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Association between gain in adiposity and diabetic kidney disease worsening in type 2 diabetes is mediated by deteriorating glycaemic control: A 3-year follow-up analysis

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ABSTRACT

Aims: Increased adiposity confers elevated risk for diabetic kidney disease (DKD) progression in type 2 diabetes mellitus (T2DM). This 3-year prospective study examined whether worsening of metabolic control e.g. development of uncontrolled diabetes mediated the relationship between increased adiposity and DKD deterioration.

Methods: T2DM subjects who had adequately controlled diabetes (HbA1c < 8%) at initial recruitment were analysed (N = 853). HbA1c ≥ 8% at follow-up was classified as development of uncontrolled T2DM. Absolute changes in body weight (Δ Weight), body mass index (Δ BMI), and body fat mass (Δ BFM) were calculated by subtracting baseline from follow-up values. DKD deterioration (outcome) was defined as an increase in the composite ranking of relative risk by glomerular filtration rate and albuminuria levels (Kidney Disease: Improving Global Outcomes 2009).

Results: Subjects with deteriorated DKD displayed lower reduction in body composition at follow-up than those who remained stable or/improved (all $P < 0.05$). In separate regression models, Δ Weight (risk ratio (RR):1.04, 95% CI:1.01–1.06), Δ BMI (RR:1.07, 95% CI:1.01–1.13), and Δ BFM (RR:1.03, 95% CI:1.01–1.06) were independently associated with worsened DKD. The associations were attenuated after accounting for the loss of glycaemic control. Binary mediation analysis revealed that the development of uncontrolled diabetes explained 41.7%, 45.4% and 39.7%, respectively, of the effects of Δ Weight, Δ BMI and Δ BFM on the outcome.

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Conclusions: Among T2DM individuals who had adequately-controlled T2DM at initial recruitment, the relationship between gain in adiposity and DKD deterioration is mediated by the development of poor glycaemic control over time. Therefore, preventing worsening adiposity and hyperglycaemia is pivotal to impede DKD progression.

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1. Introduction

Diabetic kidney disease (DKD) affects approximately 40% of patients with diabetes and is the leading cause of end-stage renal disease (ESRD) and cardiovascular morbidity and mortality [1]. Increased albuminuria is traditionally considered to be the earliest clinical manifestation that heralds the onset of DKD, followed by a progressive decline in glomerular filtration rate (GFR) and eventually ESRD over time [2]. Although the classical albuminuric pathway to renal impairment occurs in a considerable proportion of patients with diabetes, the clinical course of DKD is more heterogeneous than initially deemed [1–3]. Different presentations of DKD have increasingly been documented, including regression of albuminuria, and renal function decline not preceded by increased albuminuria [1]. Therefore, assessment of both albuminuria and GFR is critical for the diagnosis and monitoring of DKD. The Kidney Disease: Improving Global Outcomes (KDIGO) 2009 recommended clinicians and researchers to use the chronic kidney disease (CKD) classification of relative risk based on a combination of GFR and albuminuria criteria to risk-stratify patients for mortality and kidney disease outcomes [4].

Our recent study reported that gain in adiposity was associated with renal function decline and progressive albuminuria in T2DM patients [5]. Obesity is intimately linked to hyperglycaemia and other metabolic disturbances [6–8]. Hyperglycaemia is recognized to drive the onset and progression of DKD. As demonstrated by the UK Prospective Diabetes Study (UKPDS), early intensive glucose control resulted in a decrease of microvascular complications in patients with type 2 diabetes mellitus (T2DM) [9]. Similarly, intensive glycaemic control in the ADVANCE Collaborative Group reduced the risk of ESRD, development of microalbuminuria and macroalbuminuria, and progression of albuminuria among individuals with T2DM [10].

The impact of developing uncontrolled diabetes on the risk of disease progression among T2DM individuals who previously had adequately-controlled diabetes is not well-characterized. We postulate that in such patients, loss of metabolic control in response to increased adiposity elicits kidney impairment. In this analysis of T2DM subjects who presented relatively well-controlled diabetes at baseline, we examined whether subsequent development of poorly-controlled hyperglycaemia at 3-year follow-up mediated the relationship between increased adiposity and DKD deterioration. Secondarily, the role of changes in blood pressure and serum lipid profile as mediators was assessed.

2. Materials and methods

2.1. Participants

As depicted in Fig. 1, T2DM research participants (aged between 21 and 89 years) who were receiving standard medical care from the Diabetes Mellitus Centre or a primary-care polyclinic in the northern region of Singapore were recruited by the Singapore Study of Macroangiopathy and Microvascular Reactivity in Type 2 Diabetes (SMART2D) Study between August 2011 and March 2014 (N = 2057). The inclusion and exclusion criteria for recruitment were described in detail previously [5]. The cohort was recalled for a follow-up assessment after a mean 3-year interval starting from September 2014. By June 2017, all participants recruited by the SMART2D study would have been notified for follow-up; and a total of 1421 subjects returned for reassessment. Our analysis showed that people who did not return for follow-up were typically older, and had poorer blood pressure and renal function when compared with those who returned (Supplementary Table 1). In this study, follow-up data collected up to June 2017 were analysed. Subjects who had adequately controlled diabetes (HbA1c < 8% (64 mmol/mol)) at initial recruitment were included in this present analysis. Excluded from this analysis were those who were diagnosed with T2DM at age < 18 years, known to harbour disease-causative maturity-onset diabetes of the young (MODY) variants classified according to the American College of Medical Genetics 2015 guidelines [11], had missing renal data, had baseline kidney failure (GFR < 15 ml/min/1.73 m²) or had nephrotic levels of albumin-creatinine ratio (ACR ≥ 2000 mg/g) [4]. The study conformed to the Declaration of Helsinki (as revised in Brazil 2013), available at <http://www.wma.net/en/30publications/10policies/b3/index.html>; and was approved by our institution's domain specific ethics review board. Written informed consent was obtained from all the subjects.

2.2. Clinical measurements

Height and weight were taken, and body mass index (BMI) was computed as weight (in kg) divided by baseline height (in m)². Body fat mass (BFM) was estimated using the InBody-S10 body composition analyser (BioSpace, CA, USA). Study protocol to measure body composition using InBody-S10 was set up about 6 months after initial recruitment in 2011. Therefore, 1752 out of 2057 patients had available baseline readings of BFM. Creatinine was determined by enzymatic method on the Roche/Hitachi cobas c system (Roche

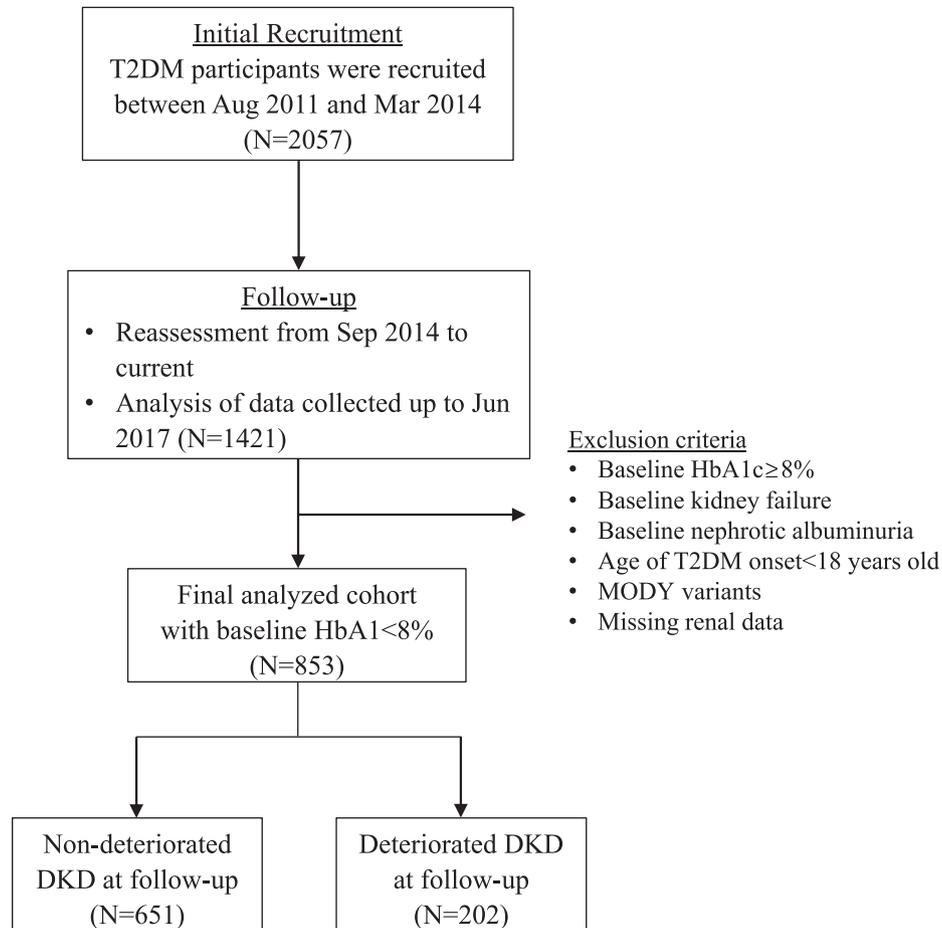


Fig. 1 – Flow diagram of patient recruitment, assignment, and follow-up.

Diagnostic GmbH, Mannheim, Germany). The estimated GFR (eGFR) was derived using the Chronic Kidney Disease Epidemiology Collaboration equation. Urinary albumin from spot urine samples was analysed using a solid phase competitive chemiluminescent enzymatic immunoassay (Immulite, Siemens Healthcare Diagnostics, NY, USA), and the urinary ACR was calculated. Systolic (SBP) and diastolic (DBP) blood pressure was measured using an automated blood pressure monitor (Dinamap Pro 100V2, Freiburg, Germany). HbA1c point-of-care testing was performed using an immunoassay analyser certified by the National Glycohaemoglobin Standardization Programme (DCA Vantage Analyser; Siemens Healthcare Diagnostics, Erlangen, Germany). Lipids were quantified by enzymatic assays using Ektachem clinical chemistry slides (Eastman Kodak, NY, USA).

2.3. Calculations and definitions

Absolute changes in body weight (Δ Weight), BMI (Δ BMI), BFM (Δ BFM), HbA1c (Δ HbA1c), SBP (Δ SBP), DBP (Δ DBP), total cholesterol (Δ TC), high density lipoprotein-cholesterol (Δ HDL-C), low density lipoprotein-cholesterol (Δ LDL-C) and triglycerides (Δ TG) were calculated by subtracting baseline values from follow-up values. Tertiles of Δ Weight (tertile 1: < -2.1 , tertile

2: -2.1 to < 0.4 , tertile 3: ≥ 0.4 kg), Δ BMI (tertile 1: < -0.80 , tertile 2: -0.80 to < 0.14 , tertile 3: ≥ 0.14 kg/m²), and Δ BFM (tertile 1: < -1.2 , tertile 2: -1.2 to < 1.2 , tertile 3: ≥ 1.2 kg) were generated for analysis. We applied the less stringent American Diabetes Association (ADA)-recommended HbA1c cut-off of $\geq 8\%$ (64 mmol/mol) as our definition for development of uncontrolled diabetes at follow-up after taking into consideration the heterogeneity of our cohort, which comprised of patients, some of whom may be elderly or with conditions such as severe hypoglycaemia, advanced stages of microvascular or macrovascular complications, extensive comorbid conditions, or long-standing suboptimal control diabetes [12]. Similarly, the International Diabetes Federation (IDF) recommended an HbA1c target of 7.5–8% (58–64 mmol/mol) for patients using multiple medications, short life expectancy, cognitive impairment, advanced chronic kidney disease or severe cardiovascular disease associated with multiple comorbidities [14]. According to the 2006 Singapore Clinical Practice Guidelines on diabetes, glycaemic control was classified into: ideal: 4.5–6.4% (26–46 mmol/mol); optimal: 6.5–7.0% (48–53 mmol/mol); suboptimal: 7.1–8%; (54–64 mmol/mol) and unacceptable: $> 8\%$ (64 mmol/mol) [15]. Taken together, HbA1c above 8% is in general regarded as an undesirable threshold for glycaemic control. The DKD status of the partic-

ipants at baseline and at follow-up was classified according to the 2009 KDIGO heat-map of “Composite Ranking for Relative Risks by GFR and Albuminuria” [4]; and deterioration of DKD was defined as an increase in risk category from baseline to follow-up by intensity of colouring (green: no/low risk, yellow: moderate risk, orange: high risk, red: very high risk, deep red: extremely high risk) [16].

2.4. Statistical analysis

Descriptive statistical-analyses were performed using SPSS version 22 (IBM Corp, NY, USA). Continuous variables were presented as mean \pm SD or median (interquartile range), while categorical data were presented as N (%). Parametric t-test and non-parametric Mann-Whitney U test were performed to compare continuous variables between groups. Chi-square test was to compare categorical variables between groups. Modified Poisson regression with a robust error variance was executed in STATA version 15 (Stata-Corp, College Station, TX, USA) to estimate the risk ratio (RR) for common binary outcome of DKD deterioration [17]. The exposures of interest including Δ Weight, Δ BMI and Δ BFM were analysed in separate regression models. The selection of covariates for statistical adjustment was based on clinical relevance and the significance of univariate associations ($P < 0.2$). Skewed data (T2DM duration and ACR) were natural log-transformed prior to regression analyses. The interaction between uncontrolled diabetes and the exposure variables was tested when modelling associations between exposure and outcomes to determine whether modifying effect existed. A more stringent definition of suboptimal glycaemic control (defined as follow-up HbA1c $\geq 7.5\%$ (58 mmol/mol)) was used in sensitivity analysis, including participants with baseline HbA1c $< 7.5\%$ (58 mmol/mol) [N = 666] in the analysis. Mediation analysis was performed in R version 3.2.4 (R Core Team, 2016) to examine the contribution of development of poor metabolic control (binary/continuous mediator M) to the relationship between adiposity gain (continuous exposure X) and DKD worsening (binary outcome Y) with full adjustment for covariates listed in Models 1–3 of Table 2. Two models were employed for mediation analysis, as adapted from Laurent et al. [18]. Firstly, the outcome model was fitted using modified Poisson regression to generate path b ($Y \leftarrow M$) and path c' ($Y \leftarrow X$). The effect of X on Y conditional on M on the RR scale defined the direct effect (DE; denoted by RR^{DE}). Secondly, the mediator model for path a ($M \leftarrow X$) either adopted a modified Poisson regression (for binary M) or linear regression (for continuous M). Using the product method [19,20], the indirect effect (IE) mediated by M was obtained by paths a and b , and defined as the product of the effect of X on M and the effect of M on Y on the RR scale (denoted by RR^{IE}). The total effect (TE; denoted by RR^{TE}) of exposure X on Y was computed as the product of DE and IE, $RR^{TE} = RR^{DE} \times RR^{IE}$. The proportion of mediated effect was calculated as $RR^{DE} \times (RR^{IE} - 1)/(RR^{TE} - 1)$ [18]. Bootstrapping with 10,000 iterations was performed to estimate the 95% confidence intervals (CI) of DE, IE, TE and mediation proportion. $P < 0.05$ indicated statistical significance.

3. Results

3.1. Characteristics of participants with deteriorated DKD versus controlled DKD

This study involved T2DM adults who had HbA1c $< 8\%$ (64 mmol/mol) at initial recruitment. Participants who returned for follow-up assessment after a mean 3-year interval were stratified according to their status of DKD deterioration. As shown in Table 1, individuals who experienced DKD deterioration at follow-up (N = 202) had older age, longer T2DM duration, higher blood pressure, and less favourable renal profile at baseline than the control group (N = 651). Additionally, the former group was more frequently treated with anti-diabetic, anti-hypertensive, and lipid-lowering medications. There was no statistical difference in baseline body composition, HbA1c and lipid parameters between the two groups. Based on the 2009 KDIGO relative risk classification, majority of individuals were at low/no risk of DKD adverse events at baseline. At follow-up, a greater proportion of people in the worsened DKD group transitioned to higher DKD risk categories as expected, and developed uncontrolled diabetes defined as HbA1c $\geq 8\%$ (64 mmol/mol).

3.2. Effect of loss of glycaemic and metabolic control on the association between changes in adiposity and DKD deterioration

Compared with individuals whose DKD status did not deteriorate, those with worsened DKD displayed lower reduction in body composition at follow-up (Table 1; all $P < 0.05$). In separate regression models, all measures of changes in adiposity (Δ Weight, Δ BMI and Δ BFM), both continuous and categorical, were significantly associated with DKD deterioration after accounting for demographics, metabolic parameters, renal function and medications (Table 2 Model 1). Baseline anthropometric measurements were not associated with DKD worsening. Notably, further adjustment for the onset of uncontrolled diabetes attenuated the relationship between the exposures of interest and outcome (Model 2). Of note, no interaction between uncontrolled diabetes and the exposure variables were observed ($P > 0.05$). Separately, adjustment of Model 1 with Δ HbA1c also weakened the association between increased adiposity and DKD worsening (Model 3). In contrast, except for Δ TG, the attenuation effect of changes in blood pressure (Supplementary Table 2) and lipids (Supplementary Table 3) on the relationship between changes in adiposity (continuous and categorical) and DKD deterioration appeared inconsistent. However, Δ TG did not emerge as a significant associate of the outcome.

3.3. Mediation analysis

Binary mediation analyses showed that the development of uncontrolled diabetes explained 41.7% (95% CI: 0.22–1.22), 45.4% (95% CI: 0.14–1.04) and 39.7% (95% CI: 0.14–0.72) of the effects of Δ Weight, Δ BMI and Δ BFM on the risk of DKD deterioration, respectively (Table 3). Similarly, Δ HbA1c mediated the relationship between Δ Weight, Δ BMI or Δ BFM and DKD wors-

Table 1 – Clinical characteristics of patients stratified by DKD deterioration status at follow-up.

Variable	No deterioration (N = 651)	Deterioration (N = 202)	P value
Baseline			
Age (years)	58 ± 10	60 ± 10	0.008
Men, N (%)	337 (51.8)	108 (53.5)	0.673
Ethnicity, N (%)			0.278
Chinese	367 (56.4)	123 (60.9)	
Malay	98 (15.1)	35 (17.3)	
Indian	161 (24.7)	37 (18.3)	
Others	25 (3.8)	7 (3.5)	
T2DM duration (years)	7 (3–12)	10 (5–18)	<0.001
Body weight (kg)	70.7 ± 14.4	71.7 ± 14.6	0.546
BMI (kg/m ²)	27.2 ± 5.1	27.6 ± 4.9	0.312
BFM (kg)*	25.8 ± 10.3	26.0 ± 9.3	0.865
	[N = 559]	[N = 171]	
SBP (mmHg)	136.8 ± 16.2	143.3 ± 18.9	<0.001
DBP (mmHg)	78.1 ± 9.0	79.6 ± 9.3	0.049
HbA1c (%)	6.9 ± 0.6	6.9 ± 0.6	0.994
HbA1c (mmol/mol)	52.2 ± 6.3	52.2 ± 6.8	0.994
TC (mM)	4.3 ± 0.9	4.2 ± 0.9	0.182
HDL-C (mM)	1.3 ± 0.4	1.3 ± 0.3	0.255
LDL-C (mM)	2.7 ± 0.8	2.6 ± 0.8	0.207
TG (mM)	1.3 (1.0–1.7)	1.4 (1.0–1.7)	0.186
eGFR (mL/min/1.73 m ²)	90.1 ± 20.8	79.5 ± 23.7	<0.001
ACR (µg/mg)	11.0 (3.0–47.0)	24.0 (13.0–147.3)	<0.001
KDIGO risk classification, N (%)			0.002
Low	404 (62.1)	105 (52.0)	
Moderate	157 (24.1)	49 (24.3)	
High	52 (8.0)	34 (16.8)	
Very high	38 (5.8)	14 (6.9)	
Extremely high	0 (0)	0 (0)	
Medications, N (%)			
Insulin*	74 (11.4)	42 (20.7)	0.001
	[N = 647]		
OHGA*	562 (86.7)	187 (92.6)	0.025
	[N = 648]		
Statins*	508 (78.3)	171 (84.7)	0.049
	[N = 649]		
RAS antagonists*	326 (50.7)	133 (66.2)	<0.001
	[N = 643]	[N=201]	
Follow-up			
ΔWeight (kg)	−0.9 (−2.9 to 0.9)	−0.4 (−2.4 to 2.2)	0.029
ΔBMI (kg/m ²)	−0.3 (−1.1 to 0.4)	−0.1 (−0.9 to 0.8)	0.027
ΔBFM (kg)*	−0.2 (−2.4 to 1.7)	0.7 (−1.1 to 2.5)	0.001
	[N = 555]	[N = 169]	
PGC, N (%)	120 (18.4)	65 (32.2)	<0.001
KDIGO risk classification, N (%)			<0.001
Low	463 (71.1)	0 (0)	
Moderate	119 (18.3)	87 (43.1)	
High	35 (5.4)	51 (25.2)	
Very high	34 (5.2)	40 (19.8)	
Extremely high	0 (0)	24 (11.9)	
T2DM: type 2 diabetes mellitus; BMI: body mass index; BFM: body fat mass; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; TG: triglycerides; eGFR: estimated glomerular filtration rate; ACR: urinary albumin-creatinine ratio; KDIGO: kidney disease: improving global outcomes; OHGA: oral hypoglycaemic agents; RAS: renin-angiotensin system; PGC: poor glycaemic control [HbA1c ≥ 8% (64 mmol/mol)].			
Data for qualitative variables are expressed as N (%) and quantitative variables as mean ± SD or median (interquartile range).			
* Variables with missing data. Number of participants available is indicated in square brackets.			

ening by 15.5% (95% CI: 0.05–0.47), 14.6% (95% CI: 0.02–0.54) and 16.3% (95% CI: 0.04–0.74), respectively. On the other hand, conditions for mediation were not satisfied when evaluating

the relationship between the exposures and outcome through changes in blood pressure (ΔSBP, ΔDBP) or lipid parameters (ΔTC, ΔHDL-C, ΔLDL-C, ΔTG), indicating no mediating effect.

Table 2 – Modified Poisson regression analysis of the effects of loss of glycaemic control on the association between changes in adiposity and DKD deterioration.

Variable	Model 1 RR (95% CI); P	Model 2 (PGC) RR (95% CI); P	Model 3 (Δ HbA1c) RR (95% CI); P
<i>Continuous</i>			
Δ Weight	1.04 (1.01–1.06); 0.004	1.03 (1.00–1.06); 0.024	1.03 (1.01–1.06); 0.009
PGC	–	1.68 (1.29–2.18); <0.001	–
Δ HbA1c	–	–	1.19 (1.11–1.28); <0.001
Δ BMI	1.07 (1.01–1.13); 0.021	1.06 (1.00–1.12); 0.069	1.06 (1.01–1.12); 0.031
PGC	–	1.71 (1.33–2.22); <0.001	–
Δ HbA1c	–	–	1.19 (1.11–1.28); <0.001
Δ BFM	1.03 (1.01–1.06); 0.015	1.03 (1.00–1.06); 0.052	1.03 (1.00–1.06); 0.045
PGC	–	1.69 (1.28–2.24); <0.001	–
Δ HbA1c	–	–	1.17 (1.09–1.27); <0.001
<i>Categorical</i>			
Δ Weight			
Tertile 1	1	1	1
Tertile 2	1.03 (0.76–1.40); 0.854	1.01 (0.74–1.37); 0.967	1.04 (0.76–1.40); 0.821
Tertile 3	1.42 (1.06–1.91); 0.017	1.34 (1.00–1.79); 0.047	1.40 (1.06–1.86); 0.019
PGC	–	1.70 (1.32–2.20); <0.001	–
Δ HbA1c	–	–	1.19 (1.11–1.28); <0.001
Δ BMI			
Tertile 1	1	1	1
Tertile 2	1.26 (0.93–1.71); 0.133	1.27 (0.94–1.73); 0.121	1.31 (0.97–1.77); 0.080
Tertile 3	1.42 (1.05–1.92); 0.024	1.35 (0.99–1.83); 0.056	1.40 (1.04–1.89); 0.028
PGC	–	1.74 (1.34–2.25); <0.001	–
Δ HbA1c	–	–	1.20 (1.12–1.29); <0.001
Δ BFM			
Tertile 1	1	1	1
Tertile 2	1.53 (1.06–2.21); 0.023	1.51 (1.05–2.16); 0.027	1.47 (1.02–2.11); 0.037
Tertile 3	1.76 (1.24–2.51); 0.002	1.66 (1.16–2.36); 0.005	1.68 (1.18–2.38); 0.004
PGC	–	1.67 (1.26–2.22); <0.001	–
Δ HbA1c	–	–	1.17 (1.09–1.27); <0.001

PGC: poor glycaemic control; RR: risk ratio; CI: confidence interval; BMI: body mass index; BFM: body fat mass.
 Model 1: Adjusted for baseline parameters including age, T2DM duration, anthropometry (weight, BMI, or BFM), gender, ethnicity, SBP, HbA1c, HDL-C, eGFR, ACR, medications (insulin, OHGA, statins, RAS antagonists).
 Model 2: Model 1 adjusted for development of poor glycaemic control (HbA1c \geq 8% (64 mmol/mol)) at follow-up.
 Model 3: Model 1 adjusted for Δ HbA1c.

3.4. Sensitivity analysis

In the sensitivity analysis, participants with baseline HbA1c < 7.5% (58 mmol/mol) were analysed (N = 666). Among them, 152 experienced DKD worsening at follow-up, of which 40.1% developed poor glycaemic control, defined as HbA1c \geq 7.5% (versus 31.3% in control group, P = 0.043). As shown in Supplementary Table 4, Δ Weight (RR: 1.04, 95% CI:

1.01–1.07), Δ BMI (RR: 1.07, 95% CI: 1.01–1.14) and Δ BFM (RR: 1.04, 95% CI: 1.01–1.07) were independently associated with DKD deterioration (Model 1). Suboptimal glycaemic control was not significantly associated with the outcome, although a trend of association was observed in the Δ BFM-based model (Model 2). On the other hand, Δ HbA1c was associated with DKD worsening and appeared to reduce the association between adiposity gain and outcome (Model 3).

Table 3 – Mediation effect of poor glycaemic control on the relationship between changes in adiposity and DKD deterioration.

	Total effect RR (95% CI)	Indirect effect RR (95% CI)	Direct effect RR (95% CI)	Mediation proportion, % (95% CI)
Δ Weight	1.05 (1.02–1.08)	1.02 (1.01–1.05)	1.03 (1.00–1.05)	41.7 (0.22–1.22)
Δ BMI	1.10 (1.04–1.18)	1.04 (1.01–1.09)	1.06 (1.00–1.12)	45.4 (0.14–1.04)
Δ BFM	1.05 (1.02–1.08)	1.02 (1.00–1.04)	1.03 (1.00–1.06)	39.7 (0.10–0.72)

RR: risk ratio; CI: confidence interval; BMI: body mass index; BFM: body fat mass.

4. Discussion

Sustained durable glycaemic control, to avoid the accumulation of metabolic memory, is important to prevent DKD progression. Here, we investigated for the first time the role of loss of glycaemic and metabolic control accrued over 3 years as mediator to explain the underlying mechanism involved in the relationship between gain in adiposity and worsening of DKD among adults who had adequately-controlled T2DM at recruitment (baseline HbA1c < 8% (64 mmol/mol)). The findings suggest that increased adiposity and resulting failure to achieve sustained and durable glycaemic control may contribute to DKD deterioration.

There is currently no consensus definition for DKD progression primarily because different definition tends to capture a different subset of the study population who experiences renal deterioration. Various definitions that are commonly used to describe worsening of DKD consist of rate of decline of eGFR, doubling of serum creatinine, ESRD and transition of albuminuria class [21–23]. However, DKD deterioration based on a combination of GFR and ACR categories is scarcely explored. In this prospective analysis, using the KIDIGO 2009 matrix to assign DKD severity, we demonstrated that all changes in measures of adiposity (Δ Weight, Δ BMI and Δ BFM) were statistically different between individuals with worsened DKD and the control group (Table 1); and were independently associated with DKD deterioration (Table 2 model 1). These observations suggest that fat accumulation may exacerbate DKD. Compared with a single static snapshot of anthropometry that does not account for changes in body composition during disease progression, more frequent data collection and assessing the changes over time may improve our understanding on the impact of increased adiposity on DKD deterioration. Measurements taken at two time points in this study are inadequate for us to ascertain whether changes in adiposity indeed exert a causal effect on the outcome, considering that worsening of DKD could also lead to increased adiposity i.e. reverse causality. Research examining the effect of gain in adiposity on DKD pathogenesis is also limited [5,24]. Nevertheless, numerous literatures have assessed the impact of weight loss on DKD outcomes. Intentional weight reduction, both by bariatric surgery and lifestyle interventions, was found to result in decreased proteinuria and albuminuria as well as normalized eGFR in obese people with CKD [25,26]. Chang et al. reported that severely obese individuals who underwent bariatric surgery had a significant 58% lower risk of an eGFR decline of $\geq 30\%$ and 57% lower risk of doubling of serum creatinine or ESRD compared to their matched non-surgery counterparts [27].

Further to our analysis, we demonstrated that the relationship between increased adiposity and deterioration of DKD was attenuated by the development of uncontrolled diabetes (Table 2 model 2), and to a lesser extent by Δ HbA1c (Table 2 model 3). Mediation analyses revealed that approximately 15–45% of the relation between the exposures and outcome analysed was mediated by the loss of glycaemic control or Δ HbA1c. The lack of full mediation suggests that other mechanisms underlying the association may be involved. Besides hyperglycaemia, obesity is linked to both hypertension and

dyslipidaemia, which are regarded as risk factors of renal impairment [28–30]. However, our data showed that neither changes in blood pressure nor changes in lipid parameters elicited a significant mediation effect on the association between increased adiposity and DKD worsening. Possible reasons for the lack of interaction are insufficient duration of follow-up to observe an association and improved metabolic control by medications. Notably, fat accumulation may directly cause kidney injury via processes that lead to deranged production of adipose tissue cytokines, increased oxidative stress and structural alterations in the kidneys, independent of mediation by metabolic aberrations [31].

In the sensitivity analysis, the HbA1c threshold to define poor glycaemic control was lowered from $\geq 8\%$ (64 mmol/mol) to $\geq 7.5\%$ (58 mmol/mol). We observed no independent relationship between development of suboptimal HbA1c and DKD deterioration among subjects with baseline HbA1c < 7.5% (58 mmol/mol). One possibility is that the reduced HbA1c threshold may increase misclassification of poor glycaemic control in our multi-ethnic heterogeneous T2DM cohort due to the variation of HbA1c. HbA1c is not a direct measure of glycaemia, and its levels can be interfered by certain medical conditions and a variety of factors such as ethnicity, age, smoking and BMI that are unrelated to glycaemia [32–34]. Our findings may also imply that the HbA1c threshold of $\geq 8\%$ (64 mmol/mol) elicits a more detrimental impact on DKD progression than HbA1c $\geq 7.5\%$ (58 mmol/mol). Evidently, a number of studies have shown that the glycaemia-dependent risk of diabetic complications is curvi-linear, which rises more steeply when HbA1c exceeds 8% (64 mmol/mol). Krolewski et al. found that the risk of microalbuminuria increased abruptly above HbA1c of 8% (64 mmol/mol) in patients with insulin-dependent diabetes mellitus [35]. Additionally, in the UKPDS, susceptibility to diabetic complications also began to demonstrate a steeper gradient primarily above the HbA1c threshold of $\geq 8\%$ (64 mmol/mol) [36]. A population-based prospective study revealed a linear positive relationship between absolute change in HbA1c and mortality among T2DM individuals with baseline HbA1c > 8% (64 mmol/mol) [37]. On the contrary, decline in HbA1c was associated with death among subgroups with baseline HbA1c $\leq 8\%$ (64 mmol/mol).

The strength of this work is the prospective study design that includes a relatively large cohort of T2DM subjects with reasonably-controlled HbA1c at initial recruitment. However, several weaknesses need to be acknowledged. Due to the research design, only two measurements (baseline and follow-up) of the clinical and biochemical parameters were recorded. It would be ideal if the patients had more measurements taken at frequent intervals to improve the accuracy of classifying disease deterioration. For example, ACR measurement is commonly associated with substantial within-person variability, therefore more measurements are required to avoid misclassification [38]. Nonetheless, analyses of the biochemical parameters were carried out by an accredited laboratory, therefore minimizing possible variance related with single measurement. The glycaemic control trajectories of the subjects prior to enrolment and in between the study periods are not available. BFM was estimated by bioimpe-

dance instead of using more accurate methods such as magnetic resonance imaging and computed tomography [39]. Having said so, our group has previously reported bioimpedance estimated adiposity and diabetes outcomes [5,40,41]. The degree of adiposity, metabolic control and DKD progression may be affected by treatment intensification, medication adherence, diet, physical activity, and sedentary behaviour. However, these confounding variables were not collected from the research participants, and thus could not be accounted for in the association analyses. This study is based on Singapore's T2DM population, and thus may not be generalizable to T2DM patients beyond the study population.

In conclusion, we showed that, in T2DM individuals who had adequately-controlled diabetes at initial recruitment, subsequent development of uncontrolled diabetes and glycaemic worsening mediated the detrimental effect of increased adiposity on DKD deterioration over time. Therefore, preventing worsening adiposity and hyperglycaemia is pivotal to impede future development and progression of DKD.

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The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.diabres.2019.107812>.

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