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EDITORIAL

Effect of proton pump inhibitors on glycemic control in patients with diabetes

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Abstract

Gastrin is a linear peptide hormone which is secreted mostly in the stomach pyloric antrum G cells. Although the main role of this hormone is the promotion of the secretion of gastric acid from the stomach parietal cells, gastrin can also behave as a growth factor and stimulate gastric cell proliferation. It is also reported that gastrin promotes β cell neogenesis in the pancreatic ductal complex, modest pancreatic β cell replication, and improvement of glucose tolerance in animal models, in which the remodeling of pancreatic tissues is promoted. These findings suggest the possibility that gastrin has the potential to promote an increase of β cell mass in pancreas, and therefore that gastrin may improve glucose tolerance. Proton pump inhibitors (PPIs) are wildly used clinically for the therapy of gastro-esophageal reflex disease, gastritis due to excess stomach acid, and gastric ulcers. PPIs indirectly elevate serum gastrin levels *via* a negative feedback effect. Recent evidence has revealed the beneficial effect of PPIs on glycemic control especially in patients with type 2 diabetes mellitus (T2DM), probably *via* the elevation of the levels of serum gastrin, although the detailed mechanism remains unclear. In addition, the beneficial effects of a combination therapy of gastrin or a PPI with a glucagon-like peptide-1 receptor agonist on glycemic control in animal models have been demonstrated. Although PPIs may be possible candidates for a new approach in the therapy of diabetes, a prospective, long-term, randomized, double-blind, placebo-controlled study is needed to establish the effect of PPIs on glycemic control in a large number of patients with T2DM.

Key words: Gastrin; Proton pump inhibitors; Glycemic control; Type 2 diabetes

Core tip: Recently, it is reported that gastrin may improve glucose tolerance mainly by the promotion of pancreatic β cell neogenesis. Proton pump inhibitors (PPIs) are wildly used clinically for the treatment such as gastric ulcers, and it is known that PPIs indirectly elevate serum gastrin levels. Recent evidence has showed the beneficial effect of PPIs on glycemic control especially in patients with type 2 diabetes, probably

via the elevation of serum gastrin levels. Therefore, PPIs may have the potential to be candidates for a new approach in the treatment of diabetes.

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INTRODUCTION

Gastrin is a linear peptide hormone which is secreted mostly in the stomach pyloric antrum G cells, in which high biologically active gastrin (gastrin-17 and gastrin-34) is formed[1,2]. The secretion of gastrin is stimulated by various factors, such as considerable distension of the stomach[3], vagal stimulation[3,4], the presence of food (especially protein, peptides, and amino acids) in the stomach[4-6], and high pH levels in the stomach cavity[5,7]. Gastrin is released into the bloodstream. The main role of this hormone is the stimulation of secretion of gastric acid from the stomach parietal cells. The gastrin receptor, cholecystokinin B (CCK-B) receptor, binds to gastrin and to cholecystokinin with a similar high affinity[8]. Gastrin can directly promote the secretion of gastric acid by binding to CCK-B receptor on parietal cells[9,10]. However, the expression of this receptor is also found on enterochromaffin-like cells, and the binding of CCK-B receptor to gastrin on these cells promotes the secretion of the histamine resulting in subsequent promotion of the release of gastric acids by parietal cells, which may be the central mechanism of gastrin-stimulated acid secretion[6,9-12]. Importantly, gastrin is also be able to behave as a growth factor and stimulate gastric cell proliferation[6,13]. It is reported that gastrin promotes β cell neogenesis in pancreatic ductal complex[14], modest pancreatic β cell replication[15], and improvement of glucose tolerance[15] in animal models in which the remodeling of pancreatic tissues is promoted. These findings suggest the possibility that gastrin has a potential promoting effect for the increase in the pancreatic β cell mass. Therefore, gastrin improves glucose tolerance, and these effects appear to occur especially during adult pancreatic tissue remodeling but not in the normal tissue state.

Proton pump inhibitors (PPIs) are wildly used clinically for the therapy of gastro-esophageal reflex disease, gastritis due to excess stomach acid, and gastric ulcers[16]. PPIs can be orally administrated as an inactive form, which enters the bloodstream from the intestine, reaches the gastric parietal cells, and is activated by crossing the cell membrane into the intracellular compartment. After converting to the active form in the unique parietal cell environment, PPIs irreversibly block the proton pump and can strongly reduce the secretion of gastric acid promoted by either gastrin, acetylcholine, or histamine. It is well known that PPIs indirectly elevate serum gastrin levels *via* a negative feedback effect[17-22]. Interestingly, in type 2 diabetes mellitus (T2DM) animal models, it has been reported that PPIs improved glycemic control, probably *via* possible effects on augmenting both serum levels of gastrin and β cell mass[23]. Although some clinical studies showed negative results on glycemic control by PPIs in patients with T2DM[24,25], most studies have demonstrated a significant improvement of glycemic control by PPI administration to these patients[26-32]. Therefore, these agents appear to have the possibility of being a new approach for the therapy of diabetes.

BASIC STUDIES ON THE EFFECT OF GASTRIN ON THE INCREASE IN β CELL MASS

Gastrin and the CCK-B receptor are transiently expressed in fetal tissues of pancreas under period of islet neogenesis[33-35], but no expression is observed in both adult pancreatic β cells[36,37] and the exocrine pancreas[34,38-40]. It has been reported that in a rat model in which the splenic portion of the pancreas is ligated (an animal model for remodeling of pancreas tissue), transdifferentitation of acinar to ductal cells is promoted, and a ductal complex consisting of a mixture transdifferented acinar and ductal cells is formed[41-44]. A similar ductal complex appeared to emerge in 95% of the pancreatectomized rats (an animal model for diabetes in which pancreatic remodeling is promoted)[15]. Although the CCK-B receptor is not expressed in adult β cells even if the pancreatic tissue is undergoing remodeling, the ductal complex shows characteristics of fetal pancreatic ductal cells in addition to those in adult, including the CCK-B receptor expression[34]. So, it appears that gastrin is able to enhance the process of β cell neogenesis, that was already induced during the remodeling state, *via* the CCK-B receptor followed by budding from the ductal complex[14,41]. In general, gastrin does not affect β cell replication probably because of a lack of the CCK-B receptor on β cells[14], but there is a report suggesting that, in 95% of the pancreatectomized rats, gastrin treatment not only increased β cells neogenesis from ductal cells but also caused both a modest increase in replication and a decrease in apoptosis in β cells with the resultant improvement of glucose tolerance. The detailed mechanism for these activities remains unclear[15]. The replication of β cells is also reported in gastrinoma patients[45] although only β cell islets located near the gastrinomas exhibited β cell turnover despite the fact that serum levels of gastrin were elevated to the degrees to induce clinically apparent gastrointestinal symptoms. Thus, it is possible that other hormones were also involved. On other hand, the synergistic effect of other hormones, such as transforming growth factor-α[46], epidermal

growth factor[47], and glucagon-like peptide-1 (GLP-1)[48], with gastrin has also been demonstrated. For example, GLP-1 induces both β cell replication with mitogens and neogenesis of β cell from ductal cells[49]. In combination with GLP-1, gastrin appears to enhance β cell neogenesis even when it is added in animal models, such as either *db/db* mice (a model of T2DM)[50] or non-obese diabetic (NOD) mice [a model of type 1 diabetes mellitus (T1DM)][48], although, in these models, pancreatic remodeling is not necessarily occurring. In addition, an effect on regulating the autoimmune response against pancreatic β cells by combination therapy was also reported in the NOD mice model[48]. Taken together, these effects of gastrin suggest that this hormone may possess a potential protective effect for the progression of diabetes, especially in combination with other hormones, such as GLP-1.

THE EFFECT OF PPIS ON GLYCEMIC CONTROL IN PATIENTS WITH TYPE 2 DIABETES: RESULTS OF CLINICAL STUDIES

Despite the possible effects of gastrin on both increasing β cell mass and improving glycemic control, gastrin treatment has not been used with the patients with T2DM mainly because of the difficulty with oral administration and the suggested side effects on the stomach. On the other hand, there are many publications describing the effects of PPIs on glycemic control in patients withT2DM.

Mefford *et al*[26] reported that a significant difference was obtained in HbA1c in patients with T2DM taking PPIs (7.0% of HbA1c, n = 65) *vs* those not taking PPIs (7.6% of HbA1c, n = 282, P = 0.002). Similarly, Boj-Carceller *et al*[27] reported that HbA1c was significantly different in T2DM patients who received PPIs (6.7% ± 1.0%, n = 54) compared with those who did not received PPIs (7.3% ± 1.4%, n = 43, P = 0.018). When these patients were assigned to two groups by the treatment of diabetes, those taking insulin and concurrent PPIs had better glycemic control, compared with those taking insulin but not PPI (-0.8% reduction, P = 0.022). In a very recent study, Barchetta *et al*[28], showed that the significantly different HbA1c and FPG levels were found in the T2DM patients with PPIs for longer than 2 years (n = 245) compared with those who did not take PPIs (n = 303) (7.1% ± 1.07% with PPIs *vs* 7.4% ± 1.4% without PPIs for HbA1c, P = 0.011; 127 ± 36.9 mg/dL with PPIs *vs* 147.6 ± 49.6 mg/dL without PPIs for FPG, P < 0.001, respectively). The increase of the differences was observed in patients treated with insulin and in those treated with combination of PPIs and GLP-1 based therapy[28]. The results of these cross-sectional studies suggest the significant association between treatment with PPIs and the improved glycemic control in patients with T2DM.

On the other hand, in a study using a retrospective analysis, patients were assigned to 2 groups: 21 patients who had taken esomeprazole (a PPI) for 11.3± 3 mo and 21 control subjects[29]. Although there was a tendency for a decline in HbA1c in the patients treated with this PPI, it was not statistically significant (8.6% to 7.9%, P = 0.054), while in a subgroup with HbA1c > 9%, the reduction was statistically significant (9.7% to 8.5%, n = 11, P = 0.004). No change in HbA1c was found in the entire control group and in a subgroup with HbA1c > 9.0% in control group (9.2% to 9.0%, P = 0.455; 10.3% to 10.0%, P = 0.287, respectively). Furthermore, Crouch *et al*[30] investigated 71 individuals with T2DM who were not taking insulin. The mean HbA1c was 7.11% during periods taking either prescription or over-the-counter PPIs, *vs* 7.7% during periods not taking PPIs (a significant difference, P = 0.001). Although there was no significant difference in mean HbA1c in a metformin monotherapy (6.81 treated with PPIs *vs* 7.10% treated without PPIs, P = 0.25), mean HbA1c was significantly lower in a concomitant therapy including metformin and/or sulfonylurea and/or glitazone (7.26 treated with PPIs *vs* 7.80 treated without PPIs, n = 27, P = 0.002). However, in another recent retrospective study of T2DM patients with relatively low levels of HbA1c, treatment with PPIs for ⩾ 2 mo (mean duration: 180 d, n = 43) did not significantly change HbA1c levels (6.86% ± 1.10% to 6.77% ± 1.07%). Metformin monotherapy did not change HbA1c compared with a combination therapy including metformin and a therapy in antidiabetic agents not including metformin[24]. Furthermore, 3 recent prospective randomized, double-blind, placebo-controlled studies using PPIs in small number of T2DM patients showed conflicting results with its effect on glycemic control. Singh *et al*[31] investigated the effect of a 12-wk pantoprazole (a PPI) therapy regimen on glycemic control in patients with T2DM[31]. Thirty one eligible patients were randomly assigned to take either pantoprazole (n = 16) or placebo (n = 15). Pantoprazole (40 mg twice daily) significantly increased both plasma levels of gastrin (54.4 ± 14.9 to 75.6 ± 15.1 pg/mL, P < 0.001) and those of insulin (10.5 ± 4.0 to 13.9 ± 4.5 μU/mL, P < 0.001) and improved the function of β cell as calculated by the homeostasis model assessment-β (HOMA-β). HbA1c significantly decreased with pantoprazole therapy (7.60% ± 1.17% to 6.80% ± 1.16%, P < 0.001). The decrease of HbA1c was positively associated with a significant elevation in both gastrin and insulin levels. González-Oritz *et al*[32] investigated the effect of pantoprazole (40 mg once daily for 45 d) on secretion of insulin in 14 drug naive patients with T2DM. Significant increases in both the late insulin phase (215 ± 127 to 308 ± 151 pmol/L, P = 0.028) and total insulin secretion (174 ± 94 to 265 ± 135 pmol/L, P = 0.028), and significant decreases in HbA1c levels (7.5% to 6.6%, P = 0.018) were found with pantoprazole administration (n =

7), while there was no significant changes in these parameters in patients treated with placebo (n = 7). On the other hand, Hove *et al*[25] investigated the effect of esomeprazole on glycemic control in 41 T2DM patients using either dietary control or treatment with oral anti-diabetic agents. These patients were randomly assigned to take either add-on esomeprazole (40 mg daily, n = 20) or placebo (n = 21) during 12 wk[25]. In the esomeprazole group, the area under the curve (AUC) for insulin did not change, while the AUC for the placebo group significantly decreased. Esomeprazole treatment caused a nine-fold elevation in the AUC for gastrin. Contrary to the expectation, HbA1c increased from 7.0% ± 0.6% to 7.3% ± 0.8% (P < 0.05) in the esomeprazole group and from 7.0% ± 0.6% to 7.4% ± 0.8% (P < 0.05) in the placebo group with no significant difference in change between both treatments (unadjusted, P = 0.297). These clinical findings from all of these studies are summarized in Table 1. Based on the published data to date, the degrees of the reduction of HbA1c by PPIs therapy in the studies with positive results appears to be approximately 0.6%-0.9%. This is somewhat milder or similar compared with those by recent available anti-diabetic drugs such as dipeptidyl peptidase-4 (DPP-4) inhibitors[51] or sodium-glucose co-transporter 2 inhibitors[52]. This suggests that the effect of PPI for glycemic control is probably moderate and that therefore PPI may have the potential for clinical benefit on glycemic control in patients with T2DM.

THE USE OF PPIS FOR THE TREATMENT OF TYPE 2 DIABETES: INTERPRETATION OF THE RESULTS AND POSSIBLE MECHANISMS OF GLYCEMIC CONTROL

As shown in the previous section, it appears that PPIs generally have a beneficial effect on glycemic control for T2DM patients with some studies showing no effect. The results of the different studies do not appear to be dependent on the type of PPI used. Based on the results of most clinical studies in which glycemic control was improved[26-32], it appears that the actual basal levels of HbA1c may be important for the PPIs to show the apparent glucose-lowing effect because PPIs significantly decreased HbA1c level only when the basal HbA1c level was high in 1 retrospective study[29]. In addition, the patients in most of the studies with negative results had a tendency to be under good glycemic control (approximate 7.0% of HbA1c)[24,25], compared with those studies that showed positive results[26-32]. In addition, treatment with PPIs and HbA1c levels were independent from possible confounders in a multivariate regression analysis in 1 study[28], suggesting the importance of baseline HbA1c levels for the glucose lowering effect of PPIs. Next, if the possible effect of PPIs on glycemic control is based on the mechanism of increase of β cell mass, treatment with PPIs for a longer period may be more effective in providing the full effect on glycemic control compared with that observed in most of the previous studies. However, in fact, the mechanism of the clinical effect of PPIs on glycemic control largely remains unclear. Because gastrin does not affect β cell neogenesis from the adult pancreatic ductal cells under a non-remodeling state as previously described[14,15], it is not apparent whether the elevation of circulating gastrin levels induced by PPIs can really promote the increase of the mass of β cell in patients with T2DM, in whom pancreatic remodeling is not necessarily occurring. Nonetheless, elevated serum gastrin levels could affect the β cell mass in animal models of T2DM although the mechanism is not fully apparent. PPI mono therapy improved glycemic control with the increase in both plasma insulin and β cells mass in *Psammomys obesus*, an animal model of T2DM[23]. In this study, a significant effect was obtained only when the PPI was used at a very high dose (lansoprazole 10-15 mg/kg); gastrin was elevated nine-fold at this dose. Since vonoprazan (a new generation PPI: potassium-competitive acid blocker) is more effective for inhibition of secretion of gastric acid and increases serum levels of gastrin (approximate six- to seven-fold with 10-40 mg of vonoprazan) compared with that of the existing PPIs[53], it would be interesting to investigate in a future study whether this agent is also more effective on glycemic control. However, it is important to note that such elevation of serum gastrin levels by PPIs is not always needed to exhibit the clinically apparent glucose-lowing effect in T2DM patients because, in the study by Singh *et al*[31], in which positive results were obtained, the increase of gastrin by a PPI (pantoprazole) was only approximately 1.5-fold[31], which was accompanied with an increase of insulin. These findings suggest the possibility that mechanisms other than the increase of β cell mass are also involved. One possible mechanism involves a gastrin-stimulated increase in insulin secretion by pancreatic β cells. It has been reported that because the secretion of the endogenous gastrin for the oral glucose tolerance test (OGTT) in healthy subjects is very small, it is unlikely that gastrin strongly promotes insulin secretion under this condition. However, an ordinary protein-rich meal (but not glucose-rich) largely increases both circulating gastrin and insulin levels[2]. Therefore, gastrin appears to significantly stimulate secretion of insulin during and after a meal, this may partially explain the effect of PPIs on glycemic control. Another mechanism may involve the interaction of gastrin with other gastric hormones, such as ghrelin, which is reported to have an important role in energy homeostasis and appetite regulation. There is a report showing that ghrelin was down-regulated in primary gastric cells during gastrin-stimulation, and that ghrelin and gastrin levels had a significant negative correlation in humans. For example, a long-term 3-fold increase of

Table 1 Studies showing glucose-lowing effect of proton pump inhibitors in patients with type 2 diabetes

Mefford *et al*[26]	Outcome measures: HbA1c levels in patients with type 2 diabetes taking PPIs (n = 65) *vs* those not taking PPIs (n = 282) was evaluated in cross-sectional design Key findings: There was a significant difference in HbA1c in patients taking PPIs *vs* those not taking PPIs (7.0% *vs* 7.6%, P = 0.002) Safety information: No information is described
Boj-Carceller *et al*[27]	Outcome measures: HbA1c levels in patients with type 2 diabetes taking PPIs (n = 54) *vs* those not taking PPIs (n = 43) was evaluated in cross-sectional design Key findings: HbA1c was significantly lower in type 2 diabetic patients who take PPIs compared with those not taking PPIs (6.7% ± 1.0% *vs* 7.3% ± 1.4%, P = 0.018) Safety information: No information is described
Barchetta *et al*[28]	Outcome measures: HbA1c and FPG levels in patients with type 2 diabetes taking PPIs for longer than 2 yr (n = 245) *vs* those not taking PPIs (n = 303) was evaluated in cross-sectional design Key findings: Patients with PPIs had significantly lower HbA1c (7.1% ± 1.07% *vs* 7.4% ± 1.4%, P = 0.011) and FPG (127 ± 36.9 mg/dL *vs* 147.6 ± 49.6 mg/dL, P < 0.001) levels than those who did not take PPIs Safety information: No information is described
Hove *et al*[29]	Outcome measures: HbA1c levels were retrospectively evaluated in patients with type 2 diabetes. Patients were assigned to 2 groups: 21 patients who had taken esomeprazole (a PPI) for 11.3 ± 3 mo and 21 control subjects Key findings: There was a tendency for a decline in HbA1c in the patients treated with this PPI (8.6% to 7.9%, P = 0.054). In a subgroup with HbA1c > 9% (n = 11), the reduction was statistically significant (9.7% to 8.5%, P = 0.004). No change in HbA1c was observed in the control group (9.2% to 9.9%, P = 0.455) Safety information: No information is described
Han *et al*[24]	Outcome measures: HbA1c was retrospectively evaluated in type 2 diabetic patients treated with PPIs for ⩾ 2 mo (mean duration: 180 d, n = 43) Key findings: There was no significant change in HbA1c levels (6.86% ± 1.10% to 6.77% ± 1.07%; P = 0.406) Safety information: No information is described
Crouch *et al*[30]	Outcome measures: 71 individuals with type 2 diabetes who were not taking insulin was retrospectively investigated for the change of HbA1c Key findings: The mean HbA1c was 7.11% during periods with either prescription or over-the-counter PPIs, *vs* 7.7% during periods without PPIs (a significant difference; P = 0.001) Safety information: No information is described
Singh *et al*[31]	Outcome measures: The effect of a 12-wk pantoprazole (40 mg twice daily) therapy regimen on HbA1c, FPG, serum insulin, serum gastrin levels was prospectively measured in patients with type 2 diabetes in randomized double-blind, placebo-controlled study design. Thirty one eligible patients were randomly assigned to receive either pantoprazole (n = 16) or placebo (n = 15) Key findings: HbA1c and FPG significantly decreased with pantoprazole therapy (7.60% ± 1.17% to 6.80% ± 1.16%, P < 0.001 for HbA1c and 126.3 ± 10.3 to 109.2 ± 13.0 mg/dL, P = 0.017 for FPG), and the differences were significant between the two groups (P = 0.004 for HbA1c, P = 0.019 for FPG). Pantoprazole significantly increased both plasma gastrin (P < 0.001) and insulin levels (P < 0.001) Safety information: Nine patients reported adverse events as nausea, vomiting, headache and myalgia, which were similar and mild in the both groups. None of the patients had hypoglycemia
González-Oritz *et al*[32]	Outcome measures: The effect of pantoprazole (40 mg once daily for 45 d) on insulin secretion in 14 drug naive patients with type 2 diabetes was prospectively investigated in a randomized, double-blind, placebo-controlled study design. Insulin secretion evaluated by hyperglycemic and hyperinsulinemic clamp technique, HbA1c, FPG and serum lipids were measured Key findings: Significant increases in total insulin secretion (P = 0.028), and significant decreases in HbA1c levels (7.5% to 6.6%; P = 0.018) but not FPG levels (P = 0.236) were found with pantoprazole therapy (n = 7), while there was no significant changes in these parameters in patients treated with placebo (n = 7). There were no significant changes in serum lipids in both groups Safety information: Two patients had mild headache (one in each group)
Hove *et al*[25]	Outcome measures: The effect of esomeprazole on glycemic control in 41 type 2 diabetic patients using either dietary control or therapy by anti-diabetic agents was prospectively examined in a randomized double-blind placebo-controlled 2 × 2 factorial study. These patients were randomly assigned to receive either add-on esomeprazole (40 mg daily, n = 20) or placebo (n = 21) for 12 wk. Insulin secretion, HbA1c levels and cardiovascular risk factors were evaluated Key findings: In the esomeprazole-treated group, the AUC (area under the curve) for insulin did not change (P = 0.838), while the AUC for the placebo group significantly decreased (P = 0.002). HbA1c increased from 7.0% ± 0.6% to 7.3% ± 0.8% (P < 0.05) in the esomeprazole-treated group and from 7.0% ± 0.6% to 7.4% ± 0.8% (P < 0.05) in the placebo group (no significant difference in change between both treatments; unadjusted, P = 0.297). The differences in cardiovascular risk factors were not significant between the two groups Safety information: Flatulence in 2 patients and diarrhea in 1 patient was reported in lansoprazole group, and flatulence in 2 patients and intermittent diarrhea in 1 patient was reported in placebo group
Takebayashi *et al*[72]	Outcome measures: The effect of alogliptin and lansoprazole (n = 46) combination therapy compared with alogliptin therapy without lansoprazole (n = 43) on glycemic control was investigated in a randomized open-label study. After 3 mo of treatment, the changes in HbA1c, FPG, serum gastrin were evaluated Key findings: A significant decrease in both HbA1c and FPG (respective 7.6% ± 0.6% to 6.8% ± 0.7%, P < 0.0001, 52.0 ± 35.6 to 127.3 ± 27.4 mg/dL, P < 0.0001 in the combination therapy group, and respective 7.7% ± 0.5% to 6.7% ± 0.5%, P < 0.0001, 153.6 ± 34.4 to 128.5 ± 26.6 mg/dL, P = 0.0001 in the alogliptin therapy group) was obtained. There were no significant differences in changes in HbA1c, FPG (P = 0.2945, P = 0.1901, respectively) and significant elevation in change in gastrin (approximate twofold, P = 0.0004) before and after therapy between the combination and the alogliptin mono therapy group Safety information: In alogliptin group, 1 patient discontinued the drug due to epi-gastric pain. In the combination group, 1 patient withdrew due to a mild cerebral infarction, and 1 patient noticed occasional hypoglycemic symptoms

PPIs: Proton pump inhibitors.

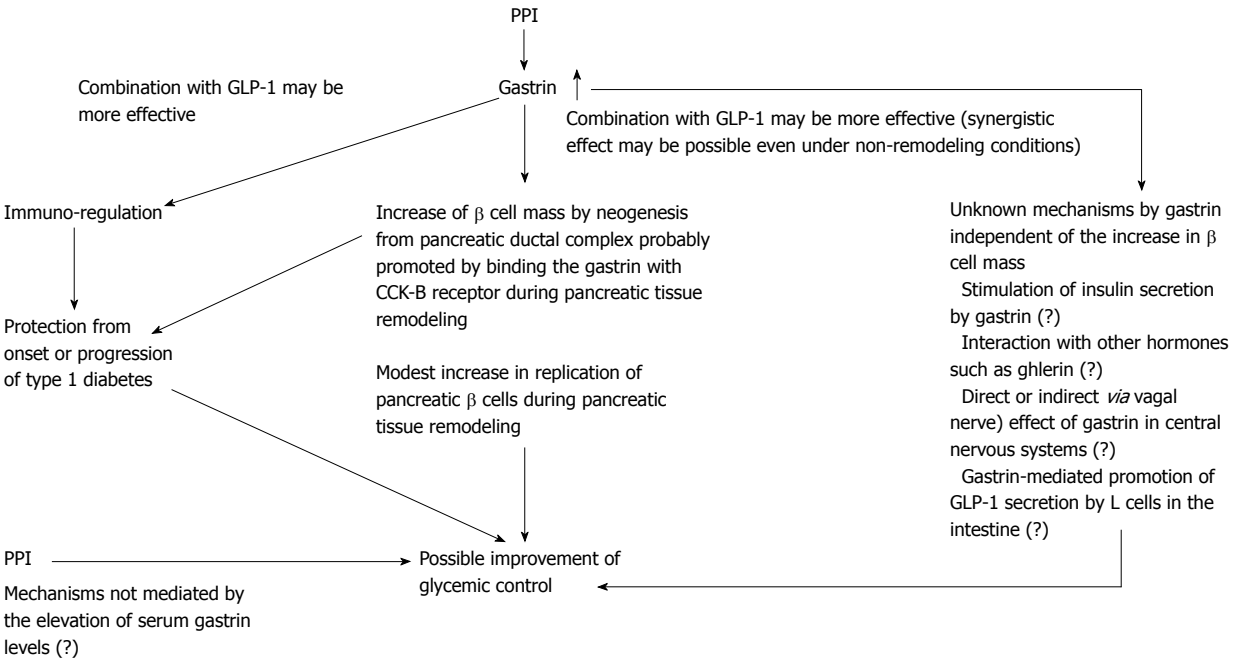

Figure 1 The possible mechanisms of proton pump inhibitors on the improvement of glycemic control. PPIs indirectly elevate serum gastrin levels. Gastrin promotes an increase in β cell mass by neogenesis of the β cells from the pancreatic ductal complex probably promoted by binding the gastrin with CCK-B receptor during pancreatic remodeling. In addition, a modest increase in the replication of pancreatic β cells during pancreatic remodeling is also reported although the mechanisms are not apparent because of the lack of a CCK-B receptor on β cells. Gastrin can enhance the effect of GLP-1 on β cell neogenesis from ductal cells. A synergistic effect may occur even under non-remodeling conditions in the pancreas. These mechanisms appear to contribute to the improvement of glycemic control in both type 1 and type 2 diabetes. Furthermore, a combination of GLP-1 and gastrin may protect from the onset or progression of type 1 diabetes by an immunoregulatory effect. Other possible gastrin-mediated mechanisms independent of the β cell mass increase may include stimulation of insulin secretion, interaction with other hormones such as ghrelin, direct or indirect (*via* vagal nerve) effects in the central nervous systems, and promotion of GLP-1 secretion by L cells in the intestine. Finally, it may be possible that PPIs affect glycemic control by unknown mechanisms independent of the elevation of serum gastrin levels. PPIs: Proton pump inhibitors; CCK-B: Cholecystokinin-B; GLP-1: Glucagon like-peptide-1.

gastrin in autoimmune gastritis significantly repressed ghrelin secretion[54]. These findings suggest the possibility that the increase of gastrin levels is associated with less appetite and improvement of glycemic control *via* the decreased ghlerin levels although there is as yet no clinical evidence. Furthermore, it is known that the CCK-B receptor exists in the brain, especially in the hypothalamic area[8,55]. Intracerebroventricular injection of gastrin decreases food intake, while inactivation of CCK-B receptor in mice changes the regulation of food-intake and body weight, and results in obesity[56]. Despite the limitation of gastrin diffusion into the brain due to the blood brain barrier (BBB)[57], there are reports suggesting that either peptide or peptide fragments might penetrate into the brain because of the lack of a BBB in the cirvumventricular organs[58], and that intravenous gastrin administration activated neurons in several portions of brain[59]. In addition, it is reported that gastrin in circulation is able to stimulate the area postrema neurons that express the CCK-B receptor and project to the nucleus of the solitary tract (NTS)[60]. Mouse brain stem NTS-proopiomelamocortin neurons are associated with feeding-induced satiety[61]. Therefore, we speculate that it might be possible that increased serum gastrin that is regulated by PPIs directly inhibits appetite *via* the central nervous system, although it may be possible that gastrin also acts indirectly brain stem *via* the vagal nerve[60]. In addition, a recent study revealed that gastrin stimulates GLP-1 secretion in L cells in the intestine[62]. This can explain the possible effect of PPIs on glycemic control at least in part. Finally, it may also be important to consider whether PPIs potentially have a beneficial effect on glycemic control *via* unknown mechanism independent of gastrin. Taken together, the mechanisms of the possible PPI effects on glycemic control largely remain unclear, and multiple mechanisms appear to be involved. These possible mechanisms are described in Figure 1.

When treating patients, it is important to consider the potentially deleterious effects of PPIs on glycemic control, which may be more serious than the possible beneficial effect and which may modify the results. It is known that diabetes occasionally occurs with gastroesophageal reflux disease (GERD)[63,64]. Because PPIs largely improve GERD clinical symptoms, it may be possible that the appetite of the patients with GERD is improved even if the elevation of gastrin levels by PPIs influences circulating ghrelin levels as previously described. These patients can thus potentially have worse glycemic control. In addition, it is reported that PPIs can induce dysbiosis[65], which is connected with metabolic syndrome. Therefore, we speculate that PPIs can worsen glycemic control in this manner as well.

THE EFFECT OF COMBINATIONAL THERAPY OF PPIS (OR GASTRIN) WITH DPP-4 INHIBITORS (OR A GLP-1 RECEPTOR AGONIST) ON GLYCEMIC CONTROL IN TYPE 1 AND TYPE 2 DIABETES IN BOTH ANIMAL AND CLINICAL STUDIES

Recent evidence suggests the greater potential beneficial effect of a combination therapy of various hormones over that of a mono hormone therapy[66]. As described in the previous section, gastrin enhances the effect of GLP-1 on β cell neogenesis, and this combination therapy more effectively improved hyperglycemia than mono therapy by each hormone in NOD mice[48]. This result is also supported in the same animal model by combination therapy with DPP-4 inhibitors, which block degradation of GLP-1 by DPP-4 resulting in the elevation of serum active GLP-1 levels, and PPIs[67]. Furthermore, Patel *et al*[68], showed that combination therapy with exendin-4 (a GLP-1 receptor agonist) and omeprazole (a PPI) had better glycemic control compared with mono therapy with these drugs in *db/db* mice. Recently, Hao *et al*[69] examined the effects of short periods of lansoprazole, sitagliptin (a DPP-4 inhibitor), and these concomitant therapy on glycemic control in mice with diet-induced obesity (DIO) and in healthy human subjects. In the DIO mice, lansoprazole therapy significantly improved glucose levels and increased both circulating insulin and C peptide levels than treatment in vehicles. Furthermore, concomitant treatment with lansoprazole and sitagliptin decreased glucose levels with higher levels in C-peptide and insulin compared to that with sitagliptin-treated mice. In a human study, the concomitant use (sitagliptin 100 mg daily and lansoprazole 30 mg daily) for 6 d resulted in significant decrease of glucose levels and increase of insulin levels in an OGTT *vs* the control, lansoprazole-, and sitagliptin-treated groups. Taken together, the results of these studies suggest the possibility that combination therapy with a GLP-1 receptor agonist (or DPP-4 inhibitors) and gastrin (or a PPI) may provide a more beneficial effect for glycemic control than each mono therapy. In addition, in *db/db* mice, a GLP-1-gastrin dual receptor agonist has showed a more continued regulatory effect of glucose with a significant increase in β-cell mass in pancreatic tissue than that of monotherapy in liraglutide (a GLP-1 receptor agonist)[70]. However, the results of recent randomized, prospective studies evaluating the combination therapy with DPP-4 inhibitors and PPIs in patients with T1DM and T2DM were basically negative. Griffin *et al*[71] reported the results of a randomized, placebo-controlled, multicenter, phase 2 trial (REPAIR-T1D) on the effect of concomitant use with sitagliptin and lansoprazole in patients with recent-onset T1DM. Patients aged 11-36 years, diagnosed with T1DM within the past 6 mo, were recruited and were randomized (2:1) to take oral sitagliptin with lansoprazole or placebo for 12 mo. At 12 mo, the 2 h C peptide AUC was similar between the combination (n = 40) and placebo (n = 18) groups. HbA1c levels were mainly constant throughout the study period for both groups (no significant difference). HbA1c adjusted by insulin-dose was also similar (no significant difference) for both groups. Although these overall results were negative, this study is still ongoing with reassessments at both 18 and 24 mo. In T2DM, we investigated the effect of alogliptin (a DPP-4 inhibitor) and lansoprazole (n = 46) combination therapy compared with alogliptin therapy without a PPI (n = 43) on glycemic control in a randomized open-label study[72] (Table 1). At 3 mo after the initiation of the therapy, the changes in HbA1c, FPG, HOMA-β, HOMA-insulin resistance (IR) and serum gastrin were evaluated. A significant decrease in both HbA1c (7.6% ± 0.6% to 6.8% ± 0.7%, $P < 0.001$ in the combination therapy group, and 7.7% ± 0.5% to 6.7% ± 0.5%, $P < 0.001$ in the alogliptin therapy group) and FPG (152.0 ± 35.6 to 127.3 ± 27.4 mg/dL, $P < 0.001$ in the combination therapy group, and 153.6 ± 34.4 to 128.5 ± 26.6 mg/dL, $P = 0.001$ in the alogliptin therapy group), and a significant increase in HOMA-β were noted in both groups. However, significant differences were not obtained in the changes in HbA1c, FPG, and HOMA-β by therapy between the combination and the alogliptin mono therapy group ($P = 0.2945$, $P = 1901$, $P = 0.3042$, respectively). The levels of serum gastrin in the concomitant group was significantly elevated compared with those in the alogliptin mono therapy group ($P = 0.0004$). With the combination therapy, the serum gastrin levels increased approximately two-fold. Apart from the issue of the period of the administration, one of the possible reasons for these negative results may be due to the use of DPP-4 inhibitors rather than a GLP-1 receptor agonist with the PPI. The elevation of GLP-1 levels by DPP-4 inhibitors is relatively small compared with that observed with the GLP-1 receptor agonist. Therefore, despite the reports with the positive results on glycemic control using a combination of a PPI and DPP-4 inhibitors[67,69], the effect may be small when compared to that observed with the combination of a PPI and a GLP-1 receptor agonist. The clinical data on the combination therapy of a PPI and a GLP-1 receptor agonist in patients with T1DM and T2DM are not available yet, but this therapy appears to be an attractive one, and future studies are warranted to confirm the effect of this combination therapy.

CONCLUSION

Although PPI therapy is attractive as a new approach for the therapy of diabetes (especially T2DM), the clinical effect on glycemic control of this drug is not yet fully established. The mechanisms of the clinical effect of PPIs on glycemic control are also not fully elucidated. A prospective, long term, randomized, double-blind,

placebo-controlled study on PPIs in a larger number of the T2DM patients is warranted to confirm the effect of PPIs on glycemic control, especially in patients with relatively poor glycemic control. The combination therapy of a PPI with a GLP-1 receptor agonist (rather than DPP-4 inhibitors) may improve glycemic control in both T1DM and T2DM. A clinical study with a large number of patients is needed to establish the potential efficacy. At present, the clinicians' concerns are whether the patients can have better glycemic control when PPIs are used for GERD or gastric ulcers in patients with T2DM, because the use of PPIs is not yet allowed for T2DM treatment in every country. If the treatment is for a long-term period, it is also important to consider the possible harmful effects of PPIs, including bone fracture[73] and small intestine bacterial overgrowth[74].

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REVIEW

Dysglycemia after renal transplantation: Definition, pathogenesis, outcomes and implications for management

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Abstract

New-onset diabetes after transplantation (NODAT) is major complication following renal transplantation. It commonly develops within 3-6 mo post-transplantation. The development of NODAT is associated with significant increase in risk of major cardiovascular events and cardiovascular death. Other dysglycemic states, such as impaired glucose tolerance are also associated with increasing risk of cardiovascular events. The pathogenesis of these dysglycemic states is complex. Older recipient age is a consistent major risk factor and the impact of calcineurin inhibitors and glucocorticoids has been well described. Glucocorticoids likely cause insulin resistance and calcineurin inhibitors likely cause β-cell toxicity. The impact of transplantation in incretin hormones remains to be clarified. The oral glucose tolerance test remains the best diagnostic test but other tests may be validated as screening tests. Possibly, NODAT can be prevented by administering insulin early in patients identified as high risk for NODAT. Once NODAT has been diagnosed altering immunosuppression may be acceptable, but creates the difficulty of balancing immunological with metabolic risk. With regard to hypoglycemic use, metformin may be the best option. Further research is needed to better understand the pathogenesis, identify high risk patients and to improve management options given the significant increased risk of major cardiovascular events and death.

Key words: Management; Epidemiology; Pathogenesis; Renal transplantation; Diabetes

Core tip: New-onset diabetes after transplantation (NODAT) carries a significant cardiovascular burden. Its pathogenesis is multifactorial and includes modifiable factors. New insights into glucose and insulin homeostasis may lead to improved ability to identify high risk patients and to the development of management strategies that do not require alteration in immunosuppression, whilst simultaneously reducing the risk of NODAT.

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INTRODUCTION

Dysglycemia post renal transplantation, encompassing new onset diabetes after transplant (NODAT), impaired fasting glucose (IGF) and impaired glucose tolerance (IGT), is a challenging clinical problem. However, despite more than two decades of research the pathogenesis of post-transplant dysglycemia is incompletely understood and a consensus on approach to screening, diagnosis and management is lacking. This review will outline the issues of defining the clinically important states, detecting and predicting their development, the progress that has been made in understanding their pathogenesis and relationship to described risk factors (particularly immunosuppression therapies) and the implications for management and further research into this significant post-transplant complication.

DEFINITION

There have been several changes in the definition of dysglycemia post transplantation over time. Initially referred to as diabetes after renal transplantation, this name failed to capture the important distinction of those who were diabetic pre-transplant from those who developed diabetes after transplant. The term post-transplant diabetes mellitus (PTDM) also failed to clearly distinguish between the two states. The most common term currently used is new-onset diabetes after transplant (NODAT); however, this too fails to capture those with new onset IGT, which is also associated with poorer outcomes (see below). Some have proposed the term "transplant associated hypergylcemia"[1], which captures the impact of dysglycemia, as opposed to the worst category of dysglycemia alone (diabetes), however it does not to make a distinction between those who came to transplant with a dysglycemic state and those who developed it after transplantation.

Prior to 2003 the most common criteria used for the diagnosis of post-transplant diabetes was use of hypoglycemic agents. However, this is reliant upon clinician awareness of the results of appropriately timed and collected glucose testing and remains an insensitive marker of NODAT. With enhanced understanding of the pathophysiology of post-transplant dysglycemia and its clinical significance a more sensitive and clinical useful definition is needed. In 2003 an international expert panel devised a consensus document[2] that adopted the World Health Organisation/American Diabetes Association (WHO/ADA) guidelines for the testing and defining of dysglycemic states post-transplant [fasting blood glucose level (F BGL) $\geqslant$ 7.0 mol/L; 2-h BGL $\geqslant$ 11.1 mmol/L], based on the definitions used for the general population. However, whilst there is consensus on the interpretation of blood glucose levels, there is no consensus on who to test, when to test and which test to use. Table 1 shows the wide range of tests used and timing of these in studies that have reported NODAT outcomes: F BGL, random blood glucose level (R BGL), 2-h 75 g oral glucose tolerance test (oGTT), HbA1c at 10 wk, 3 mo, 6 mo, 1 year and use of hypoglycemic agents at 30 d. Furthermore, there is little recognition in the literature of the importance of reporting and understanding the significance of dysglycemic states other than NODAT such as IGT or IFG. Few studies report incident rates and/or outcomes of such dysglycemic states. As a result, drawing conclusions based on research in this area has unavoidable caveats, which can only be addressed by large multi-centred well designed trials with post-transplant dysglycemia as the primary outcome.

EPIDEMIOLOGY

One of the confounders in any study of NODAT is the rate of pre-transplant unrecognised dysglycemia. Table 2 shows the rates of unrecognised dysglycemia in patients on the transplant waiting list. Bergrem *et al*[30] investigated 889 Norwegian transplant wait listed candidates who were not clinically suspected to have diabetes. The majority of patients (62%) were not on dialysis and only 12% were on glucocorticoids. All patients underwent an oGTT. Using WHO/ADA diagnostic criteria, 330 (37.1%) patients were found to have dysglycemia, in addition to which, 72 (8.1%) were found to have diabetes. Importantly, of those patients found to be diabetic on oGTT, only 22% were identified by F BGL testing alone. Further receiver operating curve (ROC) analysis demonstrated that using a cut-off of 92 mg/dL (5.1 mmol/L) for F BGL testing as the threshold for initiating an oGTT detected 90% of the diabetic patients, requiring 53% of the wait listed patients to be tested.

It is interesting to note that not all patients with dysglycemia pre-transplant develop persistent post-transplant dysglycemia (IGF, IGT or NODAT). Caillard *et al*[31] screened 243 patients at time of wait listing with oGTT and found 37 (15.2%) dysglycemic patients and eight (3.3%) newly diagnosed diabetic patients. The time from pre-transplant oGTT to transplantation was not documented; however, 50% of the dysglycemic patients developed NODAT, 23% remained dysglycemic and 14% become normoglycemic post transplantation. In 26% of those diagnosed with NODAT, this abnormality could only be detected by oGTT. A Japanese study in which patients with no known history of diabetes were administered an oGTT two weeks before receipt of a living donor transplant, found that 30.4% were dysglycemic with an additional 4.0% found to be diabetic[32]. Hornum *et al*[33] found 33% dysglycemia rate pre-transplant (n = 57) and over 12-mo follow up the pre-transplant dysglycemia was not associated with the development of NODAT. Interestingly, they too

Table 1 Selection of studies that reported rates of new-onset diabetes after transplantation or other dysglycemic states

Ref.	Criteria	*n*	Rates
Cosio *et al*[3]	Use of medications, F BGL	490	13% at 1 yr 33% dysglycemic
Hjelmesaeth *et al*[4]	Use of medications, F BGL, oGTT	201	20% at 3 mo
Vincenti *et al*[5]	oGTT	682	30% at 6 mo dysglycemic
Delgado *et al*[6]	oGTT, F BGL	374	6.7% at 4.1 yr 25.1% dysglycemic
Ramesh Prasad *et al*[7]	F BGL or R BGL	151	20.5%
Luan *et al*[8]	oGTT	203	11.8% at 10 wk 47.8% dysglycemic
Bayer *et al*[9]	Use of medications, F BGL, R BGL	640	31.4% at 1 yr
Bergrem *et al*[10]	Use of medications, F BGL, R BGL	301	13% at 10 wk
Valderhaug *et al*[11]	oGTT	1410	17% at 10 wk 38% dysglycemic
Ciancio *et al*[12]	Use of medications	150	15%-22% at 4 yr
Israni *et al*[13]	Medications, F BGL	1840	13% at 5 yr
Wauters *et al*[14]	Use of medications, F BGL	1146	14.1% at 1 mo, 11.1% at 4 mo, 13.4% at 1 yr 27%, 34.3% and 29.8% dysglycemic
Chan *et al*[15]	oGTT	292	24% at 6 mo
Vacher-Coponat *et al*[16]	Use of medications	289	16.8%-18.8% at 3 yr
Tillman *et al*[17]	oGTT	200	5% at 39 mo 30.5% dysglycemic
Bonet *et al*[18]	F BGL, R BGL, oGTT	138	13% at 6 mo
Cole *et al*[19]	Use of medications, F BGL, oGTT	49	4% at 6 mo
Nagaraja *et al*[20]	Use of medications, F BGL	118	21% at 3 mo, 37% at 1 yr
First *et al*[21]	Use of medications, F BGL, HbA1c	634	17.8%-36.5% at 1 yr
Nagaraja *et al*[22]	oGTT	76	13% at 5 yr, 24% at 11 yr 42% and 61% dysglycemic
Tokodai *et al*[23]	Use of medications, F BGL, R BGL	145	11.7% at 1 yr
Viecelli *et al*[24]	oGTT	83	17% at 3 mo, 15% at 15 mo 31% and 21% dysglycemic
Weng *et al*[25]	Use of medications, F BGL, R BGL	166	29.5%
Schweer *et al*[26]	R BGL, HbA1c	526	16.7%
Prasad *et al*[27]	oGTT	439	20% at 3 mo 33% dysglycemic
Silva *et al*[28]	HbA1c	638	21.3%-41.1% at 4 yr
Lv *et al*[29]	F BGL	428	20.3% at 5.7 yr

Definitions diabetes: F BGL ⩾ 7.0 mmol/L (126 mg/dL) or ⩾ 11.1 mmol/L (200 mg/dL) on oGTT or R BGL ⩾ 11.1 mmol/L (200 mg/dL) plus symptoms. Other dysglycemic states. IFG: ADA criteria 5.6-6.9 mmol/L (100-125 mg/dL); WHO criteria 6.1-6.9 mmol/dL (100-125 mg/dL); IGT: oGTT 7.8-11.0 mmol/L (140-199 mg/dL). F BGL: Fasting blood glucose level; R BGL: Random blood glucose level; oGTT: 2-h oral glucose tolerance test.

documented a small group of pre-transplant diabetic patients in whom the diabetic state remitted post-transplant.

The case finding described by table two highlights key differences in glucose homeostasis between end stage kidney disease (ESKD) uremic patients and the general population. Approximately 70% of general population patients can be diagnosed as diabetic *via* a F BGL[34], as compared to 22% in the Norwegian transplant wait listed cohort. Moreover, the incidence of new diagnosis of diabetes in wait listed patients on dialysis is approximately 5%-6% per year[33,35] (when using oGTT diagnostic criteria), compared with approximately 0.7%-1.3% per year in the general population[36]. These figures ought to give the reader cause to be cautious with regard to the interpretation of rates of post transplantation dysglycemia and diabetes. This is particularly the case when reviewing retrospective data, in which often only a pre-transplant F BGL is available and the time from glucose testing to transplantation may extend for many months. It may be that the denominator in the quoted rates of NODAT includes patients who were not normoglycemic at time of transplantation. This assessment is further complicated by the possibility that dysglycemia pre-transplant may not be a sufficient factor for dysglycemia post-transplant state (see below).

Further complicating the interpretation of incident rates of dysglycemia post-transplant is the spontaneous remission and normalisation of blood glucose levels observed in some patients. For example, early dysglycemia, such as in the period of hospitalisation post-transplant, is common and occurs in 75%-90% of patients within the first week[37-39]. Luan *et al*[8] in a prospective study of 203 non-diabetic patients showed the mean day 3 F BGL to be 124-134 mg/dL (6.9-7.4 mmol/L). Such dysglycemia should not be dismissed as due entirely to peri-operative factors, as some data suggests that day 7 F BGL may be predictive of NODAT at 1 year[40]. A recent clinical study measured continuous capillary blood glucose levels for the first 4 d post-transplant in 43 patients. There was a considerable

Table 2 The rates of unrecognized dysglycemia in patients on the transplant waiting list

Ref.	Unrecognised on waiting list - diabetes	Unrecognised on waiting list - dysglycemia
Ramesh Prasad *et al*[7]	-	15%
Hornum *et al*[33]	-	33%
Bergrem *et al*[30]	8.1%	45.2%
Iida *et al*[32]	4%	30.4%
Caillard *et al*[31]	3.3%	15.2%
Bonet *et al*[18]	< 0.1%	8.9%

burden on hyperglycemia with 43% having blood glucose above 7.7 mmol/L for more than 12 h per day. The incidence of NODAT at 72 mo was 18.6% and the authors suggested that the day 1 capillary blood glucose may identify those at risk[41]. Moreover, one study found that only 4% of patients normoglycemic early post-transplant later developed NODAT[42] and a normal oGTT within the first week has been shown to have a NPV of 97.6% for later NODAT development[43]. However, it is important to note that not all patients with early hyperglycemia develop permanent dysglycemic states, as there is a considerable degree of transience and variation in dysglycemic states[33]. For example, a Chinese study, employing F BGL for NODAT found an incident rate of 20.32% after a mean follow up of 5.65 years in patients who survived more than one year post transplantation. Of these, 65.5% developed NODAT within 1 year and 17.2% had transient NODAT[29]. Furthermore, such transience likely occurs within the first 3-6 mo. In an international trial comparing standard and reduced dose tacrolimus (Tac) the cumulative incidence at 6 mo of NODAT was 30.3%; however, the incidence in each group was lower at 6 mo compared to 3 mo (23.9% *vs* 28.4% and 13.2% *vs* 15.2%)[15].

Notwithstanding the notable degree of transient dysglycemia, persistent NODAT often develops within 3 to 6 mo following renal transplantation. A mean time to diagnosis of 4.3 mo has been reported[44]. This may help to determine the optimal time of testing. Using oGTT testing at 10 wk post transplantation, Valderhaug *et al*[11,45] reported an incidence of NODAT of 14%-17%. Most studies find that NODAT develops early and this is confirmed by analyses of large data sets. For instance, an analysis of the organ procurement and transplantation network (OPTN) registry data has found a cumulative incidence of NODAT of only 16.2% at 3 years (registry data is limited by the nature of reporting of outcomes), the majority had developed within the first year post transplantation[46]. Similar results have been reported in a United Sates cohort of 640 patients with a mean F BGL of less than 100 mg/dL (5.6 mmol/L) at time of transplantation. NODAT occurred in 31.4% of patients over 1 year, the majority of which had occurred within the first 6 mo (26.4% of total population by 6 mo). By 5 years post transplantation, 46.3% of previously believed to be non-diabetic patients had a diagnosis of NODAT[9].

With regard to any dysglycemia (IGT/IFG or NODAT), a moderate sized (n = 203) prospective study of the risk of developing dysglycemia post transplantation, documented a rate of 47.8% when tested at 10 wk with an oGTT and applying WHO/ADA diagnostic criteria[8]. Retrospective data has found rates of 39.7% who remained normoglycemic throughout the first year post-transplant[47]. A study specifically designed to determine the rates of pre-diabetic dysglycemia found 30.5% of patients met accepted criteria using an oGTT at a median of 39 mo post-transplant[17]. Similarly, in a large international study designed to determine the differences in diabetogenesis of cyclosporin (CsA) and Tac, at 6 mo post-transplant only 300 out of 587 patients (51.1%) remained normoglycemic[5]; however, the criteria for definition of NODAT was need for medications at greater than 30 d. A cross sectional study of multiple Spanish centres found a rate of dysglycemia of 31.8% at almost 4 years post-transplant, the majority detected by oGTT[6]. It is interesting to note that 58.8% of the dysglycemic patients had a simultaneous normal F BGL.

The above discussions reveal notable limitations when quoting rates of post-transplant dysglycemic states or NODAT alone. Whilst there is consensus with regard to blood glucose cut-off values, it is unclear which test should be employed and at which time post-transplant. Furthermore, the witnessed remission of some pre-transplant dysglycemia to normoglycemia post-transplant[19,37] (although this has not been commonly documented), further complicates analyses of rates of new-onset post-transplant dysglycemia.

RISK FACTORS

Multiple risk factors have been associated with the development of NODAT (Table 3) many of which are not modifiable. The most consistently found risk factor is advancing age appreciated since the recognition of NODAT in the early period of use of CsA[79] Increasing age has been found to be a risk factor in small and large retrospectively analysed and prospectively collected data sets, including registry datasets in which the prevalence of NODAT may have been underestimated[8,13,17,26,46,49,52-54]. Male gender, family history of diabetes and APCKD are documented as risk factors, but not consistently[46,49,54-57,61,62]. With regard to genetic risk multiple polymorphisms, including mitochondrial, have been described as contributing risk to the development of NODAT[53,54,63-67]. A closer analysis of genetic polymorphisms and their associated risk is beyond the scope of this review.

Transplant related factors: Calcineurin inhibitors

Potentially modifiable risk factors can be divided into transplant specific and generic. Of the generic, increasing body mass index (BMI) is associated with increased incidence of NODAT when categorised into intervals of 5 with < 20 as a reference, with increased

Table 3 Modifiable and non-modifiable risk factors associated with new-onset diabetes after transplantation or dysglycemic state

Variable	Ref.	Comment
ATG-divided dose	Stevens *et al*[48]	Increased dysglycemia compared to single dose in patients treated with Tac and sirolimus
African American	Kasiske *et al*[49]	OR = 1.68
	Shah *et al*[50]	RR = 1.38
	Johnston *et al*[51]	HR = 1.56
	Bayer *et al*[9]	HR = 1.35
Age	Kasiske *et al*[49]	Strong independent risk factor RR: 1.9-2.6
	Cole *et al*[52]	27707 registry patients OR: 1.33 If > 60 yr
	Ghisdal *et al*[53]	OR 1.03 of NODAT for each 6 mo of age
	Luan *et al*[8]	Increasing age associated with dysglycemia and new onset metabolic syndrome
	Luan *et al*[46]	Analysis of 25837 registry patients, increase in NODAT in each categorised group compared to reference 18-34 years old
	Israni *et al*[13]	HR: 1.33 of NODAT at 60 mo
	Tillmann *et al*[17]	Increase in dysglycemia at mean of 56 M post-transplant; RR of 1.28 for each 5 yr
	Mccaughan *et al*[54]	OR 1.4 per decade in 427 Northern Irish patients
	Schweer *et al*[26]	NODAT 56.1 yr *vs* 47.9 yr; $P < 0.01$
APCKD	de Mattos *et al*[55]	Increased 1 yr incidence in a matched cohort
	Hamer *et al*[56]	Multivariate analysis OR 2.4
	Johnston *et al*[51]	No increase found in 21564 USRDS patients
	Luan *et al*[46]	Multivariate analysis OR: 1.17
	Ruderman *et al*[57]	No increased risk found
Basiliximab	Aasebø *et al*[58]	Basilixmab (n = 134) *vs* no induction historical control; increased dysglycemic state $P = 0.017$
	Prasad *et al*[27]	In living recipients who elected to receive basiliximab OR 2.34 for NODAT at 3 mo
BMI	Kasiske *et al*[49]	Increased BMI, NODAT RR: 1.7
	Cole *et al*[52]	Multivariate analysis OR 1.76 for NODAT
	Luan *et al*[46]	Analysis of 25837 registry patients. increase in NODAT in each categorised group of BMI compared to reference < 20
	Israni *et al*[13]	BMI ⩾ 30, HR 1.69 for NODAT at 60 mo
CMV	Hjelmesaeth *et al*[59]	Asymptomatic infection OR: 4.0 for NODAT at 10 wk
CNI –	Chan *et al*[15]	NODAT 17% *vs* 31%, low dose *vs* standard dose Tac
Higher levels	Cole *et al*[19]	Single arm study of 49 patients with a 4% 6 mo incidence of NODAT. Early glucocorticoid reduction and low dose CsA
	Suszynski *et al*[60]	Higher Tac levels (plus sirolimus) compared to lower Tac (plus sirolimus) or CsA/MMF higher rates of NODAT with 10 yr FU
CNI –	Vincenti *et al*[5]	RCT. Dysglycemia at 6 mo higher in Tac/MMF *vs* CsA/MMF: $P = 0.05$
Tac *vs* CsA	Cole *et al*[52]	27707 registry patients OR 1.51 for NODAT
	Luan *et al*[46]	Analysis of 25837 registry patients. Increase in NODAT OR: 1.24
	Vacher-Coponat *et al*[16]	No difference in CsA/Aza *vs* Tac/MMF in RCT (n = 289)
	Cotovio *et al*[44]	Retrospective multivariate analysis higher Tac not CsA levels associated with NODAT
Family history of diabetes	Bora *et al*[61]	Recipients from living related donors
	Santos *et al*[62]	Retrospective (n = 303). RR: 3.6 for NODAT
Gender	Kasiske *et al*[49]	Greater risk in males in registry patients
	McCaughan *et al*[54]	OR 2.2 for male gender in 427 Northern Irish patients
Genetic polymorphisms	Ghisdal *et al*[53]	rs7903146 polymorphism of TCF7L2 OR 1.6 of NODAT at 6 mol/L, but not associated with IGT
	Ghisdal *et al*[63]	Summarises known associations
	Kurzawski *et al*[64]	Polish Caucasian patients. Increasing SNPs associated with increased risk, OR = 1.37
	Yao *et al*[65]	Fok1 vitamin D polymorphism associated with NODAT OR 11.8 $P = 0.012$
	McCaughan *et al*[54]	7 SNPs involved with β-cell apoptosis associated with NODAT
	Nicoletto *et al*[66]	Adiponectin gene polymorphism associated with NODAT
	Tavira *et al*[67]	Mitochondrial haplogroup H associated with NODAT in Tac treated patients
Glucocorticoids	Boots *et al*[68]	Early glucocorticoid withdrawal associated with reduced NODAT incidence in the first year
	Ghisdal *et al*[53]	OR 2.78 of NODAT at 6 mol/L if AR treated with glucocorticoids
	Luan *et al*[46]	Analysis of 25837 registry patients. OR 1.42 for NODAT if discharged on maintenance. Glucocorticoid only induction associated with increase in NODAT OR: 1.31
	Rizzari *et al*[69]	Significant reduction in NODAT compared with historical control when glucocorticoids rapidly tapered
	Cole *et al*[19]	Single arm study of 49 patients with a 4% 6 mo incidence of NODAT. Early glucocorticoid reduction and low dose CsA
	Schweer *et al*[26]	Pulse glucocorticoid for BPAR associated with increasing NODAT incidence
HCV +	Kasiske *et al*[49]	HCV+, NODAT RR: 1.3
	Cole *et al*[52]	27707 registry patients OR for NODAT 1.82
	Johnston *et al*[51]	21564 USRDS registry patients, HR: 1.7 for NODAT
	Baid-Agrawal *et al*[70]	14 HCV+ 24 HCV- patients. HCV+ increased insulin resistance; $P = 0.008$
	Luan *et al*[46]	Analysis of 25837 registry patients. Increase in NODAT OR: 1.43
	Lv *et al*[29]	Cohort of 428 Chinese patients. NODAT associated with HCV at mean 5.6 yr follow up, OR = 2.72
	Prasad *et al*[27]	439 Indian patients, OR = 6.37

Hyper-parathyroidism post transplant	Ivarsson *et al*[71]	PTH > 13.8 pmol/L associated with NODAT at 1 yr, OR = 4.25
Impaired glycemic state pre-transplant	Ramesh Prasad *et al*[7]	Higher within the normal range random BSL associated with NODAT
	Bora *et al*[61]	IGT at time of transplant associated with NODAT
	Hornum *et al*[33]	IGT NOT predictive of NODAT
	Cotovio *et al*[44]	Higher fasting BGL associated with NODAT
Magnesium post-transplant	Garg *et al*[72]	1 mol/L lower Mg associated with dysglycemia; no association with 1M CNI trough level
Magnesium pre-transplant	Augusto *et al*[73]	Lower magnesium immediately pre-transplant associated with NODAT; $P < 0.02$
Metabolic syndrome post-transplant	Israni *et al*[13]	MS in first 6-12 mo associated with NODAT by 60 mo, HR = 3.46
	Luan *et al*[8]	10 W dysglycemia associated with MS
	Nagaraja *et al*[22]	Development of MS predicts progressive dysglycemia
Metabolic syndrome pre-transplant	Bayer *et al*[9]	HR: 1.34 for NODAT at 1 yr
Sirolimus	Teutonico *et al*[74]	No improvement when changing from CNI to sirolimus
	Ekberg *et al*[75]	Low dose sirolimus may confer less risk than low dose Tac
	Johnston *et al*[51]	20124 registry patients. Compared to CsA + MMF/AZA: Sirolimus + CsA HR 1.61; Sirolimus + Tac HR 1.66; Sirolimus + MMF/AZA HR 1.36
	Guerra *et al*[76]	RCT (n = 150) Tac/sirolimus *vs* Tac/MMF *vs* CsA/sirolimus. No difference in NODAT
	Gyurus *et al*[77]	Retrospective (n = 514). Sirolimus HR 3.5 for NODAT over 10 yr
	Veroux *et al*[78]	21 NODAT converted to sirolimus, 80% remission of NODAT on basis of F BGL
	Suszynski *et al*[60]	Increased risk with high dose Tac/low dose sirolimus combination

F BGL: Fasting blood glucose level; oGTT: 2-h oral glucose tolerance test; NODAT: New-onset diabetes after transplantation; ATG: Antithymocyte globulin; USRDS: United States Renal Data System; BMI: Body mass index; CMV: Cytomegalovirus; CNI: Calcineurin inhibitors; Tac: Tacrolimus; MMF: Mycophenolate mofetil.

risk in the higher categories of BMI[47]. The most significant transplant specific modifiable risk factors are immunosuppressive medications specifically the use of calcineurin inhibitors (CNI - Tac and CsA) and glucocorticoids. The diabetogenic impact of CsA has been described since the early 1980s[79-82]. The introduction of Tac into clinical practice was associated with less acute rejection and improved graft function but at the expense of a greater incidence of NODAT[83]. The diabetes incidence after renal transplantation trial was first large randomised study (n = 682; not diabetic at baseline n = 567) designed primarily to investigate the increase risk posed by Tac use instead of CsA. The primary endpoint was a 6-mo composite endpoint of dysglycemia (NODAT or IFG) based on oGTT administered at 90 and 180 d. They found 6-mo cumulative incidence of 33.6% in Tac treated patients and 26% in CsA treated patients (P = 0.046). Furthermore, more patients required hypoglycemic treatment in the Tac treated group (P = 0.005) and more patients in the CsA treated group who were not treated with hypoglycemic agents had an improvement in their glycemic state by 6 mo (P = 0.067)[5]. This, however, was in the era of high trough Tac targets of approximately 10-15 in the first 3 mo.

Noting that over time target drug levels have decreased, the use of therapeutic drug monitoring may assist in the management of prevention of rejection and complications of immunosuppression. There is some evidence that dysglycemic states are related the degree of CNI exposure. For example, Chan *et al*[15] randomised 292 patients to low dose Tac (trough level 5-9 for first 3 mo then trough level 3-6 following 3 mo) or standard dose (trough level 10-15 for first 3 mo then trough level 8-12 following 3 mo). All patients received basiliximab, similar doses of MMF and glucocorticoids over the follow up period of 6 mo. Those in the low dose Tac group had significantly less NODAT incidence over 6 mo of follow up, with a tendency towards lower incidence rate of treated diabetes[15]. Similarly the dose response effect with respect to NODAT risk has also been described with the use of CsA with less dysglycemia post-transplant in those treated with low dose CsA (C2 600-800)[19]. Sub-analyses of data from larger trials, such as Efficacy Limiting Toxicity Elimination-SYMPHONY, have also suggested a dose-dependent relationship. SYMPHONY found significantly higher rates of NODAT in the low-dose Tac group, compared with low-dose CsA, low-dose sirolimus or standard dose CsA without induction agent (P = 0.02)[75]. Given the issues with choice of diagnostic test it is not surprising that when analysed according to F BGL there were no significant differences between the groups[84].

As age is commonly identified as a risk factor in univariate analysis, it is important to know if older age interacts with other risk factors. In a multivariate analysis of OPTN data there is a clear increase in risk with increasing age when grouped into age groups using 18-34 years old as a reference group[46]. Amongst the other identified risk factors use of Tac increased risk of NODAT. An analysis of the OPTN registry data compared rates of acute rejection and rates of NODAT and their impacts of graft survival. The rates of acute rejection were less in the older Tac treated patients, but the rates of NODAT were greater in the same older Tac treated group[51]. The authors comment that targeted and individualised use of immunosuppression based on the patient's risk profile may help to ameliorate worse outcomes. Part of this may be to reconsider the use of CNI, in particular Tac, in the older recipient in whom the

development of NODAT may precipitate morbidity and mortality. However, as outlined below, other strategies may be safer and more effective.

Transplant related factors: Glucocorticoids

Oral glucocorticoids form the backbone of many immunosuppressive regimens and the diabetogenic potential of these agents is well documented. The development of diabetes is related to the cumulative exposure to glucocorticoids. The data available on glucocorticoid withdrawal, glucocorticoid free or rapid glucocorticoid tapering suggests an incidence rate of 1%-22% over a 1-5 year follow-up period[12,19,26,46,60,69,85] which compares with rates of 15%-35% in regimens without glucocorticoid maintenance (Table 1). However, not all analyses find a benefit in glucocorticoids avoidance. For example, a meta-analysis of higher quality trials in which patients had glucocorticoid withdrawn within 14 d post-transplant and were treated with CNI/MMF did not find a reduction in NODAT[86]. However, the largest randomised placebo-controlled trial (n = 386) of early glucocorticoid withdrawal within 7 d of transplantation found no difference in the rate of NODAT, although fewer of the NODAT patients required insulin therapy in the early glucocorticoid withdrawal arm[83]. Furthermore, a matched cohort analysis of glucocorticoid free and maintenance therapy with glucocorticoid (n = 190 in each group) there were no differences in renal specific outcomes or any differences between F BGL or use of hypoglycemic agents. It is noteworthy that there was significantly more use of Tac and basixilimab in the glucocorticoid free group[85]. Nonetheless, many other studies do find an advantage to glucocorticoid avoidance. Analysis of United States Renal Data System (USRDS) data found that patients discharged on a glucocorticoid containing regimen had an OR of 1.42 for NODAT compared to those discharged on a glucocorticoid free regimen[46]. These results must be interpreted with caution, as it is not possible to capture the cumulative glucocorticoid exposure in the USRDS database. One small (n = 62) randomised prospective study in which glucocorticoids were ceased in one group by day 10 found a significant decrease in the incidence of NODAT when defined as used of hypoglycemic agents[68]. A more recent pilot study (n = 48) of thymoglobulin induction, MMF, low dose CsA and rapid glucocorticoid reduction in low immunological risk patients found that this protocol resulted in 42 of 48 patients being normoglycemic at 6 mo[19]. A larger single centre population (n = 1291) retrospectively analysed in which NODAT was defined as need for hypoglycemic agents found an incidence rate of only 2%-4% in the first year post transplantation in patients treated with glucocorticoid withdrawal after day 5 post-operative in combination with thymoglobulin induction, CNI plus sirolimus or MMF[69]. This was a significant improvement compared to a non-matched historical control group who received a glucocorticoid containing maintenance regimen. Despite the theoretical benefits of glucocorticoid withdrawal the studies referenced above demonstrate conflicting results[87]. The impact of glucocorticoid exposure of the development of NODAT may be answered by a current trial in which patients of low immunological risk will be randomised to one arm including thymoglobulin induction and glucocorticoid free CNI/MMF maintenance or basixilimab induction and ongoing glucocorticoid exposure[88].

The development of dysglycemia subsequent to the diagnosis and treatment of acute rejection may also disclose the risk of dysglycemia created by glucocorticoid exposure. A single centre review of 526 transplant recipients had a NODAT incidence of 16.7% when defined using ADA/WHO criteria for assessing random blood glucose or HbA1c. They found that there was a greater incidence of acute rejection in patients who developed NODAT and that intensified treatment with glucocorticoid and possible conversion to Tac was associated with increased risk of NODAT on multivariate analysis. However, the analysis did not treat rejection as a time varying co-variate[26].

Transplant related factors: Sirolimus

Sirolimus, a mammalian target of rapamycin (mTOR) inhibitor, is an immunosuppressive agent used in conjunction with, or instead of, calcineurin inhibitors. Clinical data suggests that sirolimus use is not without risk for the development of NODAT[77]. Analysis of USRDS of 2598 patients recorded as having received sirolimus, found that the combination of sirolimus with a CNI created a higher HR for cumulative 1yr incidence of NODAT compared to CNI with mycophenolate/azathioprine (MMF/AZA) or sirolimus with MMF/AZA. A sub-group multivariate analysis of USRDS data of 16861 patients known to have remained on the same immunosuppressant regimen patients treated with the combination of sirolimus and a CNI remained at increased risk of 1 year NODAT[51]. In one study of non-NODAT renal transplant recipients who were switched from CNI to sirolimus there were no improvements noted in the glycemic state of the patients when studied robustly with oGTT. Indeed higher sirolimus levels in the absence of CNI may have increased the risk of NODAT[74].

However, just as with the data on CNI and glucocorticoids there are inconsistent findings in the literature on sirolimus. A recent large (n = 440) prospectively randomised trial found that higher dose Tac, but not high or standard dose sirolimus contributed to the NODAT[60]. A further example is a recent study of patients randomised to tacrolimus/mycophenolate, TAC/sirolimus or CsA/sirolimus. The median follow up was 8 years and the quoted cumulative incidence of NODAT was 19%-32%, with no significant differences between the groups based on the use of hypoglycemic agents[76]. Lastly, as with CNI, it is likely that there is an important interaction between modifiable and non-modifiable risk factors. For example, a multivariate

analysis has found that older age and higher sirolimus trough levels were associated with increased hazard for NODAT[77], once again suggesting that drug level targets in older recipients could be reviewed, for both effect and toxicity.

Transplant related factors: Other medications

Calcineurin inhibitors and glucocorticoids are the most well studied drugs in terms of impact upon glycemic control. There is no data on the contribution of MMF or AZA to the development of dysglycemia. In the transplant literature, there does not appear to be a signal that these drugs may be implicated. Recently, there has been interest in the possibility that basiliximab, a widely used induction agent particularly in the lower immunological risk patients, may be implicated in contributing to dysglycemia; although this is based on two data sets, neither of which were prospective or randomized[27,58]. There is also little data on the contribution of thymoglobulin to the development of NODAT. A study of single dose *vs* divided dose antithymocyte globulin (ATG) induction analysed dysglycemia as a secondary outcome. In this study, fasting blood sugar levels after 1 mo to 6 mo were significantly lower (P = 0.02) in patients who received single dose ATG induction[48].

PATHOGENESIS

The pathogenesis of dysglycemia post transplantation is complex and is widely assumed to be closely aligned to the pathogenesis of type 2 diabetes mellitus. However, this assumption underestimates that the impact of end stage renal failure and dialysis on glucose homeostasis. There is also little known about the histological changes in the graft over time when exposed to persistent NODAT. Small case series have found *de novo* diabetic nephropathy within 5-10 years of diagnosis of NODAT[89,90].

Changes in both insulin resistance and insulin secretion can be shown to underlie the development of the dysglycemia post transplantation. These changes are however dynamic and sometimes transient, particularly in the early post-transplant period. Lastly, the role of changes in incretin hormones remains to be elucidated, as does the impact of the severity of chronic kidney disease (CKD) pre- and post-transplant on insulin metabolism and resistance.

Pre-transplant factors

The dynamic nature of dysglycemic states has been documented by Hornum *et al*[33]. They followed 57 patients from pre- to 12 mo post-transplant. Importantly, none were diabetic on an oGTT pre-transplant, however only 67% were normoglycemic. At 3 mo only 46% were normoglycemic and this increased to 56% by 12 mo. Pre-transplant, patients were compared with uremic controls. The transplanted patients were significantly younger (39 *vs* 47 years old) with shorter period of time on dialysis (24 mo *vs* 45 mo); however, they did not differ in terms of measure of glycemic state. These measures included F BGL, oGTT and then specific validated measures of insulin resistance and secretion. It is noteworthy that both the uremic controls and transplant patients had a worse glycemic state than a small group of healthy controls - despite normal F BGL [5.1 mmol/L (all ESKD) *vs* 5.0 mmol/L]. The normal F BGL would suggest that hepatic gluconeogenesis was not impaired by the ESKD state; however, the ESKD patients had oGTT results of 7.4-7.5mmol/L (*vs* 5.4 mmol/L) and this seemed to be accounted for by increased peripheral insulin resistance. Interestingly, the increased resistance in ESKD patients was matched by increased insulin secretion compared to healthy controls (although not statistically significant). This may have been expected for two reasons. Firstly, ESKD patients will have reduced renal clearance of insulin[91]. Secondly, as insulin resistance and insulin secretion are described as being related in a hyperbolic fashion[92], such that changes in one parameter would be expected to drive compensator changes in the other parameter. Whilst there is evidence in these cohorts of compensatory increase in insulin secretion, it can be postulated that it was insufficient as the ESKD patients had markedly higher oGTT results and 33% were found to have IGT. At 12 mo, 14% of patients had developed NODAT and this was associated with increased insulin resistance and increased insulin secretion, which nonetheless, appeared not to be sufficient to maintain normoglycemia. The development of NODAT was not associated with pre-transplant IGT. However, those who developed dysglycemia tended to be older and have a higher pre-transplant BMI, which may co-vary (although not significant in multivariate analysis) with the noted increased pre-transplant insulin resistance and, again, higher compensatory pre-transplant insulin secretion.

Insulin resistance

Increasingly, understanding the factors responsible for insulin resistance and decreasing insulin secretion is being recognised as important for determining modifiable and treatable causes of NODAT. An increase in insulin resistance would be consistent with exposure to glucocorticoids. Glucocorticoids are believed to impair peripheral glucose uptake, impair hepatic glycogen synthesis and enhance gluconeogenesis. At higher doses they may induce β-cell apoptosis[93]. Furthermore, it has been proposed the diabetogenic risk is not restricted to higher dose of glucocorticoid but also occurs with chronic exposure to low doses[94]. In addition to duration and dose of glucocorticoid, older age and higher BMI also predispose to the development of diabetes in those receiving glucocorticoid treatment[95]. Perhaps it is less well recognised that CKD and uremia may also contribute to insulin resistance. It may be that the relief from uremia, but the nonetheless persistent state of CKD post-transplant contributes to the dynamic nature of post-

transplant dysglycemia. It may also be that whilst clearly the biological stress of transplantation and exposure to diabetogenic medications is crucial in the pathogenesis, the persistence of CKD in certain older and perhaps genetically predisposed patients forms a background milieu upon which the dysglycemia can develop. There has been renewed interest in the contribution of uremia or CKD to insulin resistance and the various mechanisms are beyond the scope of this article. However, when reading literature on post-transplant dysglycemia it is important to remember that transplant patients have had periods of severe CKD/ESKD requiring dialysis and, for the most part, remain a CKD patient[96,97]. One study of 27 diabetic and 35 non-diabetic EKSD patients using a homeostatic model assessment-insulin resistance model to assess insulin resistance found increased insulin resistance in the diabetic patients. The non-diabetic patients with increased insulin resistance had elevated C-peptide levels, indicating a compensatory response maintaining non-diabetic state[98].

Other factors that may increase insulin resistance post-transplant include hepatitis C virus (HCV) and metabolic syndrome. Two studies have found that HCV-positive patients have increased insulin resistance compared to non-HCV transplant patients. One of these studies found a compensatory increase in insulin secretion[99] and one did not find such compensation[70]. On the other hand, CMV, the other recognised diabetogenic virus, seems to be associated with impaired insulin secretion; although, the exact mechanism is not well studied[59]. Whilst metabolic syndrome has been described in the general population to be associated with insulin resistance, there is a paucity of data considering metabolic syndrome and insulin resistance in transplant recipients. A recent retrospective review of 76 patients with a mean 11.1 years post-transplant follow up found that even when adjusted for age, the presence of metabolic syndrome was associated with increased risk progression of dysglycemia[22]. In a larger cohort of patients (n = 640), the presence of metabolic syndrome pre-transplant remained a significant risk factor for developing NODAT even when adjusted for age[9]; however, there is no data available on insulin resistance in any significant cohort of transplant recipients who develop metabolic syndrome and NODAT.

Insulin secretion

It seems likely that as modifiable risk factors are altered, importantly including immunosuppressive agents, that the weights of forcing factors of NODAT will also be altered. As such, studies that repeatedly measure insulin indices throughout the post-transplant period, in particular in the higher risk first year post-transplant, are particularly valuable. Nagaraja *et al*[22] has recently described insulin indices pre- and 3 and 12 mo post-transplant in non-diabetic patients (n = 118) as defined by F BGL less than 7.0 mmol/L pre-transplant. The patients defined as NODAT had increased insulin resistance at 3 and 12 mo, although less resistance at 12 mo when compared to 3 mo. By 12 mo, insulin secretion had fallen in patients with NODAT; however, despite the fall in insulin resistance the levels of secretion failed to be compensatory, suggesting that even in the face of falling doses of glucocorticoid and improving peripheral insulin sensitivity, impaired insulin secretion increasingly threatens normoglycemia[20,100]. This data is supported by previous studies in which oGTT was used for diagnosis[101,102]. Nam *et al*[102] first demonstrated impairment in insulin secretion as a necessary component in the pathogenesis. They followed 144 patients pre- and post-transplant and noted that higher, although normal, oGTT results pre-transplant were associated with increased risk of dysglycemia post-transplant. They also noted that those who developed post-transplant dysglycemia 9-12 mo post-transplant had significantly lower insulin secretion in the face of improved insulin resistance. A long term study found similar results when using oGTT at 10 wk and 6 years post-transplant. Patients who were dysglycemic at 10 wk and became normoglycemic had improvement in insulin resistance and a non-significant impairment of insulin secretion, thus retaining a compensatory response. On the other hand, those who remained diabetic or became diabetic over the follow-up period had a non-significant deterioration in insulin resistance and a significant fall in insulin secretion[103].

The mechanism of impairment in insulin secretion post-transplant is thought to be related to CNI use. The mechanism of action is believed to be the impairment of pancreatic cell function due to the binding of CNI to calcineurin. Calcineurin is a systolic phosphatase that has two targets in the β-cell: the nuclear factor of activated T cells and cyclic-AMP-responsive element-binding protein transcriptional co-activator. In mice models, normal β-cell function has been shown to be dependent upon calcineurin[104]. Calcineurin may be important for the proper response to hyperglycemia and incretin activation. Human islet cells when treated with Tac increased β-cell apoptosis, possibly mediated by the above calcineurin targets and ameliorated by the administration of incretin analogues[105,106].

Incretins

Finally, there is no data on the impact of immunosuppression in renal transplant patients on incretin hormones. It is interesting to note that in healthy volunteers the administration of glucocorticoids in the setting of being sedentary and on a high calorie diet (not unlike the initial period of time post-transplant) have impaired responses to incretin hormones[107]. In dialysis dependent patients, those with IGT have been shown to have a reduced incretin effect[108], and even normoglycemic dialysis dependent patients have reduced insulin secretion with increased incretin secretion suggesting that uremia or CKD impacts upon the proper β-cell stimulation and response[109]. However,

Table 4 Risk of mortality, cardiovascular events and graft loss associated with new-onset diabetes after transplantation or dysglycemic state

	Mortality	CV event/death	Graft loss	Ref.
Diabetes at	3 mo: 37% at 8 yr (HR = 2.1)	20% (death) at 8 yr (HR = 3.5)		Hjelmesaeth *et al*[4]
	10 wk: 34% at 6.7 yr (HR = 2.0)			Valderhaug *et al*[11]
	1 yr: 44% at 11 yr (HR = 2.2)	Death HR: 2.72		Nagaraja *et al*[20]
Dysglycemia at	10 wk: 29% at 6.7 yr (HR = 1.78) each 1 mmol/L oGTT: 5% risk increase	Events increased with increased F BGL		Cosio *et al*[3]
	4 mo: 0.5 mmol/L increase F BGL: 4% risk increase	1 mmol/L oGTT: 6% risk increase in death		Valderhaug *et al*[11]
	12 mo: 0.5 mmol/L increase F BGL: 15% risk increase	12 mo: 0.5 mmol/L increase F BGL: 11% risk increase for event		Wauters *et al*[14]
			3 mo: RR 3.6 at 6 yr	Wojtusciszyn *et al*[41]

F BGL: Fasting blood glucose level; oGTT: 2-h oral glucose tolerance test.

the dynamics of incretin hormones are yet to be described in the post-transplant setting.

OUTCOMES

There is an urgent need to develop a consensus on the best test to detect and how to manage dysglycemic states post-transplant, as there is a direct correlation with the presence of dysglycemic states and mortality predominantly from cardiovascular causes (Table 4)[3,4,11,14,20]. An analysis of the USRDS database in which NODAT was defined according to Medicare claims analysed 27707 patients with data available greater than 1 year and not diabetic pre-transplant. Death censored graft loss was more likely in those who suffered acute rejection when compared to those who developed NODAT. Conversely, those who developed NODAT had a higher hazard ratio of death with a functioning graft compared to those with episodes of acute rejection (1.41 and 1.15 respectively) compared to patients with neither exposure[51]. Analysis of earlier data from the same database found the development of NODAT associated with increased risk for acute myocardial infarction after a minimum 3 year follow up[110]. Similarly, in an analysis on the International Collaborative Transplant Study database (n = 39251) with up to 10 years of follow up, Cox regression analysis of death with a functioning graft due to cardiovascular disease revealed an increased risk for NODAT (HR = 1.6, $P < 0.001$), which was greater than episodes of rejection within the first year (HR = 1.2, $P = 0.036$) but not as great as the risk associated with pre-transplant diabetes (HR = 2.5, $P < 0.001$)[111].

The above datasets are large and their analyses robust, but what is needed are large prospective datasets with well-defined populations and sufficient duration of follow up. Smaller studies have found significant risk for mortality from the development of NODAT, but these findings have disappeared when adjusted for confounding factors. In one such study, major cardiac events occurred in 20% of persistent NODAT patients compared to 7% without NODAT and 21% with pre-transplant diabetes over a 8 year follow up[4]. The outcomes of the largest prospectively followed well defined population was described by Valderhaug *et al*[112]. They followed 1410 patients for a mean of 6.7 years, of whom 55% were dysglycemic at 10 wk post-transplant of which 17% had NODAT. They reported a significant increase in the incidence of all cause mortality between the normoglycemic and dysglycemic groups, the rates being highest in those with NODAT. After adjusting for confounding traditional and transplant associated variables, the HR for all cause mortality was 1.54 was NODAT and 1.39 for IGT ($P < 0.05$). When analysed treating glucose as a continuous variable: on adjusted analysis, for every 1 mmol/L (18 mg/dL) increase in oGTT result there was a 5% increase risk in all cause mortality ($P < 0.05$). The main cause of death was cardiovascular disease, and those with NODAT by 10 wk were at significant increased risk on adjusted analysis (HR = 1.8 $P < 0.05$). For every 1mmol/L (18 mg/dL) increase in the oGTT result there was significant 6% increase risk in cardiovascular death ($P < 0.05$). Despite the findings of the continuous glucose analysis, other dysglycemic states were not associated with cardiovascular death. Further analysis of the same cohort found a graft failure rate of 28%, 60% of which was due to death. There was no association with death censored graft loss, but for every 1 mmol/L (18 mg/dL) increase in oGTT result there was a 3% increase risk in overall graft failure[110]. This suggests similar conclusions as the large registry analyses described above: NODAT may not be associated with increased graft loss, but is associated with increased mortality.

In another large single centre prospectively followed group an increase in risk of all cause mortality and cardiovascular death according to the presence of NODAT at 1 year post-transplant was reported[14]. The 12-mo rate of dysglycemia was 29.8% and NODAT 13.4%. Continuous analysis of the glucose levels revealed that for every 10 mg/dL (0.56 mmol/L) increase in F BGL there was an increase in all cause mortality censored at graft failure over a follow up period of 90.4 mo. At 12 mo, patients with IFG had a HR of 1.7 ($P = 0.009$) and those with NODAT a HR of 3.5 ($P < 0.0001$). Of note, in this study the patients on treatment for NODAT did not have a reduced mortality risk compared to the NODAT

patients not on treatment. Given the retrospective nature of the analysis it is not possible to conclude that treatment does not affect outcomes. However, such findings indicate the importance of well-defined prospectively followed transplant population analyses and potentially the need to identify early those patients at risk of dysglycemia so that directed interventions (be they aggressive glucose or metabolic risk factor control) may ameliorate the increased risk of mortality. Furthermore, such data highlights that in the transplant population clinicians do not have targets of glycemic control that can be achieved with treatment and are associated with improved outcomes. Even in the general population there is conflicting data concerning improved macrovascular outcomes achieved by treating to more intensive targets[113,114]; however, in the transplant population, it remains unknown if meeting these same targets may improve outcomes.

SCREENING AND DIAGNOSIS

Use of oGTT remains the gold standard for diagnosis of NODAT or dysglycemia. This test, however, is not an easily completed screening test. Simple office or laboratory based tests that may be used to adequately screen for NODAT, particularly in high risk patients, include F BGL, 4 pm capillary blood glucose or HbA1c. All of these parameters have limitations. For example, a Spanish study of 374 non-diabetic pre-transplant patients found that normal F BGL in 59% of patients with an abnormal oGTT over the first 12 mo post-transplant[6]. It is well known that changes in red cell viability, need for (due to for example, drug induced bone marrow suppression) and use of erythropoeitin stimulating agents, administration of red cell transfusions and changes in hemoglobin will impact upon HbA1c levels. Notwithstanding this issue more readily encountered in ESKD, some small studies (n = 71) have shown concordance between oGTT and an HbA1c cut-off of 6.2% for the diagnosis of NODAT[115]. It would be clinically more likely to find concordance between these tests after 2-3 mo post-transplant once there has been renal function recovery and the impact of uremia on erythropoiesis has resolved. However, analysis of a much larger cohort (n = 1571) found that using if HbA1c was used as a screening tool and oGTT as the gold standard test, then the cut-off should be 5.8%[44]. More recently, when using a combined test of HbA1c $\geqslant$ 6.5% and F BGL $\geqslant$ 7.0 a Norwegian group (n = 1619) have demonstrated a negative predictive value (NPV) of 97.4% for NODAT, using oGTT as gold standard test at 10 wk post transplantation[116]. Notably, the combination of the two tests had very little additive value (NPV F BGL alone 94.2%) and the lower the HbA1c cut-off value made little difference in exclusion of NODAT (*e.g.,* $\geqslant$ 5.5% NPV 97.5 compared with $\geqslant$ 6.5% NPV 93%). However, the positive predictive value of HbA1c $\geqslant$ 6.5% or 6.2% or in combination with F BGL $\geqslant$ 7.0 mmol/L was poor (53.4%, 42.1%, 69.4% and 50.9%, respectively). Thus, while HbA1c may be of use in screening for NODAT, current evidence does not support its use as a diagnostic test in transplant patients.

Determining the best test to use in transplant patients is complicated by the need to certain of the best time to administer the test. It has recently been shown that glucocorticoid administration in the morning leads to increased afternoon or evening blood glucose levels, at approximately 7-8 h after administration of glucocorticoid. Thus, reliance on F BGL may underestimate the incidence of dysglycemia. In fact, at six weeks post transplantation a 4 pm capillary blood glucose significantly outperformed oGTT, F BGL and HbA1c in detecting NODAT. Combining the tests done at 3 and 12 mo, the cumulative incidence of NODAT with oGTT was 14% and IGT 28%. Interestingly, using an HbA1c range of $\geqslant$ 5.7 and < 6.5 to detect IGT detected an incidence of 51%; but HbA1c did not perform as well as oGTT in detecting NODAT. Hence, the authors suggested using HbA1c as a screening test from 3 mo and using oGTT to determine the presence or absence of NODAT in patients detected to have dysglycemia by HbA1c. This strategy would avoid oGTT in 49% of patients and achieve a sensitivity of 94%[117]. As yet, this data and strategy has not been replicated. Furthermore, the results of these studies suggest that the cut-offs that have been applied in the general population may not apply in CKD, ESKD or post-transplant patients. The question of the cut-off levels for any of the possible tests will only be settled by long-term large prospectively collected data sets which permit determination of the risk for poorer clinical outcomes associated with different cut-off points. Some of this data has already been described, but it is worth emphasising that only oGTT results have been shown to be associated with poorer outcomes when analysed categorically (as distinct from continuous data) and not F BGL[11].

PREDICTING NODAT

If it is difficult to develop easy to administer diagnostic tests, it is even more challenging to develop to models that may predict the development of NODAT, based either on pre- or post- transplant data. There are very few studies able to draw conclusions about predicting NODAT using pre-transplant data. Post-transplant dysglycemia is dynamic phenomenon and there are multiple physiological changes post-transplant that may impact upon insulin and glucose handling. This is emphasised by the remarked upon cases of diabetic or dysglycemic pre-transplant patients resolving their dysglycemic state post-transplant. Hence, the pre-transplant prediction of those increasingly likely to have NODAT post-transplant is fraught with multiple difficult variables that need to be taken into account.

A range of pre-transplant variables has been described as predictors of NODAT. These include age, BMI, fasting and R BGL and metabolic syndrome. For example, one study of 139 non-diabetic patients

pre-transplant found that higher (albeit normal) pre-transplant R BGL were predictive (P = 0.011) of NODAT, although this data has not been replicated[7]. A matched cohort retrospective analysis of 47 patients who developed NODAT found that a higher, albeit normal range, F BGL was associated with the development of NODAT on multivariate analysis[44].

One reasonable sized study (n = 640) with a NODAT incidence at 1 year of 31.4% found an adjusted hazard for NODAT of 1.34 (1.00-1.79, P = 0.047) for pre-transplant metabolic syndrome. On multivariate analysis, only pre-transplant low HDL remained an independent predictor[9]. Other groups have attempted to apply scores that are predictive of type 2 diabetes mellitus in the general population. A retrospectively analysed cohort of 191 patients in which 41 developed NODAT, two general population risk scores were found to have AUC-ROC of 0.756-0.807 for NODAT at 1 year, but the PPV for each test was poor (24.5%-31.2%). However, the authors point out that the NPV were high (92.5%-93.7%) perhaps allowing the identification of high risk patients[118].

There is a small body of literature considering the development of predictive models that may be more unique to the transplant patient. Analyses in the general population of the patterns of oGTT results may be predictive of future type 2 diabetes[119]; similar analyses in renal transplant patients may be useful. An analysis of a 5 time point oGTT conducted pre-transplant in 145 patients found that whilst F BGL did not predict NODAT, the AUC of the oGTT and the glucose concentrations at each time point post glucose load could be used to predict NODAT[23]. Given the logistical difficulties in studying recipients of deceased donor organs, there is little data available that would enable us to reliably assess if pre-transplant markers for NODAT can be identified. For example, one study in which 120 transplanted patients were screened with oGTT pre-transplant found that pre-transplant IGT was significantly associated with NODAT; however, these patients were screened during the 3 mo prior to being waitlisted and there was no information provided regarding the time on the waiting list. This may introduce a potential bias in that some normoglycemic patients may have developed further dysglycemia pre-transplant[31].

Chakkera *et al*[120,121] have attempted to develop and validate a model of pre-transplant factors to predict the development of NODAT. On univariate analysis they described seven pre-transplant factors associated with increased risk of NODAT, which was defined by use of HbA1c, F BGL or requirement for treatment, including dietary changes. The seven factors were: age greater than 50 years old, use of maintenance glucocorticoids, use of gout therapies, BMI $\geqslant$ 30, F BGL $\geqslant$ 5.6 mmol/L, fasting triglycerides $\geqslant$ 2.24 mmol/L and a family history of type diabetes. Insulin indices were not measured and pre-transplant oGTT were not done pre- or post-transplantation, potentially treating pre-transplant diabetic patients as normoglycemic. Complex statistical methods, including bootstrapping were used. Within the limitations of this study, there were clear differences in the 1 year incidence of NODAT for those classified as low, moderate or high risk according to seven factor risk score. The results were similar in the initial and validation groups. In the higher risk group the incidence of NODAT was 44%-56% compared to the low risk group of 11%-13%. This was a first step in attempting to develop a risk score that may assist in identifying patients who could be targeted for trials of preventive therapies.

The analysis of the data from the 5 time point oGTT points towards the possibility of identifying higher risk patients by evaluating for impaired glucose and insulin regulation pre-transplant. A test that is helpful in this regard is known as the disposition index. This is a quantification of the hyperbolic balance between insulin secretion and insulin resistance. It can be measured either *via* oral or Ⅳ glucose loads and has been shown to be associated with increased risk for developing type 2 diabetes mellitus in the general population[122,123]. There is little literature using the disposition index as a predictive pre-transplant marker. However, there are some small studies measuring insulin resistance and secretion pre-transplant and testing their relationship with NODAT. Various models that utilise data derived from oGTT or Ⅳ GTT measure insulin resistance. The homeostasis model (HOMA) is widely used, and has been validated in studies of the general population. Variations of HOMA can be used to estimate insulin resistance and secretion. There is conflicting data on whether pre-transplant insulin indices may be predictive and most studies are small[124]. A study with the primary purpose of comparing Tac and CsA (n = 150) was used to retrospectively review the risk of NODAT from pre-, 3 and 12 mo indices of insulin resistance. Pre-transplant, there were no differences in insulin resistance or secretion found between those patients who developed NODAT at 3 or 12 mo[20]. This is in contrast to an earlier study (n = 57) in which those patients more resistant at baseline (and older) had an increased odds of a dysglycemic state after 1 year follow up[33]. However, as it appears increasingly more likely that falls in insulin secretion (and thus failing to compensate for insulin resistance) is crucial in the development of NODAT, it is interesting to note that measurements of insulin secretion in non-diabetic post-transplant patients can be used to predict the future development of NODAT[98].

MANAGEMENT

The principles of management of post-transplant dysglycemia are: (1) Pre-transplant risk assessment and development of amelioration strategies; (2) Early detection and monitoring for transient or permanent dysglycemia; and (3) Appropriate therapies that may reduce the poorer outcomes in those in whom post-transplant dysglycemia develops. The issues surrounding risk assessment and detection have been discussed

above. Current advice for glucose targets during post-transplant hospitalization suggest maintaining glucose levels below diabetic range; *i.e.*, F BGL 4-7 mmol/L (72-126 mg/dL)[125]. Following discharge, current guidelines recommend that patients be screened weekly for the first four weeks, and every 3 mo for the first year and yearly after the first year. Screening should also be commenced if there is commencement of, or substantial increase in dose of, CNI, mTOR inhibitor or glucocorticoids[126]. There is no consensus on the best screening test to utilise; however, a combination of tests as discussed above would appear to be of greatest clinical use. This may involve weekly F BGL or 4 pm capillary blood glucose (although this is not currently part of guidelines). Detection of IFG would then prompt oGTT assessment[125]. Perhaps use of HbA1c after the first 3 mo is warranted in stable patients. There are also few recommendations as to what the targets for blood glucose and HbA1c ought to be, as it is not known at what ranges there is substantial reduction in poorer outcomes. At present, guidelines give an ungraded suggestion to aim for an HbA1c of 7%-7.5% in United States[126] and < 7% in Scandanavia[125].

Adjusting immunosuppression

One approach to management is amelioration of risk. It remains difficult to identify patients at risk for dysglycemia with certainty; equally, it is challenging to know what may be done should they be identified. On the basis of data available concerning modifiable risks, physicians may wish to replace, minimise or withdraw one or more agents that form part of the maintenance immunosuppression; in particular CNI or glucocorticoids. For instance, perhaps older patients with a higher BMI and a worse (if still normal) pre-transplant oGTT may be judged to be at risk and as a result not exposed to maintenance glucocorticoids, or use of CsA in preference to Tac. This approach clearly needs to balance the immunological risk of reduced immunosuppressive exposure against the higher metabolic (and ultimately cardiovascular and infection) risk. Some authors have proposed protocols to assist in balancing the metabolic and rejection risk[61]; however, there are no well validated methods for reliably making such assessments in a broad transplant population. In addition, the clinician is also faced with the complicated issue of applying risk assessments to individual patients with varying degrees of co-morbidities.

One potentially helpful immunosuppressive agent that has not been discussed above is belatacept. This co-stimulatory blockade agent, which remains available in for off-label use in many countries, can be used as part of a maintenance regimen in place of CNI, in combination with MMF and glucocorticoids. The BENEFIT and BENEFIT-EXT (extended criteria donors) trials have reported up to 5 year results, comparing belatacept with MMF and glucocorticoids with CsA, MMF and glucocorticoids. There is a concern that there may be greater early acute rejection, however, over longer follow up there is no greater rejection rate. There has also been a concern about increased risk for EBV associated post transplant lymphoproliferative disease[127-130]. With regard to NODAT, results from 1 year follow-up of BENEFIT and BENEFIT-EXT have been published. There was a significant reduction in the 1 year cumulative incidence of NODAT in the belatacept arm, with rates of NODAT in the CsA arm being comparable to that found in other studies. This was in conjunction with clinically significant reductions in blood pressure, cholesterol and triglycerides, suggesting it may have a role in management of patients at higher risk of poorer cardiovascular and metabolic outcomes[131].

Lifestyle changes

Aside from altering immunosuppressive agents, other modifiable risk factors include reduced physical activity and poor diet. There is some data to suggest that low levels of physical activity post-transplant, particularly in patients whose appetite may now be improved, are at greater cardiovascular and all-cause mortality risk[132]. Improved diets, increased physical activity and weight loss has also been shown to improve dysglycemia in renal transplant patients[133]; however, this is not a well studied therapeutic approach.

Intensive and early glycemic control

As there are many obstacles to overcome should immunosuppression be tailored to meet metabolic and immunological risk, it may be that we require strategies to "rest" β-cells in patients without changing immunosuppression in those at higher risk of metabolic complications. Hecking *et al*[38] in a proof of concept trial (n = 50) randomised patients to (non-blinded) early basal insulin or standard therapy. NODAT was defined by oGTT or need for hypoglycemic agents at study visit. All patients received maintenance Tac, glucocorticoids and MMF. Patients were given isoprene insulin if their evening blood glucose was > 140 mg/dL (7.8 mmol/L) in the treatment group; the standard of care group received short acting insulin or oral agents if their blood glucose was 180-250 mg/dL (10-13.9 mmol/L), as directed by the treating clinician. All 25 patients in the treatment group received isoprene insulin on postoperative day 3, having had high evening blood glucose the day prior. By 12 mo, no patient in the treatment group required hypoglycemic agents compared to 8 in the control group. The majority of the patients in the treatment group did not receive any hypoglycemic agent after 120 d post-operative. All patients not on hypoglycemic agents had oGTT at 3, 6 and 12 mo. By 12 mo, 5 patients in the treatment group had NODAT on oGTT compared to 4 in the control group; thus, there was a reduction in NODAT from 12 to 5. More patients in the treatment group had IGT (8 *vs* 5); but, overall, more patients in the treatment group were normoglycemic (12 *vs* 8). Furthermore, consistent with the more

recent literature on insulin secretion as a significant contributor to the pathogenesis of post-transplant dysglycemia, measures of insulin resistance between the groups did not differ at 12 mo. There was, however, a significant difference in the insulinogenic index, an oGTT derived measure of β-cell function. There was also an improvement in the disposition index (although not significant). Together these results would indicate better or more preserved insulin secretion in those whose β-cells were "rested" at time of maximal stress. Should such results be achieved in a larger study population (perhaps of higher risk patients) who are studied for a longer period of time an found to have better metabolic and cardiovascular outcomes, then it may be that early basal insulin in those with elevated evening blood glucose may become a standard of care obviating any need to tailor immunosuppression.

Standard hypoglycemic agents

Nonetheless, currently patients receive care more like the standard care administered in Hecking *et al*[38]. If these patients then develop NODAT, they receive hypoglycemic agents. The choice of agent is mostly guided by opinion and knowledge of risks associated with administration of these agents in CKD. This is due to the paucity of trial data on use of hypoglycemic agents within this population. There is only one small study (n = 48) that compares potential therapies in which a DDP IV inhibitor, vildagliptin, was compared with pioglitazone or placebo in patients with IGT at more than 6 mo post renal transplantation. Both medications reduced oGTT blood glucose levels over 3 mo, with no differences between the treatment groups[134]. As there is concern that thiazolinediones may be associated with poorer cardiovascular outcomes, such medications may not be considered as first line therapy. The incretin analogues, remain the only other hypoglycemic agent studied in transplant patients. Vildagliptin has been studied as part of a randomised placebo controlled trial, in which patients with oGTT defined NODAT at least 6 mo post-transplant were recruited. Thirty-three patients were recruited, all of whom were on a similar maintenance regimen of CNI/MMF and glucocorticoids. The follow up period was short, however, vildagliptin did significantly reduce oGTT and HbA1c results at 3 mo with no hypoglycemic events[135]. Caution should be used with vildagliptin in conjunction with ACE inhibition as there is an increased risk of angiooedema (OR = 4.57), albeit on the basis of a small absolute risk[136]. Another small study (n = 19) has shown that sitagliptin can significantly increase insulin secretion in patients known to have NODAT[137]. Sitagliptin, saxagliptin and vildagliptin should be dose reduced in renal impairment, linagliptin is not renal excreted. It is unclear if incretin analogues are ameliorating an impact upon the incretin effect or assisting β-cell function in other ways. There is no data in the transplant population concerning the incretin effect. In healthy people administered glucocorticoids the incretin effect has been noted to be impaired[103]. In favour of incretin analogues, they do not tend to produce hypoglycemia or weight gain; but they have not been shown to reduced cardiovascular events, have been associated with pancreatitis and may theoretically increase cancer risk[138].

The incretin analogues are not widely used in the transplant population, with use of sulfonylureas and more common. Metformin may not be favoured as it can contribute to gastrointestinal side effects, potentially exacerbating the same caused by MMF use. Moreover, there is no also no data on its use in transplant patients with GFR < 30 mL/min and risks of lactic acidosis. However, its lack of contribution to weight gain, its association with reduced cardiovascular events in non-transplant patients and its role as an insulin sensitiser rather than stimulating further insulin secretion from "stressed" β-cells, may make metformin more favoured than sulfonylureas[139]. Sulfonylureas do not have the cardiovascular benefits and can contribute to weight gain. However, as long as dose adjusted to prevent hypoglycemia, their use is not associated with other serious adverse events. Nonetheless, it may be that some, if not most, transplant patients with develop dysglycemia have impaired β-cell function and that potentially a treatment strategy that induces more work from the β-cells may be counter-productive in terms of relieving dysglycemia and preventing worse cardiovascular outcomes[140]. Problematically, the paucity of data on treatment (including treatment targeted at the underlying pathology) in this area of transplantation means it is not possible to make any firm recommendations on the choice of oral hypoglycemic agents.

CONCLUSION

In summary, dysglycemic states, not limited to NODAT, are associated with increased risk of mortality, principally as a result of cardiovascular disease. NODAT is better studied than other dysglycemic states. The natural history of dysglycemic states is not well characterized, apart from the recognition of transient dysglycemia and NODAT within the first 3-6 mo post transplantation. The majority of persistent NODAT develops within one over the first year post transplant. Whilst the diagnosis is made using the WHO/ADA criteria accepted in the general population, there is no consensus on which test should be employed, either for screening or diagnosis. At present, oGTT remains the most reliable diagnostic test in the post-transplant setting. However, predicting the development of NODAT remains challenging. Possibly, the small group of patients who remain normoglycemic within the first week post-transplant are at very low risk of developing NODAT. There are a few studies that may assist in developing tools for identifying those at high risk.

There are multiple risk factors, some of which are modifiable. The most consistently found risk factor is increasing age and there is a growing body of liter-

ature documenting the genetic risk factors. The most well described modifiable risk factor is the use of immunosuppressive agents, in particular CNI (Tac more than CsA) and glucocorticoids. These agents likely contribute to the development of NODAT *via* different mechanisms – glucocorticoids encouraging insulin resistance and CNI *via* β - cell failure. It seems that reduction in insulin secretion is more important in the pathogenesis than insulin resistance.

Any attempt to balance the metabolic and immunological risks by adjusting immunosuppression is complicated. It may be better to identify higher risk patients and utilise a preventive strategy, such as described by Hecking *et al*[38]. As evidence emerges of the importance of β-cell failure as a major contributor to NODAT, such as strategy appears promising. In the absence of prevention, the management of NODAT in order to prevent the poorer outcomes is important. However, it is not clear which agent is most likely to successfully treat NODAT and ameliorate the poorer outcomes. A number of options exist, and it may be that metformin is the best option if insulin is not required.

Finally, further research is needed on pathogenesis, identification of higher risk patients and development of preventive and safe treatment options. Such research needs to take into account the caveats that are identified with respect to previous research: confirming normoglycemia pre-transplant, using oGTT as the primary diagnostic test (although there may be a role for capillary blood glucose early post-transplant), using WHO/ADA to define clinical states, testing regularly to detect transient and permanent states and having adequate follow up to detect the development of permanent dysglycemic states that impact upon poorer clinical outcomes. It would be ideal if future research could also map the changes in insulin secretion, resistance and the incretin effect pre- and post- transplantation in an effort to better understand the pathogenesis and further delineate targeted prevention and treatment options.

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MINIREVIEWS

Magnesium and type 2 diabetes

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Abstract

Type 2 diabetes is frequently associated with both extracellular and intracellular magnesium (Mg) deficits. A chronic latent Mg deficit or an overt clinical hypomagnesemia is common in patients with type 2 diabetes, especially in those with poorly controlled glycemic profiles. Insulin and glucose are important regulators of Mg metabolism. Intracellular Mg plays a key role in regulating insulin action, insulin-mediated-glucose-uptake and vascular tone. Reduced intracellular Mg concentrations result in a defective tyrosine-kinase activity, postreceptorial impairment in insulin action and worsening of insulin resistance in diabetic patients. A low Mg intake and an increased Mg urinary loss appear the most important mechanisms that may favor Mg depletion in patients with type 2 diabetes. Low dietary Mg intake has been related to the development of type 2 diabetes and metabolic syndrome. Benefits of Mg supplementation on metabolic profiles in diabetic patients have been found in most, but not all clinical studies and larger prospective studies are needed to support the potential role of dietary Mg supplementation as a possible public health strategy in diabetes risk. The aim of this review is to revise current evidence on the mechanisms of Mg deficiency in diabetes and on the possible role of Mg supplementation in the prevention and management of the disease.

Key words: Magnesium; Type 2 diabetes; Metabolic syndrome; Inflammation; Aging; Hypertension; Insulin resistance; Endothelium

Core tip: Diabetes is frequently associated with Mg deficit. The fact that most but not all diabetic subjects have low magnesium (Mg) and that no large randomised controlled trial (RCT) has been specifically focused on subjects with Mg deficit, diagnosed with a reliable technique, may help explain discrepancies of the role of supplemental Mg on glycemic control, and the impact on diabetes risk in prospective epidemiological studies. Different baseline Mg, metabolic control, and age are other potential factors that may contribute. Future prospective RCTs are needed to support the potential role of dietary Mg supplementation as a possible public health strategy to reduce diabetes risk in the population.

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INTRODUCTION

Magnesium (Mg) is an electrolyte of chief physiological importance in the body, being the *most abundant* divalent intracellular cation in the cells, the second most abundant cellular ion next to potassium and the fourth cation in general in the human body[1].

Type 2 diabetes mellitus (DM2) is often accompanied by alteration of Mg status. An increased prevalence of Mg deficits have been identified in DM2 patients, especially in those with poorly controlled glycemic profiles, with longer duration of the disease and with the presence of micro- and macrovascular chronic complications[2-6].

Laboratory tests with a high sensitivity and specificity and easy to perform to allow an accurate clinical assessment of Mg status are missing. Patients are considered frankly hypomagnesemic with serum Mg concentrations $\leqslant$ 0.61 mmol/L or 1.5 mg/dL[7-9]. Mg concentrations $\leqslant$ 0.75 mmol/L or 1.8 mg/dL may be considered as preclinical hypomagnesemia[10,11].

Mg deficiency can be present without hypomagnesemia. However, hypomagnesemia, when present, is usually indicative of an important systemic Mg deficit. A depletion in intracellular and/or ionized plasma Mg can be found in individuals with normal total serum Mg[12]. However, most of the studies in the literature have measured total serum Mg instead of the free, ionized (bioactive) or the intracellular Mg concentrations, which make it a challenge to correlate Mg deficits to diseases.

We have recently confirmed that diabetic older patients are more prone to hypomagnesemia; this condition being closely related to metabolic control as measured by glycated hemoglobin even after adjustment for relevant confounders. Ionized Mg may help to identify diabetic older adults with low concentrations of blood Mg that are not evident with the only measurement of total Mg[12].

Intracellular free Mg levels are consistently reduced in subjects with DM2, when compared with nondiabetic subjects[1,13,14]. Although the mechanism has not been fully elucidated, an alteration in the mechanism(s) of the Mg uptake in the cells, and/or a deficit of ATP, may help to understand the cellular Mg deficit observed in DM2[15]. The relationship between intracellular Mg and ATP concentration is rather complex. The decrease in cellular ATP might partially explain the decrease in cellular Mg. Otherwise, a decrease in cellular ATP leads to a decreased binding of Mg to ATP in the formation of MgATP, which might increase the intracellular Mg concentration.

The aim of this review is to revise current evidence on the mechanisms of Mg deficiency in DM2. The evidence on the role of Mg supplementation in the management of DM2 will also discussed.

MECHANISMS OF MG DEFICIENCY IN DM2

Reduced Mg intake and/or augmented Mg urinary loss are among the most important causes of Mg deficits in DM2, while Mg absorption and retention seems to be maintained[16-18].

A relationship between Mg levels in the plasma and the development of DM2 in the general population has been suggested[19]. DM2 is frequently accompanied by renal calcium and Mg loss[20,21], but the mechanism(s) of this wasting is still not completely elucidated[22].

Both hyperglycemia and hyperinsulinemia may increase urinary Mg excretion. Urinary Mg excretion and fasting blood glucose have been found to be inversely related to serum Mg levels. Thus, hyperglycemia decreases Mg tubular reabsorption[20]. A good metabolic control is associated with a reduction of the urinary Mg wasting[3].

In streptozotocin-induced diabetic rats, Lee *et al*[22] found an increase in renal Mg transporters. The alteration was corrected by insulin administration. Insulin resistance and hyperinsulinemia may also affect Mg transport[21].

MG AND INSULIN SENSITIVITY

Hypomagnesemia in DM2 is present only in severe (and generally long lasting) Mg deficits. A chronic latent Mg deficiency without alteration in serum total Mg is more commonly observed[12]. These often undetected Mg insufficiencies have clinical importance, since Mg is a main co-factor in numerous enzymatic reactions (> 300 enzymatic reactions including all the enzymes of glycolysis). Mg also is deeply involved in the regulation of insulin signaling, in the phosphorylation of insulin receptor kinase, in the post receptorial action of insulin, and in insulin-mediated cellular glucose uptake[17,23].

The clinical consequence of a chronic Mg deficit is post-receptorial insulin resistance and consequent reduced glucose utilization in the cells, worsening the reduced insulin sensitivity present in DM2[18].

Another possible link between Mg deficiency and reduced insulin sensitivity is the presence of oxidative stress and/or inflammation. Thus, free radicals are often increased in DM2, hypertension, metabolic syndrome and aging, conditions also associated with Mg deficits[24,25]. In particular, we demonstrated an age-dependent deficit of cellular Mg in persons aged 65 years and over, as well as in patients with essential hypertension or DM2, independently of age[14,25].

Nevertheless, independently of the mechanisms of Mg deficits in DM2, metabolic syndrome, essential

hypertension and aging, it is apparent that this Mg deficiency may contribute to enhance the insulin resistance status of these conditions[17,18]. Mg deficit could precede and cause post-receptorial resistance of insulin and alter glucose tolerance.

MG DEFICIENCY AND CARDIO-METABOLIC DISEASES

Mg deficiency may be also a factor implicated in DM2 complications. We found a relation between ionic changes and echocardiographic indices alterations[26]. We observed an significant association of reduced cellular Mg with cardiac hypertrophy in DM2 patients[26].

Cellular Mg measured *in vivo* in skeletal muscle and in the brain with ^{31}P-NMR, was directly related to aortic distensibility[27].

Reduced Mg levels were also associated with an increased prevalence of arrhythmias in DM2 obese subjects[6], and with a more rapid decline of renal function. Thus, hypomagnesemia is currently considered an accurate predictor of progression of diabetic nephropathy[28-30]. Mg deficits have also been associated with cognitive decline[31], multimorbidity[32] and agin[25,33].

DIETARY MG DEFICIENCY MAY PREDISPOSE TO DM2

Dietary Mg deficiency may cause insulin resistance as shown by several studies both in humans and in experimental animals[34-40]. In sheeps, Mg-deficient diet caused a significant impairment of insulin-mediated glucose uptake[35]. In rats, Mg supplements were able to postpone the onset of diabetes[36]. In healthy women (without DM2), the higher was the intake of Mg, the lower were fasting levels of insulin[37]. In young, nondiabetic African Americans, low dietary Mg was associated with insulin resistance and insulin responses to an oral glucose tolerance test[38]. A low Mg diet in rats produced an increase in triglyceride and plasma glucose levels[39]. In rats, a maternal restriction of dietary Mg was able to cause insulin resistance in pups[40]. Suárez *et al*[41] suggested that the worsening of glucose metabolism induced by Mg dietary restriction in experimental rats is due to an impairment of both, insulin secretion and insulin action.

Deficiencies of Mg status including both hypomagnesemia and/or reduced dietary Mg intake have been linked to an enhanced risk to develop DM2 or glucose intolerance[19,42-44]. Higher Mg intakes were conversely associated with a reduced incidence of DM2[45].

Several studies have shown a clear association of Mg intake with DM2 and with cardio-metabolic syndrome, suggesting that a higher Mg consumption is related to a reduction of the incidence of these conditions. Two meta-analyses of prospective studies concluded that Mg intake is inversely associated with the onset of DM2[46,47]. In addition, the development of the cardio-metabolic syndrome has been linked to dietary Mg content[34,48]. Hypomagnesemia itself in a 10-year follow-up study was associated with glucose tolerance impairment[49]. Conversely, higher Mg intake was associated with increased insulin sensitivity[50] and with decreased risk of incident DM2, with a decreased risk of 0.68 in the higher compared with the lower quintiles[51,52].

Similar findings were obtained in the CARDIA study, during a 20-year follow-up, which also confirmed the reverse relationship of dietary Mg with inflammation markers[53].

POSSIBLE USE OF MG SUPPLEMENTS IN THE MANAGEMENT OF DM2

The detection and correction of altered Mg status in diabetic patients is clinically appropriate, although many physicians tend to ignore Mg status. The increased risk of developing impaired glucose tolerance and/or frank DM2 in persons with dietary or serum Mg deficits have suggested a potential benefit of Mg supplements in patients with DM2 or in the presence of risk factors for DM2. Mg supplements have been proposed as a complementary tool for the prevention of DM2 and its metabolic control[54,55]. Some benefits of Mg supplements on glycemic profiles have been found in most but not all studies.

Regrettably, results from clinical trials are still limited[56]. Thus, the clinical evidence of a clear effect of Mg supplementation on metabolic indices in persons with DM2 are controversial. Some benefit has been found in several[8,54,57,58], but not in all clinical studies[59]. The hypothesis of a role of supplemental Mg in the control of DM2 still needs to be ascertained by large randomized clinical trials[60,61]. Mg supplementation may improve glycemic concentrations in fasting and postprandial states, and insulin sensitivity. We found a significant relationship between the increase in serum and cellular Mg and insulin sensitivity[62]. We also showed that Mg supplementation is able to improve an altered endothelial function in DM2 older adults[63]. Barragán-Rodríguez *et al*[64] suggested a positive effect in the treatment of depression in older persons with DM2 and hypomagnesemia. Presumably, the main problem is that all RCTs were underpowered, partially through overestimation of the treatment effect. Differences may be related to the fact that most of the existing studies have included a small number of subjects, using different Mg doses and different Mg salts.

Several studies have linked high Mg content present in fiber with the positive action of whole grains to improve insulin sensitivity[65-68]. Oral Mg supplements have been shown to improve fasting and postprandial glucose levels and insulin sensitivity in hypomagnesemic DM2 patients[57], to improve insulin sensitivity in non-diabetic subjects with insulin resistance[8], and to decrease C-reactive protein levels in hypomagnesemic patients with prediabetes[69].

In summary, oral Mg supplements appear to be useful in persons with DM2 to restore Mg deficiencies, to improve insulin resistance, oxidative stress, and systemic inflammation.

The absence of large trials in DM2 patients specifically focusing on those with Mg deficit may help to explain the inconsistency between epidemiological (mainly positive) and clinical (mostly controversial) studies. Since most, but not all, DM2 patients have Mg deficiency, it would be useful to focus on those with deficit in order to correct it. Differences in Mg balance, glycemic control, and age are other potential factors that may help to explain the differences among the studies. Most studies used total serum Mg concentration instead of the free, ionized (bioactive) Mg concentration, which make it a challenge to correlate Mg deficiency to diseases.

Future prospective large RCTs would be important to support the possible inclusion of Mg supplements in the guidelines for the management of DM2.

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MINIREVIEWS

Vitamin paradox in obesity: Deficiency or excess?

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Abstract

Since synthetic vitamins were used to fortify food and as supplements in the late 1930s, vitamin intake has significantly increased. This has been accompanied by an increased prevalence of obesity, a condition associated with diabetes, hypertension, cardiovascular disease, asthma and cancer. Paradoxically, obesity is often associated with low levels of fasting serum vitamins, such as folate and vitamin D. Recent studies on folic acid fortification have revealed another paradoxical phenomenon: obesity exhibits low fasting serum but high erythrocyte folate concentrations, with high levels of serum folate oxidation products. High erythrocyte folate status is known to reflect long-term excess folic acid intake, while increased folate oxidation products suggest an increased folate degradation because obesity shows an increased activity of cytochrome P450 2E1, a monooxygenase enzyme that can use folic acid as a substrate. There is also evidence that obesity increases niacin degradation, manifested by increased activity/expression of niacin-degrading enzymes and high levels of niacin metabolites. Moreover, obesity most commonly occurs in those with a low excretory reserve capacity (*e.g.,* due to low birth weight/preterm birth) and/or a low sweat gland activity (black race and physical inactivity). These lines of evidence raise the possibility that low fasting serum vitamin status in obesity may be a compensatory response to chronic excess vitamin intake, rather than vitamin deficiency, and that obesity could be one of the manifestations of chronic vitamin poisoning. In this article, we discuss vitamin paradox in obesity from the perspective of vitamin homeostasis.

Key words: Obesity; Type 2 diabetes; Developmental

origin of disease; Folic acid; Vitamin D; Niacin; Oxidative stress; Insulin resistance; Vitamin fortification

Core tip: Obesity rates have dramatically increased among the United States population, including children, since the 1980s. Considering the lag time between risk exposure and the development of child obesity, the risk must have been imposed on the whole United States population around the late 1970s. Although evidence suggests that the risk is high vitamin intake due to the update of vitamin fortification in 1974 and the implementation of the Infant Formula Act of 1980, why do obese individuals paradoxically show low levels of fasting serum vitamins? In this paper, we try to give an answer to this question based on the current understanding of vitamin homeostasis.

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INTRODUCTION

Obesity, a global health problem, is associated with co-morbidities such as metabolic syndrome, diabetes, hypertension, asthma, nonalcoholic fatty liver disease, renal disease, cardiovascular disease and cancer, which are thought to be of developmental origin[1]. Since the late 1930s, when synthetic vitamins, thiamin, riboflavin and niacin (nicotinic acid and nicotinamide), were used to fortify foods or as dietary supplements, the daily intake of vitamins of the United States population has significantly increased, especially after the update of mandatory fortification in 1974[2] and the implementation of the Infant Formula Act of 1980 (without setting an upper limit for most vitamins)[3]. In fact, the introduction of synthetic vitamins into the diet was followed by a dramatic increase in the prevalence of obesity among all age groups in the United States[4,5]. Similar correlations between increased obesity and vitamin fortification were observed in other vitamin-fortified countries, such as Canada and Saudi Arabia[2]. Over the past 20-30 years, China has also been experiencing a rapid growth in the rates of obesity[6] after having shifted from a low to a high vitamin intake, due to a combination of increased intake of animal-derived foods (rich in vitamin B_1, B_2 and niacin)[7] and mandatory flour fortification with these vitamins, which was introduced in China in the late 1980s and was been mandatorily implemented in 1994[2]. Paradoxically, it is frequently reported that obesity and type 2 diabetes are associated with low levels of fasting serum vitamins, including vitamin B_1, D, and folate[8-10]. Although the mechanism of the paradox remains unclear, it is generally thought that the low vitamin status in obesity is due to inadequate intake.

Since 1998, enriched grain products in the United States have been fortified with folic acid to prevent neural tube defects. Recent studies on folic acid fortification show that obese individuals also show lower fasting serum folate concentrations, but, paradoxically, their red blood cell (RBC) folate concentrations and MeFox (5-methyltetrahydrofolate oxidation product) are significantly higher, when compared with nonobese individuals[11,12]. Moreover, obesity is also found to be associated with increased activity of cytochrome P450 (CYP) 2E1, a monooxygenase enzyme that can use folic acid as a substrate[13]. Folate content in RBC is known to reflect long-term average consumption and tissue stores because RBC only accumulates folate during erythropoiesis[14], and increased serum MeFox suggests increased degradation of folic acid. Moreover, recent evidence shows that obesity is associated with high fasting serum N^1-methylnicotinamide without significant changes in nicotinamide levels[15] and that plasma N^1-methylnicotinamide correlates with increased tissue expression of nicotinamide *N*-methyltransferase (NNMT, a major enzyme responsible for the degradation of nicotinamide to N^1-methylnicotinamide) and the degree of insulin resistance[16]. Collectively, these observations raise the possibility that the vitamin paradox in obesity may involve vitamin excess rather than deficiency. After more than seven decades of practice of vitamin fortification and painful global experience of increasing prevalence of obesity and related diseases worldwide, it is time for us to examine the relationship between vitamin fortification and vitamin paradox from the perspective of vitamin homeostasis.

VITAMIN HOMEOSTASIS AND OXIDATIVE STRESS

Vitamins are essential micronutrients needed by the body in small amounts. Vitamin homeostasis is a balance between vitamin intake and clearance. A deficiency or excess may lead to deleterious effects. Since the introduction of synthetic vitamins into food, high vitamin intake is very common during a person's lifespan from conception through to old age[2]. In this case, the removal of excess vitamins becomes particularly important in maintaining vitamin homeostasis. This depends on the efficiency of both excretory organs and drug-metabolizing enzymes.

Excretion of vitamins

The kidneys and sweat glands are the two major excretory organs responsible for the elimination of water-soluble vitamins, and the sebaceous glands excrete lipid-soluble vitamins in the sebum[17]. The excretion of vitamins is positively related to their intake. Aging is known to be associated with decreasing function

of excretory organs[18,19] and thus may reduce the clearance of vitamins. It is noteworthy that sweat excretion may be particularly important in eliminating excess water-soluble vitamins, because vitamins (*e.g.*, folate[20], nicotinic acid and nicotinamide[2,21]) are barely excreted in the urine before degradation due to the reabsorption by the renal tubules, but they can be easily excreted in the sweat[22-24]. The efficiency of sweat excretion is determined by several factors, including genetic background, intrauterine and early postnatal development, environmental temperature and physical activity. Compared with whites, blacks have a high sweating threshold, manifested by lower skin conductance (*i.e.*, low insensible perspiration)[25] and sweating rates[26] under the same ambient temperature condition, suggesting that blacks may have lower sweat excretion of vitamins than whites.

The formation of functional sweat glands begins at week 36 of gestation and completes within 10 wk of postnatal life[27,28]. This process is affected not only by gestational age but also by the environmental temperature during the early postnatal period. As demonstrated in the literature, preterm birth is associated not only with a lower renal reserve capacity[29] but also with a low sweating function[30,31]. Low temperature may cause newborn hypothermia[32], which may occur even in summer season[32]. Reduced sweat gland function (*i.e.*, low skin conductance) has been found to be associated with a winter birth in schizophrenia[33]. Therefore, preterm birth and newborn hypothermia may be associated with decreased vitamin clearance.

Ambient temperature and physical activity are two important factors affecting the excretion rates of sweat and sebum. For example, a decrease in temperature from 30 ℃ to 22 ℃ reduces insensible perspiration from about 700 mL/d to 380 mL/d in adults[34], and a one-degree decrease in local skin temperature decreases the sebum excretion rate by 10%[35]. There is evidence showing that the levels of plasma vitamin A and E are lower in summer than in winter[36], and a similar seasonal variation is found in blood drug concentrations[37]. Thus, it is conceivable that physical inactivity and winter or cold weather would decrease the tolerance to high vitamin intake.

On the other hand, it should be noted that excess sweat vitamin excretion may cause or worsen water-soluble-vitamin deficiency if there is poor vitamin intake. A good example may be pellagra, a niacin-deficiency disease that affects those who live in poverty without sufficient animal-source foods (rich in nicotinamide), with the symptoms occurring during the summer[38], a season with the highest sweat excretion rates. However, over the past decades, both natural and artificial sources (*i.e.*, vitamin fortification and supplementation) of vitamins have significantly increased[2], while sweat excretion has significantly decreased due to physical inactivity and the widespread use of air conditioning. These dietary and lifestyle changes may increase the risk of excess accumulation of vitamins in the body, especially in those with reduced excretory capacity and/or activity.

Degradation of vitamins

Besides being directly excreted, vitamins also undergo degradation through phase Ⅰ (including oxidation, reduction, and hydrolysis) and phase Ⅱ metabolisms (*e.g.*, sulpfation, methylation and glutathione conjugation), which are catalysed by phase Ⅰ and phase Ⅱ drug-metabolizing enzymes, respectively. After phase Ⅰ and/or phase Ⅱ degradation, vitamins become more water-soluble and then can be more easily excreted from the body. Excess vitamins are degraded very rapidly. For example, cumulative administration of 2000 mg nicotinic acid [166 times the estimated average daily requirement (EAR)] in 13 h 10 min is found to only increase the levels of its metabolites in the plasma, without significantly changing plasma nicotinic acid concentrations[39]. We found that, at 5 h after oral administration of 100 mg nicotinamide (8.3 times the EAR), plasma nicotinamide had returned to near baseline levels, while its metabolite N^1-methylnicotinamide remained at high levels[24]. Thus, it is clear that a transient increase in vitamin intake may not change fasting vitamin levels.

Vitamins, xenobiotics, neurotransmitters and hormones share the same drug-metabolizing enzyme system, so they may interact with one another in their metabolism by inducing and competing for the enzymes[3,40]. For example, CYP2E1, highly expressed in obesity and type 2 diabetes[13], has more than 50 compounds, including some vitamins and ethanol[41]. Thus, it is conceivable that alcohol may cause low fasting vitamin levels by induced CYP2E1.

Phase Ⅱ metabolism of vitamins consumes detoxification resources, such as methyl-group donors, sulphate donors and glutathione, which are also necessary for the degradation of neurotransmitters and hormones. Therefore, excess vitamins can disturb the phase Ⅱ metabolism of neurotransmitters and hormones by competing for the limited detoxification resources[3]. Here, we take niacin methylation as an example to explain how excess vitamins affect metabolism of neurotransmitters and hormones. Methylation is a methyl-group transfer reaction from a methyl donor to a substrate, which is mediated by the methionine-homocysteine cycle. Methyl donors, including betaine and choline, are non-renewable resources in the body, while other components in the methylation system, including methionine, folate, vitamin B_{12} and relevant enzymes, can be repeatedly used in the reaction system. Choline can be used as a methyl donor only after being converted to betaine in the liver and kidneys. According to the relationship of the components in the methylation reaction system shown in Figure 1, it is quite clear that an increase in the levels of substrates will mainly increase the demand for betaine. Since niacin is degraded mainly through

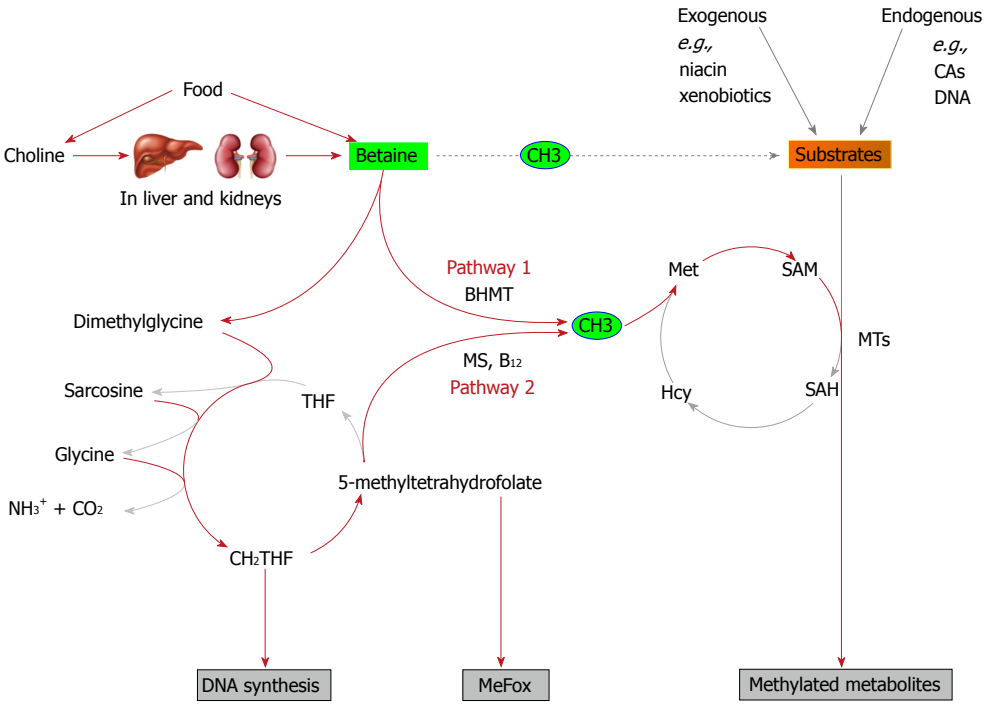

Figure 1 Relationship between methyl donors and mediators in the methylation of substrates. Methylation is a methyl-group transfer reaction from a methyl donor to a substrate, which is mediated by the methionine (Met) cycle. The deep red-arrow lines indicate the flow/transfer of methyl groups/one-carbon units from dietary sources to substrates. In this regard, methylation can be considered as a reaction between betaine and substrates (dashed line). An increase in the levels of substrates will increase the demand for betaine rather than for methylation mediators, *e.g.*, folate and vitamin B_{12} (B_{12}), because betaine is a non-renewable resource, while the mediators can be recycled if there is an adequate supply of methyl donors. Pathway 1: Betaine-dependent homocysteine (Hcy) remethylation; Pathway 2: Folate-dependent Hcy remethylation. BHMT: Betaine-homocysteine-methyltransferase; CAs: Catecholamines; CH_2-THF: 5,10-methylene tetrahydrofolate; CH3: Methyl groups; MeFox: An oxidation product of 5-methyltetrahydrofolate; MS: Methionine synthase; MTs: Methyltransferases; SAH: *S*-adenosylhomocysteine; SAM: *S*-adenosylmethionine; THF: Tetrahydrofolate.

methylation, niacin fortification/supplementation (usually using its nicotinamide form) increases the demand for methyl groups on the one hand, and on the other hand, it can reduce the utilization of choline as a methyl donor by causing hepatic and renal oxidative injury, as demonstrated in a rat model[42]. As a result, excess nicotinamide reduces the size of betaine pool and subsequently inhibits the methylation of endogenous substrates (*e.g.*, catecholamines and DNA), leading to an increase in plasma norepinephrine levels[43] and DNA hypomethylation, an important epigenetic alteration in human diseases[42,44].

Relationship between vitamin excretion and degradation

There is close cooperation between the excretory system and the drug-metabolizing enzyme system in maintaining vitamin homeostasis. If the body's excretory capacity is too low to effectively eliminate excess vitamins, the activity/expression of the drug-metabolizing enzyme system will compensatorily increase due to induction by their substrates[45]. Blacks have a lower sweat rate[2], but have a higher drug/vitamin-metabolizing activity than whites[46]. For example, compared with whites, blacks have a significantly higher catechol-*O*-methyltransferase (a phase Ⅱ enzyme that converted norepinephrine to epinephrine)[47] activity and norepinephrine clearance rate[48] and, during exercise stress, they show lower venous plasma norepinephrine and higher epinephrine[49]. Blacks are prone to low fasting serum vitamin D and folate levels[12,50] and need a higher vitamin D doses to achieve a desired serum 25-hydroxyvitamin D concentration[51]. This suggests an increase in plasma vitamin clearance. Given that the levels of plasma and urinary vitamin metabolites are linked to vitamin intake and that vitamins can induce their own degrading enzymes, the findings that increased activity/expression of drug-metabolizing enzymes (*e.g.*, CYP2E1[13,52] and NNMT[16]) and high levels of vitamin metabolites (*e.g.*, MeFox[12], N^1-methylnicotinamide[15,16] and nicotinuric acid[53]) can be considered as increased compensation for decreased vitamin excretion in response to high vitamin intake.

The degradation of vitamins is accompanied by the generation of reactive oxygen species (ROS). Although ROS at physiological levels functions as signalling molecules, at large levels they can induce cellular toxicity and insulin resistance. In our previous study, we found that co-administration of nicotinamide and glucose (like grain fortification with niacin) can induce insulin resistance due to excess ROS and subsequent reactive hypoglycaemia, demonstrating that vitamin-fortified grains can increase appetite[2,5]. This may explain the sharp increase in prevalence of obesity in the United States after the levels of vitamin fortification

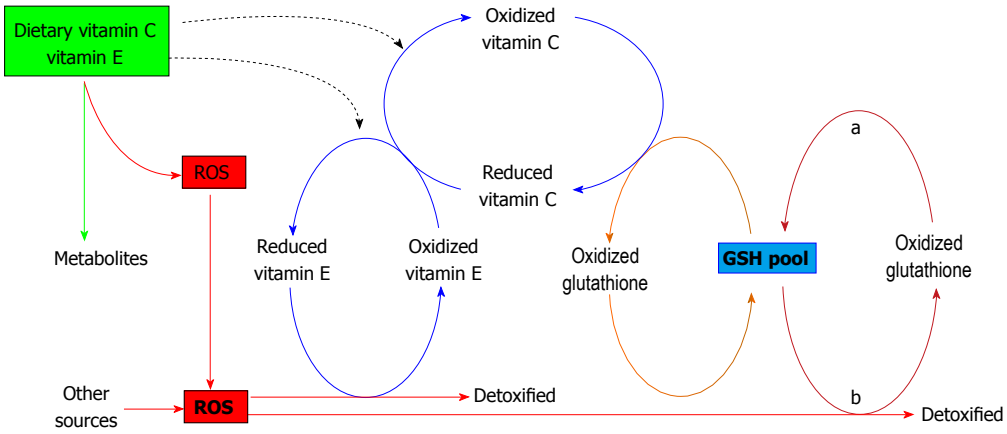

Figure 2 Glutathione-vitamin C-vitamin E interrelationship in the detoxification of reactive oxygen species. The endogenous glutathione antioxidant system maintains vitamin C and vitamin E recycling and actually determines the antioxidant effect of these vitamins. GSH: Reduced glutathione; a: Glutathione reductase; b: Glutathione peroxidase; ROS: Reactive oxygen species.

were increased in 1974[4,5]. Because decreased sweat excretion may increase enzymatic vitamin degradation and thereby ROS generation, individuals with reduced excretory capacity are at increased risk of insulin resistance, obesity and related diseases when exposed to identical high-vitamin diets.

As shown in Figure 2, it is clear that although vitamin E and C can scavenge ROS, their antioxidant effect actually depends on the capacity of the endogenous glutathione antioxidant system, by which vitamin C and vitamin E recycling is maintained[54]. Because the endogenous glutathione antioxidant system *per se* directly scavenges free radicals, high levels of supplementation of vitamin C and vitamin E are not only unnecessary but harmful due to increasing the burden of the glutathione antioxidant system. It is obvious that excess vitamin intake may provide an additional source of ROS. Thus, it is not surprising that some randomized clinical trials show that high-dosage vitamin E supplementation may increase, rather than decrease, cardiovascular events and all-cause mortality[55].

FOLIC ACID FORTIFICATION-INDUCED PARADOX

Although mandatory vitamin fortification has been implemented since the early 1940s and updated in 1974, unfortunately it is hard to determine the relationship between vitamin fortification and the increased prevalence of obesity, mainly because of the lack of studies regarding the effects of vitamin fortification and excess vitamin degradation on the metabolism of the body. Fortunately, the effects of the mandatory folic acid fortification that was started in 1998 in the United States are closely monitored based on the data from National Health and Nutrition Examination Surveys (NHANES). This provides a valuable opportunity for us to understand the vitamin paradox in obesity. The major results of studies on folic acid fortification are summarized as follows: (1) Blood folate concentrations in the United States population show first a sharp increase from pre- to postfortification (2.5 times for serum and 1.5 times for RBC folate) and then a decline over time (decreased by 17% for serum and 12% for RBC folate during 1999–2010)[56]; (2) Unmetabolized folic acid was detected in nearly all serum samples measured, and serum unmetabolized folic acid concentrations > 1 nmol/L are associated with being older, non-Hispanic black, nonfasting (< 8 h), higher total folic acid intake (diet and supplements), and higher RBC folate concentrations[57]; (3) Serum and RBC total folate concentrations, including MeFox (an oxidation product of folate), are high in older adults and individuals with low renal function[12]; (4) Body mass index is associated negatively with serum unmetabolized folic acid and 5-methyltetrahydrofolate, but positively with serum MeFox and RBC folate concentrations[12]; (5) Compared with non-Hispanic whites, non-Hispanic blacks have lower serum and RBC total folate concentrations[12]; (6) In folic acid supplement users, it was found that non-Hispanic black users have lower serum 5-methyltetrahydrofolate concentrations than non-Hispanic-white users[57]; and (7) Alcohol intake is negatively associated with serum unmetabolized folic acid, 5-methyltetrahydrofolate and MeFox, without significantly affecting RBC folate concentrations[12].

Evidently, there are significant differences in response to folic acid fortification among the United States population. From the perspective of vitamin homeostasis, the differences may actually reflect differences in folic acid excretion and degradation. Because folic acid is not a natural form of folate, the detection of unmetabolized folic acid in fasting serum suggests a folic acid overload. This overload is more evident in individuals with low excretion capacity, including either low renal function or sweat excretion (in non-Hispanic blacks), or both (in older adults).

The decline in post-fortification serum and RBC folate concentration over time in the United States

population[56], and the association between increased MeFox levels and decreased renal function[12] suggests a compensatory increase in folic acid degradation. As mentioned above, blacks may have a higher drug-metabolizing activity to compensate for their reduced sweat excretion. This may account for the finding that non-Hispanic blacks have low serum and RBC total folate concentrations. The association between unmetabolized folic acid concentrations > 1 nmol/L and non-Hispanic blacks[57] suggests that folic acid intake in this population may exceed their folic acid clearance capacity. Moreover, the low serum 5-methyltetrahydrofolate concentrations in non-Hispanic black users[57] may suggest a lack of one-carbon donors (due to the increased drug-metabolizing activity in blacks), because the formation of 5-methyltetrahydrofolate consumes one-carbon donors (Figure 1).

Many obesity risk factors, such as being blacks[11], having a low birth weight/preterm birth[58], a winter (or cold weather) birth[59,60], or physical inactivity[61], are related to decreased sweat-gland function. This is also supported by the finding that an equivalent dose of folic acid (by body weight) caused a greater increase in serum folate in obese than non-obese individuals[62]. Given that obesity is associated with folate-degrading enzyme CYP2E1[13,52], the association of increased serum MeFox and RBC folate levels and low fasting serum folate levels in obesity may reflect a severe folic acid overload. From this point of view, the finding that the inverse association between body mass index and serum folate is no longer evident among folic acid supplement users in the United States[63] can be considered as saturation of the compensatory capacity of the drug-metabolizing enzyme system in obesity.

Ethanol is known to induce drug-metabolizing enzymes[64,65], including CYP2E1[66]. This may explain the association between alcohol consumption and low fasting serum folate status. It should be pointed out that alcohol consumption-induced low fasting serum folate does not mean folate deficiency, because there is no significant decrease in RBC folate concentrations[12].

Overall, four conclusions can be reached: (1) the current folic acid intake of Americans has exceeded their excretory capacity; (2) there is increased compensation for increased folic acid intake, especially in individuals with low excretion capacity; (3) further folic acid supplementation after fortification can saturate the drug metabolizing enzyme system; and (4) the production of MeFox suggests that excess folic acid may increase the consumption of one-carbon units (Figure 1) and provide a source of ROS.

MECHANISM BEHIND LOW VITAMIN D STATUS

There is also a paradox after vitamin D is used in fortification and as a supplement. Vitamin D, although considered a vitamin, can be produced in the skin by sun exposure. Numerous studies have documented an association between low serum concentrations of 25-hydroxyvitamin D and many non-skeletal disorders. Many studies have examined the effect of vitamin D supplementation on the disorders[67], including obesity[68], diabetes[69], hypertension[70], dyslipidemia[71], cardiovascular disease[72], cancer[73], depression[74], and asthma[75]. Unfortunately, most, if not all, of published meta-analyses have failed to show a significant benefit of vitamin D supplementation with or without calcium[68-75]. It is likely that low fasting serum 25-hydroxyvitamin D status may be not the cause of these diseases.

The skin is a major determinant of 25-hydroxyvitamin D status. Besides synthesizing vitamin D, the skin also functions as a powerful excretory organ[17]. Notably, the skin functions fluctuate with seasonal temperature fluctuation, with the highest activities in summer and lowest activities in winter. Thus, it is likely that decreased skin excretory function may be a cause of human diseases. In fact, although not directly focusing on the excretory function of the skin, many studies have suggested a direct link of between the levels of plasma compounds and skin excretory function. For example, sebum excretion decreases in winter[76,77] and inhibition of sebum excretion increases the levels of blood triglycerides and cholesterol[78]. Sweat-inhibiting factors (*e.g.*, acute cold exposure[79,80]) increases plasma norepinephrine levels. Decreased sweating function is found to be closely linked to diseases, for example, skin conductance non-response in schizophrenia and depression[81], low skin conductance in hypertension[82] and type 2 diabetes[83], and the association between psoriasis and metabolic syndrome[84]. Moreover, many well-known chronic disease risk factors, such as being of black origin, having a preterm birth or winter birth, or physical inactivity, are associated with decreased skin excretory function, as mentioned above. Taken together, it can be concluded that decreased skin excretory function may play a major role in diseases, and 25-hydroxyvitamin D status may be an indicator of skin excretory function.

Interestingly, there is a graded relationship between vitamin D status and body mass index[85]. Sadiya *et al*[86] found that it is difficult to achieve target levels of 25-hydroxyvitamin D above 75 nmol/L in type 2 diabetic obese subjects with a relatively high daily dose of vitamin D_3. Recently, Didriksen *et al*[87] performed a 5-year intervention study with vitamin D_3 at a dose of 20000 IU (500 μg) per week *vs* placebo in subjects with impaired glucose tolerance and/or impaired fasting glucose, and they found that those given vitamin D_3 had significantly higher vitamin D concentration in their adipose tissue (about 6.5 times the placebo group), while their median serum 25-hydroxyvitamin D level only increased from the baseline of 61 to 99 nmol/L. This study clearly demonstrates that large amounts of vitamin D_3 are stored in adipose tissue after vitamin D_3 supplementation, and suggests that overweight and

obese subjects may store more vitamin D than normal-weight subjects because they have larger amounts of adipose tissue. Moreover, vitamin D is known to induce drug-metabolizing enzymes[88]. Thus, it seems likely that the prevalence of low 25-hydroxyvitamin D status after the introduction of vitamin D fortification may share a similar mechanism to that of low folate status: increased degradation and storage in compensation for excess intake.

THE CLINICAL SIGNIFICANCE OF THE VITAMIN PARADOX

Understanding the vitamin paradox in obesity and related diseases is crucial in determining how to manage the low vitamin status in these diseases. From the above analysis, it is apparent that the vitamin paradox in obesity may be due to increased vitamin degradation and storage in compensation for decreased vitamin excretion. This condition will continue until drug-metabolizing enzymes are saturated by their substrates, in which high expression of vitamin-degrading enzymes and elevated vitamin-metabolite levels may serve as indicators. The vitamin paradox can be resolved by reducing vitamin intake and increasing sweat rates, rather than by giving vitamin supplementation. Indeed, a recent study shows that bariatric surgery (restricting food intake) and exercise are associated with a significant reduction in NNMT expression plasma MNA levels[16]. This can be explained by decreased niacin intake and increased sweat excretion.

Excess vitamins have three major detrimental effects: (1) increasing ROS generation and subsequently leading to oxidative tissue damage and insulin resistance; (2) disturbing the degradation of neurotransmitters and hormones by competing for drug metabolizing enzymes and detoxification resources; and (3) causing epigenetic changes (*e.g.*, altered DNA methylation) by depleting the body's methyl-group pool[2,89]. Thus, fortification-induced sustained excess vitamin intake may deplete the drug-metabolizing system (*e.g.*, manifested by high levels of unmetabolized vitamins) and the antioxidant system, and eventually cause a variety of metabolic disorders and oxidative tissue damage. This may play a causal role in the increased prevalence of obesity and related diseases, as hypothesized in our previous work[2,4,5].

The association between high vitamin intake and chronic diseases can be considered as vitamin poisoning. Vitamin poisoning is dose dependent. For example, high-dosage vitamin E may increase cardiovascular events and all-cause mortality[55]. Two recent large-scale randomized niacin trials (nicotinic acid, 1500-2000 mg/d) show that nicotinic acid has many adverse effects, including loss of glycaemic control among persons with diabetes, new-onset diabetes[90,91] and increased risk of death, with borderline statistical significance (P = 0.08)[90]. There are three factors that can increase the risk of vitamin poisoning: (1) the function of excretory organs is too low to effectively remove excess vitamins from the body, for example, due to early-life malnutrition-induced renal insufficiency[92]; (2) the amount of vitamin intake has exceeded the excretory capacity of individuals without any developmental defect, which may account for excess chronic diseases in blacks and those with physical inactivity; and (3) the combination of both (1) and (2), accounting for the high rates of chronic diseases in subjects born preterm after the implementation of vitamin fortification. Because the reserve capacity of excretory/detoxifying organs has been determined in early life, whether or not chronic diseases occur will depend on whether there are chemical overloads of the excretory/detoxifying organs in late life. This may be the mechanism of the origin of chronic diseases. Excess vitamin is a kind of chemical overload, accounting for the association between the prevalence of obesity and diabetes and increased B-vitamin intake[4].

CONCLUSION

In summary, it can be concluded that the vitamin paradox in obesity may be a reflection of excess vitamin intake, rather than a vitamin deficiency. Given that there is a correlation between high vitamin intake and the increased prevalence of obesity, it can be assumed that obesity could be one of manifestations of chronic vitamin poisoning. Susceptible individuals to high vitamin intake are those with a low reserve capacity of excretory organs. Therefore, on an individual basis, prevention of obesity should focus on reducing their intake of vitamin-fortified foods, and for a country, more attention needs to be paid to the role of vitamin fortification and abuse in the increased prevalence of obesity and related diseases.

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ORIGINAL ARTICLE

Basic Study

Simvastatin, atorvastatin, and pravastatin equally improve the hemodynamic status of diabetic rats

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Abstract

AIM: To investigate if the effect of statins improving cardiovascular (CV) status of diabetics is drug-specific or class-dependent, and the underlying mechanisms involved.

METHODS: We compared the results of daily administration over a four-week period of a low dose (10 mg/kg per day) of atorvastatin (AV), simvastatin (SV), and pravastatin (PV) on cardiac performance in diabetic rats. Echocardiographic variables were tested, as well as systolic blood pressure (SBP), acetylcholine (ACh)-induced relaxation, plasma cholesterol levels, and perivascular fibrosis. Malondialdehyde (MDA) and 4-hydroxyalkenal (4-HAE), and endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) protein levels were also measured in cardiac and aortic homogenates.

RESULTS: In untreated diabetic rats, cholesterol levels were higher than in control rats (CT; $n = 8$, $P < 0.05$), and the low dose of statins used did not modify these levels. In diabetic rats, SBP was higher than in CT, and was significantly reduced by all three statins ($n = 10$, $P < 0.05$). Echocardiographic parameters (EF, SV, and COI) were all lower in untreated diabetic rats than in CT ($n = 10$, $P < 0.05$). These CV parameters were equally

improved by all three statins. The maximal relaxation (E_{Max}) induced by ACh in aortic ring from diabetic rats was also improved. Moreover, this relaxation was abolished by 1 mmol/L NG-nitro-L-arginine methyl ester, suggesting the involvement of a NO-dependent mechanism.

CONCLUSION: AV, SV, and PV are equally effective in improving CV performance in diabetic rats. All tree statins decreased media thickness, perivascular fibrosis, and both MDA and 4-HAE in the aortas of diabetic rats, without affecting eNOS and iNOS protein levels. The observed hemodynamic benefits are cholesterol-independent. These benefits appear to be secondary to the improved endothelial function, and to the reduced vascular tone and remodeling that result from decreased oxidative stress.

Key words: Statins; Diabetes; Oxidative stress; Cardiac function; Perivascular fibrosis

Core tip: Despite evidence that statins are useful therapeutic tools in treating diabetes, questions remain as to whether their effects are drug-specific or class-dependent, what mechanisms underlie these effects, and which statin is the most appropriate. We found that atorvastatin, simvastatin, and pravastatin are equally effective in improving cardiovascular performance in Type 1 diabetic rats, and that the observed benefits are likely to be secondary to the reduction of oxidative stress by these drugs.

Crespo MJ, Quidgley J. Simvastatin, atorvastatin, and pravastatin equally improve the hemodynamic status of diabetic rats. *World J Diabetes* 2015; 6(10): 1168-1178 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i10/1168.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i10.1168

INTRODUCTION

Diabetes is a group of metabolic diseases primarily characterized by hyperglycemia resulting from defects in insulin production, action, or both. This condition has been associated with an increased risk of cardiovascular (CV) deterioration, which is the major cause of death in diabetic patients[1-3]. CV complications include hypertension, ischemic heart disease, heart failure, and diabetic nephropathy. The etiology of cardiac abnormalities in diabetes has been linked to increased oxidative stress and endothelial dysfunction, although the precise mechanism for these complications remains elusive[4-6].

The addition of statins, which inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, to standard antiglycemic therapies decreases CV complications in diabetic patients. The American Diabetes Association's "Standards of Medical Care in Diabetes-2015"[7], recommends the use of statins for all diabetics under 40 years of age with additional CV risk factors, or with overt CV disease. It further recommends that diabetics over the age of 40 take statins, regardless of the absence of CV risk factors. Indeed, in Type 2 diabetics without elevated cholesterol, the risk of suffering the first CV event is reduced by atorvastatin (AV)[8]. The statin-induced improvement of cardiac function in normo-cholesterolemic patients suggests that these drugs have pleiotropic benefits that may be independent of their ability to lower cholesterol levels[9,10]. The mechanisms underlying these beneficial effects may include improvement of endothelial function through increased systemic NO bioavailability[11] and endothelial nitric oxide synthase (eNOS) expression[12], or through reduced oxidative stress[13,14].

Despite evidence that statins are useful therapeutic tools in diabetes, questions remain as to whether their effect is drug-specific or class-dependent, which statin is most appropriate, and what mechanisms underlie this effect. In the present study, we compared the effects of three different statins (AV, SV, and PV) on the CV profile of streptozotocin (STZ)-induced diabetic rats that did not receive insulin supplementation. This animal model of Type 1 diabetes is a validated model for the study of diabetic effects on the CV system. At four weeks following diabetic induction, the rats are hypertensive and have decreased cardiac output, stroke volume, and ejection fraction, when compared to age-matched controls (CT)[14,15]. To evaluate and compare the effects of these statins on cardiac function, we measured stroke volume, ejection fraction, and cardiac output with echocardiography. The effects of statins on endothelial function, cholesterol level, and vascular remodeling were also evaluated. The results from this study may help to identify the most effective statin for improving the CV profile in diabetics.

MATERIALS AND METHODS

Experimental animal model

Four-week-old male Sprague-Dawley rats (120-125 g average weight) were acquired from Hilltop Lab Animals, Inc. (Scottsdale, PA). A total of 160 rats were divided into two groups, diabetic and CT, with each group containing 80 animals. Diabetes was induced by injecting intraperitoneally (IP) streptozotocin (STZ, 65 mg/kg) dissolved in 0.1 mol/L citrate buffer (pH 4.5) after an overnight fast. Diabetic induction was confirmed with positive blood glucose tests twenty-four hours after STZ injection, (Accu-Chek Simplicity, Roche, Indianapolis, IN). Glucose was weekly monitored. The rats did not receive insulin and the experiments were performed at 4 wk after induction of diabetes.

Drug administration

After diabetic induction, each rat was treated daily

with the selected drug (AV or SV or PV) over a four-week period. The statins were suspended in corn oil and administered by gavage at a dose of 10 mg/kg per day. The volumes of all administered drugs were adjusted weekly according to each animal's weight in order to ensure a constant dose. Untreated diabetic and CT groups received by gavage only corn oil, as a placebo. Statin doses were selected based on previous studies on diabetic rats, and from our laboratory[16,17]. In order to obtain cholesterol level reductions similar to those attained in humans, a 50 mg/kg per day statin administration is needed in rats[13]. Thus, a low dose of 10 mg/kg per day allowed us to assess the effect of statins on the CV system independently of the benefits derived from cholesterol reduction.

Echocardiographic evaluation

Serial transthoracic echocardiographic evaluations were performed using an ultrasound system with a 7.5 to 9.0 MHz transducer (Sonosite Inc. WA), after anesthesia (30 mg/kg BW, IP), following a previously described protocol[17,18]. Image analysis was performed using Sitelink Image Manager (Sonosite Inc., Bothell, WA).

Noninvasive measurement of systolic blood pressure

Noninvasive systolic blood pressure (SBP) was evaluated using a RTBP-2000 system (Kent Scientific, Litchfield, CT), and analyzed with Lab View Program (National Instruments Co. Austin, TX) as previously described[19].

Evaluation of acetylcholine-induced relaxation

To evaluate endothelium-dependent relaxation, aortic rings (5 mm) from the descending thoracic aorta were placed in Krebs' bicarbonate solution (composition in mmol/L: 118 NaCl, 2.5 $CaCl_2$, 5 KCl, 1.1 $MgSO_4$, 25 $NaHCO_3$, 1.2 KH_2PO_4 and 10 glucose, pH = 7.4). The rings were suspended horizontally with a resting tension of 2.0 g, and connected to a FT03C Grass transducer, following the protocol previously described by our laboratory[19]. The effect of statins on acetylcholine (ACh)-induced relaxation was evaluated in rings pre-contracted with norepinephrine (NE, 1.0 μmol/L). Cumulative concentration-response curves (from 0.1 nmol/L to 10 μmol/L) for ACh were generated after equilibration. An additional dose response curve was then performed after a 45-min incubation period with L-NAME (1 mmol/L). For a particular ACh concentration, the relaxation was expressed as a percentage of the maximal contraction induced by 1.0 μmol/L of NE.

Cholesterol level determination

Blood samples from both untreated and treated diabetic rats and from CT were centrifuged (5000 rpm; 5 min; 4 ℃ to measure cholesterol concentration. Total cholesterol levels were quantified a cholesterol quantitation kit (Sigma-Aldrich, MAK043). A SpectraMax Microplate Reader (Molecular Devises, CA) was used to measure sample absorbance at 570 nm. A calibration curve using cholesterol standards was used to quantify cholesterol levels.

Measurement of malondialdehyde and 4-hydroxyalkenals levels

The effect of statins on lipid peroxidation, a marker of oxidative stress, was evaluated following the previously described protocol[20]. Malondialdehyde (MDA) and 4-hydroxyalkenals (4-HAE) levels were determined in cardiac and vascular homogenates at an absorbance of 586 nm.

Measurement of media thickness and perivascular fibrosis

Perivascular fibrosis and media thickness from the thoracic aorta from untreated and treated animals were determined to assess the effect of statin treatment. Tissues were stained with Azan-Mallory and Hematoxylin and Eosin (H and E) following the methodology previously described by our laboratory[20]. Results (in μm) were normalized to body weight.

Western Blot for eNOS and inducible nitric oxide synthase

Western Blot studies were performed using a modified protocol described previously[21]. Protein samples were separated by electrophoresis in a 6% SDS-PAGE gel. Proteins were transferred to a nitrocellulose membrane. Membranes were blocked with 5% Blotto for 1 h. Mouse monoclonal antibodies for eNOS (1:2000 for cardiac tissue, 1:3000 for aortic tissue; BD Biosciences, San Jose, CA), inducible nitric oxide synthase (iNOS) (1:500 for cardiac tissue, 1:750 for aortic tissue; BD Biosciences, San Jose, CA), were added to the membrane after dilution in Blotto, and incubated overnight at 4 ℃. The nitrocellulose membranes were incubated with the secondary anti-mouse antibody coupled to Horseradish Peroxidase (HRP) (1:4000; Santa Cruz Biotechnology, Santa Cruz, CA). Before exposure and development, the membranes were incubated with Super Signal West Femto Maximum Sensitivity Substrate (Thermoscientific, Waltham, MA) to enhance the HRP signal derived from the secondary antibody. The Versadoc™ Imaging System and Quantity One Software (Bio-Rad Laboratories, CA) were used to develop and analyze the membranes. eNOS and iNOS levels were standardized by comparison with the β-actin housekeeping gene detected (1:4000; Sigma-Aldrich, St. Louis, MO).

Statistical analysis

All data are expressed as the mean ± SEM (GraphPad Software, Inc., San Diego, CA). Differences between experimental groups were analyzed using Student's *t* and ANOVA, followed by Student-Newman-Keuls test for posthoc analysis. Values were considered statistically significant at a *P* value less than 0.05.

Table 1 Blood glucose (mg/dL) in diabetic and control rats treated with statins

Condition	Day 0	Day 1	Day 7	Day 14	Day 28
CT none	131.25 ± 3.14	130.50 ± 1.51	126.78 ± 4.86	112.42 ± 4.55	133.88 ± 13.66
CT + AV	137.0 ± 5.86	123.33 ± 2.85	126.8 ± 2.2	128.40 ± 7.02	180.67 ± 52.21
CT + SV	143.13 ± 1.75	128.50 ± 3.10	126.80 ± 2.22	128.40 ± 7.02	152.88 ± 18.46
CT + PV	142.63 ± 5.79	127.13 ± 3.36	122.20 ± 4.79	120.80 ± 4.47	130.63 ± 4.06
Diabetic none	133.65 ± 3.51	445.41 ± 24.11[a]	490.45 ± 34.34[a]	530.09 ± 26.65[a]	517.76 ± 18.11[a]
Diabetic + AV	133.00 ± 3.30	473.82 ± 40.23[a]	497.38 ± 47.68[a]	485.38 ± 48.73[a]	500.73 ± 32.65[a]
Diabetic + SV	133.75 ± 2.70	413.19 ± 21.22[a]	473.69 ± 27.39[a]	483.23 ± 39.90[a]	498.94 ± 30.62[a]
Diabetic + PV	126.88 ± 2.08	430.44 ± 27.31[a]	524.38 ± 19.92[a]	564.85 ± 13.57[a]	557.25 ± 12.92[a]

[a]$P < 0.05$ *vs* age-matched C. Values are means ± SEM. Rats were injected with STZ on day 0. Blood glucose for diabetic rats: n = average of 10 rats per group. AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control; STZ: Streptozotocin.

Table 2 Body weight (g) of diabetic and control rats treated with statins

Condition	Day 0	Day 1	Day 7	Day 14	Day 28
CT none	179.74 ± 4.02	182.28 ± 4.15	242.78 ± 6.18	304.06 ± 13.26	391.34 ± 9.80
CT + AV	167.17 ± 4.38	172.97 ± 4.53	234.77 ± 5.27	287.67 ± 4.67	404.10 ± 12.46
CT + SV	181.89 ± 8.26	184.19 ± 8.12	248.40 ± 7.99	291.69 ± 6.93	394.14 ± 15.92
CT + PV	193.81 ± 8.25	198.85 ± 7.66	262.99 ± 9.03	301.19 ± 8.02	389.64 ± 11.48
Diabetic none	180.22 ± 5.99	172.53 ± 5.24	198.19 ± 7.41	211.94 ± 11.11[a]	267.10 ± 27.98[a]
Diabetic + AV	176.30 ± 3.10	159.86 ± 9.93	202.32 ± 5.13	235.45 ± 8.56[a]	255.40 ± 17.09[a]
Diabetic + SV	183.14 ± 4.68	178.24 ± 4.29	207.69 ± 6.81	230.13 ± 7.23[a]	241.65 ± 12.73[a]
Diabetic + PV	187.88 ± 5.62	182.58 ± 4.60	204.85 ± 7.18	243.69 ± 5.60[a]	253.99 ± 11.82[a]

[a]$P < 0.05$ *vs* age-matched C. Values are means ± SEM. Rats were injected with STZ on day 0. Blood glucose for diabetic rats: n = average of 10 rats per group. STZ: Streptozotocin; AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control.

Table 3 Total cholesterol levels in plasma from diabetic and control rats after four weeks of statin treatment (10 mg/kg per day)

Condition	Cholesterol (mg/dL)
CT none	156.01 ± 7.32
CT + AV	143.69 ± 14.21
CT + SV	169.86 ± 12.78
CT + PV	155.53 ± 7.08
Diabetic none	248.68 ± 15.78[a]
Diabetic + AV	233.35 ± 18.44[a]
Diabetic + SV	234.40 ± 12.11[a]
Diabetic + PV	235.57 ± 18.20[a]

Values shown are the means ± SEM of an average of 8 animals per group. [a]$P < 0.05$ *vs* age-matched treated and untreated CT. AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control.

RESULTS

Blood glucose, body weight, and cholesterol levels are shown in Tables 1, 2 and 3. Twenty four-hours after diabetic induction, blood glucose levels were significantly higher in diabetic rats than in CT rats (445.41 ± 24.11 mg/dL *vs* 130.50 ± 1.51 mg/dL, respectively; $n = 10$, $P < 0.05$; Table 1). This difference was maintained throughout the course of the study and was not affected by the administration of any statin. Body weight increased in both diabetic and CT rats over the course of this study, although it was significantly lower in aged-matched diabetic rats (Table 2). This parameter also was not modified by any statin. Total cholesterol levels were significantly increased in diabetic rats when compared to aged-matched CT (248.68 ± 15.78 mg/dL *vs* 156.01 ± 7.3 mg/dL; $n = 8$, $P < 0.05$; Table 3). At 10 mg/kg per day, once again, statins did not modify plasma cholesterol levels in either diabetic or CT rats ($n = 8$, $P > 0.05$).

In diabetic rats, stroke volume (Figure 1A) increased significantly after statin treatment (from 0.20 ± 0.02 mL in untreated, to 0.51 ± 0.06 mL with AV, to 0.47 ± 0.05 mL with SV, and to 0.43 ± 0.05 mL with PV; $n = 10$, $P < 0.05$). In diabetic rats ejection fraction was lower than in CT (Figure 1B; 44.93% ± 3.03% *vs* 70.67% ± 2.11%; $n = 10$, $P < 0.05$), but also improved after statin treatment (to 59.92% ± 2.98 % with AV, to 60.13% ± 3.55% with SV, and to 56.85% ± 4.45% with PV; $n = 10$, $P < 0.05$). Similarly, cardiac output index (mL/min per 100 g BW) improved after statins treatment in diabetic rats (from 24.74 ± 3.52 in untreated to 57.65 ± 6.59 with AV, to 60.13 ± 4.10 with SV and to 53.25 ± 6.19 with PV; $n = 10$, $P < 0.05$) (Figure 1C).

SBP (Figure 2) was higher in diabetic rats than in CT (116.52 ± 3.81 mmHg in STZ *vs* 82.72 ± 2.36 mmHg in CT; $n = 10$, $P < 0.05$. Administration of statins significantly reduced this variable in diabetic rats (to 100.91 ± 5.15 mmHg with AV, 93.17 ± 3.31 mmHg with SV, and 106.44 ± 4.21 mmHg with PV; $n = 10$, $P < 0.05$).

The maximal relaxation (E_{Max}) induced by ACh (Figure 3) was significantly reduced in the aortic rings from diabetic rats compared to those from aged-matched CT (53.70% ± 4.07% *vs* 74.61% ± 3.27%; $n = 10$, $P < 0.05$). This finding confirms that, at four weeks

Table 4 Effect of chronic statin treatment on EC_{50} and E_{MAX} values following ach-induced relaxation in diabetic and control rats

Condition	E_{max} relaxation, %	EC_{50}, μmol/L
CT none	74.61 ± 3.27	0.56 ± 0.11
CT + AV	70.75 ± 3.99	0.68 ± 0.22
CT + SV	70.76 ± 4.16	1.15 ± 0.47
CT + PV	71.16 ± 4.30	0.72 ± 0.20
Diabetic none	53.70 ± 4.07[a]	0.41 ± 0.10
Diabetic + AV	82.13 ± 7.01[c]	0.84 ± 0.32
Diabetic + SV	84.63 ± 6.51[c]	0.40 ± 0.21
Diabetic + PV	83.88 ± 6.83[c]	0.66 ± 0.35

Values shown are the means ± SEM of an average of 10 animals per group. [a]$P < 0.05$ when *vs* age-matched treated and untreated CT; [c]$P < 0.05$ when *vs* age-matched untreated diabetic rats. No statistically significant differences were found between treated diabetic rats and treated CT. AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control.

following diabetes induction, endothelial dysfunction is present in the aorta of diabetic rats. The tested statins significantly improved E_{Max} values in diabetic rats (82.13% ± 7.01% with AV, 84.63% ± 6.51% with SV, and 83.88% ± 6.83% with PV; $n = 10$, $P < 0.05$), but did not modified this value in CT. Moreover, a 45-min incubation period with 1 mmol/L L-NAME completely abolished the ACh-induced relaxation, indicating that the effect of these statins on vascular relaxation is NO-mediated. EC_{50} values, by contrast, were not modified by any statin in either diabetic rats or CT (Table 4).

MDA and 4-HAE (μmol/g protein), which are oxidative stress markers were higher in aortic homogenates (Figure 4A) from diabetic rats than in those from CT (6.49 ± 1.24 *vs* 3.69 ± 0.58; $n = 8$, $P < 0.05$). In diabetic rats, but not in CT, all statins significantly reduced MDA and 4-HAE levels (2.69 ± 0.42 with AV, 3.59 ± 0.47 with SV, and 4.03 ± 0.40 with PV; $n = 8$, $P < 0.05$). In cardiac homogenates (Figure 4B), by contrast, MDA and 4-HAE levels were similar in untreated diabetic (1.42 ± 0.12) and CT (1.10 ± 0.12; $n = 8$, $P > 0.05$), and statin treatment did not modify these parameters.

Similar segments of the thoracic aorta from STZ-diabetic rats and CT were investigated to assess the effects of statins on vascular remodeling. In untreated diabetic rats, perivascular fibrosis (Figure 5A) was higher than in CT (10.59 ± 0.40 μm/100 g BW *vs* 4.21 ± 0.22 μm/100 g BW; $n = 5$, $P < 0.05$). All statins reduced perivascular fibrosis in diabetic rats (8.99 ± 0.33 μm/100 g BW with AV, 8.75 ± 0.43 μm/100 g BW with SV, and 9.04 ± 0.39 μm/100 g BW with PV; $n = 5$, $P < 0.05$). Perivascular fibrosis in CT, by contrast, was not modified by any of the statins. In addition, media thickness, which was thicker in diabetic rats than in age-matched CT (49.70 ± 1.10 μm/100 g BW *vs* 46.03 ± 0.67 μm/100 g BW; $n = 5$, $P < 0.05$), was significantly reduced by all the statins in diabetic rats (44.93 ± 0.76 μm/100 g BW with AV, 47.15 ± 0.48 μm/100 g BW with SV, and 46.78 ± 0.67 μm/100 g BW with PV), but not in CT.

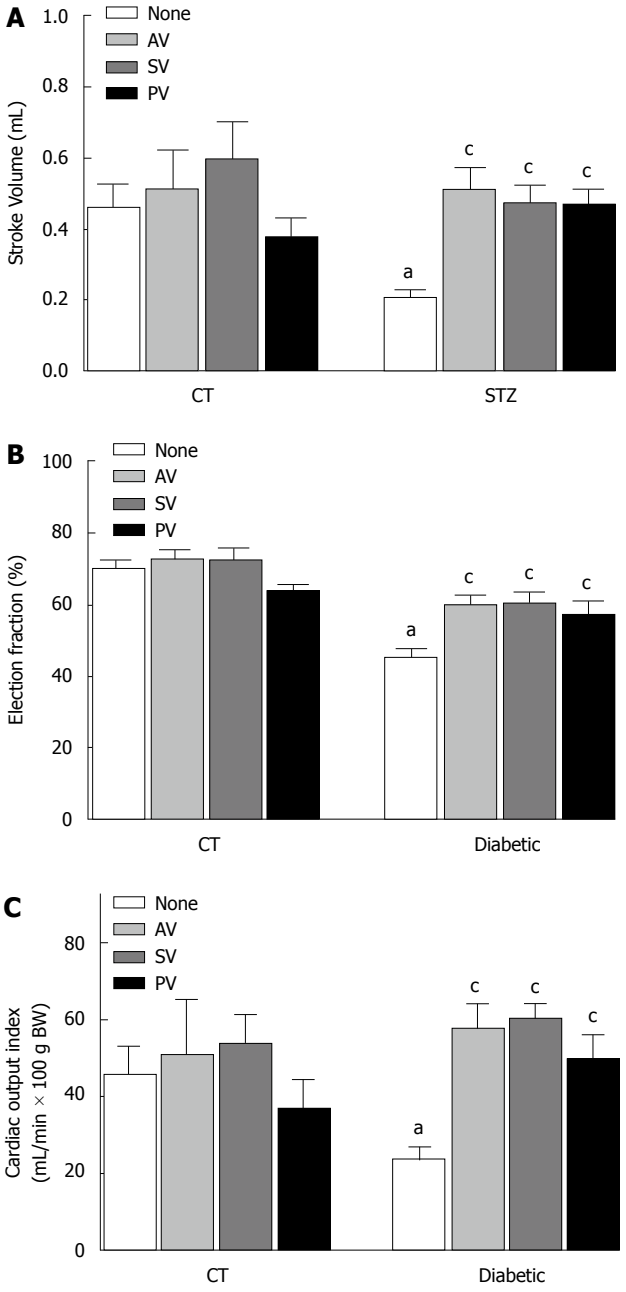

Figure 1 Effects of four weeks treatment with atorvastatin, simvastatin, and pravastain (10 mg/kg per day) on diabetic rats and control. A: Stroke volume (mL); B: Ejection fraction (%); C: Cardiac output index (mL/min x 100 g BW). The results represent the mean ± SEM of 8 animals per group. All the statins significantly improved these CV parameters in diabetic rats. [a]$P < 0.05$ for diabetic rats *vs* CT; [c]$P < 0.05$ for untreated diabetic rats *vs* treated diabetic rats. STZ: Streptozotocin; AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control.

The effect of chronic statin treatment on iNOS and eNOS protein levels (% relative to CT) was evaluated in aortic (Figure 6) and cardiac (Figure 7) tissue from diabetic rats and CT. Comparing the two groups, iNOS levels were similar in aortic tissue (115.40% ± 48.08% in diabetic *vs* 100% in CT; $n = 5$, $P > 0.05$) and in cardiac tissue (155.30% ± 54.47% in diabetic *vs* 100% in CT; $n = 5$, $P > 0.05$). Whereas eNOS levels in cardiac tissue also did not differ (92.16% ± 16.07% in diabetic

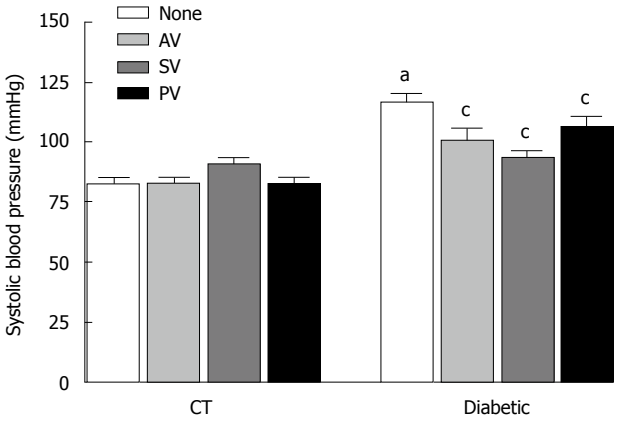

Figure 2 Effects of four weeks treatment with atorvastatin, simvastatin, and pravastain (10 mg/kg per day) on systolic blood pressure (mmHg) in diabetic rats and control. The values shown are the means ± SEM of 10 animals per group. All statins significantly decreased blood pressure in diabetic rats. $^{a}P < 0.05$ for diabetic rats *vs* CT; $^{c}P < 0.05$ for untreated diabetic rats *vs* treated diabetic rats. AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control.

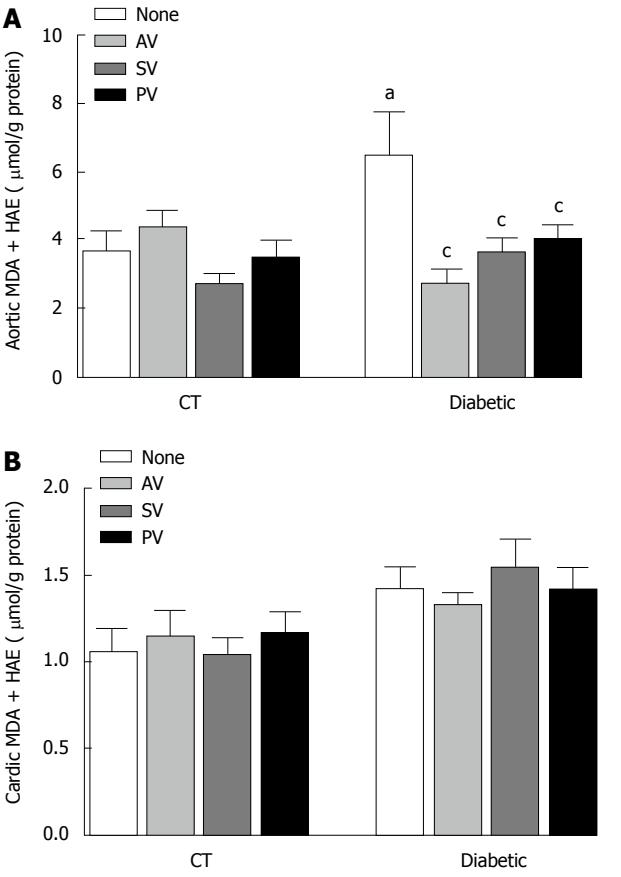

Figure 4 Effect of four weeks treatment with atorvastatin, simvastatin, and pravastatin (10 mg/kg per day) on malondialdehyde + 4-hydroxyalkenal levels in aortic homogenates (A) and in cardiac homogenates (B) from diabetic rats and control. For diabetic rats, all statins equally reduced lipid peroxidation levels in aortic homogenates, but had no effect on these levels in cardiac homogenates. For CT, no effect of statins was observed in either aortic or cardiac homogenates. The values shown are the means ± SEM of 8 animals per group. $^{a}P < 0.05$ for diabetic rats *vs* CT; $^{c}P < 0.05$ for untreated diabetic rats *vs* treated diabetic rats. AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control.

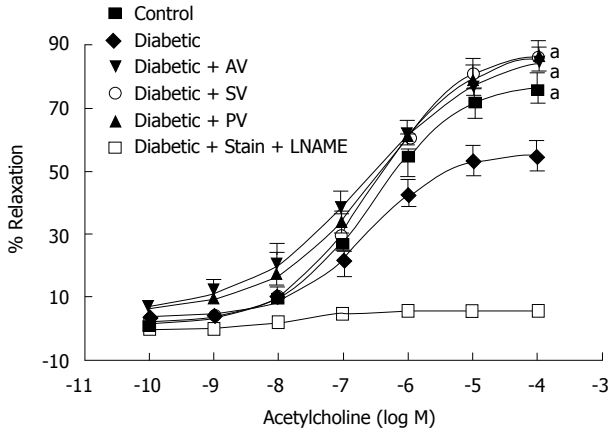

Figure 3 Cumulative concentration response curves for acetylcholine-induced relaxation of aortic rings from diabetic rats after four weeks treatment with atorvastatin, simvastatin, and pravastatin (10 mg/kg per day). Aortic rings were precontracted with 0.1 μmol/L norepinephrine (NE) before the addition of cumulative concentrations of ACh. Note that the addition of 1 mmol/L L-NAME to the incubation bath inhibited ACh-induced relaxation. The values shown are the means ± SEM of 10 animals per group. $^{a}P < 0.05$ for E_{MAX} between untreated diabetic rats and treated diabetic rats. AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; ACh: Acetylcholine.

vs 100% in CT; $n = 5$, $P > 0.05$), the levels were reduced in aortic tissue (54.37% ± 7.29% in diabetic *vs* 100% in CT; $n = 5$, $P < 0.05$). Nevertheless, statin treatment had no effect on either aortic eNOS or iNOS protein levels.

For all tested variables, no significant differences were found between the effects of the three statins. AV, SV, and PV equally improved cardiac function, vascular function, and reduced perivascular fibrosis and oxidative stress.

DISCUSSION

In this study, we compared the effects of AV, SV, and PV on CV performance of Type 1 diabetic rats. For the first time, we report that these three statins similarly improve the CV function of this animal model at a low dose of 10 mg/kg per day. Each statin improves ACh-induced relaxation and CV function, and reduces aortic oxidative stress and remodeling, without lowering cholesterol levels.

In both, patients and animal models of diabetes the beneficial effects of statins improved vascular dysfunction. In diabetic rats, a 50 mg/kg per day dose of AV improves ACh-dependent relaxation[22]. In spontaneously hypertensive rats, a lower dose of 20 mg/kg also improves vascular function[13]. Improvements of vascular function are also observed in Type 1 diabetic patients, where both AV (40 mg/d) and PV (40 mg/d per 1 mo) normalize flow-mediated dilatation[23,24]. Moreover, SV (40 mg/d per 8 wk) improves endothelial-dependent relaxation in hypercholesterolemic patients[25]. In the current study, we demonstrated that all three statins tested (AV, SV,

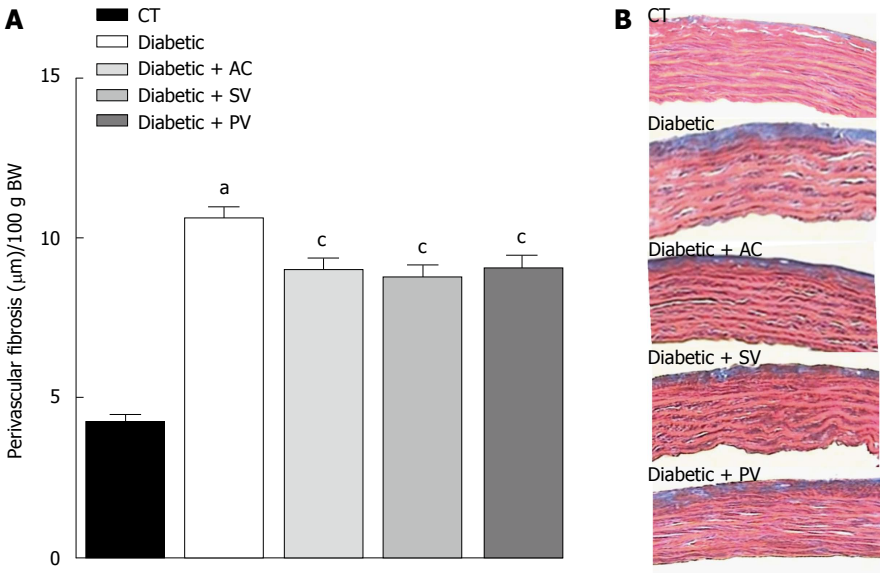

Figure 5 Representative histological sections of aortic segments from untreated and statin-treated diabetic rats, and untreated control. A: Quantified thickness of perivascular fibrosis in comparable aortic segments from treated diabetic rats and untreated diabetic rats. Perivascular fibrosis was higher in untreated diabetic rats than in CT. All statins decreased perivascular fibrosis in diabetic rats. The values shown are the means ± SEM of 5 animals per group, with the mean value for each animal based on five measurements of its aortic segment. $^{a}P < 0.05$ for untreated diabetic rats *vs* untreated CT; $^{c}P < 0.05$ for untreated diabetic rats *vs* treated diabetic rats; B: Representative histological sections (× 40, Azan-Mallory stain) of aortic segments from untreated diabetic rats and treated diabetic rats, demonstrating the typical reduction in perivascular fibrosis after treatment with each individual statin. AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control.

and PV) improve endothelium-dependent relaxation equally in the aortic rings of Type 1 diabetic rats, but at a low dose of only 10 mg/kg per day.

Nevertheless, controversy still exists regarding the beneficial effects of statins on vascular function. For example, among Type 2 diabetic patients with normal cholesterol levels, endothelial function is not restored after the administration of AV (40 or 80 mg/d per 30 wk)[26], or SV (40 mg/d per 6 wk)[27]. Similarly PV (40 mg/d per 8 wk) was ineffective in improving endothelial-induced relaxation in patients with coronary heart disease[28]. The lack of effect of statins in these cases may be due, at least in part, to differences among the experimental models, patient co-morbidities, statin doses, and treatment duration.

The EC_{50} for the ACh-induced relaxation curves is not modified by any of the three statins tested, indicating that the mechanisms by which these drugs improve endothelial function do not include changes in ACh affinity for the muscarinic receptor. The improvement, however, is fully abolished by L-NAME, suggesting that AV, SV, and PV improve vascular function by increasing NO availability. Whereas all three statins reduce lipid peroxidation markers in the aorta, none modify cardiac or vascular eNOS or iNOS protein levels. Thus, the observed CV improvements at this low dose are most likely secondary to the antioxidant properties of the statins, rather than due to their direct stimulation of NO production. In addition, although the etiology of hypertension is largely unknown, oxidative stress, endothelial dysfunction, and structural alterations of the vasculature have been associated with hypertensive pathophysiology. Thus, the reduction of oxidative stress and vascular remodeling, together with the improved endothelial dysfunction observed following statin treatment, may underlie the reduced SBP found in diabetic rats.

The results of some studies differ from ours, however. Wenzel *et al*[29] found that AV (20 mg/kg per day per 7 wk) decreases eNOS uncoupling in Type 1 diabetic rats. In addition, Ito and colleagues[30] reported that in the kidney of spontaneously hypertensive rats, AV (20 mg/kg per day per 8 wk) increases eNOS and nNOS expression. Moreover, in endothelial cell cultures from human saphenous vein SV (1 μmol/L) increases eNOS mRNA and function[31]. It is possible that statins modify NOS activity and/or expression in a dose-dependent manner. If such is the case, the lack of effect on eNOS and iNOS activity observed in the current study may be due to dosage differences. Alternatively, or in addition, experimental models (*e.g.*, *in vivo vs in vitro*) and treatment duration are likely to be major factors underlying this discrepancy.

CV status is deteriorated in diabetic rats by four weeks after induction of diabetes[19,32]. That AV, SV, and PV equally increasing ejection fraction, stroke volume, and cardiac output suggest that the cardioprotective effect of statins is class-related rather that drug-specific. In addition, this pleiotropic effect appears to be independent of the ability of these drugs to lower cholesterol levels. Improvement of systolic function may result from reductions in peripheral resistance secondary to increased endothelial function, decreased blood pressure, and the vascular remodeling regression observed with all three statins. In line with our results, SV (10 mg/kg per day per 8 wk)

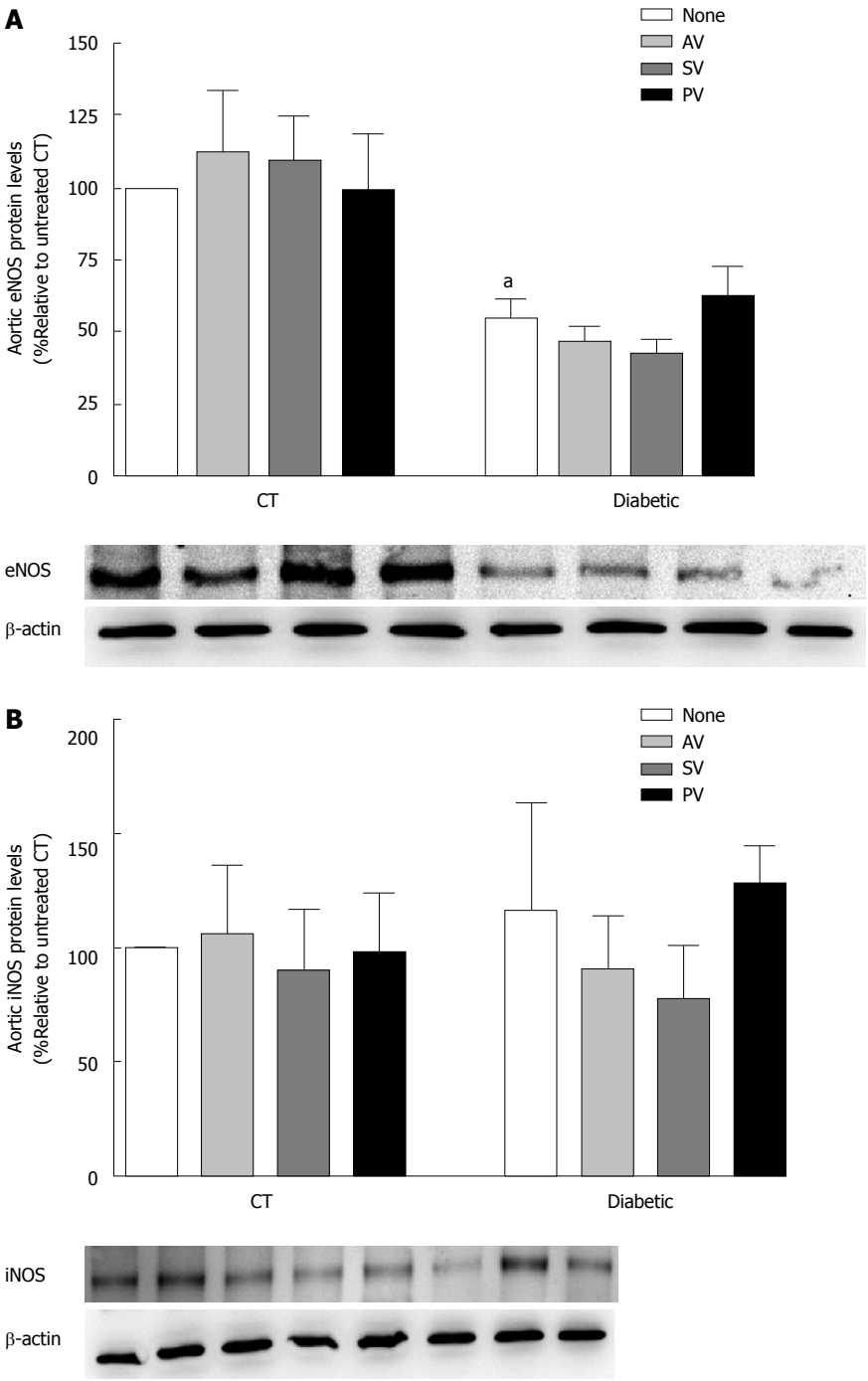

Figure 6 Effect of four weeks treatment with atorvastatin, simvastatin, and pravastatin (10 mg/kg per day) on endothelial nitric oxide synthase (A) and inducible nitric oxide synthase (B) protein levels in aortic homogenates from treated and untreated diabetic rats, and untreated control. Data represent values normalized against β-actin and expressed as percent change relative to untreated CT. The values shown are the means ± SEM of five animals per group; [a]*P* < 0.05 for untreated diabetic rats *vs* untreated CT. Bottom: Representative Western blot for eNOS and iNOS of homogenized aortic tissue; AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control; eNOS: Endothelial nitric oxide synthase; iNOS: Inducible nitric oxide synthase.

increases ejection fraction and prevents left ventricular hypertrophy and fibrosis in rabbits with non-ischemic heart failure[33]. Improved vascular function, including augmented ACh-induced relaxation and reduced perivascular fibrosis, may increase cardiac function by reducing total peripheral resistance and reducing cardiac work. Alternatively, the beneficial effects of these statins on cardiac performance may include the preservation of myocardial contractility, which is deteriorated in diabetes. Indeed, in hearts from diabetic hypercholesterolemic rats, SV (10 mg/kg per day per 5 d) improves cardiac contractility without reducing cholesterol levels[34]. Statins, however, do not appear to be effective in improving particular aspects of cardiac dysfunction associated with diabetes. The appearance of diastolic dysfunction in Type 2 diabetic rats was not prevented by 100 mg/kg AV[35]. Furthermore, although AV improves cardiac function, it does not prevent the

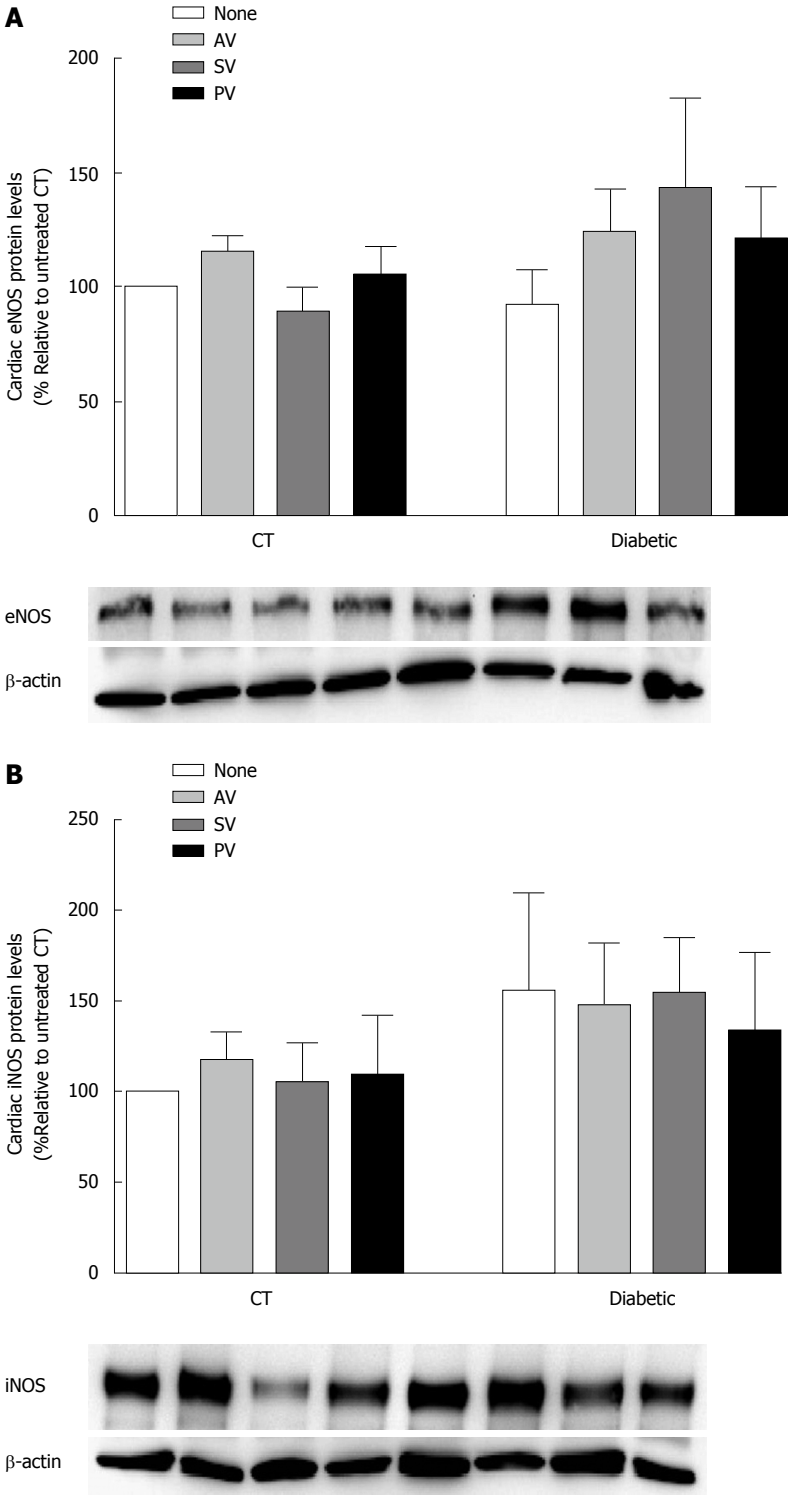

Figure 7 Effect of four weeks treatment with atorvastatin, simvastatin, and pravastatin (10 mg/kg per day) on endothelial nitric oxide synthase (A) and inducible nitric oxide synthase (B) protein levels in cardiac homogenates from treated and untreated diabetic rats, and untreated control. Data represent values normalized against β-actin and expressed as percent change relative to untreated CT. The values shown are the means ± SEM of five animals per group. No statistically significant differences were found. Bottom: Representative Western blot for eNOS and iNOS of homogenized cardiac tissue; AV: Atorvastatin; SV: Simvastatin; PV: Pravastatin; CT: Control; eNOS: Endothelial nitric oxide synthase; iNOS: Inducible nitric oxide synthase.

onset of cardiomyopathy in Type 1 diabetic rats[20].

Although the STZ-induced diabetic rat has proven to be an effective animal model for the study of Type 1 diabetes[36], it has several limitations that must be taken into consideration. Reductions in effective circulating volume due to glycosuria introduce an additional variable because cardiac and vascular RAS become activated. Autonomic dysfunction, which is present in this model, also may cause a reduction in cardiac vagal tone, without changing sympathetic tone[37].

Moreover, due to its chemical structure, STZ down-regulates glucose and lipid metabolism genes before hyperglycemia appears, suggesting that this compound can affect gene expression in a hyperglycemia-independent manner[38]. Despite these limitations, the STZ-diabetic rat is widely used in experimental studies because it replicates both Type 1 diabetes and poorly controlled Type 2 diabetic conditions, making it a useful model in the study of diabetes-related pathophysiology.

The current study demonstrates that AV, SV, and PV are equally effective in improving CV performance in Type 1 diabetic rats. The observed hemodynamic benefits are cholesterol-independent. These benefits appear to be secondary to improved vascular function which, in turn, results from reduced oxidative stress. Although the etiology of Type 1 and Type 2 diabetes is different, in both conditions oxidative stress is high. Thus, it is plausible to postulate that Type 2 diabetics also may benefit from statin treatment. If our findings for diabetic rats are applicable to humans, the benefits of statins to diabetics who are predisposed to develop cardiac complications may extend beyond cholesterol reduction. In addition, even at low doses, statins may be useful for improving the CV profile of diabetics.

COMMENTS

Background

Although there is evidence that statins are useful in the treatment of diabetes, whether cardiovascular (CV) improvement is class-related or drug-specific is unknown. To address the issue, this study tests how low doses of the class-related atorvastatin, simvastatin, and pravastatin improve CV performance in Type 1 diabetic rats.

Research frontiers

Knowledge of the mechanisms underlying statin improvement of CV function, whether these effects are drug-specific or class dependent, and which statin is most effective should result in significant advancements in the current treatment of diabetes.

Innovations and breakthroughs

The beneficial cardioprotective effect of statins is revealed to be class-related, rather than drug-specific. Moreover, this beneficial effect is secondary to reductions in oxidative stress and vascular remodeling, and appears to be independent of the ability of these drugs to lower cholesterol levels.

Applications

If these findings for Type 1 diabetic rats prove to be applicable to humans, the benefits of statins for diabetic patients who are prone to develop CV complications may extend beyond cholesterol reduction.

Terminology

Statins are a class of drugs used to lower cholesterol levels by inhibiting the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase.

Peer-review

The authors concluded the benefits appear to be secondary to the improved endothelial function, and to the reduced vascular tone and remodeling that result from decreased oxidative stress. The findings are interesting.

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