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Hydrocortisone, ascorbic acid and thiamine for sepsis: Is the jury out?

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Abstract

Sepsis and septic shock remain a major cause of morbidity and mortality among patients admitted in the intensive care unit. Diabetes is a major risk factor for the development of sepsis. The global mortality of sepsis remains high, despite significant interventions and guidelines. It has been known for decades that patients with sepsis have reduced levels of antioxidants, most notably vitamin C. Furthermore, experimental data has demonstrated multiple beneficial effects of vitamin C in sepsis. In addition, corticosteroids and thiamine may have synergistic biological effects together with vitamin C. Preliminary data suggests that therapy with hydrocortisone, ascorbic acid and thiamine improves the outcome of patients with sepsis with the potential to save millions of lives. However, this intervention has met with much resistance and has not been widely adopted. Ultimately, we await the final jury verdict on this simple, safe and cheap intervention.

Key words: Diabetes; Vitamin C; Ascorbic acid; Sepsis; Thiamine; Septic shock; Global burden of disease

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Core tip: The combination of ascorbic acid (vitamin C), hydrocortisone and thiamine hold great promise for the treatment of sepsis. Despite, significant evidence there remains reluctance among the medical community to adopt this intervention. hydrocortisone, ascorbic acid and thiamine have the potential for saving millions of lives. We await the final jury verdict on this simple, safe and cheap intervention.

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INTRODUCTION

“A new scientific truth does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die, and a new generation grows up that is familiar with it.” Max Planck, Theoretical Physicist (1858-1947).

According to the most recent estimates from the World Health Organization over 6 million citizens of the earth die from sepsis each year. As the greatest burden of sepsis is in middle- and low-income countries where accurate epidemiological data is lacking, this is likely an underestimate of the true global burden of this devastating syndrome^[1-3]. The patients with diabetes have increased risk of developing sepsis^[4]. The cornerstone of treatment of sepsis and septic shock is its early recognition, early appropriate antibiotics, early source control and a physiologic approach to fluid management^[5]. Despite this approach the mortality of septic shock in resource rich countries approximates 30%, while that in resource poor countries the mortality is estimated to be about 60%. Sepsis is the commonest cause of death in children less than 5 years of age. Imagine for one moment that a simple, cheap and readily available intervention existed that could reduce the mortality from sepsis by 20% (conservatively). Such an intervention would save in excess of 1 million lives a year. Does such an intervention exist? We believe that the combination of hydrocortisone, ascorbic acid (vitamin C) and thiamine (HAT Rx) has the potential to prevent millions of deaths from sepsis each year and to reduce the severe life altering sequelae in the sepsis survivors^[6-8]. Most importantly this intervention is cheap and without side effects (the side effects of a short course of low dose corticosteroids are minimal)^[8,9]. So why has this simple, safe and cheap intervention not been more widely adopted? To answer this question, we need to question the established medical community and their power brokers. The commonest reasons cited are that the proposed benefits are not “biologically plausible” and the “jury is not out” on the benefits of this simple intervention^[10,11].

A jury is a body of people convened to render an impartial verdict (in a legal case) on the basis of evidence submitted to them by a court. The jurors evaluate the totality of the evidence presented to them and base their findings on the preponderance of evidence. In many cases there is no smoking gun [or randomized controlled trial (RCT)] so they base their assessment on the entire body of the evidence. Furthermore, the jury needs to ponder the consequences of an incorrect verdict. In a trial, if they wrongly convict, this could result in the wrong penalty. Similarly, in the case of “HAT Rx *vs* Sepsis” concluding that such therapy is biologically implausible and unsafe could potentially cost millions of lives.

So, what does the evidence to date demonstrate? Firstly, it should be recognized that humans (anthropoid primates) are unique amongst the animals that roam the earth, in they have lost the ability to synthesize vitamin C^[12]. Vitamin C is an essential antioxidant and co-factor for many vital biological reactions. In non-human species, Vitamin C acts as a stress hormone with increased synthesis during stress which is likely protective. Secondly, over 400 basic science experiments have been performed demonstrating the benefit of vitamin C alone and in combination with hydrocortisone in attenuating the dysregulated immune response in sepsis, in limiting organ dysfunction and reducing death of the septic animals^[8]. What is remarkable is that these medications act synergistically *via* multiple overlapping pathways. *In-vitro* studies have demonstrated the synergetic effects of the combination of corticosteroids and vitamin C^[13,14]. In addition, vitamin C reverses the sepsis induced oxidation (and inactivation) of the glucocorticoid receptor, while corticosteroids increase the transcription of SVCT2, the pivotal vitamin C transporter^[15,16]. Thiamine may act synergistically with glucocorticoids and vitamin C to limit mitochondrial oxidative injury and restore mitochondrial function and energy production^[17]. Furthermore, vitamin C and thiamine may play a critical in limiting delirium and septic encephalopathy^[17,18]. The key roles of vitamin C in sepsis is provided in [Table 1](#)^[8]. Thirdly, the mortality benefit of vitamin C (alone) in reducing the mortality of septic shock and sepsis associated acute respiratory distress syndrome has recently been

demonstrated^[19,20]. Fourthly, the benefit of HAT Rx has been demonstrated in multiple observational studies^[6,20]. And fifthly, we have together treated over 2000 patients with severe sepsis and septic shock. We continue to witness the remarkable ability of this treatment strategy to reduce the dysregulated immune response and improve the outcome of patients with sepsis. We have absolutely no conflicts of interest and have no reason to provide false evidence to the court of science and public opinion. It is critical to emphasize that all three components of HAT Rx are Food and Drug Administration approved drugs, which in the recommended doses are completely devoid of side effects. We are unaware of any side-effects or complications associated with HAT Rx. It should, however, be noted that there are powerful forces at play attempting to invalidate and disprove the beneficial effects of HAT Rx^[10,11]. This includes designing clinical trials that are doomed to fail, by using absurd dosing schedules or selectively enrolling patients likely to do poorly^[21,22].

CONCLUSION

Shortly, the results of multi-center RCTs will be available^[23-25]. Many will consider these trials to be the definitive evidence on which to judge the benefit of HAT Rx and to make a jury decision. However, while RCT's are considered the gold standard, it is important to realize that most RCT's do not replicate real world experience; mainly, due to the numerous exclusion criteria, enormous patient heterogeneity, non-standardized co-interventions and delays in instituting therapy. In the end, the thoughtful juror (clinician) must weigh the totality of the evidence before rendering a verdict.

Table 1 Summary of key roles of vitamin C in sepsis

Key role	Mechanism
Antioxidant	Scavenges extracellular, intracellular and mitochondrial ROS; limits oxidation of mitochondrial proteins, enzymes, lipoproteins, cell membrane, etc.
Anti-inflammatory	Inhibits activation of NFκB, decreases HMGB1, inhibits histamine, prevents NETosis, inactivates HIF-1α
Microcirculation	Increases eNOS, decreases iNOS, preserves tight junctions
Immune function	Supports lymphocyte proliferation, increases neutrophil bactericidal action, improves chemotaxis, stimulates interferon production, decreases T regulatory cells (Tregs), prevents lymphocyte apoptosis
Anti-thrombotic	Decreases platelet activation and tissue factor expression, increases thrombomodulin
Synthesis of catecholamines	Acts cofactor in synthesis of epinephrine, dopamine and vasopressin. Increases adrenergic sensitivity
Wound healing	Hydroxylation of procollagen, increased expression of collagen mRNA

Reproduced with permission from Nutrients^[4]. ROS: Reactive oxygen species; NFκB: Nuclear factor κB; HIF-1α: Hypoxia-inducible transcription factor-1α; HMGB1: High mobility group box 1; eNOS: Endothelial nitric oxide synthetase; iNOS: Inducible nitric oxide synthetase; HO-1: Heme oxygenase-1; HIF-1α: Hypoxia-inducible transcription factor-1α.

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Age of onset of diabetes and all-cause mortality

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Abstract

Diabetes mellitus continues to present a large social, financial and health system burden across the world. The relationship between age of onset of the different types of diabetes and all-cause mortality is uncertain. In this review paper, the relationship between age of onset of the different types of diabetes and all-cause mortality will be reviewed and an update of the current evidence will be presented. There is strong evidence of the relationship between age of onset of type 2 diabetes mellitus (T2DM) and all-cause mortality, good evidence of the relationship between age of onset of T1DM and all-cause mortality and no evidence of the relationship between age of onset of gestational diabetes or prediabetes and all-cause mortality. Further research is needed to look at whether aggressive management of earlier onset of T2DM can help to reduce premature mortality.

Key words: Diabetes mellitus; Age of onset; Mortality; Type 1 diabetes; Type 2 diabetes; Gestational diabetes; Prediabetes

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Core tip: In this review paper we will look at the relationship between age of onset of the different types of diabetes (type 1 diabetes mellitus, type 2 diabetes mellitus, gestational and prediabetes) and all-cause mortality and provides an update of the current evidence.

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INTRODUCTION



Diabetes mellitus is potentially reversible^[1-3] but the disease continues to present a large social, financial and health system burden across the world^[4]. Lifetime cost of treating type 2 diabetes mellitus (T2DM) in has been estimated to range from USD 50000 to USD 130000 in United States of America^[5] and SGD 70000 to SGD 130000 in Singapore^[6] depending on age of diagnosis. A systematic review of population-based cohorts found excess mortality in type 1 diabetes mellitus (T1DM) diagnosed in childhood and adolescence^[7]. Another systematic review also found that T2DM is associated with a two-fold increase in mortality^[8]. In Hongkong, it was found that absolute and relative mortality has declined overall in diabetes patients but there is less marked improvement in patients under 45 years of age, implying a need to improve care in young onset diabetes patients^[9]. What is uncertain is the relationship between age of onset of the different types of diabetes and all-cause mortality. This is important as American Diabetes Association recommends screening for T2DM to begin at 45 years for patients with no risk factors^[10] while Singapore recommend screening starts at 40 years old with no risk factor^[11]. If earlier onset of T2DM is associated with a much higher all-cause mortality, then we must relook at the screening criteria so that we do not miss out on these high-risk group. A better risk stratification model may be needed to ensure that earlier onset T2DM patients are not overlooked to reduce premature mortality.

In this review paper we will look at the relationship between age of onset of the different types of diabetes (T1DM, T2DM, Gestational and Prediabetes) and all-cause mortality and provides an update of the current evidence. This will help to inform readers of the need to pay special attention to the group of DM patients with early onset and not delay treatment unnecessarily.

LITERATURE RESEARCH

A literature search was done as part of this narrative review which included electronic databases of PubMed and Google Scholar. Only English-Language original and review articles were reviewed. As no systematic review has been done on this topic due to insufficient original research studies, we decided to do a narrative review instead. Only relevant studies were included in this narrative review using search terms such as “Diabetes”, “Age of onset”, “Mortality” and “Death”.

AGE OF ONSET OF T1DM

T1DM is most common in youth, accounting for more than 85% of all diabetes case below 20 years of age in the world^[12,13]. A Swedish study found that patients who developed T1DM before 10 years of age had a three-fold increase in mortality compared to controls while those who developed T1DM between 26 to 30 years of age had less than two-fold increase in mortality compared to controls^[14]. Another Finnish study found that the standardised mortality ratio was 3.6 in the early onset (0-14 years) cohort and 2.8 in the late onset (15-29 years) cohort^[15]. However, an older study of 13 population-based EURODIAB registers in 12 countries did not find any significant difference in the standardised mortality ratios by age of diagnosis^[16].

Based on current evidence, it is probably worthwhile to delay the onset of T1DM if this was possible and there are some recent promising results that showed that it is possible to delay progression to clinical T1DM in high risk individuals^[17]. However, routine screening for T1DM is not recommended^[18,19] as there is no therapy currently proven to prevent or significantly delay the onset, so more research is needed in this area to warrant further discussion.

AGE OF ONSET OF T2DM

T2DM which was previously diagnosed in adults is now affecting children^[20] and adolescents. It has been found that T2DM presenting at a young age is of aggressive nature by the landmark Search for Diabetes in Youth study^[13]. Furthermore, increased lifetime exposure to hyperglycaemia is likely to be associated with higher complication risks^[21]. Two Australian studies found an inverse relationship between age of onset of Type 2 Diabetes and complication risk and mortality^[22,23]. A Swedish study also found that patients with T2DM diagnosed below 40 years had the highest excess risk for all-cause mortality and interestingly patients with T2DM diagnosed after 80 years old had no excess mortality^[24].

Based on current evidence, it is likely that younger age of onset of T2DM is likely

associated with higher all-cause mortality and it is worthwhile to prevent or delay the onset of T2DM. Furthermore, it has been found that the younger age of onset of diabetes is associated with higher levels of lifetime excess medical spending attributed to diabetes both in United States^[25] and Singapore^[6].

AGE OF ONSET OF GESTATIONAL DIABETES

Gestational diabetes is defined as glucose intolerance that begins or is first recognized during pregnancy^[26] and this is increasing in prevalence in tandem with increase in prevalence of overweight and obesity in women of reproductive age^[27]. Although gestational diabetes is a strong risk factor for developing T2DM^[28-30] in future, there are no studies that looked at the relationship between gestational diabetes and all-cause mortality. However, a recent systematic review found that young women with Gestational Diabetes have a twofold higher risk of cardiovascular events postpartum compared with their peers independent of the risk of developing T2DM^[31]. Based on current evidence, little is known of the relationship between age of onset of Gestational Diabetes and all-cause mortality, so more research is needed in this area to warrant further discussion.

AGE OF ONSET OF PREDIABETES

Prediabetes is an intermediate state of hyperglycaemia with glycaemic parameters above normal but below the diabetes threshold^[32]. A systematic review found that impaired glucose tolerance or impaired fasting glucose was associated with all-cause mortality but not mildly raised HbA1c (39-47 mmol/mol)^[33]. However, there are no studies that looked at the relationship between age of onset of Prediabetes and all-cause mortality, so more research is needed in this area to warrant further discussion. Intuitively if we manage to delay the onset of Prediabetes, we may be able to delay the onset of T2DM and there is strong evidence of the effectiveness of diabetes prevention programs^[34-36].

CONCLUSION

In this narrative review, we found strong evidence of the relationship between age of onset of T2DM and all-cause mortality, good evidence of the relationship between age of onset of T1DM and all-cause mortality and no evidence of the relationship between age of onset of Gestational Diabetes or Prediabetes and all-cause mortality. Further research is needed to look at whether aggressive management of earlier onset of T2DM can help to reduce premature mortality.

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Glargine-300: An updated literature review on randomized controlled trials and real-world studies

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Abstract

Despite the availability of a variety of insulins, rates of insulinisation and the acceptance of insulin therapy is suboptimal in real-world clinical settings. Patient and physician concerns with hypoglycaemia and weight gain are the two key issues that serve to impede appropriate insulinisation in patients with diabetes. Recently introduced second-generation basal insulin analogues [for *e.g.*, insulin glargine 300 U/mL (Gla-300) and insulin degludec] are designed to have improved pharmacokinetic profiles with an intention to deliver steady insulin levels over a longer period. Several randomised controlled and real-world studies have proven the resultant advantages of second-generation insulin analogues in lowering intra-individual variability in plasma insulin levels, flexibility in dosing, a sustained glucose-lowering effect, and decreasing the risk of hypoglycaemia. Gla-300 is one of the newer second-generation basal insulin analogues to have been approved for both type 1 and 2 diabetes. In this article, we review the currently available clinical and real-world data of Gla-300.

Key words: Insulin; Glargine-300; Type 2 diabetes; Diabetes mellitus; Hypoglycaemia; Glycaemic control

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Core tip: Despite being a crucial therapeutic option in patients with diabetes, there is a clinical inertia for use of insulin due to fear of hypoglycaemia, weight gain, and complexity of insulin regimens or dosing. Insulin intensification is perceived to be associated with disease worsening, impeding optimal insulin titration and adequate glycaemic control. Insulin glargine 300, the second-generation long-acting insulin analogue, provides an extended and stable action profile, sustained glucose lowering, reduced risk of hypoglycaemia, less weight gain, and flexibility of dosing schedule. This review illustrates the clinical efficiency and safety demonstrated by insulin glargine 300 in randomised clinical trials and real-world studies.

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INTRODUCTION

Diabetes is a growing public health problem and imparts significant burden on both healthcare resources and on society. In 2019, approximately 463 million people worldwide had diabetes, and 4.2 million deaths were due to the disease or associated complications. It is estimated that 700 million people will have diabetes by 2045. The total global health expenditure on diabetes is estimated to be 760 billion United States Dollar^[1].

Insulin is the cornerstone of therapy for patients with type 1 diabetes (T1DM). Treatment for T1DM consists of multiple daily injections of prandial insulin and basal insulin (BI) or continuous subcutaneous (SC) infusion^[2]. Hypoglycaemia risk can be reduced with use of rapid-acting insulin analogues. Prandial insulin doses should be matched with carbohydrate intake, pre-meal blood glucose levels, and anticipated physical activity. ADA recommends that the patients with T1DM, who have been successfully using continuous SC insulin infusion, should have continuous access to this therapy even after 65 years of age^[2]. A wide array of pharmacological treatment options is available for patients with T2DM. However, with progressive loss of β -cell function, exogenously administered insulin therapy becomes imperative for many patients with T2DM. In patients who show unstable T2DM or symptoms of acute decompensation despite oral antidiabetic (OAD) treatment, insulin, either alone or in combination with other OADs, is recommended^[2].

BI is required to maintain blood glucose at a consistent level during fasting periods. The goal of BI therapy is to sustain physiologic insulin levels between meals, thereby mitigating the risk of hypoglycaemia, particularly at night. When a combination of ≥ 3 OAD agents fails to lower blood glucose levels and insulin therapy needs to be commenced, an effective regimen in the first-line insulinization could be a combination of BI and OADs^[3]. Moreover, a short-term intensive insulin therapy in T2DM has been shown to salvage β -cell function^[4].

While the currently available insulins are indispensable for management of diabetes, their use in real-world settings is beset by various shortcomings. One of the biggest obstacles in using insulin for the management of diabetes is patients' fear of hypoglycaemia, which could lead to lack of patient compliance and clinical inertia and ultimately to loss of glycaemic control^[5-8]. A majority of both primary care physicians and specialists have indicated that they would treat their patients more aggressively if there was no concern about hypoglycaemia^[6]. Moreover, following insulin initiation, most patients fail to achieve glycaemic control in part due to suboptimal titration of the insulin dose^[9]. Under-titration could either be due to patient fear of hypoglycaemia or of weight gain. Furthermore, insufficient health care resources could also be responsible for the failure to assist and educate the patient on proper self-titration algorithms.

Current research on insulin therapy focusses on making it safer and more effective for patients. Newer BI formulations have provided advantages of lower intraindividual variability, flexibility in dosing, and a sustained glucose-lowering effect without an increased risk of hypoglycaemia^[10,11]. In this review article, we have summarised the clinical and real-world evidence on insulin glargine 300 U/mL (Gla-300, Toujeo®), a second-generation BI analogue approved in 2015 by the USFDA and the EMA for use in patients with T1DM/T2DM.

GLA-300, THE SECOND- GENERATION BASAL INSULIN

Glargine is a human insulin analogue that differs from the endogenous human insulin by a substitution of glycine for asparagine at position A21 and the addition of two arginine residues to the C-terminus of the B-chain. The solution of insulin glargine injection has a pH of 4, which neutralises post-injection to pH 7. The addition of arginine residues increases the isoelectric point of insulin glargine and results in formation of a microprecipitate within an amorphous SC depot, from which slow and protracted release of insulin glargine occurs^[12,13]. Gla-300 is a formulation of insulin

glargine that delivers the same amount of insulin units as insulin glargine 100 U/mL (Gla-100) in one-third of the injection volume.

Gla-300 comprises the same active glargine molecule as Gla-100 but forms a more compact SC depot with a reduced surface area than Gla-100. It is hypothesized that the size, and hence the surface area, of the SC depot determines the re-dissolution rate (Figure 1)^[13,14]. This may allow for a longer SC residence time and degradation by tissue peptidases, resulting in a reduced re-dissolution rate, lower bioavailability, and an increase in daily dose^[15]. Accordingly, Gla-300 has a more stable activity profile and a more prolonged and gradual insulin release than Gla-100, resulting in blood glucose control that lasts for up to 36 h^[16-19].

Pharmacokinetic and pharmacodynamics profile of Gla-300

Various studies have shown that Gla-300 has a more stable and prolonged pharmacokinetic/pharmacodynamics (PK/PD) profile. In a double-blind randomised study on 50 patients with T1DM, Gla-300 was shown to provide predictable and evenly distributed insulin exposure over 24 h^[16]. In another double-blind randomised study comparing Gla-300 to Gla-100 in 30 patients with T1DM, Gla-300 demonstrated a more even steady-state PK/PD profile and a longer duration of action than Gla-100^[20]. In this study, Gla-300 maintained tight blood glucose control (≤ 105 mg/dL) for a median of 30 h. In two double-blind, randomised crossover studies in Japanese ($n = 18$) and European ($n = 24$) patients with T1DM in euglycaemic clamp settings, single-dose Gla-300 injections were shown to have a more prolonged and constant PK/PD profile compared with Gla-100^[17]. In addition, blood glucose control was maintained for up to 36 h in patients receiving Gla-300. An exploratory, open-label, parallel-group, two-period crossover study on 59 patients with T1DM comparing Gla-300 with Gla-100 demonstrated reduced glucose levels (as measured by continuous glucose monitoring) in the last 4 h of the 24-h injection interval, smoother average 24-h glucose profiles regardless of injection time, and reduced nocturnal hypoglycaemia with Gla-300^[21].

The pharmacodynamic properties of Gla-300 and degludec (IDeg)-200, both at 0.4 U/kg once-daily fixed dose, were evaluated in 57 patients with T1DM in a two-period, two-treatment, two-sequence, crossover study using euglycaemic clamp^[22]. In this study, IDeg-200 showed lower day-to-day variability (approximately 4 times lower) and within-day variability (37% lesser) in glucose-lowering effect than Gla-300. In contrast, a second euglycaemic clamp study^[23] in 48 patients with T1DM demonstrated that Gla-300 had better steady-state PD profile (20% less within-day variability) and evenly distributed PK profile than IDeg-100 when administered at the same dose (0.4 U/kg/d). Though, there were several differences between both the studies such as morning versus evening injections and use of IDeg-200 *vs* IDeg-100, the difference in results was mainly attributed to parameter used for calculating the within-day variability (fluctuations) of the BIs^[24]. The latter study presented fluctuations of the BIs using absolute area under the curve values of the smoothed glucose infusion rate (GIR) curves above and below the average GIR, while, the former study presented percentage of the total glucose-lowering effect (area under the curve-GIR₀₋₂₄ hours).

Reductions in glycated haemoglobin, hypoglycaemia and weight gain

The safety and efficacy of Gla-300 has been assessed in a series of clinical trials comprising the EDITION programme which recruited patients with both T1DM and T2DM^[15,25-29]. The patients in the EDITION trials received a range of background therapies and two trials were performed specifically in Japanese populations. All EDITION trials assessed mean glycated haemoglobin (HbA_{1c}) change from baseline to 6 mo and the risk of hypoglycaemia for both Gla-300 and Gla-100. Comparable mean HbA_{1c} changes between both glargine formulations were observed across all studies (Table 1). Patients treated with Gla-300 consistently experienced a reduced risk of hypoglycaemia, including nocturnal and hypoglycaemia at any time of the day (Table 2). Rates of treatment-emergent adverse events were similar between the glargine formulations (Table 1). Similar to other insulins, the most common adverse event ($\geq 1/10$) was hypoglycaemia. Other common adverse events ($\geq 1/100$ to $< 1/10$) included lipohypertrophy and injection-site reactions. In the EDITION trials, patients continued the treatment for 6 mo in addition to the initial 6-mo of treatment. At the end of 12 mo, Gla-300 achieved better reductions in HbA_{1c} and the risk of hypoglycemia (at any time) than with Gla-100 in EDITION 1 trial and the outcomes were comparable in the other EDITION trials^[30].

A patient-level meta-analysis of the EDITION 1, 2, and 3 studies revealed comparable glycaemic control between both glargine formulations [reduced HbA_{1c} for both formulations -1.02% (standard error 0.03, 95%CI: 0.08 to 0.07)] across a large and clinically diverse population with T2DM^[31]. When compared with Gla-100, patients

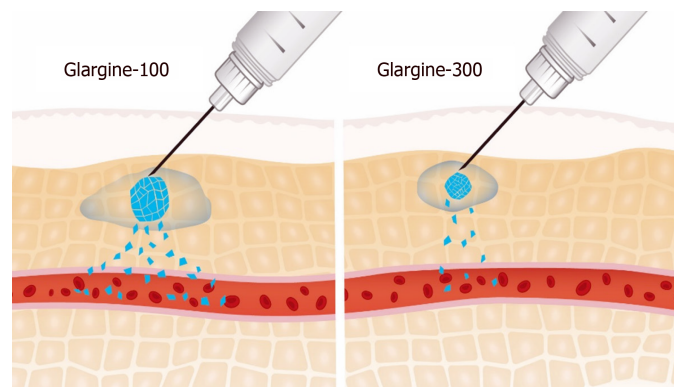


Figure 1 Surface area of subcutaneous depot: Gla-100 and Gla-300. Adapted from^[12].

treated with Gla-300 had reduced annualised rates of confirmed or severe hypoglycaemia at night (31% difference in rate ratio over 6 mo) and at any time (24 h, 14% difference). In addition, there was lower weight gain in patients treated with Gla-300 than in those receiving Gla-100 [LS mean difference 0.28 kg (95% CI: 0.55 to 0.01); $P = 0.039$]. These efficacy and safety results were further corroborated in a one-year patient-level meta-analysis of the EDITION 1, 2, and 3 studies^[32]. When compared with Gla-100, Gla-300 provided more sustained reductions in HbA_{1c} over 12 mo [LS mean difference in change from baseline 0.10% (95% CI: 0.18 to 0.02); 1.09 mmol/mol (2.01 to 0.20); $P = 0.0174$]. A lower risk of confirmed or severe hypoglycaemia was observed with Gla-300 at night [relative risk (RR): 0.85 (95% CI: 0.77 to 0.92)]; the risk was also lower at any time of day [RR: 0.94 (95% CI: 0.90 to 0.98)]. The rates of nocturnal hypoglycaemia were lower with Gla-300 versus Gla-100 [rate ratio 0.82 (95% CI: 0.67 to 0.99)] but were comparable at any time of day. When compared with Gla-100, patients treated with Gla-300 were more likely to achieve HbA_{1c} < 7.0% without nocturnal hypoglycaemia [RR: 1.24 (95% CI: 1.03 to 1.50)]. Another recently reported meta-analysis of the 6-mo pooled data from 2496 patients enrolled in the EDITION 1, 2, and 3 trials aimed at comparing safety and efficacy of Gla-300 and Gla-100 in patients with mild-to-moderate renal impairment^[33]. Results from this analysis show that while glycaemic control was comparable between the two groups, there was a reduced overall risk of confirmed or severe hypoglycaemia in both groups.

The safety and efficacy of Gla-300 has been compared in a network meta-analysis with other BI therapies in T2DM^[34]. The change in HbA_{1c} provided by Gla-300 was similar to that of detemir [difference: -0.08; 95% credible interval (CrI): -0.40 to 0.24], neutral protamine Hagedorn (NPH; difference: 0.01; 95% CI: 0.28 to 0.32), IDeg (difference: -0.12; 95% CI: 0.42 to 0.20), and premixed insulin (difference: 0.26; 95% CI: 0.04 to 0.58). A significantly lower nocturnal hypoglycaemia rate was observed with Gla-300 when compared with NPH [risk ratio/relative risk (RR): 0.18; 95% CI: 0.05 to 0.55] and premixed insulin (RR: 0.36; 95% CI: 0.14 to 0.94). No significant differences in nocturnal hypoglycaemia rate were observed between Gla-300 and detemir (RR: 0.52; 95% CI: 0.19 to 1.36) or IDeg (RR: 0.66; 95% CI: 0.28 to 1.50). There were no significant differences in documented symptomatic hypoglycaemia rates of Gla-300 versus detemir (RR: 0.63; 95% CI: 0.19 to 2.00), NPH (RR: 0.66; 95% CI: 0.27 to 1.49), and IDeg (RR: 0.55; 95% CI: 0.23 to 1.34). While comparable changes in body weight (in kg) were observed between Gla-300 and detemir (difference: 0.69; 95% CI: -0.31 to 1.71), NPH (difference: 0.76; 95% CI: 1.75 to 0.21), and IDeg (difference: 0.63; 95% CI: 1.63 to 0.35); weight gain was significantly lower compared with premixed insulin (difference: 1.83; 95% CI: 2.85 to 0.75). Another systematic review and network meta-analysis comparing 10 BIs evaluated data from > 26000 patients from 39 randomised trials lasting ≥ 12 wk in duration and reported a favourable outcome for Gla-300 in terms of HbA_{1c} reduction, change in body weight, and any hypoglycaemia^[35].

The safety and efficacy of Gla-300 has also been studied in older people with T2DM. SENIOR was an open-label, two-arm, parallel-group, multicentre phase 3b trial that compared Gla-300 with Gla-100 in 1014 randomised participants (mean age 71 years)^[36]. In the overall population, similar reductions in HbA_{1c} were observed from baseline to week 26 for Gla-300 (0.89%) and Gla-100 (0.91%) (LS mean difference: 0.02%; 95% CI: 0.092 to 0.129). The rates and incidence of confirmed or severe hypoglycaemia events were low and comparable between both treatment groups. Lower rates of documented symptomatic hypoglycaemia were observed with Gla-300. Significantly lower annualised rates of documented symptomatic hypoglycaemia were also observed [Gla-300: 1.12; Gla-100: 2.71; rate ratio: 0.45 (95% CI: 0.25 to 0.83)].

Table 1 Efficacy and safety of Gla-300 vs Gla-100 across the EDITION Phase 3 Clinical Trial Program

Study (ref.) / Registry	Diabetes type	Population	n (Gla-300 vs Gla-100)	Mean baseline A1C (%)	LS mean change in A1C from baseline to Month 6 (%)		TEAEs (%)		Serious TEAEs (%)	
					Gla-300	Gla-100	Gla-300	Gla-100	Gla-300	Gla-100
EDITION 1 ^[15] , NCT01499082	T2DM	(1) Basal insulin users (≥ 42 U/d); (2) T2DM not adequately controlled; and (3) Basal insulin (evening) + mealtime insulin	807 (404 vs 403)	8.2	-0.83	-0.83	NA	NA	6.4	5.2
					Difference: -0.00%, (95%CI: -0.11 to 0.11)					
EDITION 2 ^[25] , NCT01499095	T2DM	(1) Basal insulin users (≥ 42 U/d); and (2) Basal insulin (evening) + OADs	811 (404 vs 407)	8.2	-0.57	-0.56	58.8	50.7	3.7	3.7
					Difference: -0.01%, (95%CI: -0.14 to 0.12)					
EDITION 3 ^[26] , NCT01676220	T2DM	Insulin-naïve (evening), uncontrolled using noninsulin therapy	878 (439 vs 439)	8.5	-1.42	-1.46	NA	NA	5.5	5.9
					Difference: 0.04%, (95%CI -0.09 to 0.17)					
EDITION 4 ^[27] , NCT01683266	T1DM	Basal insulin (morning or evening) + mealtime insulin	549 (274 vs 275)	8.1	-0.42	-0.44	60.9	58.2	6.2	8.0
					Difference: 0.04%, (95%CI -0.10 to 0.19)					
EDITION JP 1 ^[28] , NCT01689129	T1DM	(1) Japanese study; and (2) Basal insulin (evening) + mealtime insulin	243 (122 vs 121)	8.1	-0.30	-0.43	62.3	64.5	2.5	2.5
					Difference: 0.13%, (95%CI -0.03 to 0.29)					
EDITION JP 2 ^[29] , NCT01689142	T2DM	(1) Japanese study; and (2) Basal insulin (evening) + OADs	240 (120 vs 120)	8.0	-0.45	-0.55	58.3	56.7	4.2	3.3
					Difference: 0.10%, (95%CI -0.08 to 0.27)					

A1C: Glycated haemoglobin A_{1c}; CI: Confidence interval; Gla-100: Insulin glargine 100 U/mL; Gla-300: Insulin glargine 300 U/mL; OADs: Oral antidiabetic drugs; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; TEAE: Treatment emergent adverse event.

In patients aged ≥ 75 years, reductions in HbA_{1c} from baseline to week 26 were comparable in both groups (LS mean difference: -0.11%; 95%CI: -0.330 to 0.106); while, the hypoglycaemia risk was lower with Gla-300 than Gla-100 [documented symptomatic hypoglycaemia (< 54 mg/dL): 1.5% *vs* 10.4%; relative risk: 0.33; 95%CI: 0.12 to 0.88]^[33].

A *post-hoc* analysis of patient-level meta-analysis of EDITION 1, 2, and 3 studies examined the extent of glycaemic control and risk of hypoglycaemia with Gla-300 *vs* Gla-100 in patients aged ≥ 65 years and with T2DM. The analysis showed comparable glycaemic control with Gla-300 and Gla-100 (LS mean difference in HbA_{1c} change from baseline to month 6: 0.00; 95%CI: -0.14% to 0.15%). A reduction in risk of hypoglycaemia was observed for Gla-300 *vs* Gla-100 (RR: 0.70; 95%CI: 0.57 to 0.85)^[37].

Gla-300: Real world evidences

Oriot *et al*^[38] evaluated the glycaemic control in patients with T1DM after switching from Gla-100 to Gla-300 in a real-world clinical practice. Patients were first treated with Gla-100, either once or twice daily, and then switched to Gla-300. Glycaemic

Table 2 Risk of hypoglycaemia for Gla-300 vs Gla-100 across the EDITION Phase 3 Clinical Trial Program (Safety population)

Study (ref.)		Confirmed [≤ 3.9 mmol/L (≤ 70 mg/dL)] or severe hypoglycaemia												At any time of the day	
		Nocturnal				At any time of the day								Severe hypoglycaemia	
		Baseline to Week 8		Week 9 to Month 6		Baseline to Month 6		Baseline to Week 8		Week 9 to Month 6		Baseline to Month 6		Baseline to Month 6	
		Gla-300	Gla-100	Gla-300	Gla-100	Gla-300	Gla-100	Gla-300	Gla-100	Gla-300	Gla-100	Gla-300	Gla-100	Gla-300	Gla-100
EDITION 1 ^[15]	Patients (%)	26.2	33.3	36.1	45.8	44.6	57.5	64.4	75.1	74.8	77.6	81.9	87.8	5	5.7
	RR (95%CI)	0.79 (0.64-0.98)		0.79 (0.67-0.93)		0.78 (0.68-0.89)		0.86 (0.78-0.94)		0.96 (0.89-1.04)		0.93 (0.88-0.99)		0.87 (0.48-1.55)	
EDITION 2 ^[25]	Patients (%)	13.2	24.6	21.6	27.9	28.3	39.9	47.1	60.1	59.3	65	70	77.3	1	1.2
	RR (95%CI)	0.53 (0.39-0.72)		0.77 (0.60-0.97)		0.71 (0.58-0.86)		0.78 (0.69-0.89)		0.91 (0.82-1.02)		0.90 (0.83-0.98)		NA	
EDITION 3 ^[26]	Patients (%)	7.4	10	15.4	17.1	17.9	23.5	24.1	29.2	39.8	46.3	46.2	52.5	0.9	0.9
	RR (95%CI)	0.74 (0.48-1.13)		0.90 (0.67-1.22)		0.76 (0.59-0.99)		0.83 (0.67-1.03)		0.86 (0.74-1.00)		0.88 (0.77-1.01)		NA	
EDITION 4 ^[27]	Patients (%)	46.7	57.1	59.1	55.6	68.6	70.2	88.3	90.2	82.1	84	93.1	93.5	6.6	9.5
	RR (95%CI)	0.82 (0.70-0.96)		1.06 (0.92-1.23)		0.98 (0.88-1.09)		0.98 (0.92-1.04)		0.98 (0.91-1.06)		1.00 (0.95-1.04)		0.71 (0.41-1.24)	
EDITION JP 1 ^[28]	Patients (%)	43.4	61.2	61.7	73.7	68.9	81	86.9	95	94.2	93.2	96.7	97.5	5.7	9.9
	RR (95%CI)	0.71 (0.56-0.91)		0.84 (0.70-1.00)		0.85 (0.73-0.99)		0.91 (0.84-0.99)		1.01 (0.95-1.08)		0.99 (0.95-1.04)		0.58 (0.24-1.42)	
EDITION JP 2 ^[29]	Patients (%)	13.3	16.7	25.4	43.7	28.3	45.8	37.5	55	60.2	72.3	65	76.7	2.5	1.7
	RR (95%CI)	0.83 (0.45-1.52)		0.58 (0.40-0.85)		0.62 (0.44-0.88)		0.69 (0.52-0.91)		0.84 (0.70-1.01)		0.86 (0.73-1.01)		1.25 (0.31-4.98)	

Relative risk of experiencing ≥ 1 confirmed (blood glucose ≤ 70 mg/dL) or severe episode of hypoglycaemia. CI: Confidence interval; Gla-100: Insulin glargine 100 U/mL; Gla-300: Insulin glargine 300 U/mL; RR: Relative risk.

control was evaluated at two-time intervals - the first (period 1) during a 2-wk period before and after switching to Gla-300 and the second (period 2) at 12 and 24 wk following the switch. Results showed that HbA_{1c} remained at pre-switch levels during period 1 but decreased from $8.0\% \pm 1.0\%$ (65.5 ± 10.5 mmol/mol) to $7.9\% \pm 1.0\%$ (62.8 ± 10 mmol/mol) by the end of period 2. The number of nocturnal hypoglycaemic events significantly reduced following the switch (22.2% before switch *vs* 12.2% after switch; RR: 0.46; 95%CI: 0.30 to 0.68; $P < 0.0001$) as was the proportion of patients with nocturnal hypoglycaemia per period (30.0% before switch *vs* 16.0% after switch; RR: 0.53; 95%CI: 0.31 to 0.86). Moreover, no perceptible weight gain was reported in study patients.

Usage patterns and clinical outcomes have been assessed before and after Gla-300 initiation in patients with T2DM starting or switching to Gla-300. A retrospective observational study using data acquired from physician survey medical records revealed a similar final titrated dose among insulin-naïve patients starting BI treatment [LS mean 0.43 units/kg (Gla-300) *vs* 0.44 units/kg (Gla-100); $P = 0.77$]^[39]. Significant reductions in HbA_{1c} levels were observed for both glargine formulations [LS mean 1.21% (Gla-300) and 1.12% (Gla-100); both $P < 0.001$]. Compared with Gla-100, Gla-300 was associated with a lower rate of hypoglycaemic events after treatment initiation (RR: 0.31; 95%CI: 0.12 to 0.81; $P = 0.018$) at similar daily doses. Significantly lower daily doses of BI were observed after switching to treatment with Gla-300 from treatment with another BI (0.73 units/kg before switch *vs* 0.58 units/kg after switch; $P = 0.02$). Mean HbA_{1c} was significantly lower after the switch to Gla-300 than before switching (adjusted difference 0.95%; 95%CI: 1.13 to 0.78; $P < 0.0001$). In addition, hypoglycaemic events per patient-year (PPY) were significantly lower in patients receiving Gla-300 (RR: 0.17; 95%CI: 0.11 to 0.26; $P < 0.0001$).

In another retrospective study conducted in routine clinical settings in Japan, 20 patients with T1DM and 62 patients with T2DM who had switched from Gla-100 to Gla-300 were evaluated for the safety and efficacy of Gla-300, 3 mo following the switch^[40]. HbA_{1c} level substantially decreased in patients with T2DM ($P < 0.01$) and while these values were lowered in T1DM patients, the magnitude of reduction was not statistically significant. Decreases in body-mass-index, an indicator of weight gain, were observed in patients with T1DM ($P = 0.06$) as well as T2DM ($P < 0.05$). Rates of hypoglycaemia were similar across all groups. These findings hint that switching the BI regimen to Gla-300 is effective in achieving glycaemic control as well as avoiding weight gain.

DELIVER 2 was a retrospective cohort study that compared real-world clinical and healthcare-resource utilisation data in patients with T2DM who were on BI treatment and who switched to Gla-300 or to another BI^[41]. Eligible patients aged ≥ 18 years and were receiving BI. Data were collected from the Predictive Health Intelligence Environment database of electronic medical records, here representing 39 integrated healthcare-delivery networks in the United States. A comparable change in HbA_{1c} from baseline was observed in both matched cohorts ($n = 1819$ in each) (0.51%, Gla-300; 0.51%, other BI; $P = 0.928$) (Figure 2). Patients in both cohorts were also equally likely to achieve HbA_{1c} $< 7.0\%$ (16.8%, Gla-300; other BI, 18.4%; $P = 0.223$) and $< 8.0\%$ (44.0% Gla-300; 44.2%, other BI; $P = 0.920$) during follow up. Significantly fewer patients treated with Gla-300 experienced hypoglycaemia during the same period (15.4%, Gla-300; 18.1%, other BI; $P = 0.015$) (Figure 3). Patients who switched to Gla-300 had a lower risk of requiring hypoglycaemia-related hospitalisation, emergency department (ED) services, and outpatient visits when compared to those who switched to other BIs [adjusted odds ratio (aOR): 0.67, hospitalization, $P = 0.037$; 0.62, ED services, $P = 0.007$; 0.77, outpatient visits, $P = 0.011$]. Considering all hypoglycaemia-related healthcare-resource utilisation data together, switching to Gla-300 resulted in an overall savings of \$1439 per patient per year.

The DELIVER 3 was a retrospective study that assessed in real-world clinical settings the glycaemic control and risk of hypoglycaemia with Gla-300 in older patients with T2DM^[42]. Patients with T2DM aged ≥ 65 years already on BI therapy, who switched to either Gla-300 or other BI (Gla-100 or detemir), were identified from the Predictive Health Intelligence Environment database (representing 39 integrated healthcare delivery networks). In this study, 1176 older adults with T2DM who switched from BI to Gla-300 were propensity score-matched to 1176 older adults who switched to a first-generation BI (Gla-100 or detemir). When compared with other BIs, switching to Gla-300 led to greater or similar changes in HbA_{1c} (Gla-300 *vs* other BI mean \pm SD - variable follow-up: $-0.45\% \pm 1.40\%$ *vs* $-0.29\% \pm 1.57\%$, $P = 0.021$; fixed follow-up: $-0.48\% \pm 1.49\%$ *vs* $-0.38\% \pm 1.59\%$, $P = 0.114$). Similar proportions of patients in each cohort (Gla-300 and other BI) achieved HbA_{1c} $< 7.0\%$ (variable follow-up: 18.5 *vs* 19.7, respectively, $P = 0.514$; fixed follow-up: 19.3% *vs* 21.3%, respectively, $P = 0.292$) and $< 8.0\%$ (variable follow-up: 49.1% *vs* 49.1%, respectively, $P = 1.000$; fixed follow-up: 50.9% *vs* 51.8%, respectively, $P = 0.773$). Patients who switched to

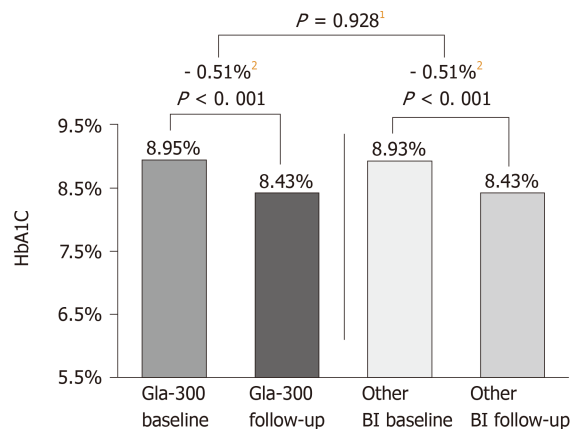


Figure 2 Glycated haemoglobin change during the 6mo followup period (DELIVER 2 Study)^[44]. DELIVER 2, a retrospective analysis of electronic medical records from the Predictive Health Intelligence Environment database.

¹Comparison of mean reduction in Gla300 vs other basal insulin. ²Magnitude of HbA1c change. BI: Basal insulin; Gla-300: Insulin glargine 300 U/mL; HbA1c: Glycated haemoglobin.

Gla-300 were less likely to have hypoglycaemia [Gla-300 *vs* other BI: 0.52 *vs* 0.80 events rate PPY; adjusted rate ratio: 0.63 (95%CI: 0.53 to 0.75); $P < 0.001$] and inpatient/ED-associated hypoglycaemia [Gla-300 *vs* other BI: 0.12 *vs* 0.27 events rate PPY; adjusted rate ratio: 0.43 (95%CI: 0.31 to 0.60); $P < 0.001$ based on variable follow-up]. The incidence of hypoglycaemia was significantly or numerically lower with Gla-300 during the fixed follow-up, Patient aged ≥ 75 years were more prone to hypoglycaemia compared with overall population^[42].

DELIVER Naïve was a retrospective study in insulin-naïve patients with T2DM who initiated Glar-300 or Glar-100. During 6-mo follow-up, HbA_{1c} reduction was significantly higher in patients who initiated with Gla300 compared with those who initiated Gla-100 (mean \pm SD: $-1.52 \pm 2.08\%$ *vs* $-1.30 \pm 2.12\%$; $P = 0.003$). More number of patients who initiated Gla-300 achieved target HbA_{1c} $< 7\%$ than Gla-100 (25.0% *vs* 21.5%; $P = 0.029$) and HbA_{1c} $< 8\%$ (55.0% *vs* 49.2%; $P = 0.002$). Gla-300 initiators had lower inpatient/ED-associated hypoglycaemia incidence (OR: 0.35; $P = 0.009$) during 3-mo follow-up, while, during 6-mo followup, numerically lower all hypoglycaemia incidence (OR 0.77; $P = 0.057$) and the inpatient/ED incidence (OR: 0.61; $P = 0.051$)^[43].

DELIVER Naïve D was a real world, retrospective, observational study in insulin naïve adults with T2DM who started receiving Gla-300 or IDeg. In matched cohorts ($n = 638$ each), the decreases in HbA_{1c} from baseline to follow-up (between 3 to 6mo from baseline) were significant in both the groups ($P < 0.001$ for both) and comparable between the groups (mean \pm SD: Gla-300 $-1.67 \pm 2.22\%$; IDeg $1.58 \pm 2.20\%$; $P = 0.51$). In addition, incidence of hypoglycaemia (overall and inpatient/EDassociated) was similar between the treatment groups. Results from study suggest that second generation BI analogues offer advantages over firstgeneration BI analogues^[44]. The results from this study highlight the real-world impact of Gla-300 were aligned with BRIGHT study, a randomized, controlled trial, that demonstrated a comparable improvement in HbA1c with Gla-300 and IDeg.

The realworld effectiveness of insulin IDeg and Gla-300was compared in insulin naïve adult patients with type 2 diabetes in a retrospective, non-interventional (CONFIRM) study^[45]. This study revealed significantly improved effects on both HbA1c and hypoglycaemia with IDeg versus Gla-300; however, the propensity score matching in this study had critical flaws^[46]. At baseline, the matched cohorts were not well-balanced in the number of hypoglycaemia episodes prior to insulin initiation (IDeg *vs* Glar-300: 6.7% *vs* 5.6%) as well as the rate of hypoglycaemia per patient years of exposure (PYE) differed (0.301 events/PYE for IDeg *vs* 0.210 events/PYE for Gla-300). Both these discrepancies have led to confounding interpretation of hypoglycaemia results (0.391 events/PYE for IDeg *vs* 0.389 events/PYE for Gla-300 post-initiation at 180 d of follow-up)^[45]. Imbalance in the cohort in terms of hypoglycaemia, before initiation of insulin treatment, may be the reason for dissimilarity of the results reported rather than the effects of treatment^[47].

The LIGHTNING study applied predictive modelling to real-world data and observed similar rates of severe hypoglycemia after switching from another BI to either Gla-300 or IDeg, in clinically vulnerable subgroups of patients with T2DM at high risk of hypoglycemia^[47]. The LIGHTNING study compared hypoglycemia rates with Gla-300 *vs* the first and second-generation BI analogues. Predictive modelling ($n = 198198$ patient-treatments) showed that rate of severe hypoglycaemia was

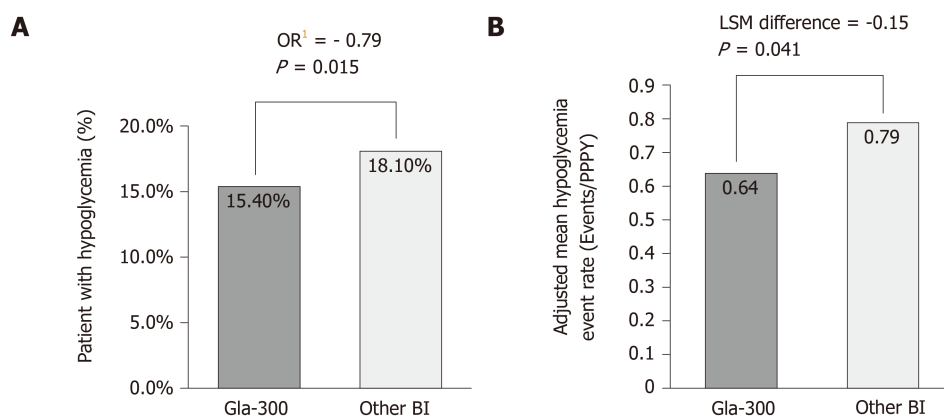


Figure 3 Hypoglycaemia incidence and event rate (DELIVER 2 study)^[41]. A: Percentage of patients with hypoglycemia¹ at 6-mo after basal insulin switch by insulin type; B: Adjusted mean hypoglycemia event rate² (Events/Per patient per year) during 6-mo follow-up. DELIVER 2, a retrospective analysis of electronic medical records from the Predictive Health Intelligence Environment database. ¹Adjusted for baseline hypoglycaemia incidence; ²Adjusted for baseline hypoglycaemia event rate. BI: Basal insulin; Gla-300: Insulin glargine 300 U/mL; HbA_{1c}: Glycated haemoglobin; OR: Odds ratio; PPPY: Per patient per year.

approximately 50% lower with Gla-300 compared with Gla-100 or insulin detemir in insulin-naïve patients, and 30% lower than insulin detemir in BI switchers (for all, $P < 0.05$). Data analysed using propensity score matching ($n = 157573$ patient-treatments) showed analogous HbA_{1c} reductions with Gla-300 compared with BI analogues (mean \pm SD in insulin naïve: Gla-300 *vs* IDeg, $-1.44 \pm 2.32\%$ *vs* $-1.56 \pm 2.26\%$; Gla-300 *vs* Gla-100, $-1.28 \pm 2.16\%$ *vs* $-1.42 \pm 2.09\%$; Gla-300 *vs* insulin detemir, $-1.27 \pm 2.15\%$ *vs* $-1.12 \pm 2.06\%$; mean \pm SD in BI switchers: Gla-300 *vs* IDeg, $-0.66 \pm 1.81\%$ *vs* $-0.60 \pm 1.73\%$; Gla-300 *vs* Gla-100, $-0.59 \pm 1.84\%$ *vs* $-0.52 \pm 1.87\%$; Gla-300 *vs* insulin detemir, $-0.59 \pm 1.84\%$ *vs* $-0.50 \pm 2.14\%$). Rate of severe hypoglycaemia was lower with Gla-300 *vs* Gla-100 or insulin detemir (both, $P < 0.05$) and similar rates versus IDeg were observed in both, insulin-naïve and BI-switcher cohorts^[48].

Clinical outcomes in the real-world scenario in T2DM patients switching from the first-generation BI analogues (Gla-100 and detemir) to the second-generation analogues (Gla-300 and IDeg) were assessed in a cohort analysis of the DELIVER D+ study^[49]. Patients who switched to Gla-300 ($n = 1592$) or IDeg ($n = 1592$) were propensity score matched for baseline characteristics and evaluated for incidence of hypoglycaemia during a 12-mo period. The HbA_{1c} change and target attainment were analysed in patients with HbA_{1c} values at baseline and during 3 to 6mo' followup (742 and 727 in matched Gla300 and IDeg cohorts, respectively). Average decreases in HbA_{1c} and target achievement rates were similar between the Gla-300 and the IDeg groups. Using an intent-to-treat approach revealed that incidence of hypoglycaemia decreased substantially in the Gla-300 group (all hypoglycaemia: 15.6% to 12.7%; $P = 0.006$; hypoglycaemia associated with inpatient/emergency room (ER) visit: 5.3% to 3.5%; $P = 0.007$) but not in the IDeg group. However, after adjusting for baseline hypoglycaemia, no difference was evident between the Gla-300 and the IDeg groups. Using the on-treatment approach replicated this finding; albeit patients receiving Gla-300 had a lower inpatient/ER visit rate (adjusted rate ratio: 0.56; $P = 0.016$).

Gla-300 vs insulin degludec - Data from randomized controlled trials

Trial-level meta-analyses of the EDITION (*vs* Gla-100) and BEGIN (*vs* IDeg) programmes compared the glycaemic control and risk of hypoglycaemia between Gla-300 and Gla-100 or IDeg. In BEGIN, while IDeg achieved a greater fasting plasma glucose reduction than Gla-100, Gla-100 offered a greater reduction in HbA_{1c} (mean difference: 0.09%; 95%CI: 0.01 to 0.18). In EDITION, no difference was observed in fasting plasma glucose and HbA_{1c} reduction between both glargine formulations. Risk of nocturnal confirmed or severe hypoglycaemia was lower with IDeg than Gla-100 (RR: 0.79; 95%CI: 0.66 to 0.94). When compared with Gla-100, Gla-300 was associated with reduced risk of both nocturnal (RR: 0.75; 95%CI: 0.61 to 0.92) and anytime (24 h) (RR: 0.81; 95%CI: 0.69 to 0.94) confirmed or severe hypoglycaemia^[50].

The BRIGHT study is the first head-to-head clinical trial that directly compared the safety and efficacy of second-generation BIs (Gla-300 and IDeg-100) in adults with T2DM who were uncontrolled on OADs (with/without GLP-1 receptor agonist at stable dose for ≥ 3 mo) and insulin naïve^[51]. In this study, patients were excluded if found with HbA_{1c} $< 7.5\%$ or $> 10.5\%$ (at screening), BMI < 25 kg/m² or > 40 kg/m², currently or previously using insulin except for a maximum of 8 consecutive days or totally 15 d (*e.g.*, acute illness, surgery) during the last year prior to screening.

BRIGHT was a phase 4, 24-wk, multinational, multicentre, open-label, two-arm, parallel-group trial that enrolled 929 insulin-naïve adults with T2DM inadequately controlled with OADs with or without a GLP-1 receptor agonist. Patients were randomised in 1:1 ratio to receive Gla-300 (0.2 U/kg) or IDeg (10 U) administered once daily using similar treat-to-target titration protocols. Non-inferiority of Gla-300 *vs* IDeg was demonstrated for HbA_{1c} change from baseline to week 24. Both BIs provided a similar reduction in fasting self-monitored plasma glucose. Hypoglycaemia incidence and rates were comparable with both insulins during the full study period but lower in favour of Gla-300 during the titration period. During the titration period of first 12-wk, the event rates of hypoglycaemia (≤ 70 mg/dL) were lower with Gla-300 *vs* IDeg by 23% (RR: 0.77; 95%CI: 0.62 to 0.96) at any time of day (24 h) and 35% (RR: 0.69; 95%CI: 0.43 to 0.98) at night^[52-54].

In a small-scale randomized cross-over study designed to compare the efficacy and safety of Gla-300 and IDeg using continuous glucose monitoring, patients were assessed for average percentage of time with sustained blood glucose levels of 70-180 mg/dL (efficacy) and incidence of hypoglycaemia defined as blood glucose level < 70 mg/dL (safety)^[55]. There was no statistically significant difference in mean percentage of time within target glucose range between the Gla-300 and IDeg groups ($77.8 \pm 19.2\%$ *vs* $76.9 \pm 18.3\%$, respectively; $P = 0.848$). However, the mean percentage of time of hypoglycaemia was substantially lower in the Gla-300 group ($1.3 \pm 2.7\%$ *vs* $5.5 \pm 6.4\%$ for IDeg; $P = 0.002$). This observation held true even for duration of severe or nocturnal hypoglycaemia. Another study also reiterated the comparable efficacy of Gla-300 and IDeg in maintaining blood glucose levels and the better safety profile of Gla-300, especially with regard to nocturnal hypoglycaemia ($P = 0.021$).^[56]

In BRIGHT study, patients with impaired renal function (eGFR < 60 mL/min/1.73 m²) demonstrated greater HbA_{1c} reduction with Gla-300 than IDeg (LS mean difference: -0.43; 95%CI: -0.74 to -0.12) and no difference in incidence of hypoglycaemia^[57]. In addition, HbA_{1c} reduction in both treatment arms was similar in patients aged < 70 years but greater with Gla-300 than IDeg-100 in those ≥ 70 years (LS mean difference: -0.34; 95%CI: -0.59 to -0.10) with no difference in incidence of hypoglycaemia^[58].

The CONCLUDE study^[59] (an open-label randomized, active-controlled, 2-arm parallel-group, multicentre, phase 3 study) compared safety and efficacy of IDeg-200 *vs* Gla-300, in European and North American adults ($n = 1609$) with T2DM, who were already taking BI (Glar-100, detemir, or NPH) with or without OAD. In this study, adults (aged > 18 years) with T2DM were included if they met the following criteria: HbA_{1c} ≤ 80 mmol/mol (9.5%), BMI ≤ 45 kg/m² and treated with BI (once or twice daily; NPH insulin, insulin detemir, glargine U100) with/without OADs at stable doses for at least 90 d. Major exclusion criteria were treatment with bolus or premixed insulin or with sulfonylureas/glinides within 90 d before the screening visit, severe renal impairment (eGFR < 30 mL/min/1.73 m), or impaired liver function (alanine aminotransferase or aspartate aminotransferase ≥ 2.5 times the upper limit of normal). The primary end point was to assess the rate of overall symptomatic hypoglycaemia in maintenance period of 36 wk. The results showed that during the maintenance period of 36 wk, the rate of overall symptomatic hypoglycaemia in patients treated with insulin degludec was not statistically significant compared to Gla-300. Since the study did not meet the primary endpoint, the secondary endpoints were considered exploratory and not conclusive^[60-62].

Role of Gla-300 in diabetes management

The ideal insulin therapy would offer the possibility of once-daily injection with flexible timing accompanied with a low risk of hypoglycaemia and ease of titration. As discussed above, Gla-300 provides a stable PK/PD profile requiring less aggressive titration not more frequently than every 3 to 4 d to achieve a steady state over the dosing period^[63,64]. Moreover, Gla-300 was associated with lower incidence of hypoglycaemia in the initial titration (the first 8 wk of treatment) and maintenance phases that would help to mitigate the fear of hypoglycaemia^[32,55]. The EDITION trials revealed that Gla-300 achieved comparable HbA_{1c} reductions as Gla-100 with a lower risk of confirmed or severe hypoglycaemia. These studies also demonstrated a reduced risk of nocturnal hypoglycaemia even during the titration phase^[15,26,27]. During the active titration period (0-12-wk) of the BRIGHT study, Gla-300 was associated with reduced risk of anytime confirmed hypoglycaemia (≤ 70 and < 54 mg/dL) than IDeg-100 and a comparable risk of nocturnal confirmed hypoglycaemia (≤ 70 mg/dL)^[55]. The safety and efficacy of Gla-300 has also been demonstrated in older populations. Gla-300 can be injected in the morning or evening and the injection device is convenient and easy-to-use^[65]. The results from the EDITION development program indicate that a range of patients with both T1DM and T2DM may benefit from Gla-300. Patients at high risk of hypoglycaemia or hypoglycaemia-related events

(such as falls) may derive significant benefit. The prolonged duration of action of Gla-300 may benefit those requiring twice-daily insulin, while the flexibility in time of dosing may improve adherence in those with rigid dosing schedules or complex regimens^[66].

Gla-300 is administered in a pre-filled, disposable injector. Each pen delivers a maximum dose of 80 units/injection. Due to the pH of the diluent, Gla-300 should not be mixed with other insulins^[10]. Patients switching from other once-daily BIs can initiate Gla-300 at the same unit-for-unit dose^[67]. Switching from Gla-100 may require dose adjustments as the two glargine formulations are not comparable. For patients switching from twice-daily BI, the recommended starting dose of Gla-300 is 80% of the previous total daily dose of BI. Gla-300 is recommended for once-daily dosing at the same time each day. Follow-on pre-defined evaluation of EDITION 1 and 2 indicate that occasional dosing flexibility (dosing interval 24 ± 3 h) is possible^[68]. In addition, patients who self-titrated Gla-300 achieved similar rates of target glucose levels without hypoglycaemia as those with clinician-titrated Gla-300^[68]. It has also been reported that the effectiveness of Gla-300 is not dependent on the duration of prior BI therapy or other concomitant anti-hyperglycaemic treatments^[58,69].

While the efficacy and safety of Gla-300 have been demonstrated in the EDITION clinical trials, a comprehensive series of both prospective (observational and interventional) and retrospective real-world evidence studies will provide further evidence on the clinical and economic benefits provided by Gla-300 in a range of diabetes populations.

Insulin glargine as active component: Other considerations

Gla-100 is a widely used BI and has been studied extensively, pre- and post-licensure, and its safety has been well-established. One of the seminal studies to investigate the outcomes of BI use in > 12000 people with type 2 diabetes presenting with cardiovascular risk factors, the ORIGIN trial, concluded that after a median follow-up of 6.2 years, Gla-100 had no discernible association with cardiovascular outcomes or cancers^[70,71]. Gla-300 comprises the same glargine molecule as Gla-100. The advantage offered by Gla-300 over Gla-100 is one of improved PK of insulin glargine release from the injected site thereby offering a smoother plasma insulin plateau for a longer duration. Following dose titration in the EDITION and BRIGHT trials, it was observed that a higher dose of Gla-300 was required to attain target HbA_{1c}^[31,55]. Importantly, despite of slight dose difference, Gla-300 demonstrated lower (*vs* Gla-100 and IDeg during titration period) or similar hypoglycemia (*vs* IDeg during the whole study and maintenance period) with similar changes in weight gain. One explanation for this could be differences in degradation of insulin glargine at the injection site due to longer residence time of the Gla-300 SC depot in comparison to Gla-100 or insulin IDeg^[15-19]. However, despite this increase in dose and comparable glycaemic control, Gla-300 was associated with a lower incidence of hypoglycaemia and lesser weight gain, the most common clinical concerns with insulinisation in people with diabetes.

Real-world study evaluating medical records of patients with T2DM from an US database showed that switching to Gla-300 from another BI lowers HbA_{1c}, hypoglycaemic events and frequency of dosing, with numerically lower daily insulin dose. This suggests that Gla-300 in the real-life settings did not lead to dose increases and was associated with less frequent daily dosing^[40].

A real-world study in Europe (France, Spain, and Germany)^[72] evaluated clinical outcomes in patients with T2DM who switched from another BI to Gla-300 or Gla-100. Switching to Gla-300 *vs* Gla-100 showed similar changes in glycaemic control and weight from baseline, a significantly greater reduction in the number of hypoglycaemia events, and no differences in weight-adjusted insulin dose change.

There is no international standard established for ascertaining the potency of insulin analogues. Moreover, “units” used to describe insulin analogues are not equivalent to ‘International Units’ that are used to describe the potency of insulins for which an international standard exists *e.g.*, human insulin. Hence, the units used to express the potency of Gla-300 are unique to insulin analogues manufactured by Sanofi (Lantus®, Apidra®, Toujeo®).

CONCLUSION

Data from clinical trials and real-world experience have shown that Gla-300 offers a number of benefits in patients with T1DM/T2DM in comparison to other available insulins and insulin analogues. Individual results from the EDITION programme and the meta-analyses of these show that Gla-300 is non-inferior to Gla-100 in attaining HbA_{1c} targets in both insulin-naïve and insulinised patients. Additionally, the

EDITION trials demonstrated a reduced risk of hypoglycaemia during the titration phase that could help to build patient confidence to initiate and properly titrate their BI with less fear of hypoglycaemia. These results were recapitulated in older people in the SENIOR trial. Furthermore, the BRIGHT study found Gla-300 comparable to insulin IDeg, another second-generation BI analogue, in reduction of HbA1c levels. The most notable advantage with Gla-300 observed in these trials was the reduction in incidence of hypoglycaemia (especially in the titration period) as well as the risk of weight gain. Evidence from the DELIVER and LIGHTNING studies indicate that these findings with Gla-300 have been translated in real-world settings. At the time of this review, various other real-world studies are either ongoing or have just been completed. When these data are collectively considered, we can conclude that Gla-300 addresses the critical issues of hypoglycaemia and weight gain and has the potential to improve rates of insulinisation in people with T1DM/T2DM.

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Novel insight into perirenal adipose tissue: A neglected adipose depot linking cardiovascular and chronic kidney disease

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Abstract

Obesity is associated with adverse metabolic diseases including cardiovascular disease (CVD) and chronic kidney disease (CKD). These obesity-related diseases are highly associated with excess fat accumulation in adipose tissue. However, emerging evidence indicates that visceral adiposity associates more with metabolic and cardiovascular risk factors. Perirenal adipose tissue, surrounding the kidney, is originally thought to provides only mechanical support for kidney. However, more studies demonstrated perirenal adipose tissue have a closer association with renal disease than other visceral fat deposits in obesity. Additionally, perirenal adipose tissue is also an independent risk factor for CKD and even associated more with CVD. Thus, perirenal adipose tissue may be a connection of CVD with CKD. Here, we will provide an overview of the perirenal adipose tissue, a neglected visceral adipose tissue, and the roles of perirenal adipose tissue linking with CVD and CKD and highlight the perirenal adipose tissue as a potential strategy for future therapeutics against obesity-related disease.

Key words: Adipose tissue; Cardiovascular diseases; Kidney diseases; Obesity; Abdominal fat; Endothelium

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Core tip: The presence of excess perirenal adipose tissue, a neglected visceral adipose tissue, is regarded as an independent risk factor for both chronic kidney disease and cardiovascular disease. We herein discuss the relationship of perirenal adipose tissue in chronic kidney disease and cardiovascular disease, and the potential mechanism for perirenal adipose tissue participated in obesity-related disease.

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INTRODUCTION

Obesity is regarded as a risk factor for adverse metabolic diseases including hypertension, type 2 diabetes, and dyslipidemia^[1-3]. These metabolic disorders cause vascular complications that primarily manifest as cardiovascular disease (CVD) and chronic kidney disease (CKD)^[4,5]. Moreover, these obesity-related diseases are strongly associated with the prevalence and severity of overweight/obesity. During the development of obesity, impaired glucose homeostasis, hypertension, dyslipidemia, and changes in blood hemodynamics may contribute to the pathogenesis of obesity-related diseases^[6]; these abnormal metabolic changes are mainly caused by excess fat accumulation in adipose tissue^[7,8]. However, there is emerging evidence that, compared with obesity itself, body fat distribution is more closely related to the pathogenesis and development of obesity-related diseases (including CVD and obesity-related CKD)^[9-11].

Adipose tissue mainly comprises a large number of adipocytes. As the main energy storage and endocrine organ, adipose tissue can maintain lipid metabolism homeostasis and energy balance by contributing to several physiological processes through secretion of various adipokines/cytokines^[12,13]. Adipose tissue is traditionally classified into visceral adipose tissue (in the trunk cavity) and subcutaneous adipose tissue (under the skin), based on morphological appearance and location^[14]. Compared with subcutaneous adipose tissue, visceral adipose tissue is reportedly more closely associated with metabolic and cardiovascular risk factors, such as insulin resistance, dyslipidemia, hypertension, and atherosclerosis^[15,16]. Perirenal adipose tissue (PRAT) is a component of visceral adipose tissue that surrounds the kidney, which was originally presumed to only provide mechanical support for the kidney. However, PRAT has been shown to demonstrate a closer relationship to renal disease among individuals with obesity, compared with other visceral fat deposits^[17]. In addition, the presence of excess PRAT has been identified as an independent risk factor for CKD and a factor associated with the development of CVD^[18-22]. Thus, PRAT may serve to connect CVD with CKD among individuals with obesity. This review will provide an overview of PRAT, a neglected visceral adipose tissue, as well as the roles of PRAT in linking CVD and CKD. Moreover, it will highlight the potential for PRAT as a therapeutic target in the treatment of obesity-related diseases.

GENERAL BACKGROUND OF PERIRENAL ADIPOSE TISSUE

PRAT is a fat pad located in the retroperitoneal space surrounding the kidney, which fills the space between the kidney and neighboring retroperitoneal tissues, renal parenchyma, and adrenal gland; the PRAT is supported by renal fascia^[23]. Fat around the renal sinus is also regarded as a component of PRAT. Notably, PRAT is the only adipose tissue that is surrounded by a multilayered fibrous membrane. Because PRAT is surrounded by fascia tissue, excess PRAT can tightly encapsulate the kidney and cause excessive renal compression. Despite its origination from preadipocytes, PRAT undergoes an unusual progressive transition from brown adipose tissue into white adipose tissue after birth^[24]. Brown adipocytes form the majority of PRAT in fetuses and newborn infants (1-11 mo), while white adipocytes comprise the outermost thin layer^[25]. With increasing age, only a small proportion of brown adipocyte areas remains within perirenal white adipose tissue in adults; thus, PRAT constitutes a combination of white adipose tissue and brown adipose tissue^[26]. One study in Siberia showed that approximately 40% of PRAT exhibited morphology typical of brown adipocytes, while approximately 30% expressed uncoupling protein 1; these findings suggest that PRAT can be converted to brown adipose tissue in cold conditions^[27].

Despite its atypical nature, PRAT has been reported to synthesize and secrete adipokines and pro-inflammatory cytokines, including adiponectin, leptin, visfatin, resistin, tumor necrosis factor- α , interleukin-6 (IL-6), and IL-1 β ^[22]. These cytokines enter nearby kidneys and serve to regulate renal function through endocrine or

paracrine pathways^[28].

Anatomically, PRAT exhibits extensive vascularization, innervation, and lymph fluid drainage. The PRAT artery originates from branches of the abdominal aorta, include branches of the inferior adrenal, dorsal, and gonadal arteries^[29]. Perirenal nerve fibers originate from the celiac superior mesenteric, ipsilateral inferior mesenteric, adrenal, aorticorenal, ovarian, testicular, and ipsilateral sympathetic chain ganglia^[30]. Perirenal lymphatic vessels communicate with renal subcapsular lymphatic vessels and then drain into para-aortic lymph nodes^[31]. These features allow interrelationships between kidney and PRAT, as well as between body function and PRAT, *via* secretion of adipokines and cytokines.

ASSESSMENT OF OBESITY AND PERIRENAL ADIPOSE TISSUE

Because of the increasing prevalence of obesity, accurate quantification of obesity has become necessary. Traditional simple indicators (*e.g.*, body mass index, waist circumference, and waist/hip ratio) have been widely used in clinical practice. Although these indicators are convenient and noninvasive for patients, they have obvious limitations. In particular, they are ethnicity specific and cannot accurately differentiate between visceral and subcutaneous fat, between muscle and adipose tissue; moreover, they cannot determine the regional distribution of adiposity throughout the body^[8,32]. Advanced imaging techniques (*e.g.*, computed tomography, magnetic resonance imaging, and positron emission tomography) have been utilized to evaluate body fat distribution, including PRAT thickness^[33-35]. Although these techniques are more accurate, widespread clinical use of computed tomography and magnetic resonance imaging for evaluation of obesity is unsuitable because of the cost and time involved, as well as the exposure to ionizing radiation. Some noninvasive ultra-sonographic methods have been reported for assessment of fat distribution^[20,36]. Armellini *et al*^[37] first described the use of ultrasonography for direct evaluation of intra-abdominal fat deposits. Subsequently, Kawasaki *et al*^[38] reported a more convenient method for quantification of visceral fat by measuring PRAT thickness on abdominal sonography. Measurement of PRAT can be performed as following: keep the patient in the supine position; place the ultrasound probe vertically to the abdominal lateral surface skin above the kidney; obtain the ultrasound longitudinal scan of the kidney which is almost parallel to the skin. Gender pressure of the probe should be noticed during image obtaining in order not to cause extra adipose tissue pressing. PRAT thickness was then measured from the kidney surface to inner side of abdominal musculature. Average measurement of the maximum thickness values of both sides by three times was regarded as ultrasound measure. Kawasaki *et al*^[38] showed that PRAT thickness was positively correlated with visceral adipose tissue area and that PRAT thickness > 10 mm could be regarded as visceral fat accumulation (area > 100 cm²). In a separate analysis, our research group found that the average of PRAT thickness in healthy people was 7.95 mm and that in obese patients was 26.54 mm^[17]. And PRAT was positively associated with body mass index and waist circumference; thus, sonographic evaluation of PRAT thickness could be used to assess visceral fat and predict early renal injury in patients with obesity^[17]. Lamacchia *et al*^[21] measured PRAT thickness in normal subjects (8 mm ± 2 mm for men and 5 mm ± 2 mm for women), which were validated by computed tomography measurements. With the same method, De pergola *et al*^[39] found an average value of PRAT in obese patients with body mass index above 30 kg/m² was 25.0 mm. Ricci *et al*^[40] verified that PRAT was statistically different between hypertensive and nonhypertensive patients, with average value of 13.6 and 11.6 mm, respectively.

PERIRENAL ADIPOSE TISSUE AND CHRONIC KIDNEY DISEASE

Relationship between PRAT and CKD

The association of obesity with CKD was first reported in 1974; obesity was linked with massive proteinuria^[41]. Thereafter, many epidemiologic studies have demonstrated that kidney disease is an independent complication of obesity; these manifestations are regarded as obesity-related glomerulopathy or obesity-related kidney disease^[42,43]. Klausen *et al*^[44] screened 2696 volunteers over a period of 10 years and found that obesity was strongly associated with proteinuria. Ejerblad *et al*^[45] observed that patients with a body mass index > 25 kg/m² had a nearly three-fold

increased risk of CKD, compared with lean patients; this risk was not affected by age. Furthermore, urinary albumin excretion was significantly greater among patients with obesity who did not have hypertension or diabetes, compared with healthy controls^[17]. Compared with overall obesity, abdominal obesity was found to be more strongly associated with kidney injury^[46,47]; notably, a retrospective study of 7676 patients without diabetes showed that lean patients who had abdominal obesity were at higher risk of CKD^[47]. As mentioned above, PRAT has been used in measurement of visceral fat deposition. PRAT thickness was found to be markedly higher among patients with obesity who exhibited microalbuminuria, compared with healthy controls and patients with obesity who did not exhibit albuminuria^[17]. The presence of excess PRAT was related to a 2.3-fold increased risk of CKD, following adjustments for body mass index and the presence of excess visceral adipose tissue^[48]. Lamacchia *et al.*^[21] found that PRAT thickness could predict reduced glomerular filtration rate in patients with type 2 diabetes. Furthermore, PRAT thickness was positively associated with microalbuminuria in patients with obesity^[17,49]. Based on these findings, the presence of excess PRAT is considered an independent predictor of renal injury in patients with obesity or diabetes.

Potential mechanism for participation of PRAT in CKD

The detailed mechanisms by which PRAT initiates and exacerbates chronic renal injury remain elusive (Table 1). First, excess PRAT surrounded by renal fascia penetrates the renal sinus, obstructing renal parenchyma and vessels, promoting sodium reabsorption, and raising blood pressure. Excess PRAT encapsulates the kidney, further increasing interstitial hydrostatic pressure and reducing renal blood flow; these effects lead to hemodynamic changes, including altered renin secretion and glomerular filtration^[50-53].

Second, hallmarks of obesity include overproduction of free fatty acids (FFAs) and chronic inflammation. Our previous study verified that PRAT thickness is positively associated with urine albumin excretion and circulating FFAs^[17]. Notably, circulating FFAs levels were found to be significantly higher in renal venous blood than in jugular venous blood, indicating that FFAs released by PRAT participate in kidney damage as direct or indirect mediators through intercellular signaling pathways^[54]. Excess FFAs produced by PRAT could escape into the kidney directly or *via* renal vascular system based on its extensive vascularization. Adeosun *et al.*^[55] verified that FFAs from PRAT cause renal lipotoxicity through uptake of FFA metabolites, such as ceramides. Then FFAs-induced renal lipotoxicity could exacerbate chronic inflammation by increasing the metabolism of intracellular fatty acids. Furthermore, our physiology-based analysis showed that FFAs could directly impair endothelial function by enhancing oxidation of tetrahydrobiopterin; this leads to L-arginine-induced production of superoxide, rather than nitric oxide (NO), by uncoupling of endothelial NO synthase^[56]. The reduction in NO level could lead to a compensatory enhancement of vascular endothelial growth factor secretion by podocytes, thereby initiating endothelial cell proliferation and permeability, which causes greater albumin leakage from glomeruli^[56]. This uncoupling of glomerular vascular endothelial growth factor-NO axis could be improved by reduction of FFAs and inflammation^[56,57]. Besides, FFAs-induced lipotoxicity can also increase renal arterial resistance, indicated by the high interlobar artery resistance in patients with microalbuminuria^[49].

Third, excess PRAT can affect kidneys by systemic or local secretion of inflammatory cytokines^[53,58]. Endothelial/vasomotor dysfunction is an early sign of vascular damage. PRAT secretes tumor necrosis factor- α , which has been shown to directly impair renal arterial endothelial dysfunction in obese swine^[59]. Potential mechanism may be due to its impairment on endothelial NO balance as mentioned before. Moreover, PRAT-related inflammation and extracellular-matrix protein can be reduced by blocking plasminogen activator inhibitor-1; this mechanism was able to attenuate renal injury in obese mice^[60]. Li *et al.*^[61] found that leptin secreted by excess PRAT could exacerbate renal vascular remodeling and glomerular endothelial cell proliferation by activation of the p38 MAPK pathway. These inflammatory adipocytes/cytokines could modulate cellular function *via* certain signaling pathways while attracting infiltrating macrophages into deposited fat, further exacerbating oxidative stress and adipocyte dysfunction^[62]. Interestingly, inhibiting the levels of inflammatory cytokines including IL-6, IL-1b and tumor necrosis factor- α in PRAT through upregulation of heme oxygenase system reduced renal inflammation and ameliorated diabetic nephropathy^[63]. However, precise mechanism still warrants further studies.

Finally, excess PRAT can act in a synergistic manner with metabolic risk factors to exacerbate renal damage. Patients with metabolic syndrome have greater PRAT thickness, as well as increased oxidative stress and renal microvascular

Table 1 Potential mechanism for participation of perirenal adipose tissue in chronic kidney disease

	Mechanistic links	Ref.
Physical compression	Suppress renal parenchyma and vessel, increase renal sodium reabsorption, renin secretion and glomerular hyperfiltration	[51-54]
FFA	Induce renal lipotoxicity and inflammation, nitric oxide decrease, endothelial dysfunction, and renal arterial resistance	[50,55-58]
Cytokines	Cause endothelial dysfunction, extracellular matrix, macrophages infiltration, oxidative stress, nitric oxide decrease, renal vascular remodeling, glomerular endothelial cells proliferation and renal sympathetic nervous activity	[60-64]
Metabolic problems	Related with insulin resistance/hyperinsulinemia, impaired fasting glucose, triglyceride and uric acid	[20,65-67,81]

FFA: Free fatty acids.

proliferation^[64]. Greater PRAT thickness has been associated with abnormal insulin levels, impaired fasting glucose, insulin resistance, increased triglyceride levels, and abnormal uric acid levels in patients with CKD^[19,65]. One possibility is that increased FFAs and inflammatory cytokines secreted from PRAT or visceral fat impair insulin-related signaling way including PI3K/Akt inhibition and activation of protein kinase C. Insulin increases sodium retention in renal tubules, stimulates the sympathetic nervous system, and acts directly on vascular structures that contribute to kidney damage^[66].

PERIRENAL ADIPOSE TISSUE AND CARDIOVASCULAR DISEASE

Relationship between CKD and CVD

Obesity is considered a strong risk factor for CVD. Since Yudkin *et al*^[67] first reported relationships between CKD and atherosclerosis, as well as between CKD and coronary heart disease, many epidemiological studies have confirmed a close association between CKD and CVD. The LIFE^[68] and PREVENT^[69] studies demonstrated that the incidence and mortality of ischemic CVD increased significantly with increasing urine albumin excretion. Furthermore, an analysis of 21050 patients in 26 countries showed that microalbuminuria was closely related to cardiovascular hazards and CVD^[70]. Among individuals without diabetes and hypertension, CVD-related mortality is significantly higher in those with microalbuminuria than in those without microalbuminuria^[71]. In the general population, the presence of microalbuminuria is predictive of the risks of diabetes, hypertension, and CVD^[72,73]. Therefore, patients with CKD have higher risks of cardiovascular events, such as hypertension, atherosclerosis, and coronary heart disease^[74]. Consistent with these observations, patients with early CKD tend to die from CVD, rather than terminal end-stage renal disease.

Relationship between PRAT and CVD

Visceral fat reportedly has a close association with CVD. As a component of visceral fat, the presence of excess PRAT has been recently identified as an emerging risk factor for CVD, independent of common metabolic parameters^[40]. De Pergola *et al*^[39] found that PRAT thickness was positively correlated with blood pressure in patients with overweight and obesity. Ricci *et al*^[40] demonstrated a close association between PRAT and high blood pressure, such that PRAT can be regarded as a predictor of hypertension. Higher levels of PRAT thickness and carotid intima-media thickness were both found in HIV-infected patients with visceral adiposity, indicating that the presence of excess PRAT is associated with atherosclerosis^[75]. Notably, the associations of PRAT with carotid intima-media thickness and diverse metabolic risk factors were present even in children^[76].

As a metabolic risk factor related to CVD, PRAT associated with diabetes or dyslipidemia may also indirectly affect CVD. A cross-sectional study showed that the presence of excess PRAT was independently associated with hyperinsulinemia and insulin resistance in patients with obesity, independent of other anthropometric and

metabolic parameters; this finding indicated that the presence of excess PRAT is a strong marker of insulin resistance^[65,77]. PRAT development and remodeling have also been associated with metabolic syndrome^[21,78]. In patients with CKD, the PRAT thickness was significantly correlated with metabolic risks, such as abnormal triglycerides and uric acid levels; patients with stages 4 and 5 CKD had the greatest PRAT thickness^[19]. Excess PRAT is related to reductions in glomerular filtration rate, regardless of other indices of adiposity, in patients with hypertension^[20]. Considering the above pathological processes, the presence of excess PRAT is believed to contribute to dysmetabolism-associated CVD. PRAT is potentially related to epicardial fat because both exhibit mesothelial layers similar to those of visceral organs, which are enriched in white adipose tissue progenitors that produce adipocytes^[65]. This finding strengthens the hypothesis that excess PRAT is a predictor of the risk of CVD, because epicardial fat has been regarded as a risk factor for CVD that predicts the tendency of cardiac dysfunction.

Potential mechanism for participation of PRAT in CVD

Although there is a potential link between CKD and CVD, the mechanisms remain unclear (Figure 1). Potential mechanisms for perirenal fat-mediated CVD regulation are closely associated with anatomical, physiological, and localization features.

Importantly, efferent nerves have been found to innervate PRAT, while afferent nerves have been found in adipose tissue; the physiological functions of PRAT neural activity are presumably related to these anatomical characteristics. Afferent nerves of adipose tissue might control the sympathetic nervous system by forming a negative feedback loop (*i.e.*, a reflex)^[24]. Renal sympathetic outflow is increased by enhancement of afferent signals from fat deposits, followed by the elevation of arterial blood pressure; this reflex phenomenon is known as the “adipose afferent reflex”^[24]. Tanida *et al*^[79] injected leptin into PRAT; this resulted in activation of adipose afferent reflex without affecting the serum levels of sympathetic-activating substances, suggesting that PRAT may directly regulate the cardiovascular system. Excess PRAT is presumed to contribute to increased hydrostatic pressure and activation of renin-angiotensin-aldosterone system through the compression of blood vessels, lymphatic vessels, and ureters; this may lead to the development of hypertension and atherosclerosis^[80]. Additionally, adipokines/cytokines synthesized by excess PRAT could regulate functions of the cardiovascular system *via* autocrine, paracrine, and endocrine pathways using similar mechanisms to those described for CKD.

Moreover, endothelial injury is typically regarded as the main initiating event for atherosclerosis and CVD. The Danish Diabetes Center proposed the Steno hypothesis, in which the emergence of microalbuminuria indicates the existence of extensive systemic endothelial dysfunction and increased systemic vascular permeability^[81]. This may explain why CKD is an indicator of increased risk of CVD and can strongly predict the occurrence of CVD. Importantly, microalbuminuria reflects the presence of microvascular disease and early damage to renal function; it is also an independent risk factor for macrovascular disease and CVD, including the occurrence and development of cardiovascular events.

PROSPECTIVE THERAPEUTIC STRATEGIES

According to current literature, lifestyle intervention including diet control and suitable exercise should be the first line of treatment of obesity. Energy-restricted diets effectively reduce fat mass and impact cardiometabolic profiles^[82]. Parrish *et al*^[83] reported that a fish oil diet reduced PRAT and limited fat hypertrophy. Interestingly, altering meal frequency to twice a day has been demonstrated to combat obesity by reducing PRAT^[84]. Additionally, intensity interval exercise (especially short time-high frequency) is proved to have beneficial effects on the body composition including reducing PRAT^[85]. Bariatric surgery is another effective intervention for morbidly obese patients. Ricci *et al*^[40] reported that PRAT in morbidly obese patients (body mass index > 35 kg/m²) significantly was reduced accompanied by a significant reduction in blood pressure after sleeve gastrectomy.

As mentioned above, PRAT is different from traditional visceral fat because it has characteristic of both brown- and white-fat. Jespersen *et al*^[86] characterized the perirenal region of humans and identified the presence of dormant brown adipose tissue in the PRAT. This property suggests that reactivating dormant BAT into active BAT by cold exposure or β 3-adrenoceptors stimulation may be a promising strategy for combatting obesity-related metabolic disease^[27,87]. However, identifying specific drugs to induce white-to-brown adipocyte and delivering them locally or selectively to PRAT require technological advances and further research.

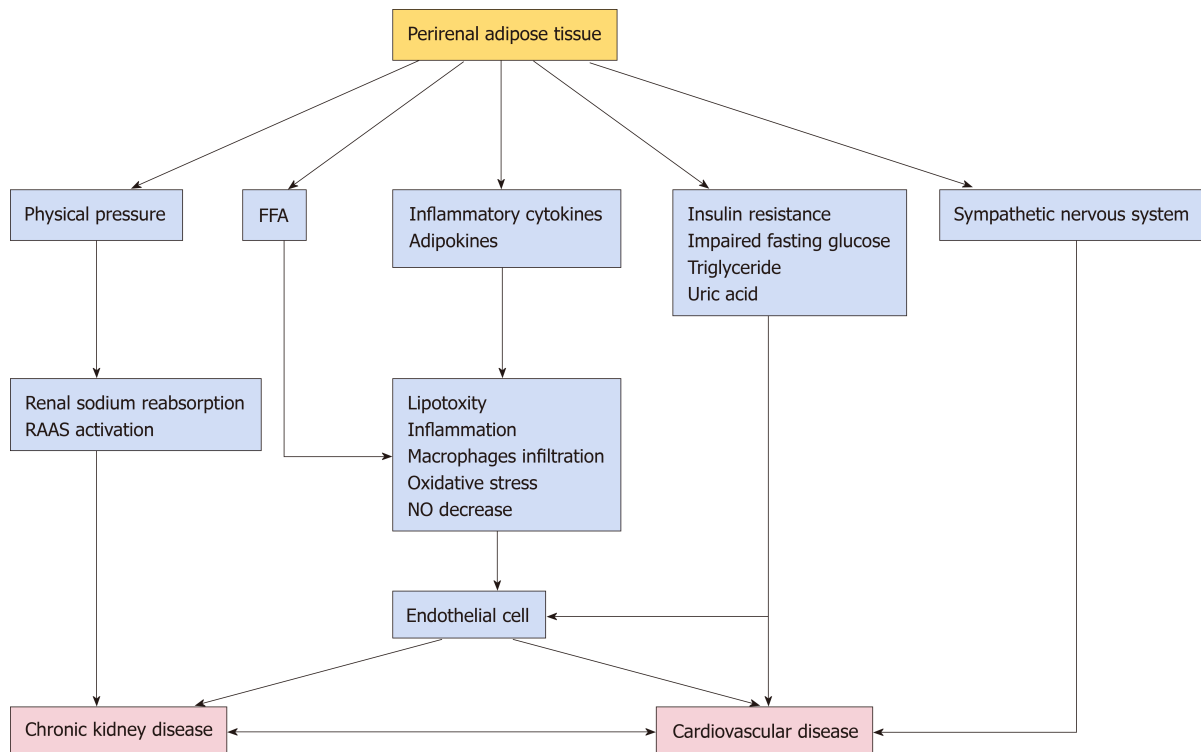


Figure 1 Potential mechanism for participation of perirenal adipose tissue in chronic kidney disease and cardiovascular disease. FFA: Free fatty acids; NO: Nitric oxide; RAAS: Renin-Angiotensin-Aldosterone System.

CONCLUSION

PRAT is a component of visceral fat that is strongly associated with adverse cardiometabolic risk factors for both CKD and CVD. Potential related mechanisms have been summarized in this review, with the aim of providing new insights and potential therapeutic targets for anti-obesity-related cardiovascular and kidney disease therapies. Further studies are needed to characterize the important roles of PRAT in the interactions between CKD and CVD. Drugs used to target CVD risk factors may influence PRAT in a manner that aids in disease prevention. The clinical implications of reducing PRAT accumulation by balanced diet, intermittent exercise, or other therapeutic interventions (*e.g.*, burning excess energy through conversion of white adipose tissue to brown adipose tissue) with regard to CVD prevention remain unclear and should be established in future studies.

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Basic Study

Lack of Syndecan-1 produces significant alterations in whole-body composition, metabolism and glucose homeostasis in mice

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Abstract

BACKGROUND

Obesity is a disease state with serious adverse metabolic complications, including glucose intolerance and type 2 diabetes that currently has no cure. Identifying and understanding roles of various modulators of body composition and glucose homeostasis is required for developing effective cures. Syndecan-1 (Sdc1) is a member of the heparan sulfate proteoglycan family that has mainly been investigated for its role in regulating proliferation and survival of epithelia and tumor cells, but little is known about its roles in regulating obesity and glucose homeostasis.

AIM

To examine the role of Sdc1 in regulating body fat and glucose metabolism.

METHODS

We used female wild type and Sdc1 knockout (Sdc1 KO) mice on BALB/c background and multiple methods. Metabolic measurements (rates of oxygen consumption, carbon dioxide production, respiratory exchange ratio and energy expenditure) were performed using an open-flow indirect calorimeter with additional features to measure food intake and physical activity. Glucose intolerance and insulin resistance were measured by established tolerance test methods.

RESULTS

Although our primary goal was to investigate the effects of Sdc1 deficiency on body fat and glucose homeostasis, we uncovered that Sdc1 regulates multiple metabolic parameters. Sdc1KO mice have reduced body weight due to significant decreases in fat and lean masses under both chow and high fat diet conditions.

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The reduced body weight was not due to changes in food intakes, but Sdc1 KO mice exhibited altered feeding behavior as they ate more during the dark phase and less during the light phase than wild type mice. In addition, Sdc1 KO mice suffered from high rate of energy expenditure, glucose intolerance and insulin resistance.

CONCLUSION

These results reveal critical multisystem and opposing roles for Sdc1 in regulating normal energy balance and glucose homeostasis. The results will have important implications for targeting Sdc1 to modulate metabolic parameters. Finally, we offer a novel hypothesis that could reconcile the opposing roles associated with Sdc1 deficiency.

Key words: Syndecan-1; High fat diet; Indirect calorimetry; Energy expenditure; IL-17; Feeding pattern

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Core tip: Obesity is a disease state with serious adverse metabolic complications that currently has no cure. Identifying key modulators is required for developing effective cures. Here we investigated Syndecan-1 (Sdc1), a member of the heparan sulfate proteoglycan family for its roles in regulating obesity and glucose homeostasis. Sdc1 knockout (Sdc1 KO) mice have reduced body weight with decreases in fat and lean masses under both chow and high fat diet conditions. The reduced body weight was not due to changes in food intakes, but Sdc1 KO mice exhibited altered feeding behavior as they ate more during the dark phase and less during the light phase than wild type mice. In addition, Sdc1 KO mice suffered from high rate of energy expenditure, glucose intolerance and insulin resistance. Our results reveal critical multisystem and opposing roles for Sdc1 in regulating normal energy balance and glucose homeostasis.

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INTRODUCTION

Obesity is a major risk factor for metabolic diseases, including insulin resistance, type 2 diabetes and cardiovascular diseases^[1,2]. Attempts to control obesity by adjusting lifestyle produce incremental or no improvement in most individuals^[3]. Significant efforts have therefore been directed towards alternative strategies to combat obesity and its complications. A major focus has been on the roles of specific cell types and molecules. Among these are different immune cells, which are being investigated for their roles in regulating adipogenesis, and chronic low-grade inflammation that fuels metabolic syndromes^[4,5]. Other cell types and molecules have received less attention, including members of the heparan sulfate proteoglycans (HSPG) family. These are glycoproteins with one or more covalently attached glycosaminoglycan^[6]. HSPG are located in the extracellular matrix, in secretory vesicles^[6], and at cell membranes where they can regulate ligand-receptor interactions. Syndecans are major components of HSPG family. The syndecan family is comprised of four transmembrane HSPGs^[7]. These four HSPGs are syndecan-1, -2, -3, and -4. There is some evidence of involvement of sdc3 and sdc4 in regulating body fat and metabolism. Syndecan-4 is implicated in angiogenesis and wound healing^[8-10]. Syndecan-3 has been implicated in regulating feeding behavior by modulating hypothalamic melanocortin activity^[11], and syndecan-3 knock out mice are resistant to diet-induced obesity^[12].

However, there is limited information about role of Sdc1 in body metabolism. Sdc1 is expressed in epithelial and other non-hematopoietic cell types^[13,14], and serves as a marker for plasma cells and developing B lymphocytes^[15]. We have recently shown that Sdc1 regulates homeostasis of natural-killer T-cells (NKT) and gamma-delta T-

cells that produce interleukin-17^[13,16]. Sdc1 has also been investigated for its role in mediating hepatic clearance of triglyceride-rich lipoproteins^[17] and regulating lipoprotein metabolism^[18,19]. Little is yet known about the role of Sdc1 to regulate body fat and glucose homeostasis. However, in one study Kasza *et al.*^[20] reported that loss of Sdc1 in mice caused depletion of intradermal fat, making those more susceptible to cold stress^[20].

In this study, we show that deficiency of Sdc1 significantly reduces body weight and adiposity, and lean mass. These effects of sdc-1 deficiency exist under normal diet and obesogenic high-fat diet conditions. Using indirect calorimetry, we show that Sdc1 knockout (Sdc1 KO) mice have increased rates of energy expenditure. Despite the decreased adiposity of Sdc1 mice, they have impaired glucose tolerance and insulin sensitivity as compared to wild type (WT) control, whether fed normal chow or high-fat diet (HFD). These results reveal critical multi-system roles for Sdc1 in regulating normal energy balance and glucose homeostasis, and inform as to potential uses, and risks, of Sdc1 immunotherapy.

MATERIALS AND METHODS

Animals

Sdc1 KO BALB/c mice were a gift from Stepp *et al.*^[21] (George Washington University). Wild-type female BALB/c mice were purchased from the Jackson Laboratory. Mice were bred and housed in approved vivaria on the Johns Hopkins University School of Medicine campus. Unless noted otherwise, mice between the ages of 8 and 10 wk were used. Except for pre-test food deprivations associated with glucose and insulin tolerance tests, mice were maintained on ad libitum normal diet (Teklad 2018, Envigo) or switched to HFD with 60% kcals from fat (D12492, Research Diets) at age of 4 wk. Experiments were conducted under protocols approved by the Johns Hopkins University Animal Care and Use Committee, in compliance with the Animal Welfare Act and principles set forth in the Guide for the Care and Use of Laboratory Animals.

Body composition measurement

Masses of fat and lean tissue, along with total body water, were measured in awake mice with an Echo-MRI-100TM QMR analyzer (Echo Medical Systems, Houston, TX, United States). Group sizes were at least $n = 12$ /group, Sdc1 KO *vs* WT, for both the studies with normal diet and with HFD. QMR measurements were performed at the Johns Hopkins University Phenotyping Core facility.

Indirect calorimetry, food intake, and physical activity measurements

After QMR for body composition, mice were moved to the Center for Metabolism and Obesity Research core facility for indirect calorimetry studies. Studies utilized an open-flow instrument [Comprehensive Lab Animal Monitoring System (CLAMS), Columbus Instruments]. Mice acclimated to the facility for one week before study. Mice were then monitored in the CLAMS for three days to confirm acclimation as indicated by stable daily body weights, food and fluid intakes, and photoperiod fluctuations of metabolic data. Data from the fourth day are presented. These calorimetry data were collected in two experiments ($n = 12$ Sdc1 KO, $n = 12$ WT per experiment), one utilizing normal diet, and the other utilizing mice made obese on HFD. The CLAMS reported rates of oxygen consumption (VO_2 , mL/kg/h) and carbon dioxide production (VCO_2), respiratory exchange ratio (RER), and rate of energy expenditure (EE, kcal/kg/h) for each mouse chamber every 25 min throughout the studies. Respiratory exchange ratio ($\text{RER} = \text{VCO}_2/\text{VO}_2$) was calculated by Oxymanx software (v.4.02) to estimate relative oxidation of carbohydrate ($\text{RER} = 1.0$) *vs* fat (RER approaching 0.7), not accounting for protein oxidation. Energy expenditure was calculated as $\text{EE} = \text{VO}_2 \times [3.815 + (1.232 \times \text{RER})]$ ^[22]. Average values for VO_2 , VCO_2 , RER and EE were calculated per subject for 24 h, and for the corresponding 12 h dark and 12 h light periods. Additional CLAMS feature permitted measurements of diet intake (powdered food in cups, on scales) and physical activity (x-axis infrared beam array). These data were also reported every 25 min throughout the studies, and are presented as 24-h sums, and sums for the 12-h light and dark phases.

Glucose tolerance tests

Glucose tolerance tests (GTT) were performed with 8-wk-old Sdc1 KO and WT mice maintained on normal diet or switched to HFD at 4 wk of age. Mice were fasted for 4 h and then D-glucose (1 g/kg and 5 g/kg body weight in 200 μL saline) was injected intraperitoneally. Glucose levels were measured by tail-snip technique with a hand-held glucometer (Bayer Contour TS, Abbott Laboratories, Abbott Park, IL) at baseline and at 15, 30, 60 and 120 min after glucose injection.

Insulin tolerance test

To determine the insulin sensitivity, insulin tolerance tests were performed with in Sdc1 KO and WT mice of similar ages. After 2 h of fasting, mice were injected with 0.5 IU/kg insulin (Humulin R, Eli Lilly, Indianapolis, IN, United States) intraperitoneally. Glucose levels were measured as in GTT at baseline, and at 15, 30, 60- and 120 min post-injection.

Statistical analyses

Statistical tests were performed using Prism 6 (Graph Pad). Data were expressed as mean \pm SE. Statistical significance was evaluated using two-tailed unpaired Student's *t*-test. One-way ANOVA was utilized to analyze body weights and body composition during the long-term diet studies (at 8 and 10 wk, respectively, on the HF diet). Statistical significance was determined as $P < 0.05$.

RESULTS**Syndecan-1 deficiency decreases both fat mass and lean mass**

We first evaluated the effect of Sdc1 deficiency on body weight and composition using Sdc1 KO and WT BALB/c female mice that were fed normal diet between the ages of 4-12 wk. Sdc1 KO female mice had significantly reduced body weight compared with WT mice at all ages examined (Figure 1A). QMR analysis of body composition at 10 wk of age showed that Sdc1 KO mice had decreases in both fat and lean masses (Figure 1B). Next, we monitored effects of Sdc1 deficiency under HFD condition. The Sdc1 KO female BALB/c mice gained less weight than WT controls during the HFD feeding period (Figure 1C). QMR analysis at 12 wk of age (8 wk on HFD) showed that HFD-fed Sdc1 KO mice had reduced body fat and lean mass as compared to HFD-fed WT mice (Figure 1D). Thus, the systemic effects of *sdc*-1 deficiency on body composition and weight are maintained under both normal diet and HFD conditions. We also measured body weights of male BALB/c mice; Sdc1 KO males on a normal diet had lower body weights than the WT controls, similar to results in female mice (Supplementary Figure S1).

Deficiency of Sdc1 affects food intake of mice

To determine whether reduced weight is due to less food intake, we measured food consumption of Sdc1 KO and WT mice fed chow diet or HFD during the indirect calorimetry trials (Figure 2). Under both conditions, the daily caloric intakes by Sdc1 KO mice were similar to those of WT mice (Figure 2A and B). However, although total food intake over a 24 h period was not different between the two genotypes, the pattern of food intake was altered. As compared to WT mice, Sdc1 KO mice ate more food during the dark phase when fed chow or HFD (Figure 2C and D). Conversely, WT mice ate more during the light phase, although the difference was statistically significant only under chow diet (Figure 2E and F). Taken together, these results exclude daily food intake reduction as a cause of the decreased body weight of Sdc1 KO mice. However, they uncovered a significant role for Sdc1 in regulating feeding patterns of mice.

Deficiency of Sdc1 increases energy expenditure

Having excluded differences in daily food consumption as a cause of decreased body weight in Sdc1 KO mice, we analyzed the effect of Sdc1 deficiency on VO_2 , RER (VCO_2/VO_2 ratio) and EE. Sdc1 KO mice had significantly elevated VO_2 compared with WT mice (Figure 3A). The RER was not significantly different between the genotypes for 24-h or for the 12-h dark phase and was in ranges expected for intake of the low-fat/high-carbohydrate diet (Figure 3B). During the light phase, the RER of Sdc1 KO mice was significantly lower than for WT mice, consistent with the decreased food intake by Sdc1 KO mice compared with WT during this period, and an increased expected reliance on body fat stores to fuel metabolism (Figure 3C). Overall, the increased VO_2 in Sdc1 KO mice drove a significant increase in the energy expenditure compared with WT (Figure 3C), and this is likely what drives the loss of body weight. Infrared beam crossing was monitored during the indirect calorimetry study to assess physical activity of WT and Sdc1 KO mice. We did similar analysis under HFD using indirect calorimetry and CLAMS. Sdc1 KO and WT mice were placed on HFD for 8 wk and then subjected to indirect calorimetry. Like Sdc1 KO mice fed chow diet, Sdc1 KO mice fed HFD showed reduced fat mass and increased energy expenditure (Figure 4A-C). Despite the overall increase in whole-body energy expenditure in Sdc1 KO mice fed either chow or HFD, their physical activity was significantly lower throughout the photoperiod compared with WT mice (Figure 5A

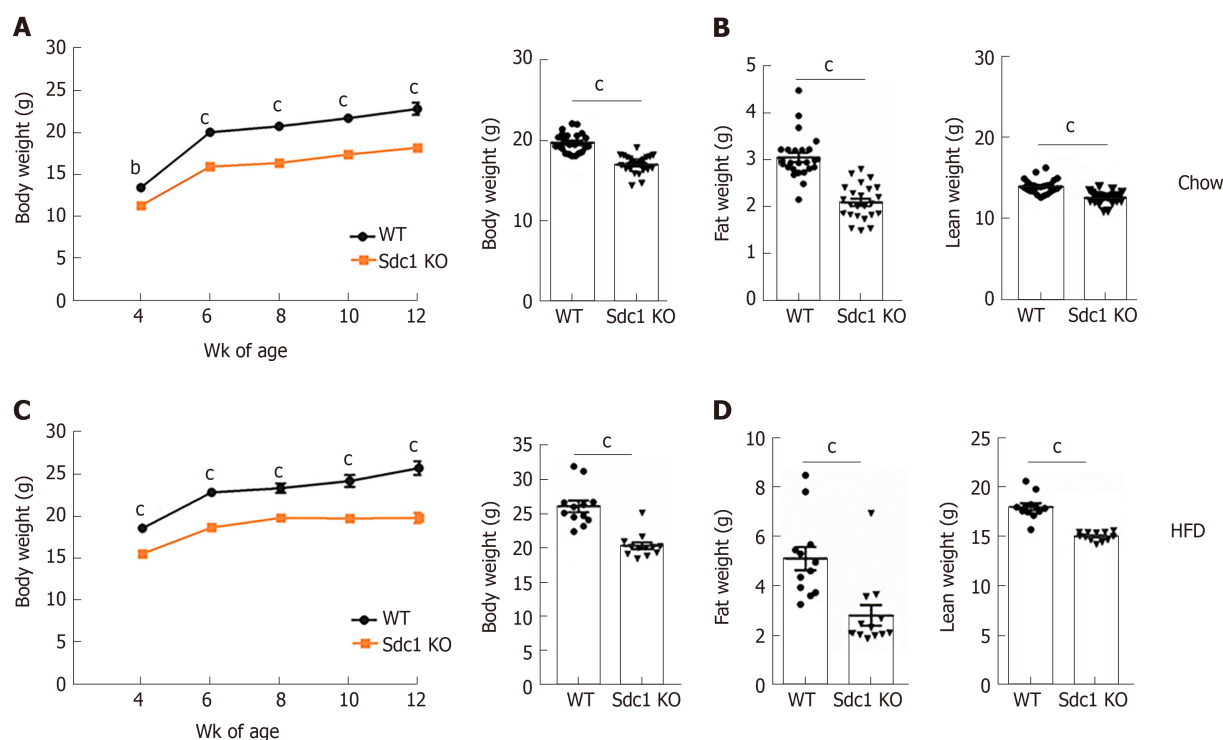


Figure 1 Effect of syndecan-1 deletion on body weight and fat content. A, B: Age-matched Sdc1 knockout (Sdc1 KO) and wild type (WT) mice were fed either chow diet or high-fat diet for indicated periods. Sdc1 KO mice maintained their body weight overtime than WT mice. There was significant difference in total body weight, fat mass and lean mass of chow-fed Sdc1 KO mice compared with WT mice; C, D: Like, chow diet fed mice, high-fat diet fed mice also maintained the difference in body weight, fat mass and lean mass. Bar graphs show cumulative data from two independent experiment of total 24 female BALB/c mice and represents mean \pm SE of 12-24 animals of each genotype. ^b $P < 0.01$, ^c $P < 0.001$. Sdc1 KO: Sdc1 knockout; HFD: High-fat diet; WT: Wild type.

and B).

Syndecan-1 deficiency impairs glucose tolerance and insulin sensitivity under both normal chow and HFD conditions

Our body composition studies showed that Sdc1 KO mice had lower fat masses than WT mice, whether maintained on chow or HFD (Figure 1A and C). No previous work, to our knowledge, has examined the role of Sdc1 in regulating glucose homeostasis. We performed GTT on 12-wk-old female BALB/c mice that were fed chow or HFD. After 4 h of fasting, we subjected WT and Sdc1 KO mice to GTT using increasing doses of glucose. Both WT and Sdc1 KO cleared 1g/kg doses of glucose at comparable rates regardless of the diet type (Figure 6A and D). They also cleared 2 g/kg to similar extent (data not shown). However, when we increased the glucose challenge to a 5 g/kg, Sdc1 KO mice, contrary to our expectation, showed diminished capacity to clear this large glucose dose as compared to WT mice, under both normal diet and HFD conditions (Figure 6B and E). We also determined whether Sdc1 deficiency alters insulin tolerance, we performed insulin tolerance tests in 12-wk-old Sdc1 KO and WT mice under normal diet and HFD conditions. Sdc1 KO mice showed significant insulin resistance under both normal diet and HFD conditions (Figure 6C and F). Thus, reduced body fat in Sdc1 KO is not associated with enhanced glucose or insulin tolerance, but rather a limited capacity to clear large dose of glucose and impaired insulin tolerance.

DISCUSSION

Our results reveal critical multisystem and opposing roles for Sdc1 in regulating normal energy balance and glucose homeostasis. The apparently contradictory effects are unusual but fit well with the properties of Sdc1. In general, cell surface receptors on each have one corresponding ligand and their expression is limited to cell types that perform similar functions. Contrary to this general rule, Sdc1 is expressed by cell types that carry non-overlapping biological functions such as epithelial cells and B cells. Furthermore, Sdc1, like other members of the HSPG family, has multiple ligands that include cytokines, chemokines, and growth factors, hence impacts different

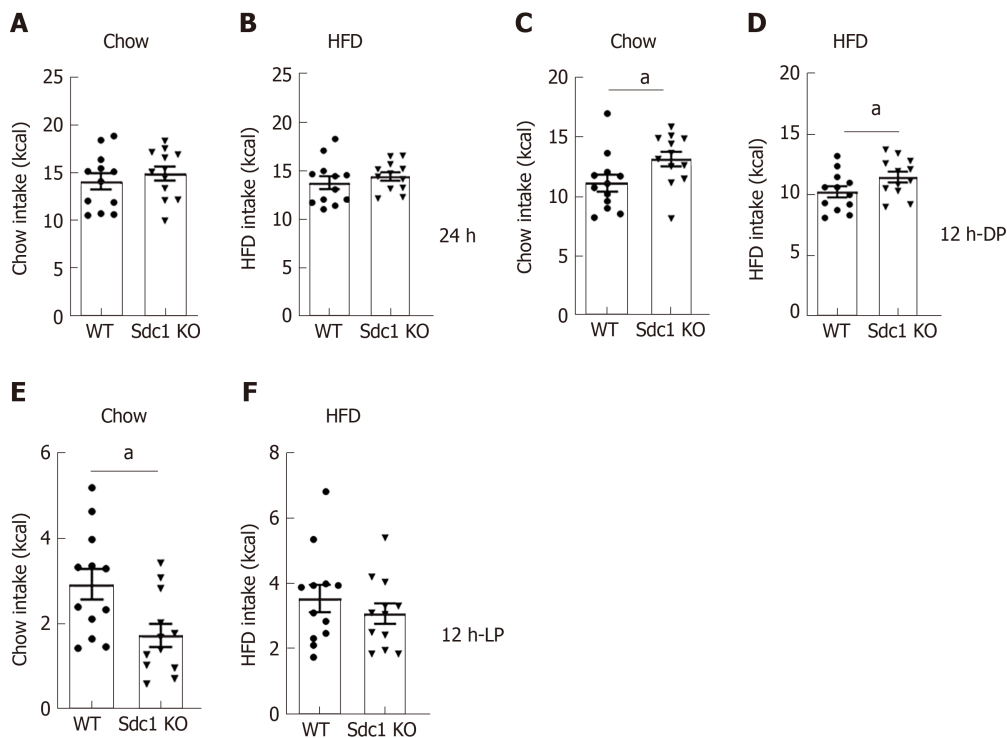


Figure 2 Energy intake on chow-fed and high fat-fed diets. Age matched Sdc1 knockout (Sdc1 KO), wild type (WT) mice kept on both chow and high-fat diet (HFD) for 8 wk and then indirect calorimetry was performed using the Oxymax/CLAMS system. Daily energy intake and body weight were monitored in each mice of each genotypes. Measurements were performed over 4 consecutive days following a 24-h acclimation period and are shown food intake, normalized to body weight (kcal/g BW) or lean mass (kcal/g lean) measured by QNMRI immediately prior to calorimetry. A, B: Graphs shows cumulative food intake (kcal) measured in Sdc1 KO and WT mice fed with chow and HFD; C, D: Cumulative food intake (kcal) in Sdc1 KO and WT mice on chow and HFD during 12 h dark; E, F: 12 h light phases of day cycle. Data represents mean \pm SE of total 12 animals of each genotype. * $P < 0.05$. Sdc1 KO: Sdc1 knockout; HFD: High-fat diet; WT: Wild type.

functions based on the ligand engaged^[6]. These unique characteristics of Sdc1 show that it plays multiple diverse functions which are reflected in the wide range and multisystem effects its deletion has on key biological functions, including body fat, lean mass, feeding behavior, energy expenditure and physical activity.

We hypothesize that the effect of Sdc1 deficiency could be due to a critical role for Sdc1 in regulating homeostasis of different cell types regardless of their specific function. Consistent with this hypothesis, our recent results show that Sdc1 plays a critical role in negatively regulating homeostasis of IL-17-producing subsets of NKT cells (NKT17) and IL-17-producing $\gamma\delta$ T cells, $\gamma\delta$ 17 cells^[13,16]. Consequently, sdc1 KO mice have higher frequency of NKT17 and $\gamma\delta$ 17 cells. These unconventional cell types reside in different non-lymphoid tissues. For example, $\gamma\delta$ 17 cells reside mainly in subcutaneous tissues, whereas NKT17 cells reside in liver and white adipose tissues. IL-17 is anti-adipogenic factors and increased IL-17 production has been linked to glucose intolerance^[23]. Therefore, it is plausible that reduced intradermal fat reported by Kasza *et al*^[20] and reduced body fat described here are linked to increased IL-17 production by unconventional T cells. On the other hand, impaired glucose tolerance could be due to high production of IL-17 by NKT cells localized in liver and white adipose tissue^[24].

Our results uncover a previously unexpected role for Sdc1 in regulating feeding pattern and links it to the circadian rhythm^[25]. This is indicated by the observation that Sdc1 KO mice ate more during the day than at night clocks with no significant change in total food consumption. Given that Sdc1 is ubiquitously expressed on intestinal epithelia and hepatocytes, whether alteration in feeding pattern is dependent on its expression on any of these cell types is currently unknown. Interestingly, lineage specification of IL-17-producing T helper cells varies diurnally by a mechanism that is linked to circadian clocks^[26]. Interestingly, Sdc1 when expressed ectopically led to hyperphagia^[27], but the study did not examined its effect on feeding behavior. Therefore, our results identify for the first time a role for Sdc1 in regulating feeding behavior. Future studies should determine whether the role of Sdc1 in feeding behavior is linked to its role in regulating homeostasis of NKT17 and $\gamma\delta$ 17 cells.

In summary, our results uncover a complex role for Sdc1 in regulating key metabolic pathways and systems. The results lay the ground for future studies and analyses of role of Sdc1 in a cell-specific mechanism that could have important

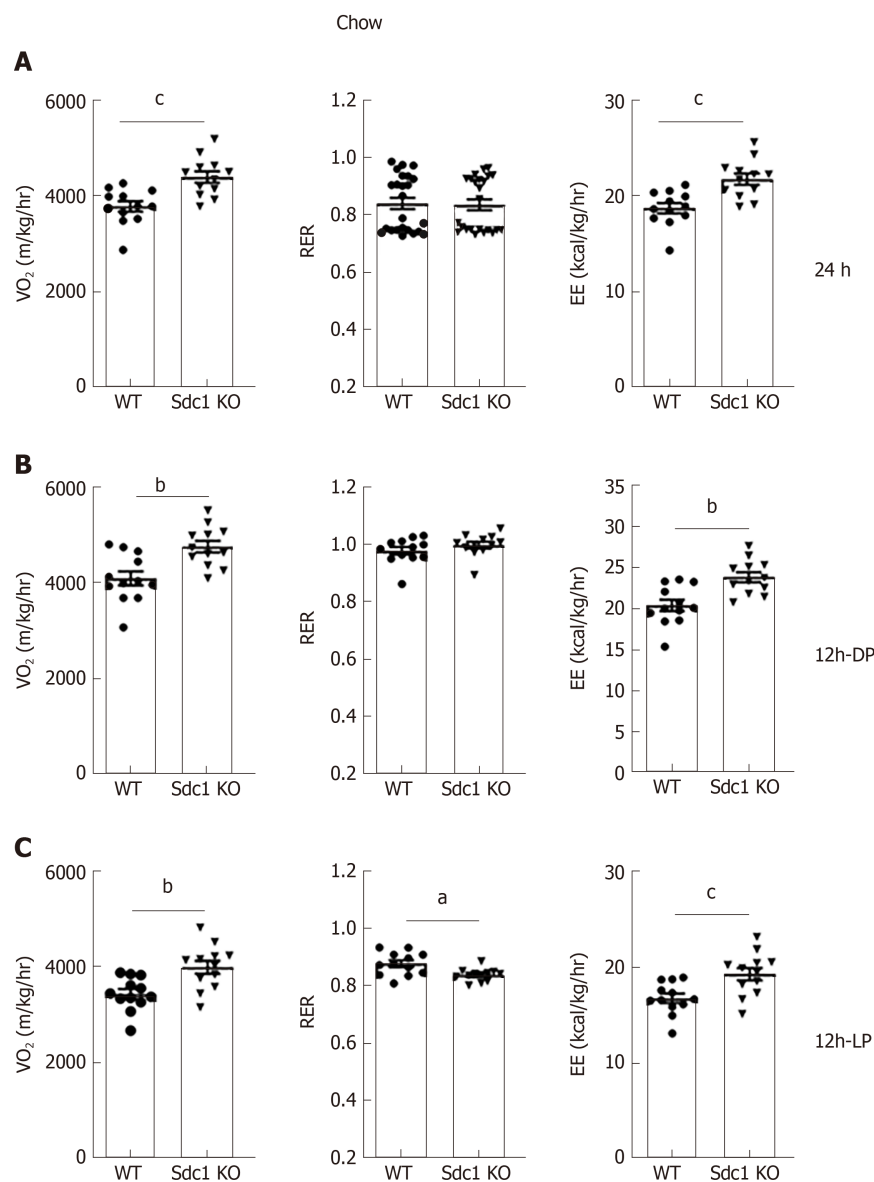


Figure 3 O₂ consumption and energy expenditure throughout light and dark phase in syndecan-1 knockout and wild type mice on chow-diet. Mice on the chow and high-fat diet were kept over 8 wk and then indirect calorimetry was performed using the Oxymax/CLAMS system. Each day O₂ consumption (VO₂) and energy expenditure was monitored in individual 8-wk-old mice of each genotypes. A: Measurements were performed over 4 consecutive days following a 24-h acclimation period and are shown as total energy expenditure and VO₂; B: Respiratory exchange ratio and VO₂ is presented as the average for the dark; C: Light phases. Data represents mean ± SE of total 12 animals of each genotype. Statistical analysis was performed using the two-tailed student's *t*-test. ^c*P* < 0.001, ^b*P* < 0.01, ^a*P* < 0.05. Sdc1 KO: Sdc1 knockout; WT: Wild type.

implications for developing strategies to target Sdc1 to manipulate specific metabolic parameters and obesity.

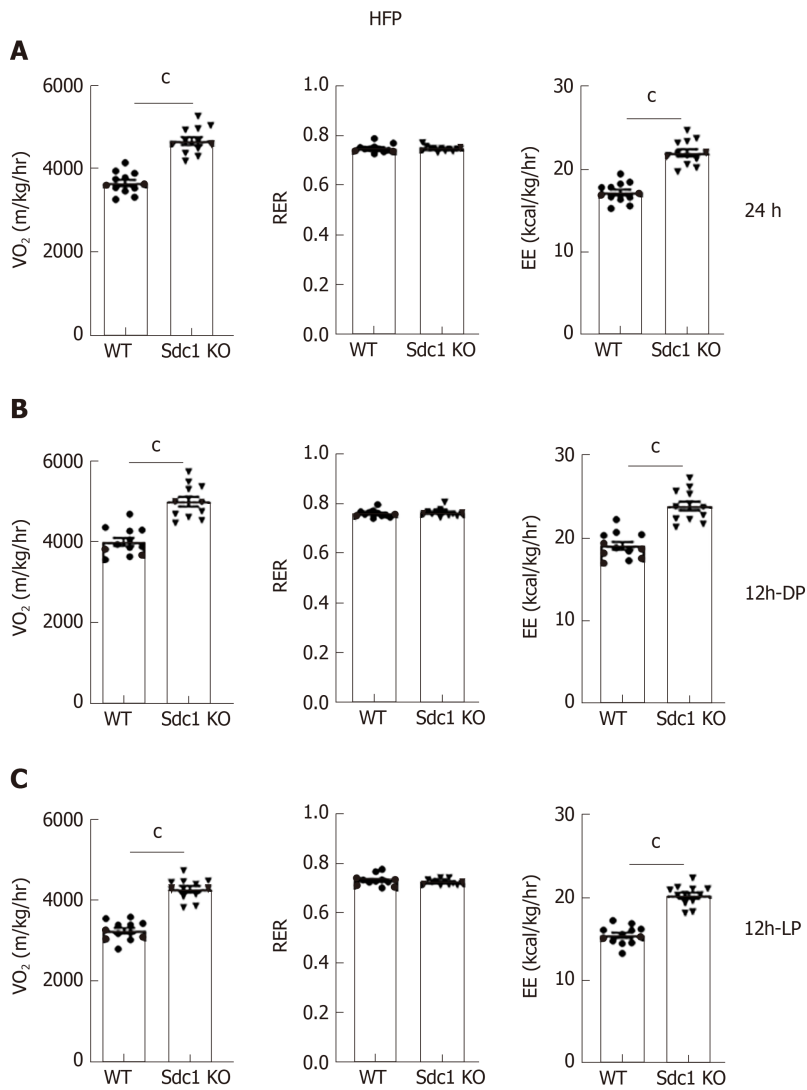


Figure 4 O₂ consumption and energy expenditure throughout light in syndecan-1 knockout and wild type mice on high-fat diet. Like chow-diet, mice were fed with high-fat diet and all parameters were monitored as previously. Data represents mean \pm SE of total 12 animals of each genotype. Statistical analysis was performed using the two-tailed student's *t*-test. ^c*P* < 0.001. Sdc1 KO: Sdc1 knockout; HFD: High-fat diet; WT: Wild type.

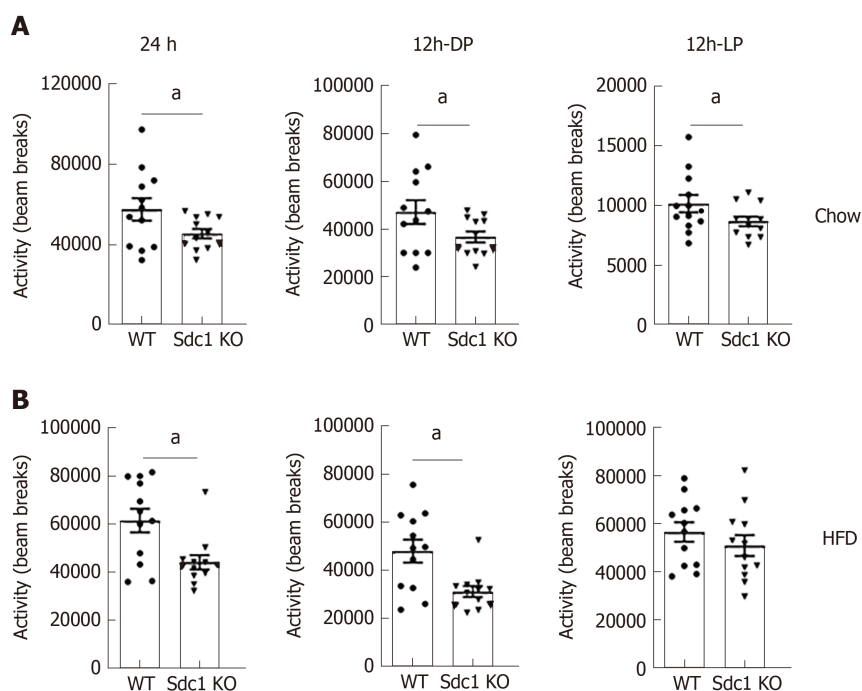


Figure 5 Syndecan-1 knockout mice have decreased locomotor activity. Mice were fed with chow or high-fat diet as described previously and physical activity (x-axis infrared beam array) was measured using the OxyMax/CLAMS system. A: The data were reported every 25 min throughout the studies, and are presented as 24-h sums; B: Sums for the 12-h dark; C: Light phases. Data represents mean \pm SE of total 12 animals of each genotype. Statistical analysis was performed using the two-tailed student's *t*-test. ^a*P* < 0.05. Sdc1 KO: Sdc1 knockout; HFD: High-fat diet; WT: Wild type.

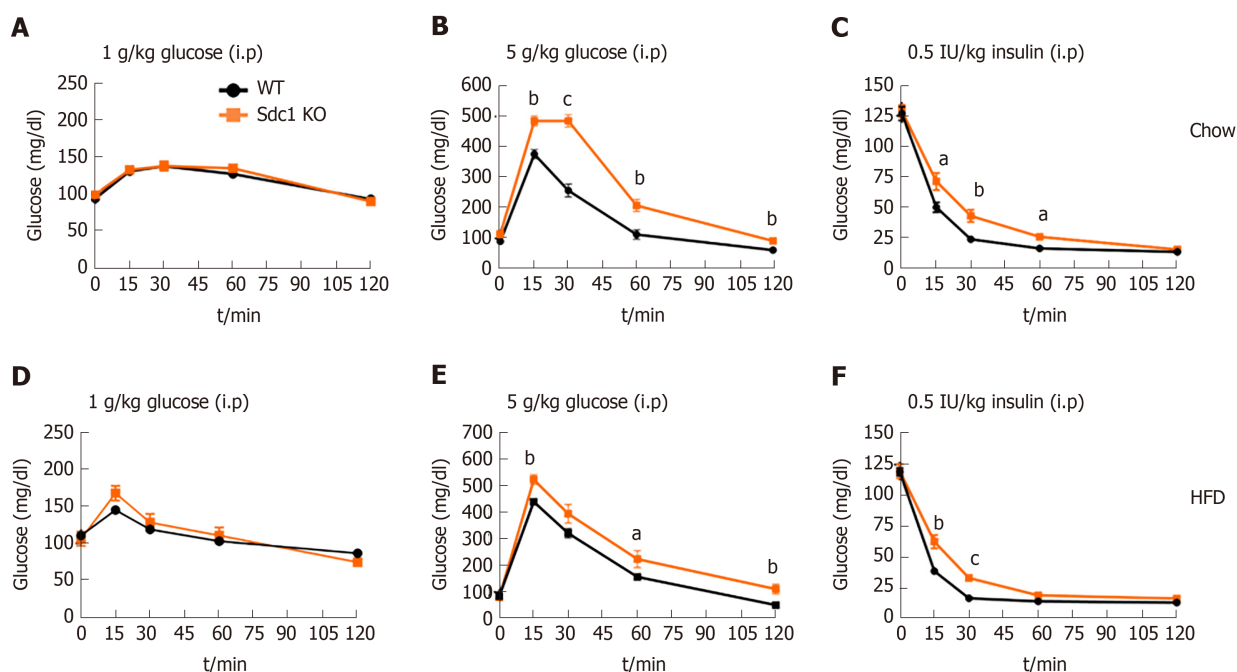


Figure 6 Glucose tolerance test and insulin tolerance test on chow and high-fat diet. A: Graph shows glucose tolerance pattern in Sdc1 knockout mice and wild type mice, when fed with chow-diet; B: High-fat diet up to 8 wks; C: Insulin tolerance test pattern when mice were fed with chow; D: High-fat diet. Graphs show cumulative data two independent experiments of total 24 female BALB/c mice (mean \pm SE). Unpaired *t* test (Two-tailed); ^c*P* < 0.001, ^b*P* < 0.01, ^a*P* < 0.05. Sdc1 KO: Sdc1 knockout; HFD: High-fat diet; WT: Wild type.

ARTICLE HIGHLIGHTS

Research background

Obesity is a disease state with serious adverse metabolic complications that currently has no cure. Attempts to control obesity by altering lifestyle has no significant improvement in most

individuals. Therefore, it is necessary to discover alternative strategies to combat obesity and its complications.

Research motivation

Syndecan-1 (Sdc1) is a member of the heparan sulfate proteoglycan family that has mainly been investigated for its role in regulating proliferation and survival of epithelia and tumor cells, but its roles in regulating obesity and glucose homeostasis are not well study.

Research objectives

The objective of this study is to examine the role of Sdc1 in regulating body fat and glucose metabolism.

Research methods

We used female wild type and Sdc1 knockout (Sdc1 KO) mice on BALB/c background and multiple methods. Metabolic measurements were performed using an open-flow indirect calorimeter with additional features to measure food intake and physical activity. Glucose intolerance and insulin resistance were measured by established tolerance test methods.

Research results

Although our primary goal was to investigate the effects of Sdc1 deficiency on body fat and glucose homeostasis, we uncovered that Sdc1 regulates multiple metabolic parameters. Sdc1 KO mice have reduced body weight due to significant decreases in fat and lean masses under both chow and high fat diet conditions. The reduced body weight was not due to changes in food intakes, but Sdc1 KO mice exhibited altered feeding behavior as they ate more during the dark phase and less during the light phase than wild type mice. In addition, Sdc1 KO mice suffered from high rate of energy expenditure, glucose intolerance and insulin resistance.

Research conclusions

These results reveal critical multisystem and opposing roles for Sdc1 in regulating normal energy balance and glucose homeostasis.

Research perspectives

The results provide important insights that will guide future strategies to target syndecan-1 for immunotherapy for obesity.

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Long-term effect of clopidogrel in patients with and without diabetes: A systematic review and meta-analysis of randomized controlled trials

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Abstract

BACKGROUND

Previous studies have shown that patients with diabetes mellitus (DM) respond poorly to clopidogrel treatment.

AIM

To systematically evaluate the efficacy of clopidogrel for the treatment of acute coronary syndromes or ischemic stroke in patients with or without DM.

METHODS

PubMed, the Cochrane Central Register of Controlled Trials, and EMBASE were searched from 1980 on 27 June 2019 to identify relevant randomized controlled trials that compared the effect of a combination of clopidogrel and aspirin with aspirin alone. A random-effects meta-analysis was used to estimate the hazard ratio (HR) and its 95% confidence interval (CI). Sensitivity analysis was performed using a fixed-effect model. The I^2 statistic was used to evaluate the heterogeneity of the study data.

RESULTS

Six randomized controlled trials, comprising 43352 participants (13491 with and

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29861 without DM) who had received antiplatelet therapy for ≥ 3 mo, were included in the meta-analysis. Compared with aspirin alone, a combination of clopidogrel and aspirin significantly reduced the risk of any cardiovascular event in patients without DM (HR = 0.78, 95%CI: 0.71–0.86, $P < 0.001$; $I^2 = 23\%$, $P = 0.26$). Clopidogrel plus aspirin also significantly reduced cardiovascular risk in patients with DM, although the effect was smaller (HR = 0.89, 95%CI: 0.81–0.99, $P = 0.030$; $I^2 = 0\%$, $P = 0.74$). Nevertheless, there was no significant difference in the efficacy of clopidogrel at reducing the risk of cardiovascular events in patients with DM *vs* those without (P for interaction = 0.062).

CONCLUSION

Thus, the present study shows that the addition of clopidogrel to aspirin significantly lowers cardiovascular risk in patients with or without DM who have experienced ischemic cardiovascular disease. The beneficial effect of the addition of clopidogrel to aspirin for patients with DM was lower than that in patients without DM, although the modifying effect of DM did not reach significance.

Key words: Clopidogrel; Diabetes; Aspirin; Meta-analysis; Randomized controlled trial

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Core tip: The long-term effects of clopidogrel in patients with and without diabetes mellitus (DM) have not been systematically reviewed. The present meta-analysis firstly investigated the modifying effect of DM on the efficacy of long-term clopidogrel treatment in patients with ischemic cardiovascular disease. Although the analysis showed that the hazard ratio reduction in patients with DM was less than that in those without, this difference was not significant. The efficacy of dual antiplatelet therapy with clopidogrel and aspirin appeared to be slightly lower in patients with DM, emphasizing the need for individualized antiplatelet treatment for patients with DM after myocardial infarction or ischemic stroke.

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INTRODUCTION

Ischemic cardiovascular disease remains the leading cause of mortality globally^[1,2]. Early initiation of dual antiplatelet therapy with a P2Y₁₂ receptor antagonist plus aspirin is recommended for patients with ischemic cardiovascular disease in the current guidelines^[3,4]. Clopidogrel remains the most widely prescribed P2Y₁₂ receptor antagonist and is recommended by the latest guidelines for the management of ischemic stroke and acute coronary syndromes (ACS)^[5,6]. Studies have shown that patients with diabetes mellitus (DM) exhibit a poorer response to clopidogrel than patients without DM^[7,8], leading to a significant difference in the incidence of recurrence of cardiovascular events and mortality associated with DM^[9].

Previous research has shown that hyperglycemia increases platelet reactivity, and the underlying mechanisms include higher platelet receptor expression, intracellular downstream signaling abnormalities, and P2Y₁₂ mutation and expression level^[7,10]. The findings of the OPTIMUS trial, which compared the efficacy of a 150-mg maintenance dose of clopidogrel with 75 mg in 40 patients with both DM and coronary artery disease, showed that the higher maintenance dose of clopidogrel is associated with greater antiplatelet effects^[11]. Hence, it is reasonable to hypothesize that clopidogrel treatment of ischemic vascular disease would be associated with a less substantial reduction in the risk of cardiovascular events in patients with DM than in those without.

Subgroup analyses of data from several randomized controlled trials (RCTs) comparing the efficacy of a combination of clopidogrel and aspirin with aspirin alone have shown that DM reduces the effect of clopidogrel on the incidence of recurrence

of cardiovascular events and all-cause mortality, although the interactions were not significant^[12-14]. A large-scale cohort study also showed that DM was associated with a lower efficacy of clopidogrel for a reduction in 1-year cardiovascular mortality in patients with myocardial infarction^[9]. However, the long-term effects of clopidogrel in patients with and without DM have not been systematically reviewed. Therefore, we hypothesized that the presence of DM modifies the long-term efficacy of clopidogrel for a reduction in cardiovascular risk, and we performed a systematic review and meta-analysis to test this hypothesis.

MATERIALS AND METHODS

This systematic review and meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement^[15].

Data sources

The literature search was conducted on 27 June 2019 using the Cochrane Library database for trials, EMBASE, and PubMed. The search strategy was: (1) PubMed (from 1980) 2019/06/27, (clopidogrel[Title/Abstract]) AND aspirin[Title/Abstract], article types: Clinical Trial; (2) EMBASE (from 1980) 2019/06/27, #2 #1 AND 'randomized controlled trial'/de, #1 'clopidogrel':ab,ti AND 'aspirin':ab,ti; and (3) Cochrane Central Register of Controlled Trials (CENTRAL, from 1980) 2019/06/27, keywords: ("clopidogrel" AND "aspirin"); search limits: Trials.

Study selection

The inclusion criteria for RCTs were double-blind design, clopidogrel and aspirin *vs* aspirin for the treatment of ischemic cardiovascular disease, age ≥ 18 years, subgroup analysis according to the presence of diabetes (if not reported, the corresponding author was contacted to obtain the data)^[16], and a duration of treatment ≥ 3 mo^[17]. We also included the CHARISMA trial, because the percentage of patients with documented vascular disease in the trial was high (78%)^[14].

Outcome

The outcomes assessed in the meta-analysis were fatal and non-fatal cardiovascular events. Table 1 summarizes the definitions of the primary outcomes of the included RCTs.

Quality assessment and data extraction

Two researchers (Liang LR and Ma Q) used a specially designed questionnaire to extract data independently. Consensus was reached by discussion. The Cochrane Collaboration's risk-of-bias table was used to assess the quality of the included RCTs independently by two authors (Feng L and Xie WX)^[18]. Figure 1 shows the risk of bias in the RCTs.

Statistical analysis

Review Manager 5.3 (Nordic Cochrane Center, Copenhagen, Denmark) was used to conduct the meta-analysis within random-effect models to estimate the pooled hazard ratio (HR) and its 95% confidence interval (CI). Sensitivity analysis was also performed using fixed-effect models. To evaluate the modifying effect of DM on the efficacy of clopidogrel, we used the Z-test to compare the difference between the two pooled HRs from the subgroup analyses, using the method proposed by Altman and Bland^[19]. We used the *I*² statistic to estimate the heterogeneity of the included RCTs, and the Egger test and funnel plots to estimate the level of publication bias in STATA (version 11; Stata Corp, College Station, TX). All analyses were two-sided, and an alpha value of 0.05 was used as the threshold for statistical significance.

RESULTS

Study characteristics

Figure 2 shows the study selection process, which was conducted according to the PRISMA statement. Two thousand and sixty-four publications were identified in the initial search, from which 598 duplicates were excluded, and a further 1432 articles were removed after reviewing their title and abstract. The full text of the remaining 34 articles was then reviewed independently by two researchers, and 28 of these were excluded because of a treatment duration < 3 mo, a lack of comparison between clopidogrel plus aspirin and aspirin alone, a lack of subgroup analysis according to

Table 1 Definitions of the primary efficacy outcome in each of the included trials

Trial	Definition of primary efficacy outcome
CHANCE 2013	Stroke recurrence (ischemic or hemorrhagic).
CHARISM 2006	The composite of myocardial infarction, stroke, or death from cardiovascular causes.
CREDO 2002	The composite of death, myocardial infarction, and stroke.
CURE 2001	The composite of myocardial infarction, stroke, or death from cardiovascular causes.
POINT 2018	The composite of ischemic stroke, myocardial infarction, or death from ischemic vascular causes.
SPS3 2012	Stroke recurrence (ischemic or hemorrhagic).

the presence of DM, a long-term *vs* short-term analysis, a high-dose *vs* low-dose analysis, ACS or ischemic stroke patients were not studied, or duplication (Table 2). Therefore, six RCTs, comprising 43352 participants (13491 with and 29861 without DM), were eligible for the present analysis^[12-14,16,20,21]. Table 3 presents the characteristics of the included RCTs, of which three had recruited patients who had experienced an ischemic stroke or transient ischemic attack, two had recruited ACS patients, and one had recruited patients with cardiovascular disease or multiple risk factors. Table 4 summarizes the antiplatelet treatments and daily doses used in these trials.

Effect of clopidogrel, according to diabetes status

As shown in Figure 3, compared with aspirin alone, the addition of clopidogrel to aspirin significantly reduced the risk of any cardiovascular event in patients without DM (HR = 0.78, 95%CI: 0.71–0.86, $P < 0.001$; $I^2 = 23\%$, $P = 0.26$) and also in patients with DM, although the benefit was less (HR = 0.89, 95%CI: 0.81–0.99, $P = 0.030$; $I^2 = 0\%$, $P = 0.74$). The presence of DM was associated with an 11% higher HR, although the difference in the efficacy of clopidogrel in patients with and without DM did not reach significance (P for interaction = 0.062).

Publication bias

Both funnel plots (Figure 4) and Egger tests ($P = 0.592$ for patients without DM, and $P = 0.296$ for patients with DM) showed no evidence of publication bias.

Sensitivity analysis

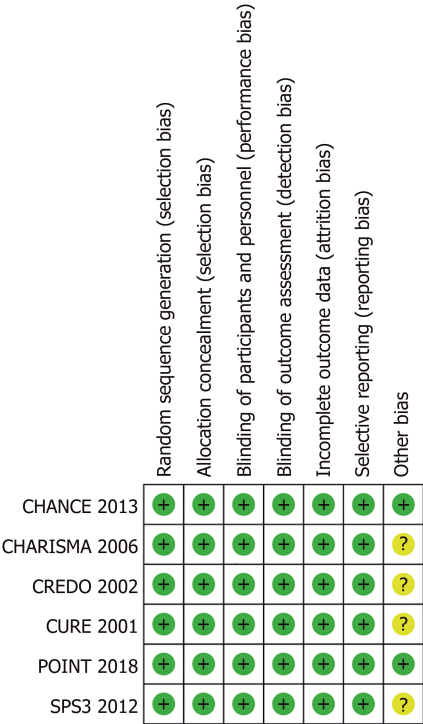
The pooled results of a meta-analysis using fixed-effect models was consistent with the principal findings of random-effect models (Figure 5). Clopidogrel treatment was associated with relative risk reductions of 11% (HR = 0.89, 95%CI: 0.81–0.99, $P = 0.030$) and 21% (HR = 0.79, 95%CI: 0.73–0.85, $P < 0.001$) for any cardiovascular event in patients with and without DM, respectively. However, the modifying effect of DM on the incidence of cardiovascular events was not significant (P for interaction = 0.064).

DISCUSSION

Our systematic review and meta-analysis found that the addition of clopidogrel to aspirin significantly reduced the risk of any cardiovascular event in patients with or without DM. In addition, the results show that the presence of DM is associated with an increase of 11% in the HR, although this difference was not significant. To our knowledge, this is the first meta-analysis to evaluate the efficacy of long-term clopidogrel treatment in patients with ischemic cardiovascular disease, according to the presence or absence of DM.

The latest evidence provided by head-to-head RCTs has led to the current guidelines for the management of ACS stating that clopidogrel is inferior to ticagrelor and prasugrel as a P2Y₁₂ inhibitor for patients with ST-segment elevation myocardial infarction or non-STE ACS^[3,4]. Recently, subgroup analyses of these RCTs have shown that the effects of ticagrelor and prasugrel are not modified by the presence of DM^[22,23]. Evidence from previous trials and the present meta-analysis indicate that it is reasonable to use ticagrelor or prasugrel in preference to clopidogrel for the treatment of ACS patients, and especially those with DM. However, it should be noted that both ticagrelor and prasugrel are associated with higher risks of bleeding than clopidogrel; therefore, clopidogrel can still be used if ticagrelor and prasugrel are not available or are contraindicated^[3,4]. Furthermore, data regarding the clinical efficacy and safety of ticagrelor or prasugrel for the treatment of ischemic stroke are limited. The SOCRATES trial, which compared the efficacy and safety of ticagrelor and aspirin in

A



B

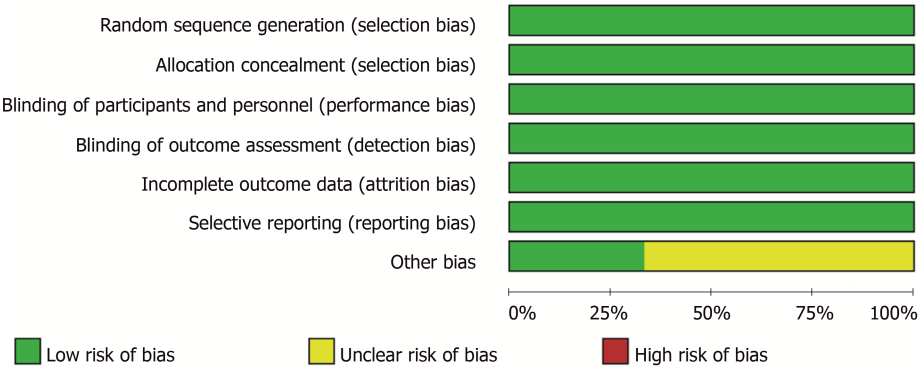


Figure 1 Risk-of-bias of included trials. A: Judgements about each source of bias in each study; B: Review authors' judgements regarding the risk of each source of bias. The data are presented as percentages across all the included studies and are classified as Low, Unclear, or High. We followed the recommended approach for assessing the risk of bias in studies included in the Cochrane Handbook for Systematic Reviews of Interventions, version 5.3.0. This addresses seven specific domains: Random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias. Each domain includes one or more specific entries in a "Risk of bias" table. The tool involves assigning a judgement relating to the risk of bias for that entry. This is achieved by answering a pre-specified question about the adequacy of the study in relation to the entry, such that a judgement of "Yes" indicates a low risk of bias, "No" indicates a high risk of bias, and 'Unclear' indicates an unclear or unknown risk of bias.

13199 patients who had experienced a mild ischemic stroke or high-risk transient ischemic attack, found that ticagrelor was no more effective than aspirin at lowering cardiovascular risk over 90 days^[24]. Therefore, clopidogrel remains the first choice of P2Y₁₂ inhibitor for the treatment of ischemic stroke^[6]. In the present study, a weaker effect of clopidogrel was identified in patients with DM, which implies that a new antiplatelet agent is needed for ischemic stroke patients with DM.

A prospective cohort study of 58851 patients who had experienced a myocardial infarction confirmed this hypothesis^[9]. Andersson and colleagues reported that clopidogrel treatment was associated with lesser reductions in 1-year all-cause mortality and cardiovascular mortality in patients with DM, compared to those without^[9]. The results of the large-scale cohort study were consistent with the present findings, and we consider that one of the most important reasons why the present meta-analysis did not demonstrate a modifying effect of DM was the relatively small number of RCTs included. Therefore, a second meta-analysis should be performed when data from additional RCTs become available.

Table 2 Characteristics of the excluded trials

Title	Reason for exclusion
A randomised, blinded, trial of clopidogrel <i>vs</i> aspirin in patients at risk of ischaemic events (CAPRIE)	Not clopidogrel + aspirin <i>vs</i> aspirin
Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation	Treatment duration < 3 mo; Not report subgroup results
Addition of clopidogrel to aspirin in 45852 patients with acute myocardial infarction: Randomised placebo-controlled trial	Treatment duration < 3 mo; Not report subgroup results
Aspirin and clopidogrel compared with clopidogrel alone after recent ischaemic stroke or transient ischaemic attack in high-risk patients (MATCH): Randomised, double-blind, placebo-controlled trial	Not clopidogrel + aspirin <i>vs</i> aspirin
Aspirin and extended-release dipyridamole <i>vs</i> clopidogrel for recurrent stroke	Not clopidogrel + aspirin <i>vs</i> aspirin
Aspirin plus clopidogrel therapy increases early venous graft patency after coronary artery bypass surgery	Treatment duration < 3 mo
Aspirin plus clopidogrel <i>vs</i> aspirin alone after coronary artery bypass grafting: The clopidogrel after surgery for coronary artery disease (CASCADE) Trial	Not report subgroup results
Clinical outcomes of patients with diabetic nephropathy randomized to clopidogrel plus aspirin <i>vs</i> aspirin alone	Duplicate trials
Clopidogrel with aspirin in acute minor stroke or transient ischemic attack (CHANCE) trial: One year outcomes	Duplicate trials
Dose comparisons of clopidogrel and aspirin in acute coronary syndromes	High dose <i>vs</i> low dose
Duration of Dual Antiplatelet Therapy after Implantation of Drug-Eluting Stents	Long-term <i>vs</i> short-term
Efficacy and safety outcomes of ticagrelor compared with clopidogrel in elderly Chinese patients with acute coronary syndrome	Not clopidogrel + aspirin <i>vs</i> aspirin
Effect of clopidogrel pretreatment before percutaneous coronary intervention in patients with ST-elevation myocardial infarction treated with fibrinolytics	Treatment duration < 3 mo
Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: The PCI-CURE study	Duplicate trials
Fast assessment of stroke and transient ischaemic attack to prevent early recurrence (FASTER): A randomised controlled pilot trial	Not report subgroup results
Prasugrel or double-dose clopidogrel to overcome clopidogrel low-response - The TAILOR randomized trial	Not clopidogrel + aspirin <i>vs</i> aspirin
Prasugrel <i>vs</i> clopidogrel for acute coronary syndromes without revascularization	Not clopidogrel + aspirin <i>vs</i> aspirin
Prasugrel <i>vs</i> clopidogrel in patients with acute coronary syndromes	Not clopidogrel + aspirin <i>vs</i> aspirin
PROCLAIM: Pilot study to examine the effects of clopidogrel on inflammatory markers in patients with metabolic syndrome receiving low-dose aspirin.	Treatment duration < 3 mo
Randomized clinical trial of the antiplatelet effects of aspirin-clopidogrel combination <i>vs</i> aspirin alone after lower limb angioplasty	Treatment duration < 3 mo
Randomized comparison of prasugrel (CS-747, LY640315), a novel thienopyridine P2Y ₁₂ antagonist, with clopidogrel in percutaneous coronary intervention	Not clopidogrel + aspirin <i>vs</i> aspirin
Results of the randomized, placebo-controlled clopidogrel and acetylsalicylic acid in bypass surgery for peripheral arterial disease (CASPAR) trial	Not report subgroup results
Ticagrelor <i>vs</i> aspirin in acute stroke or transient ischemic attack	Not clopidogrel + aspirin <i>vs</i> aspirin
Ticagrelor <i>vs</i> clopidogrel in patients with acute coronary syndromes	Not clopidogrel + aspirin <i>vs</i> aspirin
Ticagrelor <i>vs</i> clopidogrel after fibrinolytic therapy in patients with ST-elevation myocardial infarction	Not clopidogrel + aspirin <i>vs</i> aspirin
Ticagrelor <i>vs.</i> clopidogrel in Japanese, Korean and Taiwanese patients with acute coronary syndrome	Not clopidogrel + aspirin <i>vs</i> aspirin
Twelve or 30 mo of dual antiplatelet therapy after drug-eluting stents	Long-term <i>vs</i> short-term
Effect of Clopidogrel Added to Aspirin in Patients with Atrial Fibrillation	Not ACS or ischemic stroke patients

Several potential mechanisms that might explain the variability in the response to clopidogrel have been suggested. Firstly, a pharmacokinetic study showed that variability in intestinal absorption is the principal cause^[25]. Second, polymorphisms in

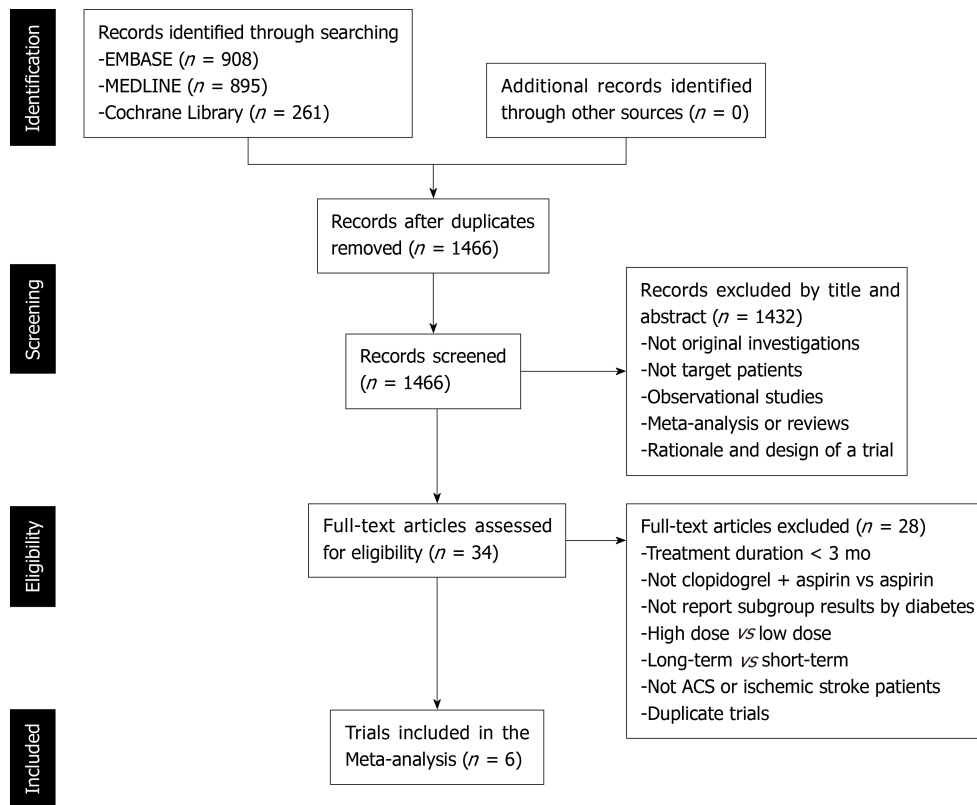


Figure 2 Study selection flow chart.

hepatic cytochrome (CYP) P450 3A, including in CYP3A4 and CYP3A5, may also be important: An inverse correlation has been shown between the percentage platelet aggregation and CYP3A4 activity in clopidogrel users^[26]. In particular, of the multiple polymorphisms of CYP3A4, the IVS10 + 12G>A variant has been shown to regulate clopidogrel responsiveness^[27]. Furthermore, a CYP3A5 polymorphism has been shown to be associated with atherothrombosis after clopidogrel treatment^[28], although there is new evidence that challenges the existence of the association between CYP3A5 genotype and clopidogrel response^[29]. Third, genetic platelet receptor polymorphisms have been implicated. Single-nucleotide polymorphisms of platelet receptors, such as GP IIIa L33P (= P1A1/2) and GP Ia 807 C/T have been suggested to be risk factors for thrombosis^[30]. Fourth, the use of concurrent medication, such as calcium channel blockers, has recently been demonstrated to be a predictor of atherothrombotic events after clopidogrel treatment^[29]. These mechanisms might also explain the effect of DM on the clopidogrel response.

Our study had several strengths: The inclusion of high-quality RCTs, large samples, no significant heterogeneity among the studies, and no evidence of publication bias. However, the study also had some limitations. First, the primary outcomes of the included RCTs were not entirely consistent, and secondary outcome data cannot be collected, because publications describing RCTs only show subgroup data for the primary outcomes. The present study only pooled the results of the primary outcomes of the included trials, which may have caused bias. Second, we estimated the pooled HRs on the basis of trial-level data, whereas pooled HRs derived from individual-level data would be more accurate. Third, although we conducted a thorough literature search to identify relevant RCTs, it is possible that some appropriate RCTs were not included in this study, implying the possibility of selection bias.

In conclusion, the present study found that the addition of clopidogrel to aspirin significantly reduced cardiovascular risk in patients with and without DM who had experienced ischemic cardiovascular disease. The beneficial effect of a combination of clopidogrel and aspirin for patients with DM appeared to be lower than that for patients without DM, although this difference did not reach significance.

Table 3 Baseline characteristics of participants in the included trials

Trial	Country	Centers	Patients	Sample size	Male %	Mean age Y	Diabetes %	Treatment duration M	Lost to follow-up %	Blinding	ITT analysis
CHANCE 2013	China	114	Minor ischemic stroke or high-risk TIA	5170	66	63	21	3	0.7	Double-blind	Yes
CHARISM 2006	Worldwide	768	CVD or multiple risk factors	15603	70	64	42	28	0.5	Double-blind	Yes
CREDO 2002	United States and Canada	99	Those would undergo elective PCI	2116	71	62	26	12	1.1	Double-blind	Yes
CURE 2001	Worldwide	482	ACS without ST-segment elevation	12562	62	64	23	12	0.1	Double-blind	Yes
POINT 2018	Worldwide	269	Acute ischemic stroke or high-risk TIA	4881	55	65	27	3	4.1	Double-blind	Yes
SPS3 2012	Worldwide	82	Recent symptomatic lacunar infarcts	3020	63	63	36	41	2.0	Double-blind	Yes

ITT: Intention-to-treat; TIA: Transient ischemic attack; CVD: Cardiovascular disease; PCI: Percutaneous coronary intervention; ACS: Acute coronary syndromes.

Table 4 Antiplatelet treatments and the daily doses used in the included trials

Trial	Drug (daily doses, mg)	
	Clopidogrel + Aspirin	Aspirin
CHANCE 2013	Clopidogrel (day 1: 300; day 2-90: 75) + Aspirin (day 1: 75-300; day 2-21: 75; day 22-90: placebo)	Aspirin (day 1: 75-300; day 2-90: 75)
CHARISM 2006	Clopidogrel (75) + Aspirin (75-162)	Aspirin (75-162)
CREDO 2002	Clopidogrel (day 1: 300; day 2 to 12 mo: 75) + Aspirin (day 1: 325; day 2 to 12 mo: 81-325)	Clopidogrel (day 2-28: 75) + Aspirin (day 1: 325; day 2 to 12 mo: 81-325)
CURE 2001	Clopidogrel (day 1: 300, and then 75) + Aspirin (75-325)	Aspirin (75-325)
POINT 2018	Clopidogrel (day 1: 600, and then 75) + Aspirin (50-325)	Aspirin (50-325)
SPS3 2012	Clopidogrel (75) + Aspirin (325)	Aspirin (325)

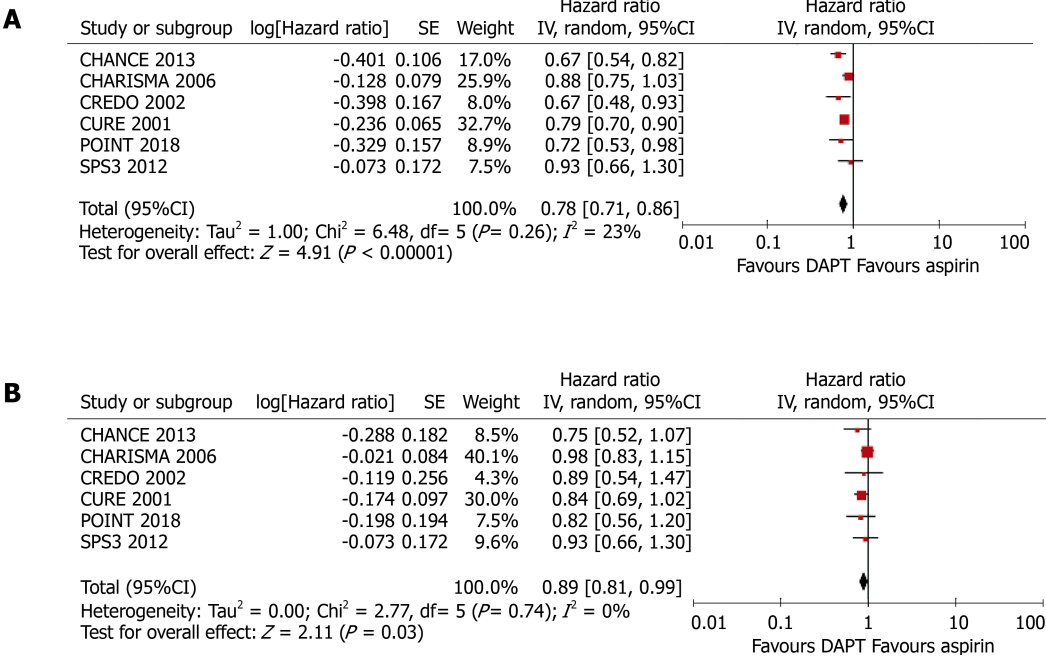


Figure 3 Meta-analyses using random-effect models conducted on the pooled data describing the effect of clopidogrel treatment on the incidence of any cardiovascular event. A: In patients without diabetes mellitus; B: In patients with diabetes mellitus. DAPT: Dual antiplatelet therapy.

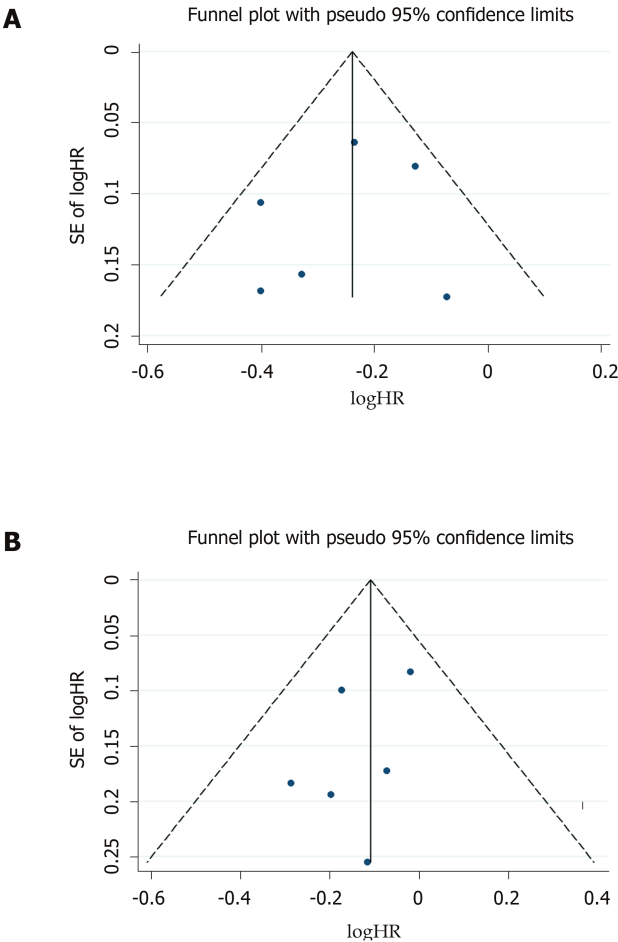


Figure 4 Funnel plot for the included studies, used to evaluate the effect of clopidogrel on the incidence of any cardiovascular event. A: In patients without diabetes mellitus; B: In patients with diabetes mellitus.

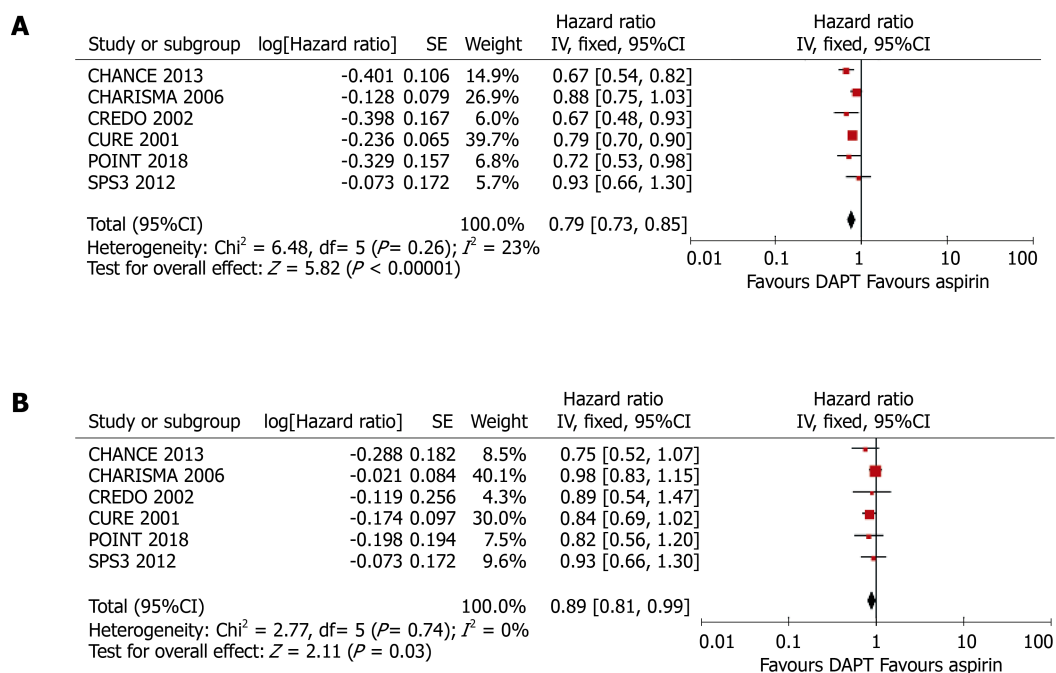


Figure 5 Meta-analyses using fixed-effect models conducted on the pooled data describing the effect of clopidogrel treatment on the incidence of any cardiovascular event. A: In patients without diabetes mellitus; B: In patients with diabetes mellitus. DAPT: Dual antiplatelet therapy.

ARTICLE HIGHLIGHTS

Research background

Clopidogrel remains the most widely prescribed P2Y₁₂ receptor antagonist and is recommended by the latest guidelines for the management of ischemic stroke and acute coronary syndromes. Studies have shown that patients with diabetes mellitus (DM) exhibit a poorer response to clopidogrel than patients without DM, leading to a significant difference in the incidence of recurrence of cardiovascular events and mortality associated with DM. However, the long-term effects of clopidogrel in patients with and without DM have not been systematically reviewed.

Research motivation

We hypothesized that the presence of DM modifies the long-term efficacy of clopidogrel for a reduction in cardiovascular risk. To our knowledge, this is the first meta-analysis to evaluate the efficacy of long-term clopidogrel treatment in patients with ischemic cardiovascular disease, according to the presence or absence of DM.

Research objectives

This study aimed to systematically evaluate the efficacy of clopidogrel for the treatment of acute coronary syndromes or ischemic stroke in patients with or without DM.

Research methods

A systematic review and meta-analysis of randomized controlled trials.

Research results

Six randomized controlled trials, comprising 43,352 participants (13,491 with and 29,861 without DM) who had received antiplatelet therapy for ≥ 3 mo, were included in the meta-analysis. Compared with aspirin alone, a combination of clopidogrel and aspirin significantly reduced the risk of any cardiovascular event in patients without DM (HR = 0.78, 95%CI: 0.71–0.86, $P < 0.001$; $I^2 = 23\%$, $P = 0.26$). Clopidogrel plus aspirin also significantly reduced cardiovascular risk in patients with DM, although the effect was smaller (HR = 0.89, 95%CI: 0.81–0.99, $P = 0.030$; $I^2 = 0\%$, $P = 0.74$). Nevertheless, there was no significant difference in the efficacy of clopidogrel at reducing the risk of cardiovascular events in patients with DM *vs* those without (P for interaction = 0.062).

Research conclusions

The present study found that the addition of clopidogrel to aspirin significantly reduced cardiovascular risk in patients with and without DM who had experienced ischemic cardiovascular disease. The beneficial effect of the addition of clopidogrel to aspirin for patients with DM was lower than that in patients without DM, although the modifying effect of DM did not reach significance. In the present study, a weaker effect of clopidogrel was identified in patients with DM, which implies that a new antiplatelet agent is needed for ischemic stroke patients with DM. We hypothesized that the presence of DM modifies the long-term efficacy of

clopidogrel for a reduction in cardiovascular risk. Cardiologist and neurologist should pay attention to the presence of DM when provide clopidogrel for patients with ischemic cardiovascular disease.

Research perspectives

A systematic review and meta-analysis of randomized controlled trials to summarize the evidence on this topic is important. A new antiplatelet agent is needed for ischemic stroke patients with DM.

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