAMA* 2018; **319**: 1580-1591 [PMID: 29677303 DOI: 10.1001/jama.2018.3024]

51 **DeVries JH**, Rosenstock J. DPP-4 Inhibitor-Related Pancreatitis: Rare but Real! *Diabetes Care* 2017; **40**: 161-163 [PMID: 28108536 DOI: 10.2337/dci16-0035]

52 **Dicembrini I**, Montereggi C, Nreu B, Mannucci E, Monami M. Pancreatitis and pancreatic cancer in patientes treated with Dipeptidyl Peptidase-4 inhibitors: An extensive and updated meta-analysis of randomized controlled trials. *Diabetes Res Clin Pract* 2020; **159**: 107981 [PMID: 31870827 DOI: 10.1016/j.diabres.2019.107981]

53 **U.S. Food and Drug Administration**. FDA Drug Safety Communication: FDA adds warnings about heart failure risk to labels of type 2 diabetes medicines containing saxagliptin and alogliptin. [cited 25 April 2021]. In: U.S. Food and Drug Administration [Internet]. Available from: https://www.fda.gov/

54 **Inzucchi SE**, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR; American Diabetes Association (ADA); European Association for the Study of Diabetes (EASD). Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 2012; **35**: 1364-1379 [PMID: 22517736 DOI: 10.2337/dc12-0413]

55 **Athanasakis K**, Prodromiadou E, Papazafiropoulou A, Koutsovasilis A, Driva S, Ziori M, Georgopoulos E, Gougourelas D, Sotiropoulos A, Bousboulas S, Melidonis A, Liatis S. Twenty-year trends in the prescription costs of Type 2 diabetes: Real world data and empirical analysis in Greece. *Diabetes Res Clin Pract* 2020; **162**: 108095 [PMID: 32112790 DOI: 10.1016/j.diabres.2020.108095]

56 **Liao EP**. Patterns of medication initiation in newly diagnosed diabetes mellitus: quality and cost implications. *Am J Med* 2012; **125**: S1-S2 [PMID: 22998891 DOI: 10.1016/j.amjmed.2012.05.001]

57 **Kostev K**, Rockel T, Jacob L. Prescription Patterns and Disease Control in Type 2 Diabetes Mellitus Patients in Nursing Home and Home Care Settings: A Retrospective Analysis in Germany. *J Diabetes Sci Technol* 2018; **12**: 136-139 [PMID: 28539088 DOI: 10.1177/1932296817710477]

58 **Tatosian DA**, Guo Y, Schaeffer AK, Gaibu N, Popa S, Stoch A, Langdon RB, Kauh EA. Dipeptidyl peptidase-4 inhibition in patients with type 2 diabetes treated with saxagliptin, sitagliptin, or vildagliptin. *Diabetes Ther* 2013; **4**: 431-442 [PMID: 24163113 DOI: 10.1007/s13300-013-0045-8]

59 **Baranov O**, Kahle M, Deacon CF, Holst JJ, Nauck MA. Feedback suppression of meal-induced glucagon-like peptide-1 (GLP-1) secretion mediated through elevations in intact GLP-1 caused by dipeptidyl peptidase-4 inhibition: a randomized, prospective comparison of sitagliptin and vildagliptin treatment. *Diabetes Obes Metab* 2016; **18**: 1100-1109 [PMID: 27300579 DOI: 10.1111/dom.12706]

60 **Alsalim W**, Göransson O, Tura A, Pacini G, Mari A, Ahrén B. Persistent whole day meal effects of three dipeptidyl peptidase-4 inhibitors on glycaemia and hormonal responses in metformin-treated type 2 diabetes. *Diabetes Obes Metab* 2020; **22**: 590-598 [PMID: 31789451 DOI: 10.1111/dom.13934]

61 **American Diabetes Association**. 9. Pharmacologic Approaches to Glycemic Treatment: *Standards of Medical Care in Diabetes-2019*. *Diabetes Care* 2019; **42**: S90-S102 [PMID: 30559235 DOI:

10.2337/dc19-S009]

62 **Del Prato S**, Rosenstock J, Garcia-Sanchez R, Iqbal N, Hansen L, Johnsson E, Chen H, Mathieu C. Safety and tolerability of dapagliflozin, saxagliptin and metformin in combination: Post-hoc analysis of concomitant add-on *vs* sequential add-on to metformin and of triple *vs* dual therapy with metformin. *Diabetes Obes Metab* 2018; **20**: 1542-1546 [PMID: 29446523 DOI: 10.1111/dom.13258]

63 **Gallwitz B**. A safety evaluation of empagliflozin plus linagliptin for treating type 2 diabetes. *Expert Opin Drug Saf* 2017; **16**: 1399-1405 [PMID: 28934557 DOI: 10.1080/14740338.2017.1382471]

64 **Matthaei S**, Catrinoiu D, Celiński A, Ekholm E, Cook W, Hirshberg B, Chen H, Iqbal N, Hansen L. Randomized, Double-Blind Trial of Triple Therapy With Saxagliptin Add-on to Dapagliflozin Plus Metformin in Patients With Type 2 Diabetes. *Diabetes Care* 2015; **38**: 2018-2024 [PMID: 26324329 DOI: 10.2337/dc15-0811]

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ORIGINAL ARTICLE

Clinical and Translational Research

Altered spontaneous brain activity patterns in patients with diabetic retinopathy using amplitude of low-frequency fluctuation

Wen-Qing Shi, Mou-Xin Zhang, Li-Ying Tang, Lei Ye, Yu-Qing Zhang, Qi Lin, Biao Li, Yi Shao, Yao Yu

ORCID number: Wen-Qing Shi 0000-0002-9153-5865; Mou-Xin Zhang 0000-0001-8005-6167; Li-Ying Tang 0000-0002-9887-2135; Lei Ye 0000-0002-0303-812X; Yu-Qing Zhang 0000-0001-8096-0789; Qi Lin 0000-0002-9742-1674; Biao Li 0000-0002-7123-0321; Yi Shao 0000-0003-1571-2433; Yao Yu 0000-0002-0033-1016.

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Wen-Qing Shi, Lei Ye, Yu-Qing Zhang, Qi Lin, Biao Li, Yi Shao, Yao Yu, Department of Endocrinology and Ophthalmology, The First Affiliated Hospital of Nanchang University, Jiangxi clinical research center for endocrine and metabolic disease, Nanchang 330006, Jiangxi Province, China

Mou-Xin Zhang, Xiamen Eye Center of Xiamen University, School of Medicine, Xiamen University, Xiamen 361001, Fujian Province, China

Li-Ying Tang, Department of Ophthalmology, Zhongshan Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen 361001, Fujian Province, China

Corresponding author: Yao Yu, MD, Department of Endocrinology and Ophthalmology, The First Affiliated Hospital of Nanchang University, Jiangxi clinical research center for endocrine and metabolic disease, No. 17 Yongwaizheng Street, Donghu, Nanchang 330006, Jiangxi Province, China. 375135747@qq.com

Abstract

BACKGROUND

Diabetes mellitus is a metabolic disorder characterized by prolonged elevation of blood glucose due to various causes. Currently, the relationship between diabetic retinopathy (DR) and altered connectivity of brain function is unclear.

AIM

To investigate the relationship between this brain activity and clinical manifestations and behaviors of DR patients by using the amplitude of low-frequency fluctuation (ALFF) technique.

METHODS

Twenty-four DR patients and 24 healthy controls (HCs) matched for age and gender were enrolled. We measured and recorded average ALFF values of DR patients and HCs and then classified them using receiver operating characteristic (ROC) curves.

RESULTS

ALFF values of both left and right posterior cerebellar lobe and right anterior cingulate gyrus were remarkably higher in the DR patients than in the HCs; however, DR patients had lower values in the bilateral calcarine area. ROC curve analysis of different brain regions demonstrated high accuracy in the area under

the curve analysis. There was no significant relationship between mean ALFF values for different regions and clinical presentations in DR patients. Neuronal synchronization abnormalities in some brain regions of DR patients were associated with cognitive and visual disorders.

CONCLUSION

Abnormal spontaneous brain activity was observed in many areas of DR patients' brains, which may suggest a possible link between clinical manifestations and behaviors in DR patients.

Key Words: Amplitude of low-frequency fluctuation; Functional magnetic resonance imaging; Diabetic retinopathy; Resting-state; Diabetes mellitus; Spontaneous activity

Core Tip: We found that patients with diabetic retinopathy (DR) may have multiple low-frequency amplitude frequency changes in the brain, and the generation of this change may be related to the alteration of patients' visual cortex and anxiety, which may help us to explore the pathological mechanism and disease progression in DR patients.

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INTRODUCTION

Chronic complications of diabetes are the main causes of death and disability with diabetes, and its morbidity may be related to many factors, among which diabetic retinopathy (DR) is the main cause of blindness and visual impairment[1]. The incidence of DR is increasing worldwide mainly because of the longevity of diabetes patients[2]. A large proportion of adults who are over the age of 40 are affected by DR. Early manifestations of DR are mainly aneurysms, bleeding spots, hard exudation, cotton buds, venous beading, intravascular microvascular abnormalities, and macular edema[3]. According to the presence of retinal neovascularization, we can divide DR into proliferative DR (PDR) and non-proliferative DR (NPDR). In PDR, retinal damage stimulates neovascular growth. Neovascular growth is detrimental to the retina, which can cause fibrosis and even retinal detachment. Neovascularization can also enter the vitreous, which will lead to vitreous hemorrhage[4] (Figure 1). Recently, there has been increasing evidence that similar microvascular lesions happen in the brains of diabetics. Autopsy results in patients with chronic diabetes have shown that their brains developed a severe microvascular disease and neurological disease[5]. Pearce *et al*[6] pointed out that a diagnosis of DR suggests an increased risk of brain parenchymal disease in diabetic patients. These diabetic vascular and neurological complications interact for a long time, and we believe that the effects of diabetic complications on the brain's microvasculature are often overlooked. The previous gold standard for a DR diagnosis relied mainly on fundus fluorescence imaging, but it is not suitable for people with skin test allergies and poor liver and kidney function. However, there has been little analysis of changes in brain function in DR and its relationship to clinical manifestations in the eye to date. We hypothesized that the presence of DR may suggest central damage to the brain neural network caused by the existence of a microvascular system, while central damage in the brain neural network is an early predictor of DR.

Functional magnetic resonance imaging (fMRI) makes it possible to observe specific differences in the brain neural network[7] and studying these changes can enhance our knowledge of diseases. Thus, fMRI neuroimaging is of great interest for exploring the function of the central nervous system, considering it can monitor neural activity in

Nanchang University (Ethics approval number: 2017035), following the principles of the Declaration of Helsinki. Notify all subjects of the purpose, content and potential risks of the study, and provide written informed consent.

Clinical trial registration statement: This study is registered at Medical Ethics Comitee of the First Affiliated Hospital of Nanchang University trial registry. The registration identification number is JX2017035.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: This was not an industry supported study. The authors report no conflicts of interest in this work.

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the brain and can also provide some new explanations for the pathophysiological mechanism and pathogenesis of disease[8]. Resting-state fMRI (rs-fMRI) is a functional magnetic resonance technique used in resting-state functional network research. It explores the human nervous system through magnetic resonance imaging, which is simpler than task-state fMRI[9]. By combining electroneurophysiological records with fMRI, studies have demonstrated that low frequency (0.0-0.08 Hz) fluctuations (LFF) in blood oxygen level dependent (BOLD) fMRI signals are closely associated with spontaneous neuronal activity[10]. ALFF is one of the methods used to evaluate fMRI analysis of resting brain activity[11,12]. It reflects the intensity of local spontaneous brain activity at rest and brain endogenous/background neurophysiological processes. We have used the ALFF method to assess the neurological status and brain changes in some patients with eye diseases; for example, optic neuritis[13], glaucoma and strabismus[14,15], Parkinson's disease[16], in our previous studies.

MATERIALS AND METHODS

Subjects

We randomly selected 24 DR patients who visited the First Affiliated Hospital of Nanchang University, Nanchang, China. All the subjects met the following inclusion criteria: (1) Diagnosis of type 2 diabetes; (2) Diagnosis of PDR; (3) Fasting blood glucose controlled at approximately 7-10 mmol/L and blood glucose after a meal at 10-14 mmol/L; (4) No abnormality of the cerebral parenchyma on cranial MRI; (5) Without other ocular diseases bilaterally (*e.g.*, retinal detachment, glaucoma, amblyopia, ocular trauma, optic nerve disease); (6) Right-handed; and (7) Untreated PDR. The exclusion criteria were: (1) Smokers; (2) No other eye diseases; (3) Pregnant or lactating women; (4) Other diabetes complications; (5) Congenital systemic disease; (6) Mental illness (such as depression, memory impairment, cognitive impairment, and schizophrenia); and (7) Cerebral infarction diseases or cerebral vascular malformations. Twenty-four healthy controls (HCs) matched in age, educational status, and sex were enrolled. Both the DR group and HCs met the following criteria: (1) MRI showed no obvious damage or deformity of the brain parenchyma; (2) No history of brain infarction, cardiovascular disease or cerebral hemorrhage; (3) No evidence of drug or alcohol addiction; and (4) Were able to tolerate an MRI examination.

This study was conformed with the Declaration of Helsinki and had formal approval from the Medical Ethics Committee of the First Affiliated Hospital of Nanchang University. All the volunteers signed informed consent forms and were allowed to ask questions after learning about the purpose, content, and potential risks of this research.

Diagnostic criteria

The International Council of Ophthalmology in Sydney defined the international classification standard of DR in 2002, and the details are as follows: (1) In the early stage of the disease, after mydriasis, ophthalmoscopy revealed diffuse microaneurysms and small petechia in the posterior pole of the retina; some patients exhibited white or yellow exudate with complaints of blurry vision; (2) Retinopathy was found in the fundus under fundus angiography machine; (3) Using fundus fluorescein angiography, the number of microadenomas in the fundus was obviously increased and were beyond the results of fundus microscopy and there was capillary dilation around the retina, increased permeability, and increased abnormalities such as bleeding or neovascularization[17]. A patient with any of these symptoms was diagnosed as DR.

MRI parameters

In this research, we performed MRI scans using 3-Tesla MRI scanners (Siemens, Munich, Germany). All participants were asked to close their eyes and maintain natural breathing until the end of the scan. We applied a gradient echo sequence of pulses of the 3D variation to obtain function data, and the parameters were as follows: 176 structural images (acquisition matrix = 256 × 256, field of view = 250 mm × 250 mm, TR = 2 s, TE = 2.26 ms, cycle time = 1900 ms, thickness = 1.0 mm, gap = 0.5 mm, flip angle = 9°). We acquired 240 functional images in total (acquisition matrix = 64 × 64, field of view = 220 mm × 220 mm, thickness = 4.0 mm, gap = 1.2 mm, cycle time = 2000 ms, echo time = 30 ms, flip angle = 90°, 29 axial). A typical scan took 15 min to complete.

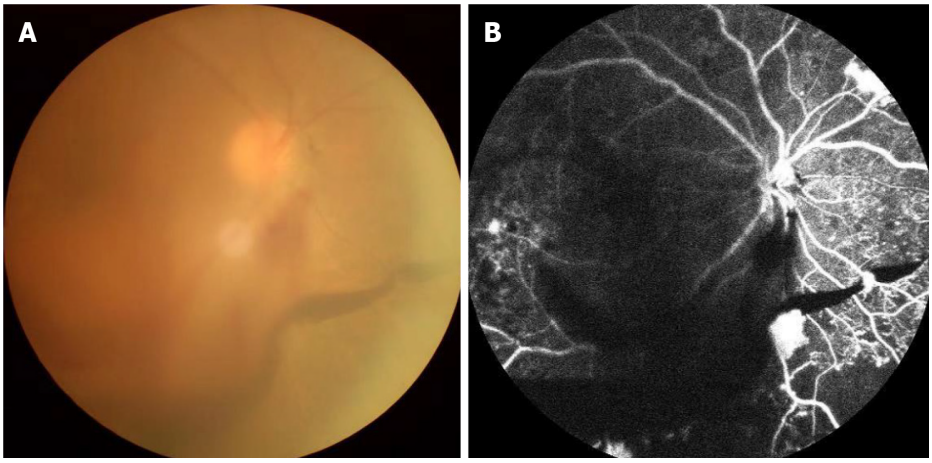

Figure 1 Example of diabetic retinopathy shown on fundus photography and fluorescein angiography.

fMRI data analysis

Our previous reports described how to analyze fMRI data. First, we used MRIcro software (www.mricro.com) to expel broken data. During magnetization equilibration, the first ten time points were discarded. Data Processing Assistant for the advanced edition of Resting-State fMRI (DPARSFA 4.0, http://rfmri.org/DPARSF) software was used for the form conversion of digital imaging communications in medicine (DICM), the correction of head motion, slice timing, realignment, spatial normalization, full-width smoothing with a Gaussian kernel of 6 mm × 6 mm × 6 mm at half-maximum, detrending, and nuisance covariates regression, based on the rs-fMRI data analysis toolkit (REST, http://www.restfmri.net). The other images were corrected time differences and micromotions during scanning. During the fMRI examination, subjects were excluded if they had more than three degrees of motion or if the maximum excursion in the x, y or z direction exceeded 3 mm.

We used linear regression analysis to remove spurious covariates and their time derivatives from various other sources, including the signal from the region of interest (ROI) to the central white matter region[18]. It should be noted that, in this study, the data showed that the global signal did not shrink, as was the case in our former study [19,20], which may have been caused by the elimination of global signals during preprocessing of the data at rest[21,22]. The fMRI images were unified to the Montreal Neurological Institute spatial standard using a standard echoplanar imaging template after correction for head motion, while the images were resampled to a resolution of 3 mm × 3 mm × 3 mm. To reduce the effect of diversity between participants, we divided the ALFF of each voxel by the average whole-brain ALFF value for each subject.

Brain-behavior correlation analysis

According to the results of ALFF, REST software was applied to divide different brain areas of these groups into areas of interest. The mean ALFF values in each region of interest were obtained by calculating the average ALFF values of all voxels. Using GraphPad Prism 9.0 (Graph Pad Software Inc., San Diego, CA, United States), the correlation between the average ALFF values of multiple brain regions in the DR group and the clinical data was evaluated ($P < 0.05$).

Statistical analysis

We used the SPSS version 22.0 (IBM Corporation, Armonk, NY, United States) with an independent samples *t*-test to analyze the cumulative clinical variables between HCs and the DR group. A P value < 0.05 showed statistical significance. We compared functional data with a two-sample *t*-test using REST software. Through Gaussian random field theory, the statistical thresholds for multiple comprehensively compared voxel levels were set as $P < 0.001$. Alphasim was corrected for cluster sizes > 30 voxels at a $P < 0.01$ level.

RESULTS

Demographics and behavioral results

No statistically significant differences were found between the two groups in weight (P = 0.982) or age (P = 0.975). The mean ± SD of DR duration was 253.18 ± 76.22 d (Table 1).

ALFF differences

Compared with the results in the HCs, the ALFF values of the DR patients were remarkably lower in the bilateral calcarine fissures, but higher in the left and right posterior lobes of the cerebellum as well as the right anterior cingulate gyrus (Figures 2 and 3 and Table 2).

Receiver operating characteristic curves

Receiver operating characteristic (ROC) curves were applied to analyze the mean ALFF values of different cerebral areas. The diagnosis rate was displayed in the area under the curve (AUC). The AUCs of ALFF values for different cerebral areas were as follows: Right anterior cingulate gyrus (0.080, P < 0.001), left posterior lobe of the cerebellum (0.938, P < 0.001), right posterior lobe of the cerebellum (0.947, P < 0.001; Figure 4A), and the bilateral calcarine fissures (0.893, P < 0.001; Figure 4B).

Correlation analysis

There was a positive correlation between the best-corrected visual acuity (BCVA) values of the affected eyes in the DR group and bilateral calcarine signal values (r = 0.938, P = 0.001). Figure 5 shows the specific details.

DISCUSSION

DR comprises microvessel damage, which is caused by diabetes mellitus. It is one of the most important causes of visual impairment in young adults[23]. DR is found in 33% of the diabetic patients in the world and is considered an increased risk for systemic vascular complications[24]. The retinal anatomy, physiology and embryological features are similar to cerebral small blood vessels of the brain[25]. Research has shown fundus arteriosclerosis has a positive correlation with cerebral atherosclerosis (P < 0.01)[26], and many diabetes neuroimaging markers of brain abnormalities have a relationship with microvascular disease. Previous studies have shown that the retinal pathological changes in the microcirculation (for example, the Gunn arteriovenous phenomenon, microaneurysms, soft exudate, and retinal hemorrhages) may be associated with markers of cerebral microvascular disease[27].

To our knowledge, this is the first study designed to explore spontaneous cerebral activity changes in DR patients using the ALFF method. Compared with the values in the HCs, the DR patients showed significantly lower ALFF values in the bilateral calcarine fissures, but higher values in the left and right posterior lobes of the cerebellum and the right anterior cingulate gyrus (Figure 6). The ALFF method has already been successfully applied in several eye diseases (Table 3) and it is expected that the application has good prospects for the future.

Analysis of the decreased ALFF values of DR patients

The calcium troponin crack is a deep groove on the inside surface of the occipital lobe. The upper and lower parts of the sulcus are the main cortical projection areas of vision. Impulses from the upper retina are transmitted above the sulcus, and visual information from the lower retina is projected below the sulcus. Impulses from the macular area are transmitted above and below the posterior third of the sulcus. This structure is an anatomical marker or landmark.

Previous reports[28] have shown that diabetes affects the microvascular system and leads to cerebral small vascular disease (SVD; Figure 7). The underlying mechanism of visual impairment in DR may involve a compromised arterial blood supply to the visual region that results from SVD. As fMRI has shown, bilateral activation of the visual cortex and eyeball movement can be caused by stimulation of the visual system [29]. Previous studies[30] have shown that prolonged poor glycemic control manifests with basement membrane thickening, tight junction disruption, and pericyte loss, leading to dysregulation of the vascular tone and endothelial cell proliferation, which can lead to microaneurysm formation and ultimately spot and speckle hemorrhages.

Table 1 Demographics and clinical measurements by groups

Condition	DR	HC	t	*P* value
Male/female	10/14	10/14	N/A	> 0.99
Age (yr)	54.31 ± 5.96	53.08 ± 5.33	0.093	0.975
Weight (kg)	53.67 ± 9.64	59.86 ± 9.93	0.095	0.982
Handedness	24R	24R	N/A	> 0.99
Duration of DR (d)	253.18 ± 76.22	N/A	N/A	N/A
Best-corrected VA-right eye	0.19 ± 0.08	1.12 ± 0.36	-0.883	0.009
Best-corrected VA-left eye	0.22 ± 0.09	1.09 ± 0.42	-0.743	0.014
IOP-R (mmHG)	15.63 ± 2.93	17.56 ± 2.74	0.092	0.815
IOP-L (mmHG)	16.13 ± 2.32	16.54 ± 2.63	0.088	0.843

$P < 0.05$ independent *t*-tests comparing two groups. DR: Diabetic retinopathy; HC: Healthy control; N/A: Not applicable; R: Right; IOP: Intraocular pressure.

Table 2 Brain areas with significantly different amplitude of low-frequency fluctuation values between groups

Condition	L/R/B	Brain regions	MNI coordinates			BA	Peak voxels	*t* value	*P* value
			X	Y	Z				
DRs > HCs									
1	L	Cerebellum posterior lobe	-27	-72	-36	/	238	5.4338	< 0.001
2	R	Cerebellum posterior lobe	33	-57	-42	/	111	4.6875	< 0.001
3	R	Anterior cingulate	9	48	3	32	32	-4.4176	< 0.001
DRs < HCs									
1	B	Calcarine	3	-66	21	/	46	-4.3494	< 0.001

$P < 0.05$, $P < 0.001$, independent *t*-test, *P* values between diabetic retinopathies and healthy controls. BA: Brodmann area; DR: Diabetic retinopathy; HC: Healthy control; MNI: Montreal Neurological Institute; L: Left; R: Right; B: Bilateral.

In this study, we found that there were decreased ALFF values bilaterally in the calcarine fissures of DR patients, which may be related to the impaired vision in these patients. We examined BCVA in both eyes of the patients and found that patients with DR had significantly lower BCVA compared to HCs ($P < 0.05$; Table 1). Our study observed that there was a positive correlation between the BCVA values of the eyes of the DR group and the signal values of the bilateral calcarine ($r = 0.938$, $P = 0.001$). It may be speculated that the decreased signal values of the bilateral calcarine fissures may reflect damage to visual processing in the patients.

Analysis of the increased ALFF values in DR

Traditionally, the cerebellar function is considered to involve physical balance and motor coordination[31]. However, because of the development and application of neuroimaging technology in recent years, we have a better understanding of the specific effect of the cerebellum, especially its posterior lobe, in emotional processing [32].

The cerebellum's posterior lobe is located between the primary fissure and the posterolateral fissure. In earlier studies, lesions of the posterior lobe could cause severe damage to spatial memory, emotional regulation, and executive functions[33,34]. Nitschke *et al*[35] showed that the cerebellum is associated with the movement of the eyeballs. Kresyun[36] found that using a transcranial magnetic to stimulate the cerebellum of DR patients helped with the recovery of the retina's ability to respond to light stress. Previous studies employing positron emission tomography found that patients with social anxiety had abnormal cerebellum signals with increasing cerebral

Table 3 Amplitude of low-frequency fluctuation method applied in ophthalmological diseases

Ref.	Disease	Brain areas	
		PG > HG	PG < HG
Guo *et al*[46], 2010	High myopia	LCN; Thalamus; Cuneus	LOL; BFL; RIPL; RPL; RMTL
Li *et al*[14], 2014	Glaucoma	RPG	LPG; BMFG; BSFG; RP; RAG
Liu *et al*[13], 2018	Optic neuritis	LCPL; RCPL; RITG; LPG; RFG; LFG; LCF; LIPL; LC	RCPL; RCAL; RP; RIFG; RI;LMFG; LSTG; RSG; BAC;BP;RIPL
Xi *et al*[15], 2010	Strabismus	BT	POS
Liang *et al*[48], 2016	Amblyopia	BC; LMOG; LPG	BPC
Tan *et al*[47], 2016	Open-globe injury	LC; LMCC; BP	
Pan *et al*[49], 2018	Acute eye pain	LPG; RPG; LC	LPG; RPG; LP

HG: Healthy group; PG: Patient group; LOL: Left occipital lobe; BFL: Bilateral frontal lobe; RIPL: Right inferior parietal lobule; RPL: Right parietal lobe; RMTL: Right middle temporal lobe; LCN: Left caudate nucleus; LPG: Left precentral gyrus; BMFG: Bilateral middle frontal gyrus; BSFG: Bilateral superior frontal gyrus; RP: Right precuneus; RAG: Right angular gyrus; RPG: Right precentral gyrus; LCPL: Left cerebellum posterior lobe; RCPL: Right cerebellum posterior lobe; RITG: Right inferior temporal gyrus; LPG: Left parahippocampal gyrus; RFG: Right fusiform gyrus; LFG: Left fusiform gyrus; LCF: Left calcarine fissure; LIPL: Left inferior parietal lobule; LC: Left cuneus; RCAL: Right cerebellum anterior lobe; RP: Right putamen; RIFG: Right inferior frontal gyrus; RI: Right insula; LMFG: Left medial frontal gyrus; LSTG: Left superior temporal gyrus; RSG: Right supramarginal gyrus; BAC: Bilateral anterior cingulate; BP: Bilateral precuneus; BCPL: Bilateral cerebellum posterior lobe; LAG: Left angular gyrus; BMG: Bilateral medialfrontal gyrus; BC: Bilateral calcarine; LMOG: Left middle occipital gyrus; LPG: Left postcentral gyrus; BPC: Bilateral precuneus cortex; LMCC: Left middle cingulum cortex; BP: Bilateral precuneus; RPG: Right parahippocampal gyrus; LPG: Left precentral gyrus; LC: Left caudate; RPG: Right precentral/postcentral gyrus; LP: Left precuneus; BT: Bilateral thalamus; POS: Parieto-occipital sulcus.

blood flow, which were illustrative. All these studies showed that abnormal activity in the cerebellum was related to anxiety[37,38]. In this study, we found an increase in the ALFF values of the left and right posterior cerebellar lobe. While we cannot prove that DR patients have anxiety, we can propose the hypothesis that anxiety may occur in DR patients with visual and even cognitive disorders.

The anterior cingulate cortex (ACC) is a vital part of the limbic system of the brain. It has extensive and numerous fibrous connections to the cortex and subcortical structures and is involved in the regulation of emotional and motor functions[39]. In a previous study, researchers observed structural defects in the ACC in many depressed patients[40]. Yu *et al*[41] evaluated diabetic patients using the SF-36 Health-Related Quality of life (HRQL) and anxiety disorders, and they found that there was a statistically significant difference in impaired HRQL (SF-36 summary score) in DR patients compared to the control group ($P < 0.05$). Clinically, DR patients may also have serious psychosocial problems[42]. Depression is not uncommon in DR patients and it has a negative impact on their condition. However, in recent years, an increasing amount of evidence from electrophysiology[43], functional imaging[44], and behavioral studies [45] have shown that the ACC is closely related to the management of pain. The ACC can be activated by nociceptive and contextual stimuli, and it can participate in pain management, especially affective pain. The neural mechanisms of the ACC's involvement in effective pain have not been clarified. The specific neural mechanism remains to be studied in the future.

CONCLUSION

This study demonstrated that there are some abnormal spontaneous brain activities in DR patients. The findings using these new techniques offer information that may help to explain the nerve mechanisms underlying the clinical manifestations of DR patients and contribute to improved clinical diagnoses.

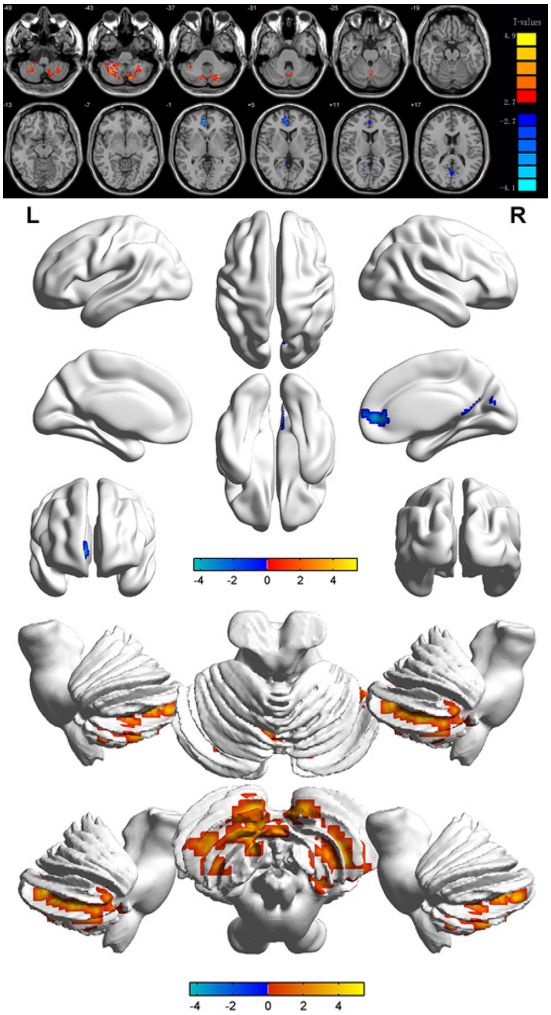

Figure 2 Significant differences in spontaneous brain activity between the diabetic retinopathy group and healthy controls. The different brain regions were observed in the left cerebellum posterior lobe, right cerebellum posterior lobe, right anterior cingulate and bilateral calcarine. The red areas denote higher amplitude of low-frequency fluctuation (ALFF) brain regions, and the blue areas denote lower ALFF brain regions [$P < 0.001$ for multiple comparisons using Gaussian random field theory (z.2.3, $P < 0.01$, cluster > 30 voxels, Alphasim corrected)].

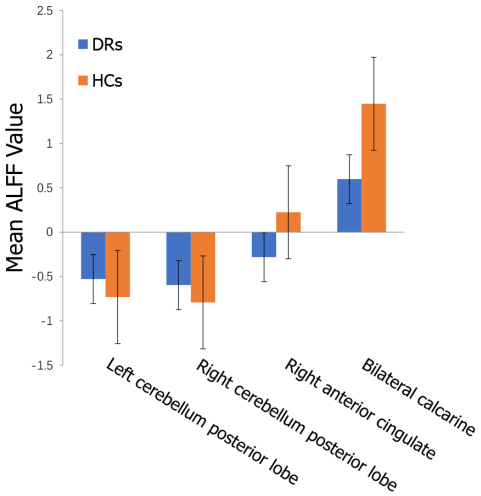

Figure 3 Means of altered spontaneous brain activity between the diabetic retinopathy group and healthy controls. DR: Diabetic retinopathy; HCs: Healthy controls; ALFF: Amplitude of low-frequency fluctuation.

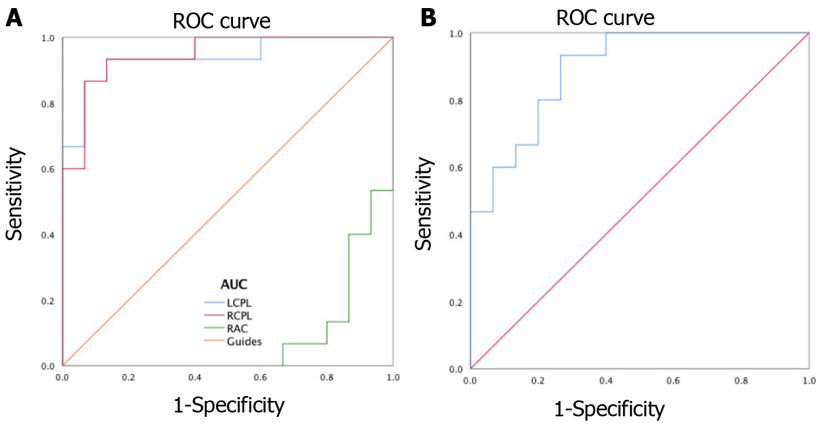

Figure 4 Receiver operating characteristic curve analysis of the mean amplitude of low-frequency fluctuation values for altered brain regions. A: The area under the ROC curve were 0.080, [$P < 0.001$; 95% confidence interval (CI): 0.000 to 0.177] for RAC, LCPL 0.938 ($P < 0.001$; 95%CI: 0.849 to 1.000), RCPL 0.947 ($P < 0.001$; 95%CI: 0.871 to 1.000) [diabetic retinopathies (DRs) > healthy controls (HCs)]; B: The area under the ROC curve were 0.893 ($P < 0.001$; 95%CI: 0.782 to 1.000) for BC (DRs < HCs). ROC: Receiver operating characteristic; AUC: Area under the curve; LCPL: Left cerebellum posterior lobe; RCPL: Right cerebellum posterior lobe; RAC: Right anterior cingulate.

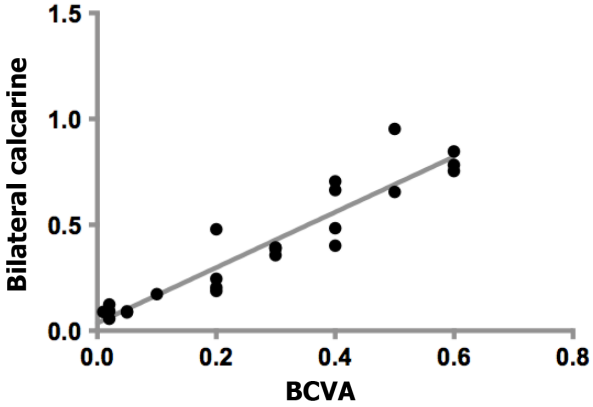

Figure 5 Correlations between the best-corrected visual acuity values and signal values in the bilateral calcarine. The best-corrected visual acuity value of the eyes of the diabetic retinopathy group showed a positive correlation with the signal value of the bilateral calcarine ($r = 0.938$, $P = 0.001$). BCVA: Best-corrected visual acuity.

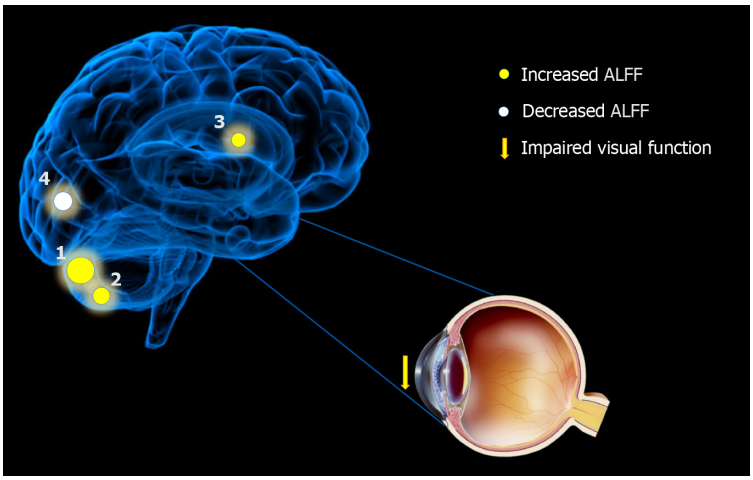

Figure 6 The amplitude of low-frequency fluctuation results of brain activity in the diabetic retinopathy group. Compared with the healthy controls, the amplitude of low-frequency fluctuation (ALFF) of the following regions in diabetic retinopathies were increased to various extents: 1-left cerebellum posterior lobe (t = 5.4338), 2-right cerebellum posterior lobe (t = 4.6875) and 3-right anterior cingulate (t = -4.4176), and decreased ALFF values in the 4-bilateral calcarine (t = -4.3494). The sizes of the spots denote the degree of quantitative changes. ALFF: Amplitude of low-frequency fluctuation.

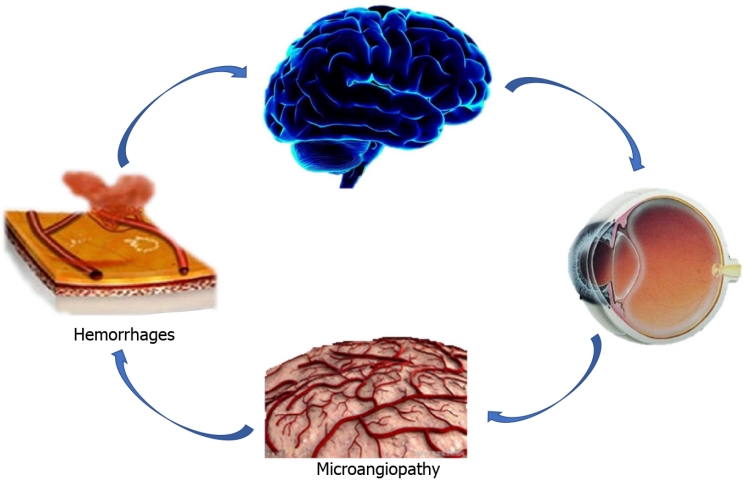

Figure 7 Relationship between magnetic resonance imaging images and clinical manifestations in diabetic retinopathy.

ARTICLE HIGHLIGHTS

Research background

Diabetic retinopathy (DR) is one of the most common complications of diabetes mellitus; however, to date, there has been little analysis of the changes in brain function in patients with DR and their relationship to the clinical manifestations in the eye. This study is the first to examine brain changes in patients with DR using the amplitude of low-frequency fluctuation (ALFF).

Research motivation

The current diagnosis of DR mainly involves fundus fluorescein imaging for examination, and the direct connection between eyes, other manifestations, and the brain is still unknown. In this study, we employed the ALFF technique to investigate abnormal brain activity in DR patients and its relationship with clinical characteristics. Our research may help with understanding how DR disease develops.

Research objectives

We investigated the underlying ALFF of local characteristics of spontaneous brain activity in DR patients and their relationship with behavioral performance. Our findings suggested possible mechanisms of clinical manifestations and behavior in DR patients.

Research methods

Twenty-four DR patients and 24 healthy controls (HCs) matched for both age and sex were recruited. We measured and recorded the average ALFF values of DR patients and HCs and then classified them using receiver operating characteristic (ROC) curves.

Research results

We found that the ALFF values of both the left and right cerebellum posterior lobe and the right anterior cingulate gyrus were remarkably higher in the DR patients compared with the HCs, but DR patients also had lower values in the bilateral calcarine. ROC curve analysis of different brain regions demonstrated high accuracy of area under the curve analysis. However, there was no remarkable relationship between ALFF mean values for different regions and clinical presentations of DR patients.

Research conclusions

We hypothesized that DR may lead to alterations in visual cortical activity. Our results showed altered spontaneous activity in three regions of the brain in patients with DR. Abnormalities in low-frequency amplitudes in the brain may be associated with alterations in contralateral best-corrected visual acuity and depression in DR patients. These findings may suggest possible mechanisms of clinical manifestations and behaviors in DR patients.

Research perspectives

Our finding that DR may lead to multiple low-frequency amplitude frequency changes in the brain may facilitate our exploration of pathological mechanisms and disease progression in DR patients. However, the drawback is that the sample size was too small. Future studies should increase the sample size in order to ensure the validity of our findings.

REFERENCES

1 **Gunasekeran DV**, Ting DSW, Tan GSW, Wong TY. Artificial intelligence for diabetic retinopathy screening, prediction and management. *Curr Opin Ophthalmol* 2020; **31**: 357-365 [PMID: 32740069 DOI: 10.1097/ICU.0000000000000693]

2 **Pascolini D**, Mariotti SP. Global estimates of visual impairment: 2010. *Br J Ophthalmol* 2012; **96**: 614-618 [PMID: 22133988 DOI: 10.1136/bjophthalmol-2011-300539]

3 **Zhu XR**, Yang FY, Lu J, Zhang HR, Sun R, Zhou JB, Yang JK. Plasma metabolomic profiling of proliferative diabetic retinopathy. *Nutr Metab (Lond)* 2019; **16**: 37 [PMID: 31160916 DOI: 10.1186/s12986-019-0358-3]

4 **Su JB**, Zhao LH, Zhang XL, Cai HL, Huang HY, Xu F, Chen T, Wang XQ. HbA1c variability and diabetic peripheral neuropathy in type 2 diabetic patients. *Cardiovasc Diabetol* 2018; **17**: 47 [PMID: 29598819 DOI: 10.1186/s12933-018-0693-0]

5 **Usuelli V**, La Rocca E. Novel therapeutic approaches for diabetic nephropathy and retinopathy. *Pharmacol Res* 2015; **98**: 39-44 [PMID: 25447794 DOI: 10.1016/j.phrs.2014.10.003]

6 **Pearce I**, Simó R, Lövestam-Adrian M, Wong DT, Evans M. Association between diabetic eye disease and other complications of diabetes: Implications for care. A systematic review. *Diabetes Obes Metab* 2019; **21**: 467-478 [PMID: 30280465 DOI: 10.1111/dom.13550]

7 **Liu H**, Wang X. Correlation of iron deposition and change of gliocyte metabolism in the basal ganglia region evaluated using magnetic resonance imaging techniques: an *in vivo* study. *Arch Med Sci* 2016; **12**: 163-171 [PMID: 26925133 DOI: 10.5114/aoms.2016.57593]

8 **Meijer FJ**, Goraj B. Brain MRI in Parkinson's disease. *Front Biosci (Elite Ed)* 2014; **6**: 360-369 [PMID: 24896211 DOI: 10.2741/E711]

9 **Fox MD**, Greicius M. Clinical applications of resting state functional connectivity. *Front Syst Neurosci* 2010; **4**: 19 [PMID: 20592951 DOI: 10.3389/fnsys.2010.00019]

10 **Cakir Y**. The effects of Alzheimer's disease related striatal pathologic changes on the fractional amplitude of low-frequency fluctuations. *Comput Methods Biomech Biomed Engin* 2020; **23**: 1347-1359 [PMID: 32749154 DOI: 10.1080/10255842.2020.1801653]

11 **Xing XX**, Zheng MX, Hua XY, Ma SJ, Ma ZZ, Xu JG. Brain plasticity after peripheral nerve injury treatment with massage therapy based on resting-state functional magnetic resonance imaging. *Neural Regen Res* 2021; **16**: 388-393 [PMID: 32859803 DOI: 10.4103/1673-5374.290912]

12 **Rosenbaum D**, Int-Veen I, Kroczek A, Hilsendegen P, Velten-Schurian K, Bihlmaier I, Fallgatter AJ, Ehlis AC. Amplitude of low frequency fluctuations (ALFF) of spontaneous and induced rumination in major depression: An fNIRS study. *Sci Rep* 2020; **10**: 21520 [PMID: 33299001 DOI: 10.1038/s41598-020-78317-y]

13 **Liu Y**, Jiang X, Butzkueven H, Duan Y, Huang J, Ren Z, Dong H, Shi FD, Barkhof F, Li K, Wang J. Multimodal characterization of gray matter alterations in neuromyelitis optica. *Mult Scler* 2018; **24**: 1308-1316 [PMID: 28741987 DOI: 10.1177/1352458517721053]

14 **Li T**, Liu Z, Li J, Tang Z, Xie X, Yang D, Wang N, Tian J, Xian J. Altered amplitude of low-frequency fluctuation in primary open-angle glaucoma: a resting-state FMRI study. *Invest Ophthalmol Vis Sci* 2014; **56**: 322-329 [PMID: 25525176 DOI: 10.1167/iovs.14-14974]

15 **Xi S**, Yao J, Zhang S, Liu R, Wu L, Ye X, Zhang P, Wen W, Zhao C. Disrupted neural signals in patients with concomitant exotropia. *Ophthalmic Physiol Opt* 2020; **40**: 650-659 [PMID: 32672862 DOI: 10.1111/opo.12715]

16 **Kwak Y**, Peltier SJ, Bohnen NI, Müller ML, Dayalu P, Seidler RD. L-DOPA changes spontaneous low-frequency BOLD signal oscillations in Parkinson's disease: a resting state fMRI study. *Front Syst Neurosci* 2012; **6**: 52 [PMID: 22783172 DOI: 10.3389/fnsys.2012.00052]

17 **Alzner E**. International Congress of Ophthalmology — The World Meeting of Ophthalmologists, 21.–25. April 2002, Sydney — Australien. *Spektrum Der Augenheilkunde* 2002; 16: 189-190 [DOI: 10.1007/BF03164300]

18 **Fox MD**, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc Natl Acad Sci U S A* 2005; **102**: 9673-9678 [PMID: 15976020 DOI: 10.1073/pnas.0504136102]

19 **Kang HH**, Shu YQ, Yang L, Zhu PW, Li D, Li QH, Min YL, Ye L, Zhou Q, Shao Y. Measuring abnormal intrinsic brain activities in patients with retinal detachment using amplitude of low-frequency fluctuation: a resting-state fMRI study. *Int J Neurosci* 2019; **129**: 681-686 [PMID: 30499735 DOI: 10.1080/00207454.2018.1554657]

20 **Wu YY**, Yuan Q, Li B, Lin Q, Zhu PW, Min YL, Shi WQ, Shu YQ, Zhou Q, Shao Y. Altered spontaneous brain activity patterns in patients with retinal vein occlusion indicated by the amplitude

of low-frequency fluctuation: A functional magnetic resonance imaging study. *Exp Ther Med* 2019; **18**: 2063-2071 [PMID: 31410162 DOI: 10.3892/etm.2019.7770]

21 **Saad ZS**, Gotts SJ, Murphy K, Chen G, Jo HJ, Martin A, Cox RW. Trouble at rest: how correlation patterns and group differences become distorted after global signal regression. *Brain Connect* 2012; **2**: 25-32 [PMID: 22432927 DOI: 10.1089/brain.2012.0080]

22 **Zang YF**, He Y, Zhu CZ, Cao QJ, Sui MQ, Liang M, Tian LX, Jiang TZ, Wang YF. Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. *Brain Dev* 2007; **29**: 83-91 [PMID: 16919409 DOI: 10.1016/j.braindev.2006.07.002]

23 **Polack S**, Yorston D, López-Ramos A, Lepe-Orta S, Baia RM, Alves L, Grau-Alvidrez C, Gomez-Bastar P, Kuper H. Rapid assessment of avoidable blindness and diabetic retinopathy in Chiapas, Mexico. *Ophthalmology* 2012; **119**: 1033-1040 [PMID: 22342012 DOI: 10.1016/j.ophtha.2011.11.002]

24 **Koch EAT**, Nakhoul R, Nakhoul F, Nakhoul N. Autophagy in diabetic nephropathy: a review. *Int Urol Nephrol* 2020; **52**: 1705-1712 [PMID: 32661628 DOI: 10.1007/s11255-020-02545-4]

25 **Tso MOM**, Jampol LM. Pathophysiology of hypertensive retinopathy. *Ophthalmol* 1982; 89: 1132-1145 [DOI: 10.1016/S0161-6420(82)34663-1]

26 **Reid IR**, Bolland MJ. Controversies in medicine: the role of calcium and vitamin D supplements in adults. *Med J Aust* 2019; **211**: 468-473 [PMID: 31680267 DOI: 10.5694/mja2.50393]

27 **Wong TY**. Is retinal photography useful in the measurement of stroke risk? *Lancet Neurol* 2004; **3**: 179-183 [PMID: 15029894 DOI: 10.1016/s1474-4422(04)00682-9]

28 **Szcześniak D**, Rymaszewska J, Zimny A, Sąsiadek M, Połtyn-Zaradna K, Smith EE, Zatońska K, Zatoński T, Rangarajan S, Yusuf S, Szuba A. "Cerebral small vessel disease and other influential factors of cognitive impairment in the middle-aged: a long-term observational cohort PURE-MIND study in Poland". *Geroscience* 2021; **43**: 279-295 [PMID: 33074422 DOI: 10.1007/s11357-020-00271-4]

29 **Dieterich M**, Bauermann T, Best C, Stoeter P, Schlindwein P. Evidence for cortical visual substitution of chronic bilateral vestibular failure (an fMRI study). *Brain* 2007; **130**: 2108-2116 [PMID: 17575279 DOI: 10.1093/brain/awm130]

30 **Fang M**, Selvin E. Thirty-Year Trends in Complications in U.S. Adults With Newly Diagnosed Type 2 Diabetes. *Diabetes Care* 2021; **44**: 699-706 [PMID: 33419932 DOI: 10.2337/dc20-2304]

31 **Ferrari C**, Oldrati V, Gallucci M, Vecchi T, Cattaneo Z. The role of the cerebellum in explicit and incidental processing of facial emotional expressions: A study with transcranial magnetic stimulation. *Neuroimage* 2018; **169**: 256-264 [PMID: 29246845 DOI: 10.1016/j.neuroimage.2017.12.026]

32 **Adamaszek M**, D'Agata F, Ferrucci R, Habas C, Keulen S, Kirkby KC, Leggio M, Mariën P, Molinari M, Moulton E, Orsi L, Van Overwalle F, Papadelis C, Priori A, Sacchetti B, Schutter DJ, Styliadis C, Verhoeven J. Consensus Paper: Cerebellum and Emotion. *Cerebellum* 2017; **16**: 552-576 [PMID: 27485952 DOI: 10.1007/s12311-016-0815-8]

33 **Stoodley CJ**, Schmahmann JD. Functional topography of the human cerebellum. *Handb Clin Neurol* 2018; **154**: 59-70 [PMID: 29903452 DOI: 10.1016/B978-0-444-63956-1.00004-7]

34 **Schmahmann JD**. The cerebellum and cognition. *Neurosci Lett* 2019; **688**: 62-75 [PMID: 29997061 DOI: 10.1016/j.neulet.2018.07.005]

35 **Nitschke MF**, Arp T, Stavrou G, Erdmann C, Heide W. The cerebellum in the cerebro-cerebellar network for the control of eye and hand movements--an fMRI study. *Prog Brain Res* 2005; **148**: 151-164 [PMID: 15661188 DOI: 10.1016/S0079-6123(04)48013-3]

36 **Kresyun NV**. The influence of deltalycyn and transcranial cerebellar stimulation upon recovery of retina after photo stress in patients with diabetic retinopathy. *Rev Med Chir Soc Med Nat Iasi* 2014; **118**: 1068-1073 [PMID: 25581971]

37 **Kilts CD**, Kelsey JE, Knight B, Ely TD, Bowman FD, Gross RE, Selvig A, Gordon A, Newport DJ, Nemeroff CB. The neural correlates of social anxiety disorder and response to pharmacotherapy. *Neuropsychopharmacology* 2006; **31**: 2243-2253 [PMID: 16525417 DOI: 10.1038/sj.npp.1301053]

38 **Olexová L**, Štefánik P, Kršková L. Increased anxiety-like behaviour and altered GABAergic system in the amygdala and cerebellum of VPA rats - An animal model of autism. *Neurosci Lett* 2016; **629**: 9-14 [PMID: 27353514 DOI: 10.1016/j.neulet.2016.06.035]

39 **Bush G**, Luu P, Posner MI. Cognitive and emotional influences in anterior cingulate cortex. *Trends Cogn Sci* 2000; **4**: 215-222 [PMID: 10827444 DOI: 10.1016/s1364-6613(00)01483-2]

40 **Webb CA**, Weber M, Mundy EA, Killgore WD. Reduced gray matter volume in the anterior cingulate, orbitofrontal cortex and thalamus as a function of mild depressive symptoms: a voxel-based morphometric analysis. *Psychol Med* 2014; **44**: 2833-2843 [PMID: 25066703 DOI: 10.1017/S0033291714000348]

41 **Yu Y**, Feng L, Shao Y, Tu P, Wu HP, Ding X, Xiao WH. Quality of life and emotional change for middle-aged and elderly patients with diabetic retinopathy. *Int J Ophthalmol* 2013; **6**: 71-74 [PMID: 23549357 DOI: 10.3980/j.issn.2222-3959.2013.01.15]

42 **Crosby-Nwaobi RR**, Sivaprasad S, Amiel S, Forbes A. The relationship between diabetic retinopathy and cognitive impairment. *Diabetes Care* 2013; **36**: 3177-3186 [PMID: 23633523 DOI: 10.2337/dc12-2141]

43 **Yu W**, Wu X, Chen Y, Liang Z, Jiang J, Misrani A, Su Y, Peng Y, Chen J, Tang B, Sun M, Long C, Shen J, Yang L. Pelvic Pain Alters Functional Connectivity Between Anterior Cingulate Cortex and Hippocampus in Both Humans and a Rat Model. *Front Syst Neurosci* 2021; **15**: 642349 [PMID: 34149369 DOI: 10.3389/fnsys.2021.642349]

44 **Ito T**, Tanaka-Mizuno S, Iwashita N, Tooyama I, Shiino A, Miura K, Fukui S. Proton magnetic resonance spectroscopy assessment of metabolite status of the anterior cingulate cortex in chronic pain patients and healthy controls. *J Pain Res* 2017; **10**: 287-293 [PMID: 28203104 DOI: 10.2147/JPR.S123403]

45 **Boccard SG**, Pereira EA, Moir L, Van Hartevelt TJ, Kringelbach ML, FitzGerald JJ, Baker IW, Green AL, Aziz TZ. Deep brain stimulation of the anterior cingulate cortex: targeting the affective component of chronic pain. *Neuroreport* 2014; **25**: 83-88 [PMID: 24100411 DOI: 10.1097/WNR.0000000000000039]

46 **Guo MX**, Dong HH, Zhang Y T. ALFF Changes in Brain Areas of Human with High Myopia Revealed by Resting-state Functional MRI. *IEEE* 2010; 1: 91-94

47 **Tan G**, Huang X, Ye L, Wu AH, He LX, Zhong YL, Jiang N, Zhou FQ, Shao Y. Altered spontaneous brain activity patterns in patients with unilateral acute open globe injury using amplitude of low-frequency fluctuation: a functional magnetic resonance imaging study. *Neuropsychiatr Dis Treat* 2016; **12**: 2015-2020 [PMID: 27570455 DOI: 10.2147/NDT.S110539]

48 **Liang M**, Xie B, Yang H, Yu L, Yin X, Wei L, Wang J. Distinct patterns of spontaneous brain activity between children and adults with anisometropic amblyopia: a resting-state fMRI study. *Graefes Arch Clin Exp Ophthalmol* 2016; **254**: 569-576 [PMID: 26245338 DOI: 10.1007/s00417-015-3117-9]

49 **Pan ZM**, Li HJ, Bao J, Jiang N, Yuan Q, Freeberg S, Zhu PW, Ye L, Ma MY, Huang X, Shao Y. Altered intrinsic brain activities in patients with acute eye pain using amplitude of low-frequency fluctuation: a resting-state fMRI study. *Neuropsychiatr Dis Treat* 2018; **14**: 251-257 [PMID: 29386898 DOI: 10.2147/NDT.S150051]

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ORIGINAL ARTICLE

Retrospective Study

Large-scale functional connectivity predicts cognitive impairment related to type 2 diabetes mellitus

An-Ping Shi, Ying Yu, Bo Hu, Yu-Ting Li, Wen Wang, Guang-Bin Cui

ORCID number: An-Ping Shi 0000-0003-1080-9623; Ying Yu 0000-0002-6537-5159; Bo Hu 0000-0002-1135-0669; Yu-Ting Li 0000-0001-9334-5582; Wen Wang 0000-0001-6473-4888; Guang-Bin Cui 0000-0002-6908-0788.

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An-Ping Shi, Ying Yu, Bo Hu, Yu-Ting Li, Wen Wang, Guang-Bin Cui, Department of Radiology, Department of Radiology and Functional and Molecular Imaging Key Lab of Shaanxi Province, The Affiliated Tangdu Hospital of Air Force Medical University (Fourth Military Medical University), Xi'an 710038, Shaanxi Province, China

Corresponding author: Guang-Bin Cui, BMed, Doctor, PhD, Academic Research, Chief Doctor, Chief Physician, Professor, Research Dean, Department of Radiology, The Affiliated Tangdu Hospital of Air Force Medical University, No. 569 Xinsi Road, Xi'an 710038, Shaanxi Province, China. cuigbtd@163.com

Abstract

BACKGROUND

Large-scale functional connectivity (LSFC) patterns in the brain have unique intrinsic characteristics. Abnormal LSFC patterns have been found in patients with dementia, as well as in those with mild cognitive impairment (MCI), and these patterns predicted their cognitive performance. It has been reported that patients with type 2 diabetes mellitus (T2DM) may develop MCI that could progress to dementia. We investigated whether we could adopt LSFC patterns as discriminative features to predict the cognitive function of patients with T2DM, using connectome-based predictive modeling (CPM) and a support vector machine.

AIM

To investigate the utility of LSFC for predicting cognitive impairment related to T2DM more accurately and reliably.

METHODS

Resting-state functional magnetic resonance images were derived from 42 patients with T2DM and 24 healthy controls. Cognitive function was assessed using the Montreal Cognitive Assessment (MoCA). Patients with T2DM were divided into two groups, according to the presence (T2DM-C; n = 16) or absence (T2DM-NC; n = 26) of MCI. Brain regions were marked using Harvard Oxford (HOA-112), automated anatomical labeling (AAL-116), and 264-region functional (Power-264) atlases. LSFC biomarkers for predicting MoCA scores were identified using a new CPM technique. Subsequently, we used a support vector machine based on LSFC patterns for among-group differentiation. The area under the receiver operating characteristic curve determined the appearance of the classification.

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RESULTS

CPM could predict the MoCA scores in patients with T2DM (Pearson's correlation coefficient between predicted and actual MoCA scores, r = 0.32, P=0.0066 [HOA-112 atlas]; r = 0.32, P=0.0078 [AAL-116 atlas]; r = 0.42, P=0.0038 [Power-264 atlas]), indicating that LSFC patterns represent cognition-level measures in these patients. Positive (anti-correlated) LSFC networks based on the Power-264 atlas showed the best predictive performance; moreover, we observed new brain regions of interest associated with T2DM-related cognition. The area under the receiver operating characteristic curve values (T2DM-NC group *vs.* T2DM-C group) were 0.65-0.70, with LSFC matrices based on HOA-112 and Power-264 atlases having the highest value (0.70). Most discriminative and attractive LSFCs were related to the default mode network, limbic system, and basal ganglia.

CONCLUSION

LSFC provides neuroimaging-based information that may be useful in detecting MCI early and accurately in patients with T2DM.

Key Words: Connectome-based predictive modeling; Large-scale functional connectivity; Mild cognitive impairment; Resting-state functional magnetic resonance; Support vector machine; Type 2 diabetes mellitus

Core Tip: Large-scale functional connectivity (LSFC) patterns show unique characteristics. Abnormal LSFC patterns have been observed in patients with dementia or mild cognitive impairment. Patients with diabetes may develop mild cognitive impairment that could potentially progress to dementia. We assessed the applicability of LSFC-related discriminative features to predict the cognitive level of patients with type 2 diabetes mellitus using a connectome-based predictive modeling and support vector machine. We found that the application of these two techniques, based on LSFC patterns, to predict neurocognitive abilities, can complement conventional neurocognitive assessments and aid the management of type 2 diabetes mellitus.

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INTRODUCTION

Diabetes is a common and frequently occurring disease in clinical practice. It is a non-communicable disease that has gradually attracted increased attention worldwide, and its incidence is increasing with each passing year[1]. Mild cognitive impairment (MCI) occurs in nearly a quarter of patients with type 2 diabetes mellitus (T2DM) and is related to a significantly increased risk of developing dementia[2-5]. Patients with T2DM may present with deteriorated memory, attention, reagency, and execution[5]. In a cross-sectional study, Biessels *et al*[2] indicated that cognitive function was up to 0.3-0.5 SD lower than that of healthy controls (HC). T2DM leads to a variety of complications, as well as social health and economic problems[3]. In addition, 8.7% of patients with MCI rapidly progress to dementia each year[4]. In patients with T2DM who have a strong tendency to develop MCI and dementia, elucidating the neural mechanisms underlying cognitive dysfunction may assist in clinical identification and intervention, which can mitigate the progress of MCI. However, the mechanism underlying MCI in patients with T2DM warrants further exploration.

Previous studies using neuroimaging measures including the amplitude of low-frequency fluctuation[6], regional homogeneity[7], and functional connectivity[8,9] have reported potential neurobiological underpinnings in patients with T2DM and MCI. However, most of these studies focused on predetermined regions or networks,

including the default mode network (DMN), frontoparietal network (FPN), *etc*[9-12]. Additionally, most of these studies investigated group-wise differences among healthy participants, patients with T2DM with or without MCI, and patients with MCI alone, which limits the provided cognitive information.

Whole-brain functional connectivity, also known as large-scale functional connectivity (LSFC), presents vast functional interaction information between all pairs of brain nodes, which facilitates individual phenotypic prediction and the elucidation of individual differences in cognitive ability[13,14]. There are robust and reliable patterns of LSFC within several brain networks. Therefore, analyzing LSFC patterns may help elucidate the neural mechanisms underlying MCI. Abnormal LSFC patterns were reported in patients with Alzheimer's disease or MCI[15,16]. Additionally, recent functional magnetic resonance imaging (fMRI) studies have used LSFC to successfully predict individual behavioral and cognitive phenotypes, including psychiatric disorders[17], attention ability[18,19], intelligence ability[13], and treatment outcomes [20]. Zeng *et al*[17] used LSFC to discriminate patients with major depressive disorder from matched HC through machine learning (ML) based on LSFC. Similarly, Li *et al* [21] used ML and LSFC to classify patients with schizophrenia and HC.

Similar to fingerprints, individual LSFC patterns are highly unique and reliable, and could be applicable to the recognition of individual characteristics and cognitive function[13,18]. Therefore, some LSFC patterns could be considered as potential biomarkers for evaluating or identifying T2DM-related MCI. However, few studies have used LSFC combined with ML for assessing T2DM[8]. Thus, this study aimed to predict T2DM-related MCI at an individual level using connectome-based predictive modeling (CPM) and a support vector machine (SVM) combined with LSFC.

MATERIALS AND METHODS

Participants

All participants' informed consent forms were signed before the experiment began. LSFC was examined using resting state (rs)-fMRI data obtained from 42 patients with T2DM and 24 HC at Tangdu Hospital, Xi'an, Shaanxi, China, between October 1, 2016, and December 30, 2018. All participants were native Chinese speakers. T2DM was diagnosed based on the fasting blood glucose test (FBG; ≥ 7.0 mmol/L) and oral glucose tolerance test (2 h blood glucose ≥ 11.1 mmol/L after the test)[22], with the diagnosis being confirmed by clinical endocrinologists. Additionally, we administered the Chinese version of the Montreal Cognitive Assessment (MoCA)[23] and Mini-Mental State Examination (MMSE)[24] to classify the cognitive levels of all participants during this experiment. Trained physicians checked for MCI in patients with T2DM, who were divided into the T2DM-C (MoCA score ≤ 23 or MMSE score < 27, n = 16) and T2DM-NC (MoCA score ≥ 26 or MMSE score ≥ 27, n = 26) groups. A MoCA score ≤ 23[25] or MMSE score < 27[24] is indicative of cognitive impairment, whereas a MoCA score ≥ 26[25] or MMSE score ≥ 27[24] is considered cognitively normal. The exclusion criteria were as follows: other types of diabetes (type 1 diabetes or gestational diabetes); a history of severe encephalopathy (injury, tumor, inflammation, hemiplegia, or infarction) or myocardial infarction; central nervous system dysfunction or medical diseases that considerably affect neurological function, including acquired immune deficiency syndrome; taking drugs within 3 mo, such as psychoactive and steroid drugs; alcohol or drug addiction; pregnancy; contraindications for MRI examination, including cardiac pacemakers, artificial heart valves, and claustrophobia; body mass index (BMI) >35 kg/m^2 (because obesity impairs cognition); and unfavorable image quality or lack of coordination (head movement: translation >3.0 mm or rotation in any direction >3°). Similar exclusion criteria were adopted for the HC group.

MoCA scores

The MoCA is a quick evaluation scale for screening MCI[23,25]. Compared to the MMSE, the MoCA is more suitable for the screening and monitoring of MCI and dementia[23,25]. In our study, we used the Chinese version of the MoCA Basic (MoCA-BC) to assess the cognition level in patients with T2DM. MoCA-BC is recognized as a reliable test in cognitive screening, especially for milder forms of cognitive impairment across the education of all levels, especially in older Chinese adults, which has higher acceptance and better reliability[26]. It has good standard correlation validity (Pearson correlation coefficient MoCA-BC *vs*. MMSE = 0.787) and credible internal consistency (Cronbach alpha = 0.807)[26]. MoCA is scored on a 30-

point scale and comprises 11 items assessing orientation, attention, calculation, recall, and language. A MoCA score ≤ 23 indicates cognitive impairment and one ≥ 26 is considered to indicate cognitively normal.

Image acquisition and preprocessing

MRI data were obtained using a GE Discovery MR750 3.0T scanner (GE Medical Systems) with a brand-new coil system and high scanning speed. Foam pads were used to minimize head movement and earplugs were used to silence the scanner noise. During the acquisition phase, the participants were asked to relax, including at rest, to close their eyes, not think about anything, not allow being disturbed by others, and not to sleep. We recorded the blood oxygen level-dependent signals of spontaneous fluctuations during wakeful rest to assess brain activity. Three-dimensional brain volume (3D-BRAVO) and blood oxygen level-dependent sequences were used to obtain structural (including high-resolution T1-weighted images) and functional images, respectively. For details regarding the scanning parameters, see the Supplementary Material.

Data processing was conducted using DPABI (http://www.rfmri.org/)[27] and SPM (http://www.fil.ion.ucl.ac.uk/spm/), as well as homemade codes in MATLAB 2018a (MathWorks, Inc., Natick, MA). For details regarding rs-fMRI data preprocessing, see the Supplementary Material.

Functional connectivity network construction

Figure 1A shows the procedure for constructing functional brain networks. Brain regions were marked using three templates; namely, the Harvard Oxford (HOA-112) atlas[28], Automated Anatomical Labeling (AAL-116) atlas[29], and 264-region functional (Power-264) atlas introduced by Power *et al*[30]. We used the Pearson correlation analysis to calculate the mean time series of any two brain regions. Fisher's r-to-z transformation was applied to convert correlation coefficients to z-values. For each participant, an N × N (HOA-112 atlas, n = 112; AAL-116 atlas, n = 116; Power-264 atlas, n = 264) symmetric matrix was obtained.

We defined network nodes using the HOA -112, AAL-116, and Power-264 atlases. As previously described[31], 112 nodes were used to divide the brain into eight functional networks and the 116 nodes into nine macroscale brain regions. The eight functional networks included the visual (VN), sensory-motor (SMN), dorsal attention (DAN), ventral attention (VAN), limbic system, FPN, DMN, and basal ganglion (BG) networks. The nine macroscale brain regions included the VN, SMN, DAN, VAN, limbic system, FPN, DMN, BG, and cerebellar networks. Additionally, 264 nodes were divided into 14 Large-scale regions[30]. These nodes belonged to the DMN, salience, Cingulo-opercular Task Control (COTC), Fronto-parietal Task Control (FPTC), DAN, VAN, VN, auditory, Sensory/somatomotor Hand (SSH), Sensory/somatomotor Mouth (SSM) subcortical, Memory retrieval, cerebellar and uncertain networks. Details regarding the three templates can be found in Supplementary Tables 1, 2 and 3, as well as Supplementary Figure 1. Additionally, we calcula-ted the group mean functional connectivity matrices (FCMs) based on the three atlases for all three groups. Pairwise connectivity among the network nodes was described as a two-dimensional matrix using the functional connectivity matrix (FCM). In Supplementary Figure 2, the various regions of high (redder) and low (bluer) synchronization levels represent the FCM patterns of both patient groups, which were complex and similar. However, no evident between-group differences were found in the highlighted areas. Generally, the patient groups had fewer but stronger connections than the HC group.

Feature selection and connectome-based predictive modeling

Machine learning-based classification and prediction can allow the identification of clinically feasible neuroimaging biomarkers for cognitive decline in T2DM patients. We used CPM and SVM to obtain neuroimaging-based information potentially facilitating the clinical diagnosis of T2DM-C. Both analytical methods established links between the LSFC and several behavioral measures to generate a predictive model of behavioral data obtained from LSFC. However, SVM used the participants' group labels (*i.e.*, T2DM-C, T2DM-NC, and HC) as behavioral data while CPM used the MoCA scores in patients with T2DM.

Figure 1B illustrates the key CPM steps. Step 1: For each participant, CPM inputs comprised a set of M × M FCMs based on three atlases and a set of behavioral measures (here, MoCA scores). In the set of M × M FCMs, the number of brain regions or nodes is denoted by M; moreover, the between-node connection strength is associated with the matrix elements. Step 2 (feature selection): The Pearson's

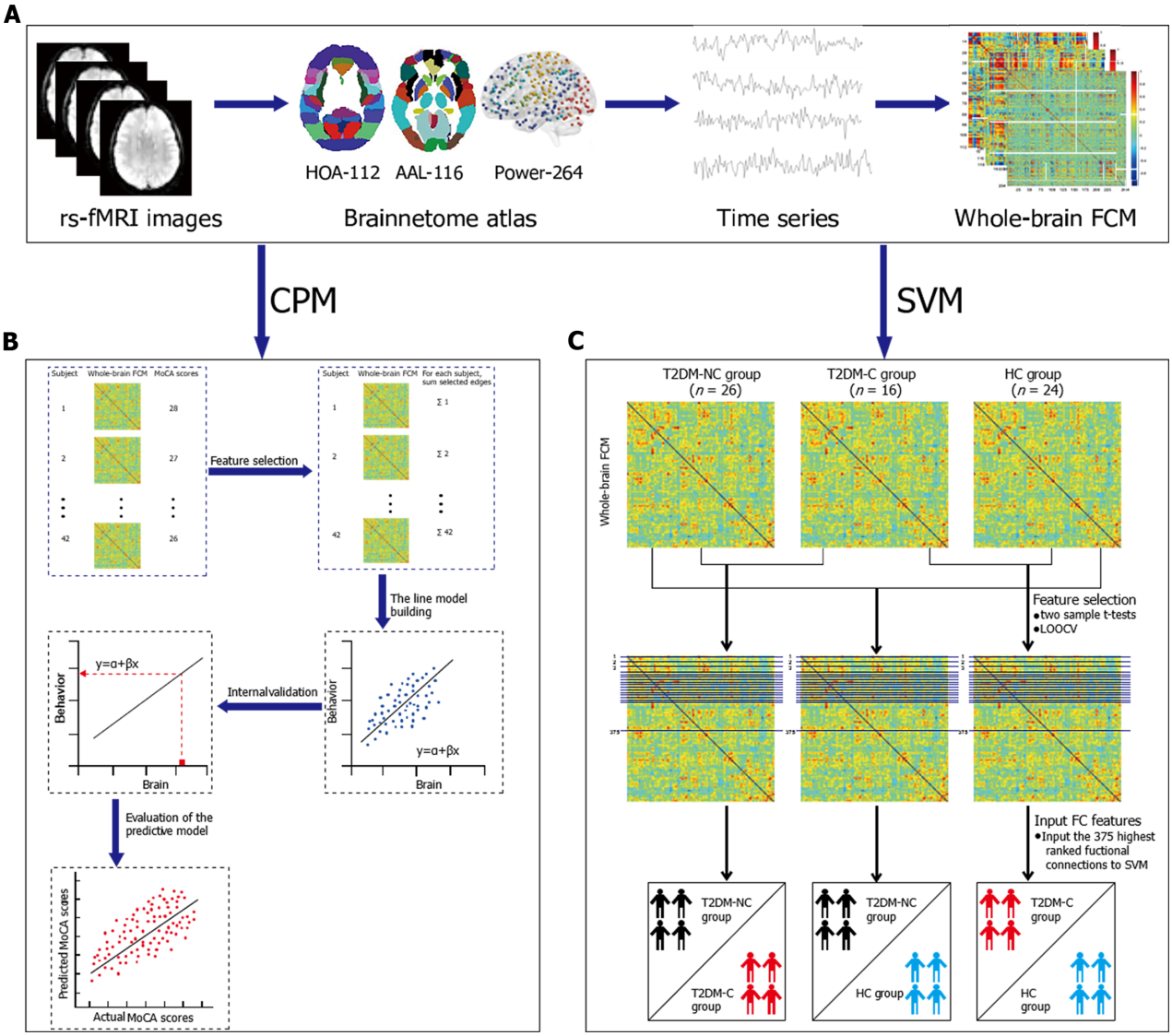

Figure 1 The prediction and classification flowchart. A: Relevant information from image preprocessing to feature identification; B: Detailed steps of connectome-based predictive modeling; C: Detailed steps of support vector machine. rs-fMRI: Resting state functional magnetic resonance imaging; HOA -112: Harvard Oxford atlas; AAL-116: Automated Anatomical Labeling; Power-264: 264-region functional atlas introduced by Power *et al*; FCM: Functional connectivity matrix; MoCA: Montreal Cognitive Assessment; T2DM: Type 2 diabetes mellitus; T2DM-C/T2DM-NC: Patients with T2DM with the presence/absence of mild cognitive impairment; HC: Healthy controls; LOOCV: Leave-one-out cross validation; FC: Functional connectivity.

correlation of each edge in the FCMs with the MoCA scores was computed. The most significant edges were pitched on by linear regression and subsequently merged into a single value for each participant. Based on the sign of the resultant r values with respect to a threshold of $P < 0.01$, they were separated into positive and negative tails (*i.e.*, positive and negative correlations, respectively, between the edge strength and MoCA scores)[18,32]. Subsequently, the positive and negative network strengths were computed by summing the edge strengths (*i.e.*, Z scores) for all edges in the positive and negative tails, respectively. Finally, we assessed the correlations of the positive and negative network strengths with the MoCA scores. Step 3 (line model building): Next, once the assumption of a linear relationship between the summary value of the connectivity data (independent variable) and the behavioral variable (dependent variable) was true, the predictive model was built; this was done separately for the positive and negative edge sets. Step 4 (prediction of novel participants): For each participant, the positive and negative edge sets were predicted by the behavioral measures. Given the limited sample size, leave-one-out cross validation (LOOCV) was applied separately to training and test data. The training and test datasets comprised N-1 and one participant, respectively. Step 5 (evaluation of predictive model): The comparison between the predicted and observed values can effectively evaluate the predictive model. Predictive accuracy was assessed using Pearson correlation analysis of the predicted and actual scores (r predicted-actual). Prediction performance was

assessed using permutation tests.

Feature selection and support vector machine

An SVM model was used to identify LSFC biomarkers for differentiating between the T2DM-NC/T2DM-C, T2DM-NC/HC, and T2DM-C/HC groups. SVM is the most used classification algorithm in ML[33]. For instance, we trained an SVM model using the training dataset to map the set of features of respective labels when given a specific feature (*e.g.*, LSFC) and label (*e.g.*, T2DM and HC). Therefore, given a new dataset, the SVM can be used to predict its label (group). The performance of these models was estimated through LOOCV using measures of accuracy, sensitivity, the receiver operating characteristic (ROC) curve, and the area under the ROC curve (AUC). The use of SVM was dependent on the Statistics and Machine Learning Toolbox in MATLAB 2018a. Figure 1C illustrates the detailed steps of SVM. Step 1: For further selection, some lower triangle elements of each FCM were extracted. The feature space spanned $(112 \times 111)/2 = 6216$, $(116 \times 115)/2 = 6670$, and $(264 \times 263)/2 = 34716$ dimensional functional connections for the HOA-112 atlas, AAL-116 atlas, and Power-264 atlas, respectively. Step 2 (feature selection): As reported previously[17,21], the analysis mentioned above was performed *via* two-sample t-tests and LOOCV. Specifically, 66 observations (FCMs with among-group differences) were subdivided into 66 folds. For each fold, the features were ranked in descending order based on the absolute between-group t values, followed by selection of the most discernible connections (from 1 to 375). Step 3 (input the classification features): We input the 375 highest-ranked functional connections into the SVM classifier model trained by LOOCV using the training data. Step 4 (evaluate the appearance of the SVM model using ROC curves and AUC): Sensitivity and specificity refer to the proportion of true positive and negative samples, which are associated with the diagnostic values.

Statistical analysis

SPSS (version 20.0; SPSS, Chicago, IL, USA) was used for statistical analysis. $P < 0.05$ indicated statistical significance. Grouped non-continuous data, including sex, were compared using chi-squared tests. We used one-way analysis of variance to evaluate normally distributed quantitative data, including education, HbA1c (%), BMI, self-rating anxiety scale (SAS) scores, and self-rating depression scale (SDS) scores. The SDS is a simple, 20-question scale that reflects depressive mood, physical symptoms, psychomotor behavior, and psychological symptom experience based on how one feels over the course of a week. Since it is self-administered, the test is widely used and does not require others' participation. The SAS is a self-rating scale containing 20 items (hoping to elicit 20 symptoms) divided into 4 grades. The main evaluation item is the frequency of the occurrence of the defined symptoms. The criteria are: "1" the symptoms occur a little or none of the time; "2" a small part of the time; "3" a lot of the time; "4" most or all the time. The SAS is intended for adults with symptoms of anxiety. At the same time, it has a wider applicability than the SDS. For values with significant among-group differences, the least significant difference was used to perform post hoc comparisons between each group pair. Non-normally distributed continuous quantitative data, including age, FBG, waist-to-hip ratio (WHR), systolic pressure, diastolic pressure, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, urinary microalbumin, duration of diabetes, MMSE scores, and MoCA scores, are expressed as the median (minimum, maximum). Between-group and among-group differences in non-normally distributed data were evaluated using the Mann-Whitney U test and Kruskal-Wallis non-parametric comparisons, respectively. However, the Kruskal-Wallis test could not perform pairwise comparisons among the three groups, which were performed directly through SPSS version 20.0.

P-values were corrected, and multiple comparison issues were addressed by permutation tests[34] performed by randomly assigning participants to two groups 5,000 times. When regional volume and eigenvector centrality values did not belong to the 95% of the null distribution of permutation tests ($P < 0.05$, corrected), the differences were considered significant. All the analyses mentioned above were performed using MATLAB.

RESULTS

Demographic and clinical characteristics

Table 1 summarizes the clinical and demographic characteristics of the T2DM-NC, T2DM-C, and HC groups. No significant between-group differences were found in age, sex distribution, BMI, blood pressure, total cholesterol, triglycerides, urinary microalbumin, duration of diabetes, SAS scores, and SDS scores. Compared with the T2DM-C group, the T2DM-NC and HC groups had higher levels of education (P = 0.040 and P = 0.015, respectively), MMSE scores (P < 0.001 and P = 0.002, respectively), and MoCA scores (P < 0.001 and P = 0.001, respectively). No significant differences were found in education, MMSE scores, and MoCA scores between the T2DM-NC and HC groups. Furthermore, compared with the HC group, the T2DM-C/NC groups had higher levels of HbA1c (P < 0.001), HDL cholesterol (P = 0.005, P = 0.006), FBG (P = 0.001, P < 0.001), and WHR (P = 0.017, P = 0.002, respectively). No significant differences were found in the levels of HbA1c, HDL cholesterol, FBG, and WHR between the T2DM-C/NC groups.

Individualized prediction of T2DM outcome

Based on the fMRI data, we found that the CPM, which was based on positive network strength, could significantly predict the participants' MoCA scores (Pearson's correlation of predicted and observed MoCA scores, r = 0.32, P = 0.0066 [HOA-112 atlas]; r = 0.32, P = 0.0078 [AAL-116 atlas]; r = 0.42, P = 0.0038 [Power-264 atlas]; see Table 2 and Figure 2). However, the predictions were not significant in the negative network model. Compared to the random label (P < 0.01), permutation tests (repetition times: 5000) indicated the higher actual classification accuracy.

For the HOA-112 atlas, between-network connectivity in the VAN, DMN, and SMN was crucially involved in predicting the MoCA scores in patients with T2DM. For the AAL-116 atlas, significantly discriminative LSFCs were mainly located across the limbic system, DMN, VN, BG, and cerebellum. For the Power-264 atlas, the most significantly predictive LSFCs were those between the VN and SSH. Overall, highly discriminative LSFCs were mainly located in the DMN, limbic system, BG, and VN.

Network anatomy predicts MoCA scores

Next, we investigated the neuroanatomy of positive MoCA networks. Figure 3A-C show a circle plot visualization for edges, which comprises the positive MoCA networks. These figures present the general neurocognitive composition of positive MoCA networks, which are indicative of the advanced descriptions of the brain regions involved. Figure 3D-F show glass brain plots displaying the above LSFCs localized in the 3D brain space. These figures indicate that these LSFCs, which were used to predict the differences between MoCA scores, were not located in specific brain regions but distributed throughout the brain.

Individualized classification of T2DM outcomes

Table 3 and Figure 4 show the ROC curves and AUC values. We selected 375 functional connections using the LOOCV after achieving the highest performance. Although the SVM model did not achieve good performance in three two-category classifications, the highest performance was achieved in discriminating between the T2DM-C/NC groups using the 375 highest-ranked functional connections (HOA-112 atlas: AUC=0.70, specificity = 0.69, sensitivity = 0.73, P = 0.0144; AAL-116 atlas: AUC = 0.65, specificity = 0.69, sensitivity = 0.65, P = 0.0556; Power -264 atlas: AUC = 0.70, specificity = 0.63, sensitivity = 0.77, P = 0.0160).

For the HOA-112 atlas, between-network connectivity in the BG, SMN, and FPN was crucially involved in discriminating between the T2DM-C/NC groups. For the AAL-116 atlas, the most discriminative and attractive LSFCs were located between the limbic system and the BG, as well as between the DMN and cerebellum. For the Power-264 atlas, the most significantly predictive functional connections were between the DMN and FPTC network. Overall, the DMN and BG were crucially involved in differentiating between the T2DM-C/NC groups.

Network anatomy in the classification of T2DM-C and T2DM-NC

Next, we visualized the neuroanatomical location of the network identified by classification (T2DM-C group *vs*. T2DM-NC group). Figure 5A-C demonstrate the network identified by classification after grouping the edges into macroscale brain regions. Figures 5D-F show glass brain plots displaying the same LSFCs localized in the 3D brain space; these figures indicate that these LSFCs, which were also used to predict

Table 1 Demographic and clinical characteristics of these three groups

Characteristics	T2DM-NC (*n* = 26)	T2DM-C (*n* = 16)	HC (*n* = 24)	*P* value
Age (yr)[2]	51 (34, 65)	54 (39, 67)	49 (26, 59)	0.227
Female/Male	4/22	6/10	9/15	0.153
Education (yr) [1]	12.88 ± 2.55	10.81 ± 2.76	13.38 ± 3.88	0.040
HbA1c (%)[1]	8.13 ± 1.87	9.06 ± 1.77	5.66 ± 0.33	0.000
FBG (mg/dL) [2]	7.85 (4.20, 15.80)	7.60 (3.60, 11.70)	5.20 (4.80, 6.80)	0.000
BMI (kg/m2) [1]	25.26 ± 2.43	24.90 ± 2.97	23.80 ± 2.41	0.779
WHR[2]	0.91 (0.76, 0.96)	0.91 (0.86, 0.96)	0.87 (0.78, 0.93)	0.004
Blood pressure (mmHg)				
SP[2]	128.00 (105.00, 150.00)	120.00 (101.00, 150.00)	128.00 (100.00, 181.00)	0.836
DP[2]	80.00 (60.00, 90.00)	80.00 (60.00, 90.00)	80.00 (67.00, 118.00)	0.432
Total cholesterol[2]	4.04 (2.76, 6.69)	4.21 (2.63, 5.71)	4.43 (3.69, 5.39)	0.407
HDL cholesterol[2]	1.35 (0.43, 6.60)	1.26 (0.53, 8.08)	0.94 (0.71, 1.64)	0.001
Triglycerides (mg/dL)[2]	1.75 (0.43, 6.60)	1.26 (0.53, 8.08)	2.06 (0.87, 6.41)	0.457
UMA (μg/min)[2]	12.45 (1.00, 342.70)	15.95 (7.00, 299.00)	13.65 (0.40, 58.60)	0.706
Duration of diabetes (mo)[2]	96.00 (0.25, 180.00)	24.00 (0.25, 228.00)		0.515
MMSE[2]	29.00 (27.00, 30.00)	26.00 (23.00, 29.00)	28.00 (27.00, 30.00)	0.000
MoCA[2]	27.00 (25.00, 30.00)	24.00 (18.00, 30.00)	27.00 (24.00, 30.00)	0.000
SAS[1]	41.62 ± 7.12	43.75 ± 7.26	39.54 ± 7.00	0.190
SDS[1]	46.12 ± 6.87	45.31 ± 8.46	41.71 ± 10.07	0.172

[1]Data are presented as mean ± SD.
[2]Data are presented as median (minimum, maximum).
P < 0.05 was considered significant. T2DM: Type 2 diabetes mellitus; T2DM-C/T2DM-NC: Patients with T2DM with the presence/absence of mild cognitive impairment; HC: Healthy controls; HbA1c: Glycosylated hemoglobin A1c; FBG: Fasting blood glucose; BMI: Body mass index; WHR: Waist-to-Hip Ratio; SP: Systolic pressure; DP: Diastolic pressure; HDL: High density lipoprotein; UMA: Urinary microalbumin; MMSE: Mini-mental state examination; MoCA: Montreal cognitive assessment; SAS: Self-rating anxiety scale; SDS: Self-rating depression scale.

Table 2 Prediction outcome

Brain atlas	Correlation coefficient	*P* value
HOA-112 atlas	0.32	0.0066
AAL-116 atlas	0.32	0.0078
Power-264 atlas	0.42	0.0038

HOA-112: Harvard Oxford atlas; AAL-116: Automated Anatomical Labeling; Power-264: 264-region functional atlas introduced by Power *et al*.

the differences between MoCA scores, were not located in specific brain regions but distributed throughout the brain.

DISCUSSION

The present study examined whether we could adopt LSFC patterns as discriminative features to classify and predict cognitive impairment related to T2DM with a high degree of accuracy. Compared to neuropsychological scales, which may be unreliable and subjective, it is evident from our results that LSFC is useful in the early detection of MCI related to T2DM. Our results indicate that functional networks contain clinically relevant cognition-related information, which is defined in a data-driven

Table 3 Classification outcome

Group	Brain atlas	AUC	Specificity	Sensitivity	*P* value
T2DM-NC *vs* T2DM-C	HOA-112 atlas	0.70	0.69	0.73	0.0144
	AAL-116 atlas	0.65	0.69	0.65	0.0556
	Power-264 atlas	0.70	0.63	0.77	0.0160
T2DM-NC *vs* HC	HOA-112 atlas	0.54	0.75	0.42	0.3122
	AAL-116 atlas	0.53	0.58	0.54	0.3804
	Power-264 atlas	0.56	0.58	0.58	0.2478
T2DM-C *vs* HC	HOA-112 atlas	0.54	0.63	0.56	0.3152
	AAL-116 atlas	0.72	0.67	0.75	0.0096
	Power-264 atlas	0.70	0.79	0.63	0.0184
T2DM *vs* HC	HOA-112 atlas	0.67	0.63	0.69	0.0144
	AAL-116 atlas	0.63	0.58	0.64	0.0444
	Power-264 atlas	0.50	0.50	0.67	0.4898

T2DM: Type 2 diabetes mellitus; T2DM-C/T2DM-NC: Patients with T2DM with the presence/absence of mild cognitive impairment; HC: Healthy controls; AUC: The area under the receiver operating characteristic curve; HOA-112: Harvard Oxford atlas; AAL-116: Automated Anatomical Labeling; Power-264: 264-region functional atlas introduced by Power *et al*.

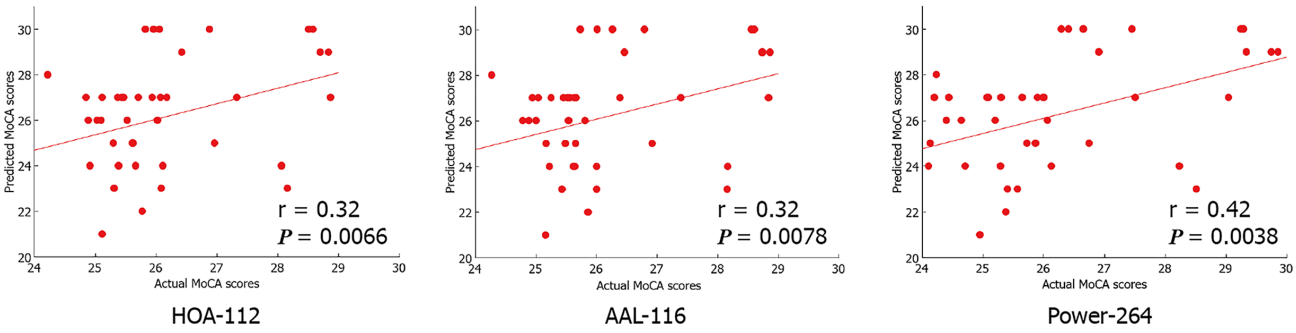

Figure 2 The connectome-based predictive modeling predicted the Montreal Cognitive Assessment scores. Scatterplot of predicted the Montreal Cognitive Assessment (MoCA) scores vs. actual MoCA scores. Predicted scores were derived from edges positively correlated with prediction (positive network). *r*: The *r* value of Pearson's correlation of predicted the MoCA scores and actual MoCA scores; *P*: *P* values from permutation tests (5000 times); T2DM: Type 2 diabetes mellitus; T2DM-C/ T2DM-NC: Patients with T2DM with the presence/absence of mild cognitive impairment; HOA -112: Harvard Oxford atlas; AAL-116: Automated Anatomical Labeling; Power-264: 264-region functional atlas introduced by Power *et al*.

manner and has the potential to be a biomarker to assess the degree of cognitive decline related to T2DM.

T2DM is often associated with cognitive impairment and a higher dementia risk. Patients with T2DM may present with deteriorated memory, attention, reagency, and execution[2-5]. However, the exact pathophysiological mechanisms underlying T2DM-related cognitive dysfunction remain unclear, which impedes the development of preventive treatments. We analyzed resting-state fMRI data using the CPM and SVM. We computed the LSFC patterns using three types of functional brain atlases that separately comprised 112, 116, and 264 nodes covering the whole brain. The SVM-based classification results were not as expected; the exact reasons for which remain unclear. However, the CPM-based prediction results were positive, with exciting prospects. There have been no previous CPM studies on patients with T2DM; moreover, this is the first study to identify LSFC as an imaging biomarker for predicting T2DM-related MCI using CPM. CPM can reliably predict the participants' MoCA scores, which was based on positive network strength (r = 0.32, P = 0.0066 [HOA-112 atlas]; r = 0.32, P = 0.0078 [AAL-116 atlas]; r = 0.42, P = 0.0038 [Power-264 atlas]). Highly discriminative and attractive LSFCs were mainly located within the DMN, limbic system, BG, VN, or across these regions. Our findings suggest that the resting-state LSFC can reveal T2DM-related MCI, which could be more reliable than

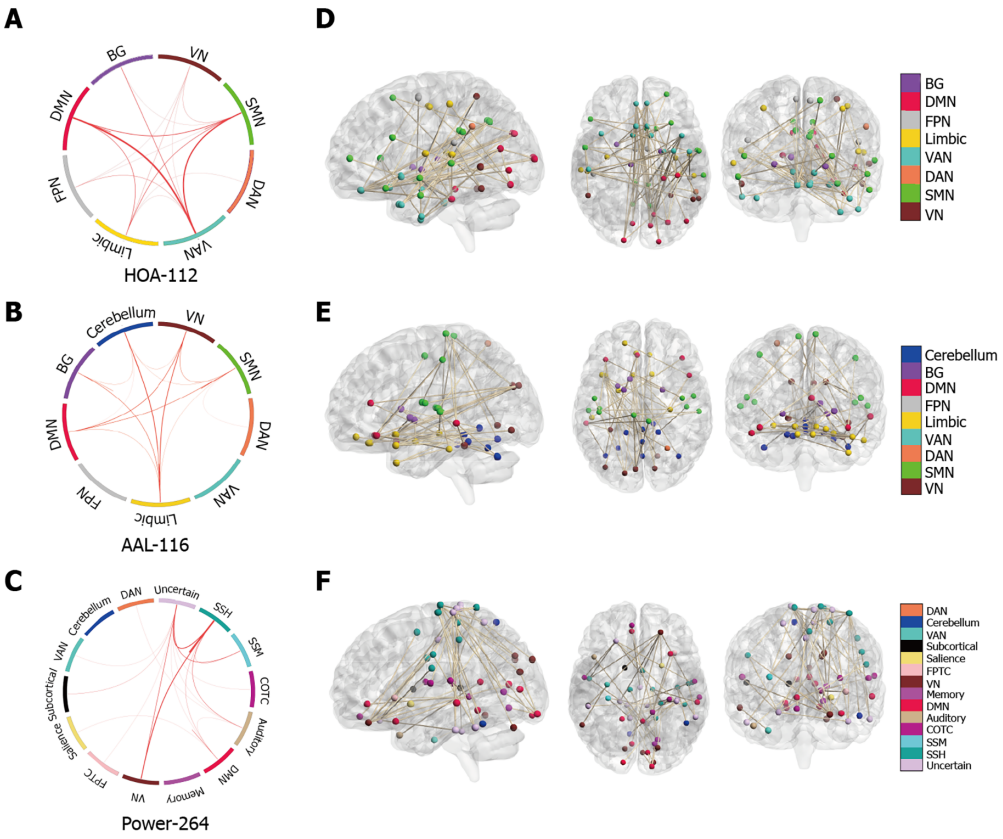

Figure 3 Functional connections predicting individual Montreal Cognitive Assessment scores based on three atlases. A-C: On the far left of the image above, edges were classified as macroscale brain regions, and visualized by circle plots, in which nodes are grouped based on their anatomic location. The resting-state network (RSN) of the brain based on three templates is represented by a rectangle on the circumference of the big circle. The lines connecting two rectangles represent the connections between the corresponding one or two RSNs, including inter-network connections and intra-network connections. The thickness of the line represents the weight (*i.e.,* connectome-based predictive modeling weight) of the connection. The thicker the line, the larger the weight. This visualization was created using Circos (http://circos.ca/). D-F: On the right of the image above, the same edges are visualized in the brain. The lines represent edges connecting the spheres, which in turn represent nodes. A legend indicating the approximate anatomic 'lobe' is shown in the far right side of the figure. HOA -112: Harvard Oxford atlas; AAL-116: Automated Anatomical Labeling; Power-264: 264-region functional atlas introduced by Power *et al*; VN: Visual Network; SMN: Sensory-motor Network; DAN: Dorsal Attention Network; VAN: Ventral Attention Network; Limbic: Limbic System; FPN: Fronto-parietal network; DMN: Default mode network; BG: Basal ganglia; SSH: Sensory somatomotor hand; SSM: Sensory somatomotor mouth; COTC: Cingulo-opercular task control; Memory: Memory retrieval; FPTC: Fronto-parietal task control.

standardized neuropsychological scales. There is significant interest in using the LSFC to predict human behavior. We found that the LSFC-based CPM could effectively predict the MoCA scores in patients with T2DM. The prediction of neurocognitive abilities from CPM can complete the conventional assessments. The CPM-related positive network was used as a T2DM-related MCI connectivity measure and showed favorable results based on the Pearson correlation coefficient. CPM can predict individua behaviors or characteristics by LSFC, which is novel and data-driven[13,35]; moreover, it can successfully predict the number of psychiatric and psychological phenotypes[32,36]. CPM can isolate brain "fingerprints" that identify individual participants from a group[13], as well as predict personality traits[32], sustained attention[18,37], treatment outcomes[20], and cognitive dysfunction[8,38]. However, unlike previous studies on fluid intelligence[13] and attention[18], where the positive and negative networks showed comparable predictive performance, we found that the negative network showed an unfavorable predictive performance.

Regarding the functional anatomy of the edges, which is most relevant to individual differences in the degree of cognition, we paid more attention to the CPM-positive network. Moreover, lower MoCA scores were associated with higher network strength, indicating more severe cognitive dysfunction. This suggests that the cognitive decline in T2DM patients may involve abnormal connectivity among these different resting-state networks. For both prediction and classification, most significantly discriminative functional connections were related to the DMN, limbic system, and the BG.

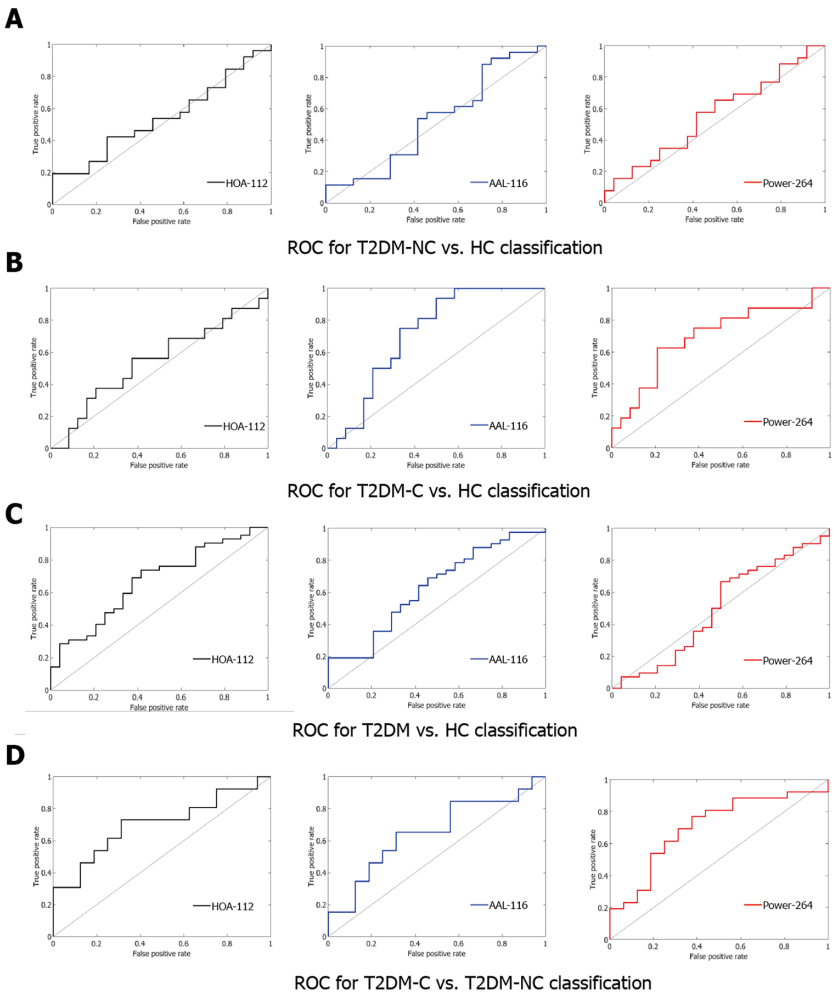

Figure 4 Classification efficiency of support vector machine based on three atlases. The classification effect was not very ideal. A: The area under curve (AUC) value of patients with type 2 diabetes mellitus (T2DM) with the absence of mild cognitive impairment (T2DM-NC) Verus healthy controls (HC) group was 0.54 [Harvard Oxford (HOA-112) atlas], 0.53 [Automated Anatomical Labeling (AAL-116) atlas], 0.56 [264-region functional (Power-264) atlas]; B: the AUC value of patients with T2DM with the presence of mild cognitive impairment (T2DM-C) Verus HC group was 0.54 (HOA-112 atlas), 0.72 (AAL-116 atlas), 0.70 (Power-264 atlas); C: the AUC value of T2DM Verus HC group was 0.67 (HOA-112 atlas), 0.63 (AAL-116 atlas), 0.50 (Power-264 atlas); D: the AUC value of T2DM-C Verus T2DM-NC group was 0.70 (HOA-112 atlas and Power-264 atlas), 0.65(AAL-116 atlas). ROC: receiver operating characteristic curve.

The DMN is activated during wakeful rest and deactivated during cognitive task execution; further, it is involved in cognitive processing[8,11]. The DMN comprises several brain regions, including the anterior cingulate cortex; medial prefrontal cortex; and the medial, lateral, and inferior parietal cortices[39], which are involved in constructing self-related mental simulations, including recalling the past, thinking about the future, and understanding others' perspectives[8,11]. Cognitive impairment in T2DM is related to reduced connectivity in cognition-related networks, most prominently in the DMN[40]. Changes in brain structure and function are associated with the deterioration of cognition; moreover, blood glucose fluctuations (hyperglycemia or hypoglycemia) may be related to T2DM-related brain changes[41]. Repeated hyperglycemia and hypoglycemia can lead to a variety of metabolic and molecular changes that eventually lead to widespread changes in brain cells. However, the exact causes of T2DM-related changes in the DMN are unclear. Specific alterations in functional connectivity may contribute to cognitive decline in patients with T2DM and may represent a promising biomarker.

The cingulate/paracingulate gyrus and parahippocampal gyrus are indispensable to the functioning of the limbic system. They are crucially involved in learning, emotion, memory, and other processes. A recent meta-analysis, including 15 structural studies and 16 functional studies, reported decreased global and regional gray matter volume in the limbic system of patients with T2DM, which could be associated with poor cognitive performance[42]. The results from some studies indicate that the changes in limbic regions, especially in dendritic structures, inhibit the formation of the spinal cord due to the chronic hyperglycemia; moreover, they may also disrupt the

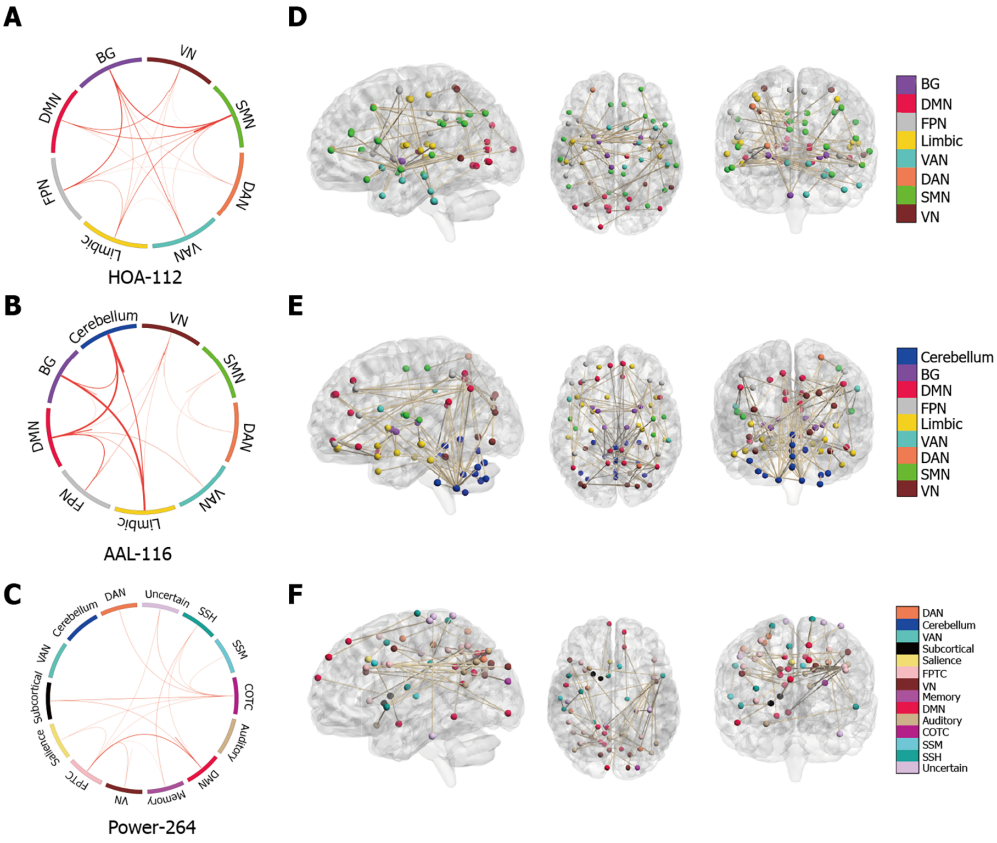

Figure 5 Functional connections classifying patients with type 2 diabetes mellitus with the present of mild cognitive impairment and patients with type 2 diabetes mellitus with the absence of mild cognitive impairment based on three different atlas. A-C: On the far left of the image above, edges were classified as macroscale brain regions and were visualized using circle plots, in which nodes are grouped based on anatomic location. The resting-state network (RSN) of brain based on three atlas is represented by a rectangle on the circumference of the big circle. The lines connecting two rectangles represent the connections between the corresponding one or two RSN, including inter-network connections and intra-network connections. The thickness of the line represents the weight (*i.e.*, support vector classification weight) of the connection. The thicker the line, the larger the weight. This visualization was created using Circos (http://circos.ca/); D-F: On the right of the image above, the same edges are visualized on brains. The lines represent edges connecting the spheres, which represent nodes. A legend indicating the approximate anatomic 'lobe' is shown in the far right side of picture. VN: Visual network; SMN: Sensory-motor network; DAN: Dorsal attention network; VAN: Ventral attention network; Limbic: Limbic system; FPN: Fronto-parietal network; DMN: Default mode network; BG: Basal ganglia; SSH: Sensory Somatomotor hand; SSM: Sensory somatomotor mouth; COTC: Cingulo-opercular task control; Memory: Memory retrieval; FPTC: Fronto-parietal task control.

processes of memory and learning[43,44]. In addition, multi-timescale variability of abnormal glucose regulation may be associated with poor cognitive function in patients with T2DM, which may be attributed to the gray matter atrophy in the limbic region[45].

Different structures within the basal ganglia, which is involved in movement regulation, play different roles in various diseases. Lesions in the basal ganglia region mainly result in abnormal movement (increased or decreased movement) and changes in muscle tone (increased or decreased). The basal ganglia represent an important neural functional area, closely related to sensory, motor, visual, behavioral and other functions. This area has a high incidence of stroke. Parkinson's disease and Huntington's disease are among the most studied diseases in the area[46]. There is no adequate evidence regarding a relationship between the basal ganglia and T2DM; however, patients with T2DM-C have been shown to have severely impaired overall network efficiency, with decreased lymph node efficiency and connections in multiple regions, including the limbic system and BG[47]. Additionally, a meta-analysis reported reduced overall brain volume and BG atrophy in patients with T2DM[48]. Basal ganglia changes in diabetics typically occur in hyperglycemic osmotic states in older Asian women[49]. Attributable causes of dyskinesia in diabetic patients include hyperglycemia, high viscosity, changes in brain gamma aminobutyric acid metabolism, diabetic angiopathy, and cytotoxic edema. High blood sugar and viscosity can break down the blood-brain barrier, leading to ischemia. Taken together, T2DM-related cognitive impairment may involve abnormal connection patterns across the DMN, limbic system, and BG.

Overall, our study has three main features. First, this is the first study to successfully apply CPM in patients with T2DM for identifying neuroimaging biomarkers associated with cognitive impairment. Second, unlike most previous studies that performed between-group comparisons using *a priori* defined brain regions/networks[8,9,11,40], we performed whole-brain bottom-up analyses. Therefore, our method could facilitate the identification of crucial features for predicting cognitive performance at an individual level. Third, we used three brain templates, namely the HOA-112, AAL-116, and Power-264 atlases, to demonstrate the predictive utility of CPM for determining T2DM-related cognitive impairment from different perspectives. Still, there are some limitations in this study. First, doubling our sample size might have increase the generalizability of our results. Second, we only used rs-fMRI data, whereas other modalities like structural MRI and diffusion-weighted imaging might provide complementary information to improve the quantification of brain networks. Finally, according to our findings, the neurobiological changes of T2DM can be reflected by the resting-state brain network. More in-depth and longitudinal studies are required to elucidate the specific influence on T2DM pathogenesis, especially T2DM-related problems in thought processing.

CONCLUSION

This study used the CPM method to identify LSFC patterns, including connections across the DMN, limbic system, and BG, as potential biomarkers for overall cognitive status in patients with T2DM. LSFC provided neuroimaging-based information that could clinically predict the MoCA scores in patients with T2DM. Applying CPM based on LSFC for predicting neurocognitive abilities can complement conventional neurocognitive assessments and facilitate the management of patients with T2DM.

ARTICLE HIGHLIGHTS

Research background

Whole-brain functional connectivity patterns, or large-scale functional connectivity (LSFC) patterns, are both highly unique and reliable in each individual, and similar to a fingerprint, can identify individual differences in personality traits or cognitive functions. Abnormal LSFC patterns have been found in patients with dementia, as well as in those with mild cognitive impairment (MCI), which predicted their cognitive performance. It has been reported that patients with type 2 diabetes mellitus (T2DM) may develop MCI that could progress to dementia. We assessed the applicability of LSFC-related discriminative features to predict the cognitive level of patients with T2DM using a connectome-based predictive modeling (CPM) and support vector machine (SVM).

Research motivation

Whether machine learning techniques like CPM and SVM could utilize LSFC patterns to predict T2DM-related MCI with a high degree of accuracy remains unclear.

Research objectives

To investigate the utility of LSFC for more accurately and reliably predicting the cognitive impairment related to T2DM.

Research methods

Resting-state functional magnetic resonance images were derived from 42 patients with T2DM and 24 healthy controls. Cognitive function was assessed using the Montreal Cognitive Assessment (MoCA). Patients with T2DM were divided into two groups, according to the presence (T2DM-C; n = 16) or absence (T2DM-NC; n = 26) of MCI. Brain regions were marked using the Harvard Oxford (HOA-112), automated anatomical labeling (AAL-116), and 264-region functional (Power-264) atlases. LSFC biomarkers for predicting MoCA scores were identified using a new CPM technique. Subsequently, we used the SVM based on LSFC patterns for among-group differentiation. The area under the receiver operating characteristic curve determined the classification appearance.

Research results

CPM could predict MoCA scores in patients with T2DM, indicating that LSFC patterns represent cognition-level measures in these patients. Positive (anti-correlated) LSFC networks based on the Power-264 atlas showed the best predictive performance (r=0.42, *P*=0.0038); moreover, we observed new brain regions of interest associated with T2DM-related cognition. The area under the receiver operating characteristic curve values (T2DM-NC group *vs*. T2DM-C group) were 0.65-0.70, with LSFC matrices based on HOA-112 and Power-264 atlases having the highest value (0.70). Most discriminative and attractive LSFCs were related to the default mode network, limbic system, and basal ganglia.

Research conclusions

LSFC provides neuroimaging-based information that may be useful in detecting MCI early and accurately in patients with T2DM and therefore assist with T2DM management.

Research perspectives

Our study provides promising evidence that LSFC can reveal cognitive impairment in patients with T2DM, although further development is needed for clinical application.

REFERENCES

1 **Dong Y**, Gao W, Zhang L, Wei J, Hammar N, Cabrera CS, Wu X, Qiao Q. Patient characteristics related to metabolic disorders and chronic complications in type 2 diabetes mellitus patients hospitalized at the Qingdao Endocrine and Diabetes Hospital from 2006 to 2012 in China. *Diab Vasc Dis Res* 2017; **14**: 24-32 [PMID: 27941053 DOI: 10.1177/1479164116675489]

2 **Biessels GJ**, Strachan MW, Visseren FL, Kappelle LJ, Whitmer RA. Dementia and cognitive decline in type 2 diabetes and prediabetic stages: towards targeted interventions. *Lancet Diabetes Endocrinol* 2014; **2**: 246-255 [PMID: 24622755]

3 **Roberts RO**, Knopman DS, Geda YE, Cha RH, Pankratz VS, Baertlein L, Boeve BF, Tangalos EG, Ivnik RJ, Mielke MM, Petersen RC. Association of diabetes with amnestic and nonamnestic mild cognitive impairment. *Alzheimers Dement* 2014; **10**: 18-26 [PMID: 23562428 DOI: 10.1016/j.jalz.2013.01.001]

4 **Ma F**, Wu T, Miao R, Xiao YY, Zhang W, Huang G. Conversion of mild cognitive impairment to dementia among subjects with diabetes: a population-based study of incidence and risk factors with five years of follow-up. *J Alzheimers Dis* 2015; **43**: 1441-1449 [PMID: 25159674]

5 **Kodl CT**, Seaquist ER. Cognitive dysfunction and diabetes mellitus. *Endocr Rev* 2008; **29**: 494-511 [PMID: 18436709 DOI: 10.1210/er.2007-0034]

6 **Wang ZL**, Zou L, Lu ZW, Xie XQ, Jia ZZ, Pan CJ, Zhang GX, Ge XM. Abnormal spontaneous brain activity in type 2 diabetic retinopathy revealed by amplitude of low-frequency fluctuations: a resting-state fMRI study. *Clin Radiol* 2017; **72**: 340.e1-340.e7 [PMID: 28041652 DOI: 10.1016/j.crad.2016.11.012]

7 **Qi N**, Cui Y, Liu JC, Yu M, Teng GJ. [Follow-up of resting-state brain function with magnetic resonance imaging in patients with type 2 diabetes mellitus]. *Zhonghua Yi Xue Za Zhi* 2017; **97**: 3057-3061 [PMID: 29081148 DOI: 10.3760/cma.j.issn.0376-2491.2017.39.004]

8 **Liu Z**, Liu J, Yuan H, Liu T, Cui X, Tang Z, Du Y, Wang M, Lin Y, Tian J. Identification of Cognitive Dysfunction in Patients with T2DM Using Whole Brain Functional Connectivity. *Genomics Proteomics Bioinformatics* 2019; **17**: 441-452 [PMID: 31786312 DOI: 10.1016/j.gpb.2019.09.002]

9 **Tan X**, Liang Y, Zeng H, Qin C, Li Y, Yang J, Qiu S. Altered functional connectivity of the posterior cingulate cortex in type 2 diabetes with cognitive impairment. *Brain Imaging Behav* 2019; **13**: 1699-1707 [PMID: 30612339 DOI: 10.1007/s11682-018-0017-8]

10 **Chen Y**, Liu Z, Wang A, Zhang J, Zhang S, Qi D, Chen K, Zhang Z. Dysfunctional organization of default mode network before memory impairments in type 2 diabetes. *Psychoneuroendocrinology* 2016; **74**: 141-148 [PMID: 27611859 DOI: 10.1016/j.psyneuen.2016.08.012]

11 **Liu H**, Liu J, Peng L, Feng Z, Cao L, Liu H, Shen H, Hu D, Zeng LL, Wang W. Changes in default mode network connectivity in different glucose metabolism status and diabetes duration. *Neuroimage Clin* 2019; **21**: 101629 [PMID: 30573410 DOI: 10.1016/j.nicl.2018.101629]

12 **Fang F**, Lai MY, Huang JJ, Kang M, Ma MM, Li KA, Lian JG, Wang Z, Yin DZ, Wang YF. Compensatory Hippocampal Connectivity in Young Adults With Early-Stage Type 2 Diabetes. *J Clin Endocrinol Metab* 2019; **104**: 3025-3038 [PMID: 30817818 DOI: 10.1210/jc.2018-02319]

13 **Finn ES**, Shen X, Scheinost D, Rosenberg MD, Huang J, Chun MM, Papademetris X, Constable RT. Functional connectome fingerprinting: identifying individuals using patterns of brain connectivity. *Nat Neurosci* 2015; **18**: 1664-1671 [PMID: 26457551 DOI: 10.1038/nn.4135]

14 **Jiang R**, Calhoun VD, Zuo N, Lin D, Li J, Fan L, Qi S, Sun H, Fu Z, Song M, Jiang T, Sui J. Connectome-based individualized prediction of temperament trait scores. *Neuroimage* 2018; **183**:

366-374 [PMID: 30125712 DOI: 10.1016/j.neuroimage.2018.08.038]
15 **Liu Z**, Zhang Y, Bai L, Yan H, Dai R, Zhong C, Wang H, Wei W, Xue T, Feng Y, You Y, Tian J. Investigation of the effective connectivity of resting state networks in Alzheimer's disease: a functional MRI study combining independent components analysis and multivariate Granger causality analysis. *NMR Biomed* 2012; **25**: 1311-1320 [PMID: 22505275 DOI: 10.1002/nbm.2803]
16 **Liu Z**, Zhang Y, Yan H, Bai L, Dai R, Wei W, Zhong C, Xue T, Wang H, Feng Y, You Y, Zhang X, Tian J. Altered topological patterns of brain networks in mild cognitive impairment and Alzheimer's disease: a resting-state fMRI study. *Psychiatry Res* 2012; **202**: 118-125 [PMID: 22695315 DOI: 10.1016/j.pscychresns.2012.03.002]
17 **Zeng LL**, Shen H, Liu L, Wang L, Li B, Fang P, Zhou Z, Li Y, Hu D. Identifying major depression using whole-brain functional connectivity: a multivariate pattern analysis. *Brain* 2012; **135**: 1498-1507 [PMID: 22418737 DOI: 10.1093/brain/aws059]
18 **Rosenberg MD**, Finn ES, Scheinost D, Papademetris X, Shen X, Constable RT, Chun MM. A neuromarker of sustained attention from whole-brain functional connectivity. *Nat Neurosci* 2016; **19**: 165-171 [PMID: 26595653 DOI: 10.1038/nn.4179]
19 **Yoo K**, Rosenberg MD, Hsu WT, Zhang S, Li CR, Scheinost D, Constable RT, Chun MM. Connectome-based predictive modeling of attention: Comparing different functional connectivity features and prediction methods across datasets. *Neuroimage* 2018; **167**: 11-22 [PMID: 29122720 DOI: 10.1016/j.neuroimage.2017.11.010]
20 **Lichenstein SD**, Scheinost D, Potenza MN, Carroll KM, Yip SW. Dissociable neural substrates of opioid and cocaine use identified *via* connectome-based modelling. *Mol Psychiatry* 2021; **26**: 4383-4393 [PMID: 31719641 DOI: 10.1038/s41380-019-0586-y]
21 **Li J**, Sun Y, Huang Y, Bezerianos A, Yu R. Machine learning technique reveals intrinsic characteristics of schizophrenia: an alternative method. *Brain Imaging Behav* 2019; **13**: 1386-1396 [PMID: 30159765 DOI: 10.1007/s11682-018-9947-4]
22 **American Diabetes Association**. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2014; **37** Suppl 1: S81-S90 [PMID: 24357215 DOI: 10.2337/dc14-S081]
23 **Freitas S**, Simões MR, Alves L, Santana I. Montreal cognitive assessment: validation study for mild cognitive impairment and Alzheimer disease. *Alzheimer Dis Assoc Disord* 2013; **27**: 37-43 [PMID: 22193353 DOI: 10.1097/WAD.0b013e3182420bfe]
24 **Li H**, Jia J, Yang Z. Mini-Mental State Examination in Elderly Chinese: A Population-Based Normative Study. *J Alzheimers Dis* 2016; **53**: 487-496 [PMID: 27163822]
25 **Luis CA**, Keegan AP, Mullan M. Cross validation of the Montreal Cognitive Assessment in community dwelling older adults residing in the Southeastern US. *Int J Geriatr Psychiatry* 2009; **24**: 197-201 [PMID: 18850670 DOI: 10.1002/gps.2101]
26 **Chen KL**, Xu Y, Chu AQ, Ding D, Liang XN, Nasreddine ZS, Dong Q, Hong Z, Zhao QH, Guo QH. Validation of the Chinese Version of Montreal Cognitive Assessment Basic for Screening Mild Cognitive Impairment. *J Am Geriatr Soc* 2016; **64**: e285-e290 [PMID: 27996103 DOI: 10.1111/jgs.14530]
27 **Yan CG**, Wang XD, Zuo XN, Zang YF. DPABI: Data Processing & Analysis for (Resting-State) Brain Imaging. *Neuroinformatics* 2016; **14**: 339-351 [PMID: 27075850 DOI: 10.1007/s12021-016-9299-4]
28 **Makris N**, Meyer JW, Bates JF, Yeterian EH, Kennedy DN, Caviness VS. MRI-Based topographic parcellation of human cerebral white matter and nuclei II. Rationale and applications with systematics of cerebral connectivity. *Neuroimage* 1999; **9**: 18-45 [PMID: 9918726 DOI: 10.1006/nimg.1998.0384]
29 **Tzourio-Mazoyer N**, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 2002; **15**: 273-289 [PMID: 11771995 DOI: 10.1006/nimg.2001.0978]
30 **Power JD**, Cohen AL, Nelson SM, Wig GS, Barnes KA, Church JA, Vogel AC, Laumann TO, Miezin FM, Schlaggar BL, Petersen SE. Functional network organization of the human brain. *Neuron* 2011; **72**: 665-678 [PMID: 22099467 DOI: 10.1016/j.neuron.2011.09.006]
31 **Schaefer A**, Kong R, Gordon EM, Laumann TO, Zuo XN, Holmes AJ, Eickhoff SB, Yeo BTT. Local-Global Parcellation of the Human Cerebral Cortex from Intrinsic Functional Connectivity MRI. *Cereb Cortex* 2018; **28**: 3095-3114 [PMID: 28981612 DOI: 10.1093/cercor/bhx179]
32 **Hsu WT**, Rosenberg MD, Scheinost D, Constable RT, Chun MM. Resting-state functional connectivity predicts neuroticism and extraversion in novel individuals. *Soc Cogn Affect Neurosci* 2018; **13**: 224-232 [PMID: 29373729 DOI: 10.1093/scan/nsy002]
33 **Bellec P**, Chu C, Chouinard-Decorte F, Benhajali Y, Margulies DS, Craddock RC. The Neuro Bureau ADHD-200 Preprocessed repository. *Neuroimage* 2017; **144**: 275-286 [PMID: 27423255 DOI: 10.1016/j.neuroimage.2016.06.034]
34 **Smith SM**, Vidaurre D, Beckmann CF, Glasser MF, Jenkinson M, Miller KL, Nichols TE, Robinson EC, Salimi-Khorshidi G, Woolrich MW, Barch DM, Uğurbil K, Van Essen DC. Functional connectomics from resting-state fMRI. *Trends Cogn Sci* 2013; **17**: 666-682 [PMID: 24238796 DOI: 10.1016/j.tics.2013.09.016]
35 **Shen X**, Finn ES, Scheinost D, Rosenberg MD, Chun MM, Papademetris X, Constable RT. Using connectome-based predictive modeling to predict individual behavior from brain connectivity. *Nat Protoc* 2017; **12**: 506-518 [PMID: 28182017 DOI: 10.1038/nprot.2016.178]

36 **Lake EMR**, Finn ES, Noble SM, Vanderwal T, Shen X, Rosenberg MD, Spann MN, Chun MM, Scheinost D, Constable RT. The Functional Brain Organization of an Individual Allows Prediction of Measures of Social Abilities Transdiagnostically in Autism and Attention-Deficit/Hyperactivity Disorder. *Biol Psychiatry* 2019; **86**: 315-326 [PMID: 31010580 DOI: 10.1016/j.biopsych.2019.02.019]

37 **Rosenberg MD**, Scheinost D, Greene AS, Avery EW, Kwon YH, Finn ES, Ramani R, Qiu M, Constable RT, Chun MM. Functional connectivity predicts changes in attention observed across minutes, days, and months. *Proc Natl Acad Sci U S A* 2020; **117**: 3797-3807 [PMID: 32019892 DOI: 10.1073/pnas.1912226117]

38 **Lin Q**, Rosenberg MD, Yoo K, Hsu TW, O'Connell TP, Chun MM. Resting-State Functional Connectivity Predicts Cognitive Impairment Related to Alzheimer's Disease. *Front Aging Neurosci* 2018; **10**: 94 [PMID: 29706883 DOI: 10.3389/fnagi.2018.00094]

39 **Anticevic A**, Cole MW, Murray JD, Corlett PR, Wang XJ, Krystal JH. The role of default network deactivation in cognition and disease. *Trends Cogn Sci* 2012; **16**: 584-592 [PMID: 23142417 DOI: 10.1016/j.tics.2012.10.008]

40 **Macpherson H**, Formica M, Harris E, Daly RM. Brain functional alterations in Type 2 Diabetes - A systematic review of fMRI studies. *Front Neuroendocrinol* 2017; **47**: 34-46 [PMID: 28687473 DOI: 10.1016/j.yfrne.2017.07.001]

41 **Seaquist ER**. The final frontier: how does diabetes affect the brain? *Diabetes* 2010; **59**: 4-5 [PMID: 20040482 DOI: 10.2337/db09-1600]

42 **Yao L**, Yang C, Zhang W, Li S, Li Q, Chen L, Lui S, Kemp GJ, Biswal BB, Shah NJ, Li F, Gong Q. A multimodal meta-analysis of regional structural and functional brain alterations in type 2 diabetes. *Front Neuroendocrinol* 2021; **62**: 100915 [PMID: 33862036 DOI: 10.1016/j.yfrne.2021.100915]

43 **Martínez-Tellez R**, Gómez-Villalobos Mde J, Flores G. Alteration in dendritic morphology of cortical neurons in rats with diabetes mellitus induced by streptozotocin. *Brain Res* 2005; **1048**: 108-115 [PMID: 15916754 DOI: 10.1016/j.brainres.2005.04.048]

44 **Sánchez F**, Gómez-Villalobos Mde J, Juarez I, Quevedo L, Flores G. Dendritic morphology of neurons in medial prefrontal cortex, hippocampus, and nucleus accumbens in adult SH rats. *Synapse* 2011; **65**: 198-206 [PMID: 20665725 DOI: 10.1002/syn.20837]

45 **Cui X**, Abduljalil A, Manor BD, Peng CK, Novak V. Multi-scale glycemic variability: a link to gray matter atrophy and cognitive decline in type 2 diabetes. *PLoS One* 2014; **9**: e86284 [PMID: 24475100 DOI: 10.1371/journal.pone.0086284]

46 **Troncoso-Escudero P**, Sepulveda D, Pérez-Arancibia R, Parra AV, Arcos J, Grunenwald F, Vidal RL. On the Right Track to Treat Movement Disorders: Promising Therapeutic Approaches for Parkinson's and Huntington's Disease. *Front Aging Neurosci* 2020; **12**: 571185 [PMID: 33101007 DOI: 10.3389/fnagi.2020.571185]

47 **Li C**, Zhang J, Qiu M, Liu K, Li Y, Zuo Z, Yin X, Lai Y, Fang J, Tong H, Guo Y, Wang J, Chen X, Xiong K. Alterations of Brain Structural Network Connectivity in Type 2 Diabetes Mellitus Patients With Mild Cognitive Impairment. *Front Aging Neurosci* 2020; **12**: 615048 [PMID: 33613263 DOI: 10.3389/fnagi.2020.615048]

48 **Moulton CD**, Costafreda SG, Horton P, Ismail K, Fu CH. Meta-analyses of structural regional cerebral effects in type 1 and type 2 diabetes. *Brain Imaging Behav* 2015; **9**: 651-662 [PMID: 25563229 DOI: 10.1007/s11682-014-9348-2]

49 **Das L**, Pal R, Dutta P, Bhansali A. "Diabetic striatopathy" and ketoacidosis: Report of two cases and review of literature. *Diabetes Res Clin Pract* 2017; **128**: 1-5 [PMID: 28431304 DOI: 10.1016/j.diabres.2017.03.008]

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LETTER TO THE EDITOR

Inflammatory bowel disease and diabetes: Is there a link between them?

Miao-Miao Sang, Zi-Lin Sun, Tong-Zhi Wu

ORCID number: Miao-Miao Sang 0000-0003-2477-1662; Zi-Lin Sun 0000-0001-7936-0196; Tong-Zhi Wu 0000-0003-1656-9210.

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Miao-Miao Sang, Zi-Lin Sun, Department of Endocrinology, Zhongda Hospital, Institute of Diabetes, School of Medicine, Southeast University, Nanjing 210009, Jiangsu Province, China

Tong-Zhi Wu, Adelaide Medical School and Centre of Research Excellence in Translating Nutritional Science to Good Health, The University of Adelaide, Adelaide, SA 5000, Australia

Corresponding author: Tong-Zhi Wu, Doctor, MD, PhD, Associate Professor, Senior Research Fellow, Adelaide Medical School and Centre of Research Excellence in Translating Nutritional Science to Good Health, The University of Adelaide, Level 6, Adelaide Health and Medical Science Building, North Terrace, Adelaide, SA 5000, Australia. tongzhi.wu@adelaide.edu.au

Abstract

Patients with inflammatory bowel disease (IBD) are reported to have an increased risk of diabetes. IBD therapies may also modulate blood glucose substantially. These observations are indicative of mechanistic connection(s) between IBD and diabetes.

Key Words: Inflammatory bowel disease; Abnormal glucose metabolism

Core Tip: Inflammatory bowel disease is associated with an increased risk of diabetes. Mechanistic insights into their common pathogenesis may render novel therapeutic targets for these major chronic disorders.

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TO THE EDITOR

We read with interest the recent review, entitled "Effect of inflammatory bowel disease treatments on patients with diabetes mellitus", by Bower *et al*[1], which

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provided an excellent summary on the effects of different agents recommended for the treatment of inflammatory bowel disease (IBD) on glucose metabolism. These findings highlight the need for clinicians to consider the impact of IBD-related drugs on blood glucose control among IBD patients with diabetes, and also provide strong impetus to understand the potential mechanistic connection(s) between IBD and the onset of diabetes mellitus.

IBD refers to nonspecific chronic intestinal inflammatory conditions, including Crohn's disease (CD) and ulcerative colitis (UC). In the pursuit of the pathogenesis underlying IBD, 99 susceptibility loci/genes have been found to be related to IBD *via* genome-wide association studies. Interestingly, among those loci/genes, many are also associated with the risk of metabolic diseases, including type 1 and 2 diabetes[2]. A recent nationwide Danish cohort study has reported an increased risk of type 2 diabetes in patients with CD and UC, independent of glucocorticoid use[3]. Similarly, an elevated risk of type 1 diabetes was reported in pediatric patients with UC[4]. More recently, Jasser-Nitsche *et al*[5]. observed that in the German and Austrian population, children and adolescents with type 1 diabetes are at increased risk of IBD. These observations are, therefore, indicative of shared pathway(s) of pathogenesis between IBD and diabetes.

It is now widely appreciated that the gastrointestinal tract plays an important role in glucose homeostasis[6]. In recent years, there is mounting evidence that the gut microbial metabolites and their ensuing effects on the intestinal and systemic inflammation are associated with the occurrence and progression of diabetes; approximately 90% of type 2 diabetes is related to the disrupted gut microbiota, *i.e.* dysbiosis[7], a phenomenon also seen in IBD[8]. In the Danish cohort of IBD, specific abnormal microbial features are linked to the risk of type 2 diabetes[3]. Accordingly, dysbiosis may represent a common pathogenic factor of both IBD and dysglycemia.

Intestinal and metabolic homeostasis is also regulated by a number of gut-derived hormones, as a result of complex interactions between the ingesta and enteroendocrine cells. The incretin hormone glucagon-like peptide (GLP-1) is secreted from L-cells, which predominate in the distal small and large intestine[9]. GLP-1 regulates blood glucose metabolism *via* pleotropic actions, including stimulation of insulin secretion, suppression of glucagon secretion and energy intake, and slowing of gastric emptying [10]. In rodents, GLP-1 was reported to attenuate intestinal mucositis induced by chemotherapy[11]. In both patients with UC and CD and mice with colitis, the expression of GLP-1 receptor of intestinal biopsies was found to be reduced[12]; treatment with the GLP-1 receptor agonist, liraglutide, reduced levels of colonic inflammation in mice with colitis[12]. Accordingly, the reduction in the expression of dipeptidyl peptidase-4 - the enzyme that inactivates endogenously released GLP-1 – in the inflammatory bowel of patients with CD may have reflected a compensatory response of the gut to the development of inflammation[13].

Despite the reported association between the onset of IBD and diabetes, and the potential influence of IBD therapies on glucose metabolism, the common pathogenesis of IBD and diabetes remains elusive. Understanding the latter may provide novel therapeutic opportunities for these major chronic disorders.

REFERENCES

1 **Bower JAJ**, O'Flynn L, Kakad R, Aldulaimi D. Effect of inflammatory bowel disease treatments on patients with diabetes mellitus. *World J Diabetes* 2021; **12**: 1248-1254 [PMID: 34512890 DOI: 10.4239/wjd.v12.i8.1248]
2 **Lees CW**, Barrett JC, Parkes M, Satsangi J. New IBD genetics: common pathways with other diseases. *Gut* 2011; **60**: 1739-1753 [PMID: 21300624 DOI: 10.1136/gut.2009.199679]
3 **Jess T**, Jensen BW, Andersson M, Villumsen M, Allin KH. Inflammatory Bowel Diseases Increase Risk of Type 2 Diabetes in a Nationwide Cohort Study. *Clin Gastroenterol Hepatol* 2020; **18**: 881-888.e1 [PMID: 31394285 DOI: 10.1016/j.cgh.2019.07.052]
4 **Kappelman MD**, Galanko JA, Porter CQ, Sandler RS. Association of paediatric inflammatory bowel disease with other immune-mediated diseases. *Arch Dis Child* 2011; **96**: 1042-1046 [PMID: 21903597 DOI: 10.1136/archdischild-2011-300633]
5 **Jasser-Nitsche H**, Bechtold-Dalla Pozza S, Binder E, Bollow E, Heidtmann B, Lee-Barkley YH, Raile K, de Sousa G, Schramm U, Holl RW. Comorbidity of inflammatory bowel disease in children and adolescents with type 1 diabetes. *Acta Paediatr* 2021; **110**: 1353-1358 [PMID: 33119925 DOI: 10.1111/apa.15643]
6 **Holst JJ**, Gribble F, Horowitz M, Rayner CK. Roles of the Gut in Glucose Homeostasis. *Diabetes Care* 2016; **39**: 884-892 [PMID: 27222546 DOI: 10.2337/dc16-0351]
7 **Arora A**, Behl T, Sehgal A, Singh S, Sharma N, Bhatia S, Sobarzo-Sanchez E, Bungau S.

Unravelling the involvement of gut microbiota in type 2 diabetes mellitus. *Life Sci* 2021; **273**: 119311 [PMID: 33662428 DOI: 10.1016/j.lfs.2021.119311]

8 **Joossens M**, Huys G, Cnockaert M, De Preter V, Verbeke K, Rutgeerts P, Vandamme P, Vermeire S. Dysbiosis of the faecal microbiota in patients with Crohn's disease and their unaffected relatives. *Gut* 2011; **60**: 631-637 [PMID: 21209126 DOI: 10.1136/gut.2010.223263]

9 **Xie C**, Jones KL, Rayner CK, Wu T. Enteroendocrine Hormone Secretion and Metabolic Control: Importance of the Region of the Gut Stimulation. *Pharmaceutics* 2020; **12** [PMID: 32825608 DOI: 10.3390/pharmaceutics12090790]

10 **Meier JJ**. GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. *Nat Rev Endocrinol* 2012; **8**: 728-742 [PMID: 22945360 DOI: 10.1038/nrendo.2012.140]

11 **Kissow H**, Hartmann B, Holst JJ, Poulsen SS. Glucagon-like peptide-1 as a treatment for chemotherapy-induced mucositis. *Gut* 2013; **62**: 1724-1733 [PMID: 23086829 DOI: 10.1136/gutjnl-2012-303280]

12 **Bang-Berthelsen CH**, Holm TL, Pyke C, Simonsen L, Søkilde R, Pociot F, Heller RS, Folkersen L, Kvist PH, Jackerott M, Fleckner J, Vilién M, Knudsen LB, Heding A, Frederiksen KS. GLP-1 Induces Barrier Protective Expression in Brunner's Glands and Regulates Colonic Inflammation. *Inflamm Bowel Dis* 2016; **22**: 2078-2097 [PMID: 27542128 DOI: 10.1097/MIB.0000000000000847]

13 **Moran GW**, O'Neill C, Padfield P, McLaughlin JT. Dipeptidyl peptidase-4 expression is reduced in Crohn's disease. *Regul Pept* 2012; **177**: 40-45 [PMID: 22561447 DOI: 10.1016/j.regpep.2012.04.006]

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