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Clinical Diabetes/Therapeutics

O234
Correlation between DPP4 inhibitors and the risk of skin cancer and liver cancer: a meta-analysis of randomized clinical trial
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Objectives: Currently, some clinical trials have reported the incidence of skin cancer and liver cancer in type 2 diabetes patients after using DPP4 inhibitors, but the exact correlation between DPP4 inhibitors and the risk of skin cancer and liver cancer in type 2 diabetic patients remains unclear because of the limitations of sample size and inconsistent results. To resolve the aforementioned problems, we adopted a meta-analysis of systematic reviews to increase the sample size and improve the credibility of the data, which may provide an accurate conclusion regarding the using of DPP4 inhibitors.

Methods: We retrieved all of the randomized clinical trial literature published in English in the Embase, PubMed and Cochrane databases that discussed the efficacy and safety of long-term treatment with DPP4 inhibitors in type 2 diabetes. The results of unpublished but completed clinical trials were retrieved from The Clinical Trials Database. The retrieval date was up to 30 April 2013. Carcinogenicity data for the DPP4 inhibitors were extracted, and data from the clinical trials lasting more than 24 weeks were evaluated. The sample size and the general baseline characteristics are presented in tables. A random effects model was used to calculate the odds ratio (OR) value to assess whether DPP4 inhibitors increased the risk of skin cancer and liver cancer in patients with type 2 diabetes.

Results: Lastly, 26 clinical experimental studies were included in our study. Cancer cases were reported in both the control group and the experimental group, with a total of 113 cases (72 in the experimental group, 41 in the control group) in which eight studies reported skin cancer cases and four studies reported liver cancer cases. The combined results showed no heterogeneity in skin cancer ($I^2 = 0\%$, $P = 0.53$), and the incidence of skin cancer did not increase significantly after taking DPP4 inhibitors between the experimental group ($n = 2743$) and the control group ($n = 1978$) [OR = 1.00, 95% confidence interval (CI) 0.39–2.54]. Additionally, the combined results showed no heterogeneity in liver cancer ($I^2 = 0\%$, $P = 0.91$), and the incidence of liver cancer did not increase significantly between the experimental group ($n = 1506$) and the control group ($n = 1522$) (OR = 0.85, 95% CI 0.17–4.22). To exclude the possible interference of combination therapy, we also recalculated all of the non-combination therapy studies. The results revealed no heterogeneity in cancer ($I^2 = 16\%$, $P = 0.23$; OR = 0.98, 95% CI 0.59–1.61). In addition, there were no significant differences between the DPP4 inhibitor subgroups.

Conclusions: We found that patients with type 2 diabetes who were using DPP4 inhibitors to control blood sugar did not experience an increased risk of skin cancer and liver cancer, and no significant influence on the incidence of general cancer was observed. However, because the current research on the clinical safety of DPP4 inhibitors is inconsistent, and because of the limitations of the research included in this study, and possible publication bias, this conclusion must be validated by more high quality cohort studies.

O264
Continuous glucose monitoring effects on maternal glycemic control and pregnancy outcomes in patients with gestational diabetes mellitus: a prospective cohort study
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Background: Clinical evidence of the consequential effects of continuous glucose monitoring (CGM) on pregnancy outcomes in women with gestational diabetes mellitus (GDM) is scarce. The aim of the current study was to evaluate the effectiveness of CGM on maternal glycemic control and pregnancy outcomes in patients with GDM.

Methods: This was a prospective cohort study in the Department of Obstetrics at Guangdong Women and Children Hospital in China. Recruitment was initiated in April 2011 and terminated in August 2012. In total, 340 pregnant Chinese women with GDM were allocated to either the routine care group (n = 190) or the CGM group (n = 150). A 72-h continuous glucose monitoring system was used as a supplementary tool to monitor glucose in the CGM group. The parameters of glycemic variability, which included mean blood glucose, standard deviation of blood glucose (SDBG), mean amplitude of glycemic excursions (MAGE) and mean of daily differences (MODD), were measured. Maternal outcomes (preeclampsia and caesarean delivery) and composite neonatal outcomes were analysed.

Results: The SDBG, MAGE and MODD values were significantly lower in the CGM group compared with the routine care group ($P < 0.001$). Subjects in the CGM group had a lower risk of preeclampsia and primary caesarean delivery compared with the routine care group ($P < 0.05$). The mean infant birth weight of women in the CGM group was lower than infants of women in the routine care group ($P < 0.001$). The MAGE was associated with birth weight ($\beta = 0.196, P < 0.001$), and it was an independent factor for preeclampsia [odds ratio (OR), 3.66; 95% confidence interval (CI) 2.16–6.20] and composite neonatal outcome (OR, 1.34; 95% CI 1.01–1.77).
Conclusions: The use of supplementary CGM combined with routine antenatal care can improve glycemic control and pregnancy outcomes in patients with GDM.

O279
Analysis of risk factors for adverse pregnancy outcomes in women with gestational diabetes mellitus
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Objectives: To evaluate clinical features, insulin sensitivity and β-cell function in pregnant women with different glucose tolerance statuses and to investigate possible risk factors predicting adverse pregnancy outcomes in women with gestational diabetes mellitus (GDM).

Methods: We retrospectively analysed the clinical data of 360 pregnant women with positive results for the 50 g glucose challenge test at 24–28 gestational weeks from Jan 2009 to Jun 2012 at Peking Union Medical College Hospital. Three hundred and sixty pregnant women were divided into three groups after the 100 g oral glucose tolerance tests: 83 women in the GDM group, 75 women in the impaired glucose tolerance (IGT) group and 202 women in the normal glucose tolerance (NGT) group. All of the women had blood glucose that was controlled within the normal range for the gestational period. The clinical data were recorded in detail. We compared the general clinical data and biochemical results obtained for the three groups. We calculated HOMA-IR and insulin sensitivity index (ISI)-Matsuda to evaluate insulin sensitivity, and HOMA-β, first-phase and second-phase insulin secretion, and ISSI to evaluate β-cell function. We also compared pregnancy outcomes among the three groups.

Results: Compared with the NGT group, pregnant women in the GDM group were older and had elevated systolic blood pressure and diastolic blood pressure, a positive family history of diabetes in the first degree relative, were receiving insulin therapy, and had increased serum triglyceride, free fatty acid and C-reactive protein levels (P < 0.05). Compared with IGT and NGT, pregnant women in the GDM group had a higher pre-pregnancy body mass index (BMI) (P < 0.01). Pregnant women with GDM had the highest levels of plasma glucose in GCT, plasma glucose, insulin, area under the curve (AUC) glucose and AUC insulin in oral glucose tolerance test, haemoglobin A1c (HbA1c), and HOMA-IR, followed by those with IGT and NGT (P < 0.01). Pregnant women with NGT had the highest ISI-Matsuda and ISSI, followed by those with IGT and GDM (P < 0.01). The results of HOMA-β and first-phase and second-phase insulin secretion were comparable to those in the IGT and NGT. Pregnant women with GDM had a shorter gestational week and higher positive rate of adverse pregnancy outcomes than those with IGT and NGT (P < 0.01). There were seven risk factors predicting adverse pregnancy outcomes in women with GDM, including pre-pregnancy BMI, 0-h BG, 1-h BG, 2-h BG, 3-h INS, HbA1c and CRP (P < 0.05). Among these factors, 1-h BG had the largest coefficient (odds ratio = 2.767).

Conclusions: Pregnant women with GDM had higher blood pressure and dyslipidemia, and increased inflammatory cytokines. Women with GDM and IGT showed impaired insulin secretion and insulin sensitivity, and these impairments were more severe in women with GDM. Higher pre-pregnancy BMI and blood glucose levels during pregnancy were associated with adverse pregnancy outcomes in women with GDM.

O298
Clinical features in different age groups of patients with diabetic ketoacidosis
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Objectives: To characterize the clinical features and biochemical status at presentation in different age groups of patients with diabetic ketoacidosis (DKA) and to analyse the outcomes of a specific treatment protocol.

Methods: We reviewed the records of patients with DKA who were admitted to our hospital between June 2008 and January 2014. Patients were divided into three subgroups according to age, and the results were compared between these groups.

Results: Eighty-six patients (51 males, 35 females) were analysed. In group A (12–30 years old, 20 patients), the level of haemoglobin A1c (HbA1c; 13.27 ± 0.745%) was higher compared with the other groups. The diabetic history of the patients was 1 month, which is the shortest duration among the groups. The level of β-hydroxybutyric acid was 5.03 ± 1.84 mmol/L. Patients with new-onset diabetes represented 55% (11/20) of the population. In group B (31–60 years old, 54 patients), the patients had higher levels of β-hydroxybutyric acid (6.33 ± 2.24 mmol/L) and lower levels of P (7203 ± 0.109; P < 0.01) compared with the other groups. The level of HbA1c was 11.75 ± 2.25%, and the diabetic history was 66 months.

Group C (over 61 years old, 12 patients) had the longest diabetic history at 174 months (P < 0.01) and the highest level of β-hydroxybutyric acid (4.11 ± 1.69 mmol/L) and PH (7.242 ± 0.153). The incidence rate in males in groups A and B were 70.0% (14/20) and 64.8% (35/54), which was higher than that in group C at 16.7% (2/12) (P < 0.01).

Conclusion: Our study indicates that younger patients with DKA should receive more diabetic education because of their shorter diabetic history. Middle-aged patients had severe metabolic acidosis. Acid–base disorders should be rectified quickly. The elderly patients had a longer diabetic history. They should receive more attention in terms of system evaluation and treatment by synthesis. In particular, men younger than 60 years old should strengthen the life way rationalization and reduce the incidence rate of DKA.
**Aims:** Insulin glargine is a long-acting insulin analog that can be administered by physician-led or patient-led titration, although its safety and efficacy require further study in China and other Asian diabetic populations.

**Methods:** Type 2 diabetic patients uncontrolled by oral anti-diabetics (OADs) were examined in this randomized, open-label, multicentre Asian Treat to Target Lantus Study (552 patients in six countries), including 161 patients in China. Patients were initiated with fixed-dose insulin glargine (10 U/day) followed by 24 weeks of physician-led (Arm A; n = 81) or patient-led (Arm B; n = 80) titration with basal insulin, using the same algorithm for fasting blood glucose (FBG) targeting 110 mg/dL. The primary endpoint was the change in haemoglobin A1c (HbA1c) from baseline. Results obtained for Chinese and overall patients were compared.

**Results:** The demographic and baseline data for the Chinese and overall population are shown in Table 1. More Chinese than overall patients had baseline HbA1c values of 7–8% (38.5% vs 29.0%). Significant least squares mean changes in HbA1c from baseline were achieved in both Arms (Arm B: −1.34%, Arm A: −1.27%, P < 0.001), but the changes were similar in both Arms (difference −0.007%, P = 0.581) in Chinese patients, consistent with the overall patients. The proportions of Chinese patients achieving HbA1c < 7.0% without severe hypoglycemia were slightly increased compared with the overall arms (Arm B vs A: 42.5% vs 45.7% in Chinese and 40.0% vs 32.9% in overall population). The proportions of Chinese patients with decreases in HbA1c ≥ 1% (71.3% vs 61.7%) and mean FBG changes (−3.0 vs −2.6 mmol/L) were larger in Arm B than in Arm A, consistent with the overall patients. The final daily dose of insulin was significantly higher in Arm B than in Arm A in both Chinese and overall populations (22.7 ± 11.99 vs 19.5 ± 9.10 U and 27.7 ± 1.26 vs 21.1 ± 0.68 U, respectively; P < 0.001). Severe hypoglycemia was rare and similar in both Arms in the Chinese and overall populations during the entire study (1 vs 0 episode and 2 vs 2 episodes, respectively). Slightly fewer Chinese patients experienced at least one adverse event (29.8% vs 33.6%) compared with the overall population. No study-related deaths were reported.

**Conclusions:** Patient-led or physician-led insulin titration allows safe and effective glycemic control in Chinese patients uncontrolled by OADs. Thus, simple patient-administered titration of insulin glargine can improve glycemic control with a low incidence of severe hypoglycemia in Chinese patients.

**Table 1. The demographic characteristics and baseline data for the Chinese and overall population**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Chinese population (N = 161)</th>
<th>Overall population (N = 552)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.4 (8.25)</td>
<td>57.2 (8.55)</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>77 (47.8%)</td>
<td>285 (51.6%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.4 (3.45)</td>
<td>27.4 (4.69)</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>9.5 (5.79)</td>
<td>9.7 (6.19)</td>
</tr>
<tr>
<td>OAD treatment duration (years)</td>
<td>7.2 (5.86)</td>
<td>7.6 (5.60)</td>
</tr>
<tr>
<td>Biguadines + SU</td>
<td>104 (64.6%)</td>
<td>435 (78.8%)</td>
</tr>
<tr>
<td>Biguadines + glinides</td>
<td>22 (13.7%)</td>
<td>30 (5.4%)</td>
</tr>
<tr>
<td>SU + alpha glucosidase inhibitors</td>
<td>19 (11.8%)</td>
<td>49 (8.9%)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.56 (1.123)</td>
<td>8.73 (1.047)</td>
</tr>
<tr>
<td>7</td>
<td>5 (3.1%)</td>
<td>10 (1.8%)</td>
</tr>
<tr>
<td>&gt;7 to ≤8</td>
<td>62 (38.5%)</td>
<td>160 (29.0%)</td>
</tr>
<tr>
<td>&gt;8 to ≤9</td>
<td>47 (29.2%)</td>
<td>181 (32.8%)</td>
</tr>
<tr>
<td>&gt;9 to ≤10</td>
<td>24 (14.9%)</td>
<td>124 (22.5%)</td>
</tr>
<tr>
<td>&gt;10 to ≤11</td>
<td>23 (14.3%)</td>
<td>77 (13.9%)</td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
<td>8.94 (2.054)</td>
<td>9.02 (2.105)</td>
</tr>
<tr>
<td>PPG (mmol/L)</td>
<td>12.40 (3.032)</td>
<td>12.28 (2.875)</td>
</tr>
</tbody>
</table>

BMI, body mass index; OAD, oral anti-diabetics; HbA1c, haemoglobin A1c; FBG, fasting blood glucose; PPG, postprandial blood glucose.

**Disclosure of interests:** Sanofi-Aventis supported the study.
O581
Patient experience in physician-led and patient-led insulin glargine titration in Chinese type 2 diabetic patients

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Background: Patient satisfaction and quality of life (QoL) for either patient-led or physician-led titration of insulin glargine, a long-acting insulin analog, have been under-assessed in Chinese and other Asia-Pacific diabetic populations.

Methods: Patients with type 2 diabetes uncontrolled by oral anti-diabetics were examined in this randomized, open-label, multicentre Asian Treat to Target Lantus Study (552 patients in six countries) from Aug 2010 to Dec 2011, including 161 patients (mean age of 57.4 ± 8.25, 40–75 years; 47.8% male) in China. Patients were initiated with a fixed-dose of insulin glargine (10 U/day) followed by 24 weeks of physician-led (Arm A; n = 80) or patient-led titration with basal insulin (Arm B; n = 80), using the same algorithm to achieve a target fasting blood glucose of 110 mg/dL. Patient satisfaction and QoL were assessed using the Diabetes Treatment Satisfaction Questionnaire status (DTSQs) and change (DTSQc) as well as the EuroQol Questionnaire-5 Dimension (EQ-5D) and visual analog scale (EQ-VAS) scores.

Results: Similar mean DTSQs total scores were observed at baseline (25.5 ± 0.51), with improvements observed as early as week 6 (30.5 ± 0.41) and by week 24 (31.6 ± 0.39) in Chinese patients, consistent with the overall patients who exhibited significant improvements in the final mean DTSQ total scores compared with baseline (25.7 ± 0.27 vs 30 ± 0.25; P < 0.001). In both Chinese and overall patients, mean DTSQc scores revealed no significant changes in perceived frequency of hyper-glycemia and hypo-glycemia and improved overall scores, which were similar in both Arms. The EQ-5D improved in Chinese and overall patients in both treatment arms, but this change was not significant. In Chinese patients, however, slight improvements in mean EQ-VAS scores from baseline (73.3 ± 1.16) were apparent by week 6 (80.27 ± 1.143) and week 24 (83.48 ± 1.212). Slightly fewer Chinese patients experienced at least one adverse event (29.8% vs 33.6%) compared with the overall population. No study-related deaths were reported.

Conclusions: DTSCs/c and EQ-5D/VAS scores were indicative of good health at baseline, which was improved by both patient-led and physician-led insulin glargine titration. Both therapies produced high patient satisfaction with good tolerance.

Disclosure of interests: Sanofi-Aventis supported the study.

O644
Applicability of the new diagnostic criteria for gestational diabetes from the outcomes of postpartum years 5–6 in patients with prior gestational diabetes in China

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Background: IADPSG diagnostic criteria have been adopted by more and more countries and regions including China. From an obstetric perspective, this standard is suitable for our country. However, what about the perspective of long-term postpartum outcomes? This study will estimate the applicability of the new diagnostic criteria for gestational diabetes from the outcomes at postpartum years 5–6 in patients with prior gestational diabetes in China.

Methods: In total, 84 women were diagnosed with hyperglycemia during pregnancy and delivered in our hospital from February 2007 to December 2009. They were divided into two groups based on their gestational glucose levels. Women in group A could be diagnosed with gestational diabetes mellitus (GDM) using both NDDG and IADPSG criteria. Women in group B were diagnosed with a gestational impaired glucose test using NDDG criteria but with GDM using IADPSG criteria. The postpartum years 5–6 prognoses were compared.

Results: Women in the two groups had no significant differences in glucose metabolism, lipid metabolism, β-cell function or insulin resistance at postpartum years 5–6.

Conclusions: The diagnostic criteria of IADPSG could include more patients for GDM management and follow-up. The application of IADPSG criteria is appropriate in China.

O648
Efficacy and mechanism of insulin secretagogues and acarbose in newly diagnosed type 2 diabetes

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Objectives: The two representative drugs glibenclamide and repaglinide were used as insulin secretagogue monotherapy in newly diagnosed patients with type 2 diabetes mellitus (T2DM). Sitagliptin and acarbose monotherapy were compared with the control in newly diagnosed T2DM in terms of hypoglycemic effect. Concomitantly, the impact of therapy on glucagon-like peptide-1 (GLP-1) and GC was assessed.

Methods: Research subjects will be randomly divided into sitagliptin (SG) (n = 30; 20 men, 10 women), acarbose (AG) (n = 31; 18 men, 13 women), glibenclamide (GG) (n = 34; 23 men, 11 women), RG32 (n = 32; 20 men, 12 women) and repaglinide treatment groups. In addition, there were 20 persons with normal glucose tolerance (13 men, 7 women) aged 51.80 ± 6.41 years. Prior to treatment, body mass index, blood pressure, fasting blood glucose
(FBG) and haemoglobin A1c (HbA1c) were measured in all of the patients. Venous blood was collected at fasting 30, 60, 120 and 180 min during oral glucose tolerance test. GLP-1, glucagon, insulin, lipids, liver function and kidney function were also detected. These indicators were retested 12 weeks after treatment. Longitudinal comparison of the groups was performed. Differences between the aforementioned indicators were compared between the groups before and after medication administration.

**Results:** After treatment, FBG, 2-h BG and HbA1c were significantly reduced compared with baseline in SG, AG, GG and RG (P < 0.01). Compared with SG, the decrease in HbA1c was lower in GG and RG was (P < 0.01). Compared with AG, the decrease in HbA1c was lower in GG and RG (P < 0.05). After administration of sitagliptin, HbA1c ≤ 7% was observed in 19 cases and HbA1c > 7% in 11 cases. The compliance rate was 63%. After administration of acarbose, HbA1c ≤ 7% was observed in 21 cases and HbA1c > 7% in 10 cases. The compliance rate was 71%. After administration of repaglinide, HbA1c ≤ 7% was observed in 22 cases and HbA1c > 7% in 9 cases. The compliance rate was 72%. After administration of glibenclamide, HbA1c ≤ 7% was observed in 24 cases and HbA1c > 7% in 10 cases. The compliance rate was 71%. After administration of repaglinide, HbA1c ≤ 7% was observed in 22 cases and HbA1c > 7% in 9 cases. The compliance rate was 72%. FGC and 2-h GC decreased, and FGLP-1 and 2-h GLP-1 increased significantly after treatment in AG (P < 0.01). FGC decreased significantly after treatment in AG (P < 0.05). The 2-h GC decreased significantly after treatment in AG (P < 0.01). FGLP-1 and 2-h GLP-1 increased significantly after treatment in AG (P < 0.01). FGC, 2-h GC, FGLP-1 and 2-h GLP-1 were not significantly different after treatment in AG and RG. Compared with SG, the increase in FGLP-1 and 2-h GLP-1 in AG was reduced, and this difference was statistically significant (P < 0.05). In addition, the reduction of FGC in AG was reduced, and this difference was statistically significant (P < 0.01).

**Conclusions:** Sitagliptin, acarbose, glibenclamide and repaglinide can effectively lower blood glucose in patients newly diagnosed with T2DM. The hypoglycemic effect of glibenclamide and repaglinide is better than that of sitagliptin and acarbose. Sitagliptin can lower blood glucose caused by elevated plasma levels of GLP-1, reducing the level of GC. The effects of acarbose on GLP-1 and GC may be one of the mechanisms by which blood glucose is lowered. Glibenclamide and repaglinide do not regulate GLP-1 and GC. Sitagliptin or acarbose monotherapy in patients with newly diagnosed T2DM is better than glibenclamide in reducing weight, improving lipid metabolism and lowering blood pressure.

**Background:** It is widely accepted that timely initiation and titration of basal insulin is effective to improve glycemic control. However, a specific guideline for basal-supported oral therapy (BOT) management has not yet been established in China. This study was designed to explore geographical differences in the current management of BOT in type 2 diabetes mellitus (T2DM) with inadequate glucose control on oral hypoglycemic agents (OADs) in China.

**Methods:** This was a nationwide, 12-week observational study. Patients with T2DM with inadequate glucose control on OADs who were to start BOT with insulin glargine at outpatient clinics were enrolled from 134 hospitals in China. Patients were divided into five groups according to the geographical locations of the hospitals in which they were managed (Group 1: Northern district, Group 2: Northeastern district, Group 3: Eastern district, Group 4: Southern district and Group 5: Western district). All of the patients were followed up for 12 weeks. The dosage of insulin glargine was titrated by the physicians to achieve the target fasting plasma glucose (FPG) of ≤ 7.0 mmol/L.

**Results:** A total of 11 192 patients finished the 12-week visits and were included in the analysis. Patients initiated BOT at a mean haemoglobin A1c (HbA1c) of 9.1% and FPG of 11.0 mmol/L, with substantial variation between groups (mean HbA1c range of 8.9–9.6%; P < 0.001; mean FPG range of 10.5–11.9 mmol/L, P < 0.001). Both the HbA1c and FPG level at glargine initiation were highest in Group 3 and lowest in Group 1 (P < 0.001). However, the starting dose of insulin glargine was highest in Group 4 (0.201 ± 0.091 U/kg/day) and lowest in Group 5 (0.185 ± 0.067 U/kg/day) (P < 0.001). Dose titration also varied between the groups, with the most aggressive titration in Group 1 (0.378 ± 0.984 U/week) and the least aggressive in Group 3 (0.092 ± 0.989 U/week). Fifty-nine per cent of the patients achieved FPG ≤ 7.0 mmol/L, ranging from 53.7% to 68.2% (P < 0.001) between groups, with the highest observed in Group 2 and the lowest in Group 1. These differences were maintained after adjustment for significant covariates of baseline age, body weight and sex.

**Conclusions:** BOT is still being delayed in China. Large geographical differences in the current management of BOT in China lead to different clinical outcomes of glucose control. A standard and specific guideline for BOT management is needed for Chinese patients with T2DM.

**O761**

**Geographic differences in basal-supported oral therapy management in Chinese type 2 diabetic patients with inadequate glycemic control on oral anti-diabetic drugs**

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C-peptide (CPmax) after breakfast, body mass index and waist were submitted for analysis. Receiver operating characteristic (ROC) and Fisher’s discriminant analysis were conducted.

Results: We assessed whether FCP or CPmax of patients who cannot stop using insulin was much lower than in patients who can stop using insulin. The area under the ROC curve of CPmax was the largest. The appropriate cut-offs of FCP and CPmax were 0.86 and 2.78 ng/dL. In a joint analysis of multiple indicators, the area under the ROC curve was slightly increased.

Conclusions: Prior to improving hyperglycemia, the oral C-peptide release test is helpful to judge whether patients require a long-term insulin treatment.

**O873**

Clinical validation of the dual-modes of action of glucokinase activator HMS5552 for type 2 diabetes

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Background: Glucokinase (GK) is a glucose-sensing enzyme that is predominantly expressed in pancreatic beta cells and liver. HMS5552 is a novel allosteric GK activator (GKA) that targets both the pancreas and liver and leads to enhanced glucose-stimulated insulin release (GSIR) and suppressed hepatic glucose production. Hua Medicine licensed HMS5552 from Roche for the first time in a Phase I single ascending-dose study in healthy Chinese subjects.

Methods: HMS5552 was investigated in preclinical studies of biochemical, cellular and tissue/organ assays, and in seven animal models to elucidate its mechanism of action. The drug safety was evaluated in 4-week and 13-week glucagon-like peptide (GLP) studies together with tox-kinetic and drug metabolism tests. HMS5552 was studied in a double-blind, randomized, placebo-controlled single ascending dose study in healthy Chinese volunteers to investigate its safety, tolerability, pharmacokinetics and pharmacodynamics, as well as biomarkers related to its mechanism of action.

Preclinical results: HMS5552 activated recombinant human GK in vitro by decreasing the S0.5 and increasing the Vmax of GK with minor effects on the Hill coefficient, preserving the positive cooperativity of GK for glucose. It possessed favourable physiochemical properties and PK profiles. HMS5552 enhanced GSIR in rodent pancreatic islets and increased glucose uptake in cultured rodent primary hepatocytes. In vivo, HMS5552 showed dose-related effects on fasting and basal and post-prandial glucose levels in rodent models of type 2 diabetes. No significant toxicity and safety findings were observed in acute toxicity studies (up to 4 weeks) in rodent and dog, and in longer toxicity studies (up to 3 months) in the same species.

Results: Sixty healthy Chinese volunteers received either placebo or a single dose of HMS5552 orally under fasting conditions in six dose cohorts (5, 10, 15, 25, 35 and 50 mg). HMS5552 was well tolerated at all doses. The incidence of overall adverse events was comparable with placebo. HMS5552 showed a dose proportional increase in plasma exposure and good PK properties that supported BID therapeutic dosing. The Cmax and area under the curve displayed dose proportionality. HMS5552 resulted in a dose-dependent reduction in fasting glucose. The reduction of glucose exposure (median change from baseline of –7% at 5 mg to –21% at 50 mg) increased with ascending dose. Significant increases in insulin after meals but not during fasting periods were observed, consistent with a role for GK in mediating GSIR and lowering fasting glucose by reducing hepatic glucose production.

Conclusions: The novel GKA HMS5552 demonstrated dual functions, mediating the secretion of hormones that control glucose homeostasis and hepatic glucose utilization in animals and humans. These properties led to a dose-dependent reduction of plasma glucose. It was well tolerated and showed dose-proportional PK properties in healthy Chinese volunteers.

**O1117**

Altered B lymphocyte subset frequency in peripheral blood in autoimmune diabetes

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Objective: To investigate the frequency of B lymphocyte subsets in peripheral blood in autoimmune diabetes.

Methods: Patients with type 2 diabetes mellitus (T2DM) (n = 95), latent autoimmune diabetes in adults (LADA) (n = 82) and type 1 diabetes mellitus (T1DM) (n = 81) were recruited from the Institute of Metabolism and Endocrinology of the Second Xiangya Hospital, Central South University. Healthy individuals (n = 218) with normal glucose tolerance (NGT) were enrolled at the same time. Peripheral blood samples were obtained, and peripheral blood mononuclear cells from 476 individuals were separated and stained. The frequency of B lymphocyte subsets in peripheral blood was measured by multicolour flow cytometry.

Results: There were no significant differences in the frequency of B lymphocytes among different types of diabetes and controls. The frequency of B10 (CD19+CD5-CD1d+) cells gradually decreased in the NGT, T2DM and T1DM groups (P < 0.05). The frequency of B10 cells gradually decreased in the NGT, LADA and T1DM groups (P < 0.05). The frequency of marginal zone B (MZB, CD19+CD21+CD23+) cells in T1DM and LADA increased compared with NGT and T2DM (P < 0.05). The frequency of follicular B (FoB, CD19+CD23+CD21+) cells in T1DM and LADA decreased compared with NGT and T2DM. These results were adjusted by age and body mass index. The frequency of B10 cells in all subjects was positively correlated with fasting C peptide (FCP) and 2-h postprandial C-peptide (PCP) (P < 0.05 for both). The frequency of MZB cells in all of the subjects was negatively correlated with FCP and PCP and positively correlated with fasting blood glucose and 2-h postprandial blood glucose (P < 0.05 for each).
Conclusions: The frequency of B10 cells decreased, while the frequency of MZB cells increased in LADA and T1DM. These results highlight the altered frequency of peripheral blood B lymphocyte subsets in autoimmune diabetes and suggest that similar immunological alterations may be correlated with the development of autoimmune diabetes.

O1298
Comparison of prepregnant body mass index and fasting plasma glucose in predicting gestational diabetes mellitus in Chinese subjects
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Aims: This study aimed to evaluate prepregnant body mass index (preBMI) and fasting plasma glucose (FPG) to predict gestational diabetes mellitus (GDM) in Chinese subjects.

Methods: A total of 327 pregnant women were recruited from our hospital in Guangzhou, China, from Apr 2013 to Oct 2013. PreBMI, FPG (16–18 weeks of gestation) and 75-g oral glucose tolerance test (OGTT) (24–28 weeks of gestation) were measured in each subject. GDM was diagnosed according to IADPSG guidelines (2010).

Results: The subjects had an average age of 29 years (interquartile range: 27–31 years), mean preBMI of 20.8 ± 2.5 kg/m² and FPG of 5 ± 0.4 mmol/L. Both preBMI and FPG were positively related to fasting and 1-h and 2-h plasma glucose during OGTT (all P < 0.05) but were negatively correlated with high-density lipoprotein cholesterol (P < 0.001). Forty-eight subjects (14.7%) were diagnosed with GDM. The area under the receiver operating characteristic curve was 0.628 (0.573–0.718) for FPG in the diagnosis of GDM (P < 0.001). Thirty-one subjects (9.5%) were diagnosed with GDM according to IADPSG guidelines (2010).

Conclusions: PreBMI or FPG could be used as an index to predict GDM in Chinese subjects. Compared with preBMI, FPG may be suitable for predicting GDM because of its higher sensitivity.

O1377
Altered CD4 central-memory T (CD4Tcm) cell frequency in peripheral blood in latent autoimmune diabetes in adult patients
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Objectives: We aimed to investigate the frequency of CD4Tcm (CD4+CCR7+CD45RA−) cells in peripheral blood of latent autoimmune diabetes in adult (LADA) patients and to explore its relationship with age, islet function and haemoglobin A1c (HbA1c) levels.

Methods: We recruited 40 LADA patients and 80 healthy individuals with normal glucose tolerance (NGT). Patients with LADA were enrolled into the study according to the following criteria: (1) diabetes diagnosed according to the criteria of the World Health Organization in 1999 at age ≥30 years, (2) glutamic acid decarboxylase antibody positive, (3) no ketosis in the first 6 months after the diagnosis of diabetes and (4) insulin independence for 6 months after diagnosis. Peripheral blood mononuclear cells were separated by density gradient centrifugation. The frequency of CD4Tcm cells was measured by flow cytometry.

Results: (1) Compared with NGT, the frequency of CD4Tcm was higher in LADA patients after adjustment for age and sex (18.33 ± 7.52 vs 13.15 ± 7.21, P = 0.003); there was no significant association between CD4Tcm frequency and age in NGT. (2) The CD4Tcm frequency was positively correlated with age in LADA patients (r = 0.301, P = 0.013). (3) The CD4Tcm frequency was not associated with fasting C-peptide (FCP), 2-h postprandial C-peptide or HbA1c levels in LADA patients. The CD4Tcm frequency was not associated with FCP in NGT.

Conclusions: LADA patients have an increased CD4Tcm frequency in peripheral blood. This result suggests that alteration of CD4Tcm frequency may be associated with the development of LADA.
considered for the study, and another 65 healthy people were enrolled in the control group. Blood was collected, and serum was separated. Serum 8-oxo-dG was measured by using a competitive enzyme-linked immunosorbent assay (ELISA) kit specially developed to optimize the cross-reaction of 8-oxo-dG antibody with serum guanosine.

**Results:** The average serum 8-oxo-dG levels in the diabetic and control groups were 0.72 ± 0.41 and 0.24 ± 0.14 ng/mL, respectively (P < 0.001). The 8-oxo-dG value was significantly higher in diabetic women than in diabetic men (P = 0.028), but it did not correlate with age. The sensitivity and the specificity of the 8-oxo-dG ELISA assay were 0.80 and 0.96, respectively, and the receiver operating characteristic value was 0.93.

**Conclusion:** This study indicates that increased oxidative stress has a primary role in the pathogenesis of diabetes. Serum 8-oxo-dG is a useful clinical biomarker for the early diagnosis of diabetes and its management.

**P718**

**Effects of sitagliptin on beta cell function and insulin sensitivity in patients with latent autoimmune diabetes in adults**

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**Objective:** This study aimed to investigate the effects of the DPP-4 inhibitor sitagliptin on β-cell function and insulin sensitivity in patients with recent-onset latent autoimmune diabetes in adults (LADA).

**Methods:** We conducted a randomized controlled trial in nine subjects with recent-onset LADA who received insulin therapy with 100 mg/day sitagliptin (n = 5) or without sitagliptin (n = 4) for 24 months. Hyperglycemic and hyperinsulin euglycemic clamp tests were performed at baseline and after 12 and 24 months of treatment to determine β-cell function and insulin sensitivity. The study was approved by the local ethics committee of our institution.

**Results:** At 12 and 24 months, the first-phase insulin response during hyperglycemic clamp was better with sitagliptin plus insulin than with insulin alone (140.4 ± 12.3 mU/L vs 118.9 ± 6.3 mU/L, P = 0.021, and 148.5 ± 15.4 mU/L vs 116.8 ± 6.5 mU/L, P = 0.009, respectively). However, it was not the case with regard to second-phase insulin response or maximum insulin secretion during the 24-month follow-up (58.6 ± 6.8 mU/L vs 54.8 ± 2.0, P = 0.324, and 69.8 ± 5.8 mU/L vs 68.1 ± 4.6 mU/L, P = 0.667, respectively). Moreover, during euglycemic clamp, there was no significant difference between the M values and insulin sensitivity index of the two groups at 24 months [9.76 ± 1.3 mg/kg/min vs 8.53 ± 1.12 mg/kg/min, P = 0.183, and 5.92 ± 1.67 mg/ml/(kg min uU) vs 4.65 ± 0.86 mg/ml/(kg min uU), P = 0.181, respectively].

**Conclusions:** The pilot trial suggested that sitagliptin plus insulin improved acute-phase insulin release of β-cell function but did not improve insulin sensitivity in recent-onset LADA. An extended study is warranted.

**P965**

**A clinical and neuropathological study of patients with diabetic peripheral neuropathy**

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**Objective:** To examine whether neuropathological and metabolic changes in peripheral nerves are correlated to clinical features in diabetes mellitus type 2 patients with peripheral neuropathy.

**Methods:** Fasting plasma glucose, haemoglobin A1c (HbA1c), and red blood cell sorbitol (RBC sorbitol) in addition to nerve conduction velocity (NCV) were assessed in 147 type 2 diabetic patients with signs/symptoms of diabetic peripheral neuropathy (DPN) aged 53.4 ± 12.3 years and 134 healthy volunteers aged 55.5 ± 11.7 years. Among the 147 diabetic patients, 10 patients underwent superficial peroneal nerve biopsy for light and electron microscopy.

**Results:** In the experimental group, the levels of HbA1c and RBC sorbitol increased significantly compared with the control group, whereas motor nerve conduction velocity (MNCV) and sensory nerve conduction velocity (SNCV) of both the median (MNCV 44.40 ± 5.07 vs 54.28 ± 5.32; SNCV 34.28 ± 8.83 vs 52.37 ± 5.42) and common peroneal nerves (MNCV 41.14 ± 6.42 vs 49.77 ± 3.14; SNCV 29.70 ± 8.88 vs 52.07 ± 4.12) showed a statistically significant reduction in the DPN group compared with the control group, and SNCV decreased to a greater extent. Morphologically, there were various degrees of nerve fibre loss associated with axon degeneration and capillary luminal narrowing in 10 patients who underwent nerve biopsy.

**Conclusions:** The metabolic change in sorbitol and subsequent observed changes in NCV and histopathology of peripheral nerves were positively correlated with the duration of diabetes and overall level of blood glucose.

**P1361**

**Glycemic status and its associated influence in patients with type 2 diabetes in Chinese communities**

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**Objectives:** To evaluate the level of glycemic management among diabetic patients in 11 communities of Nanjing, China, and to assess the possible correlations with unsatisfactory glucose control, with the goal of improving glucose management.

**Methods:** The study included community-dwelling Chinese patients with type 2 diabetes who were recruited from the ‘5 + 1’ Diabetes Community Management Research (five goals: blood glucose, blood pressure, lipids, stopping smoking, and antiplatelet therapy; one, complication screening once a year). All of the data were collected from patients who were willing to participate in our community management research, including glycosylated haemoglobin.
(HbA1c) status and glucose-lowering treatment options. Associations between not achieving the glycemic target and potential related factors were tested using bivariate and multivariate analyses.

Results: Of 2454 patients (mean age, 65.17 years; 64.6% male), 76.37% used oral antidiabetes drugs and/or insulin, and 55.26% achieved the target of HbA1c <7%. In both bivariate and multivariate analyses, male gender, fasting blood glucose, low-density lipoprotein cholesterol (LDL-c), triglyceride and increasingly complex anti-diabetic treatment programmes were significantly associated with HbA1c ≥7%. However, smoking, drinking, diabetes duration, body mass index (BMI), waist circumference and systolic blood pressure were significantly associated with not accomplishing the glycemic target only in bivariate analyses. In addition, in male but not in female patients, BMI and LDL-c were significantly associated with HbA1c ≥7%.

Conclusions: Glycemic control in diabetic patients in Chinese communities of Nanjing was suboptimal. In our consecutive Diabetes Community Management Study, corresponding interventions can be applied to factors that can be modified. In addition, we may need to focus more attention on BMI and LDL-c in male patients to raise the standard rate of achievement of glycemic control.

Diabetes-related Diseases

Effects of fluctuant high blood glucose on apoptosis in intestinal mucous membrane cells, the distribution of L cells and changes in glucagon-like peptide-1 in type 2 diabetic rats

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Objective: To investigate the changes in intestinal mucous membrane cells, apoptosis and the expression of Caspase-3 BCL-2 and Bax in response to fluctuations in blood sugar, as well as whether the changes in glucagon-like peptide-1 (GLP-1) are related to the reduced number of L cells or apoptosis in the intestinal mucous membrane of diabetic rats.

Methods: Forty-five male Sprague–Dawley (SD) rats were randomly divided into two groups: 30 in the experimental group and 15 in the normal group. Intraperitoneal injection of streptozotoein (35 mg/kg) was used to establish a diabetic model in SD rats after feeding them a high-sugar and high-fat diet for 4 weeks. Two random blood glucose measurements in 2 days over 16.7 mmol/L indicated the successful establishment of a diabetic rat model. The rats were then divided into two groups: the sustained hyperglycemia (group S) and diabetic blood fluctuant group (group F). A normal group (group N) was also established. The rats in group F were induced by intraperitoneal injection of insulin and glucagon at different time points daily. The insulin dosage was adjusted based on blood glucose levels to maintain daily maximum fluctuations of blood glucose equal to or greater than 20 mmol/L and minimum blood sugar fluctuations equal to or less than 10 mmol/L. Concomitantly, the same volume of saline was administered to rats in groups S and N. A high-sugar and high-fat diet was still given to rats in groups F and S during the feeding, while a normal diet was given to rats in group N. Blood glucose was tested four times per day, twice per week. After 12 weeks, blood glucose, haemoglobin A1C (HbA1C) and pancreatic island function were determined. H&E staining was performed to observe pathological intestinal tissue in rats, and intestinal tissue damage was assessed by Haglund grading. Enzyme-linked immunosorbent assay was used to assay serum levels of GLP-1. The numbers of apoptotic intestinal epithelial cells were determined by TUNEL, and revealed that in China, glucocorticoid administration was the main therapeutic method (51.4% vs 11.2%, *P* < 0.01), and a lower proportion of patients was treated only by withdrawal of the incriminating drugs and diet modification (22.9% vs 82.2%, *P* < 0.01).

Conclusions: The average age of onset of IAS was earlier in Chinese than in Japanese patients, and most of the cases were associated with Graves’ disease and had taken an incriminating drug. In the majority of Chinese cases, a low dose of glucocorticoid was administered based on withdrawal of the incriminating drug and diet modification.

Epidemiology and clinical features of Chinese patients with insulin autoimmune syndrome

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Background: Insulin autoimmune syndrome (IAS) is a rare disease characterized by hyperinsulinemic hypoglycemia and a high titer of insulin autoantibodies to native insulin in an individual who has never received exogenous insulin. The majority of IAS cases were reported in Japan, but the epidemiological characteristics of Chinese IAS patients have rarely been described. Here, we performed a meta-analysis of the epidemiological characteristics of Chinese IAS patients.

Methods: We used ‘Insulin autoimmune syndrome’ as keywords and searched the China Knowledge Resource Integrated Database for case reports published from 1992 to 2013. We retrospectively reviewed the information for all IAS cases and then compared these data with those from Japan.

Results: The 105 IAS patients (47 male and 58 female subjects) ranged in age from 9 to 82 years. The incidence of IAS peaked at age 30–39 and 60–69 years, respectively, in China. The average age of onset of IAS occurred earlier in Chinese than in Japanese patients (48.3 ± 18.4 years vs 55.3 ± 16.6 years; *P* < 0.01).

The proportion of IAS patients associated with Graves’ disease was 57.1%, of whom 71% had taken drugs before the onset of IAS. These proportions were significantly higher than those in Japan (63.8% vs 43.7%, *P* < 0.01). The most common drugs included methimazole (49.5%), tiopronin (7.6%), PTU (4.8%), captopril (1.9%), and penicillamine (1%). Among the patients, 46.7% had severe hyperglycemic coma or consciousness disorders as initial symptoms. Comparison with the Japanese data revealed that in China, glucocorticoid administration was the main therapeutic method (51.4% vs 11.2%, *P* < 0.01), and a lower proportion of patients was treated only by withdrawal of the incriminating drugs and diet modification (22.9% vs 82.2%, *P* < 0.01).

Conclusions: The average age of onset of IAS was earlier in Chinese than in Japanese patients, and most of the cases were associated with Graves’ disease and had taken an incriminating drug. In the majority of Chinese cases, a low dose of glucocorticoid was administered based on withdrawal of the incriminating drug and diet modification.

immunohistochemistry was used to detect the expression of gene Bax, Bcl-2, Caspase-3 and the distribution of L cells in the intestine.

Results: Four weeks after feeding SD rats a high-sugar and high-fat diet, a diabetic rat model was successfully established after intraperitoneal injection of streptozotocin (35 mg/kg). The weight of the diabetic rats was greater than that of the rats in group N \( (P < 0.05) \). At the end of the trial, the weight of rats in groups S and F decreased compared with those in group N \( (P < 0.05) \). The fluctuation in blood glucose was greater in group F compared with groups N and S. In addition, there were significant changes in CV compared with group N or S \( (P < 0.05) \). There was no significant difference in HbA1c between groups F and S, which both displayed values greater than those in group N \( (P > 0.05) \). Compared with group N, the secretory function of insulin islands decreased in groups F and S \( (P < 0.05) \). Compared with group N, the serum levels of GLP-1 were reduced in group F \( (P < 0.05) \). Compared with group S, group F showed intestinal mucosal villus structures that were fragmented, a decreased villus height with tissue congestion, inflammatory cell infiltration of the mucosa and submucosa, and irregular nuclei in the mucosal villus. In the TUNEL analysis, a few apoptotic cells were observed in intestinal mucous membranes in the diabetes mellitus group compared with group N \( (P < 0.05) \), while there were more apoptotic cells in group F than in group S \( (P < 0.05) \). Immunohistochemistry revealed Caspase-3, Bax and Bcl-2 expression on intestinal mucous membranes; compared with group S, Caspase-3 and Bax were increased, and Bcl-2 was further increased in group F. Compared with group N, the distribution of L cells in the intestinal mucous membrane of rats in groups F and S was decreased, and compared with group S, it was further decreased in group F \( (P < 0.05) \).

Conclusions: The fluctuation of blood glucose in diabetic rats can significantly accelerate apoptosis of intestinal mucosal cells and increase the expression of Bax, Bcl-2 and Caspase-3. Meanwhile, it decreases the distribution of L cells. Compared with sustained hyperglycemia, fluctuations in blood sugar result in decreased GLP-1 secretion. Apoptosis of intestinal mucosal cells may induce enteroendocrine effects. I-cell reduction or apoptosis may correlate with the decrease in GLP-1 secretion.

O178
Studies of AMP-activated protein kinase expression in the breast cancer MCF-7 cell line cultured with high glucose, high insulin and metformin
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Objectives: To investigate the effects of high glucose and insulin on MCF-7 breast cancer cells, and to uncover the effect of metformin on the expression of adenosine monophosphate-activated protein kinase (AMPK) in MCF-7 cells treated with glucose and (or) insulin.

Methods: The expression of AMPK with high glucose and/or high insulin treatment and different concentrations of metformin were assessed by Western blot and reverse transcriptase-polymerase chain reaction. The cell cycle was detected by flow cytometric analysis.

Results: The expression levels of AMPK\(_{\alpha 1}\) and p-AMPK\(_{\alpha 1/2}\) protein in MCF-7 cells treated with high glucose plus high insulin were significantly decreased compared with those in the control group \( (P = 0.02 \text{ and } 0.01, \text{ respectively}) \). In the high glucose group, the expression of p-AMPK\(_{\alpha 1/2}\) and AMPK\(_{\alpha 1}\) mRNA in the metformin-treated group \( (2.5, 5, 10 \text{ and } 20 \text{ mM}) \) were significantly increased compared with those in the control group. In the high insulin group, the expression levels of p-AMPK\(_{\alpha 1/2}\) protein in the metformin-treated group \( (2.5, 5, 10 \text{ and } 20 \text{ mM}) \), AMPK\(_{\alpha 1}\) in the metformin-treated group \( (10 \text{ and } 20 \text{ mM}) \) and AMPK\(_{\alpha 1}\) mRNA in the metformin-treated group \( (2.5, 5, 10 \text{ and } 20 \text{ mM}) \) were significantly increased compared with those in the control group. In the glucose plus high insulin group, the expression levels of p-AMPK\(_{\alpha 1/2}\) protein in the metformin-treated group \( (5, 10 \text{ and } 20 \text{ mM}) \), AMPK\(_{\alpha 1}\) in the metformin-treated group \( (10 \text{ and } 20 \text{ mM}) \) and AMPK\(_{\alpha 1}\) mRNA in the metformin-treated group \( (2.5, 5, 10 \text{ and } 20 \text{ mM}) \) were significantly increased compared with those in the control group. Compared with the control group, cells in G1 were decreased in high glucose plus high insulin-treated cells \( (P = 0.042) \); metformin-treated \( (20 \text{ mM}) \) cells accumulated at the G1 stage.

Conclusions: High glucose and high insulin can decrease the expression of AMPK. Metformin can increase the expression of AMPK and block the cell cycle at G1 stage in MCF-7 breast cancer cells treated with high glucose and (or) high insulin.

O184
Fibroblast growth factor 21 is expressed in and protects the diabetic heart from cell death and cardiomyopathy via the Erk1/2-dependent signalling pathway
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Aims: To study fibroblast growth factor 21 (FGF21)-induced cardiac protection against cell death and dysfunction caused by diabetic lipotoxicity in vitro and in vivo, and to explore the protective mechanism.

Methods: Cardiac FGF21 mRNA expression was examined at different times in streptozotocin-induced type 1 diabetic mice using real-time polymerase chain reaction. After pre-incubation of palmitate-treated H9c2 cells with FGF21 for 15 h, apoptosis and possible FGF21-induced cell survival signalling pathways were determined using RNA interference. We also examined apoptosis and cardiac function in wild-type and FGF21-KO mice at the early or late stage of diabetes.

Results: Type 1 diabetes up-regulated cardiac FGF21 expression by approximately 40-fold at 2 months and 3-fold to 1.5-fold at 4 and 6 months after diabetes onset. FGF21 at a dose range of 25–250 μM significantly reduced palmitate-induced cardiac cell apoptosis. Mechanistically, palmitate down-regulated, but
FGF21 up-regulated the phosphorylation levels of Erk1/2, p38 MAPK and adenosine monophosphate-activated protein kinase (AMPK). Inhibition of each kinase with its specific inhibitor or siRNA revealed that FGF21 prevents palmitate-induced apoptosis by up-regulating the Erk1/2-dependent p38 MAPK/AMPK signalling pathway. In vivo administration of FGF21, but not FGF21 along with an ERK1/2 inhibitor, to diabetic or fatty acid-infused mice significantly prevented cardiac apoptosis and reduced the inactivation of Erk1/2, p38 MAPK and AMPK; it also prevented cardiac remodelling and dysfunction in late-stage diabetes. Mice lacking the FGF21 gene were more susceptible to diabetes-induced cardiac apoptosis.

Conclusions: FGF21 prevented lipid-induced or diabetes-induced cardiac cell apoptosis and cardiac dysfunction by activating the Erk1/2 P38 MAPK-AMPK signalling pathway.

O411 Rosiglitazone attenuates cognitive function by altering hippocampal IRS-1/P3K/Akt signalling pathways in ob/ob mice

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Objective: Alzheimer's disease (AD) and type 2 diabetes mellitus share many common pathologic features, including impaired glucose metabolism, hyperinsulinemia and insulin resistance. It has been reported that the peroxisome-proliferator-activated receptor gamma (PPARγ) agonist rosiglitazone (RSG) improves hippocampus-dependent cognition in a mouse model of AD, but whether cognition in diabetes with AD was affected by RSG remains unknown. Therefore, the purpose of this study was to identify alterations of key molecular components related to memory formation and insulin signalling in the hippocampus after RSG was injected into ob/ob mice to test whether cognitive dysfunction was pharmacologically reversed by regulation of PPARγ.

Methods: Wild type (WT) and leptin-deficient ob/ob mice were maintained on a standard chow diet under a 12 h light/dark cycle with free access to water. The age-matched mice were divided into three groups (n = 6): PBS-treated WT mice (WT-PBS); PBS-treated ob/ob mice (ob/ob-PBS) and RSG-treated ob/ob mice (ob/ob-RSG), with intraperitoneal injection of 10 mg/kg RSG daily for 2 weeks. Body weight was also monitored during the experiment. The glucose levels were measured during the intraperitoneal injection period. The Morris water-maze test was performed before the mice were sacrificed. Mice were killed by decapitation, and the brain was rapidly removed from the skull for hippocampal dissection. Western blot analysis was used to evaluate the following proteins: Bace1, p-Tau, p-IRS-1, IRS-1, P3K, p-Akt and Akt in hippocampal tissues. The Aβ1-40 and Aβ1-42 levels were detected by enzyme-linked immunosorbent assay.

Results: Blood glucose levels were significantly reduced in ob/ob-RSG compared with ob/ob-PBS, suggesting improved glucose metabolism. RSG treatment led to an increase in hippocampus-dependent cognition of ob/ob mice according to the Morris water-maze test. The protein levels of Bace1, p-Tau and Aβ, which are critical hallmarks of cognitive dysfunction and amyloid deposits, were lowered in RSG-treated ob/ob mice. Furthermore, RSG treatment up-regulated the hippocampal P-IRS-1/IRS-1 and P-Akt/Akt ratio and PI3K protein expression, demonstrating that insulin signalling pathways were enhanced in ob/ob-RSG mice.

Conclusions: In the present contexts, we concluded that a PPARγ agonist ameliorates cognitive deficits in ob/ob mice by up-regulating insulin signalling pathways in the hippocampus.

O418 Role and mechanism of DPP-4 inhibitors in promoting the migration and invasion of cancer cells

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Background: Type 2 diabetes has been associated with cancer. Some of the medications used to treat hyperglycemia are associated with increased or decreased risk of cancer. The present study shows the effects of DPP-4 inhibitors (DPP-4i) on the migration and invasion of cancer cells and examines possible mechanisms underlying these effects.

Methods: Here, five types of cancer cells were examined, and all of them were closely related to type 2 diabetes: colon cells (SW480 and HCT116), hepatocellular carcinoma cells (HuH7), lung cancer cells (A549), melanoma cells (A375), and glioma cells (CHG5). Each cell type was divided into two groups: a control group and a treatment group. The control group was treated with saline, and the treatment group was treated with saxagliptin and sitagliptin. The ability of the cells to migrate and invade was evaluated using a Transwell experiment, and the persistence of cancer cell migration was detected using a time-lapse microscope and track-plot analysis. Reactive oxygen species were detected in cultured cancer cells by flow cytometry, and oxidative DNA damage was measured by the amount of 8-hydro-2'-deoxyguanosine (8-oxo-dG). A GSH/GSSG-Glo™ assay was used to examine the oxidative state of cancer cells. The relative levels of Nrf2, NQO1, HIF-1, Cox-2, APRIL, and Cortactin protein were analysed by Western blot analysis.

Results: In the Transwell experiments, DPP-4i was found to promote the migration and invasion of cancer cells (P < 0.05). The effect of DPP-4i may have been induced by the increased persistence of cancer cell migration, as indicated by time-lapse microscopy and Transwell analysis. It was even found to promote pseudopod formation in cells, as observed under the microscope. Enzyme-linked immunosorbent assay analyses showed that DPP-4 was inhibited in cancer cells (P < 0.05). Reactive oxygen species (ROS) levels were significantly lower after DPP-4i treatment (P < 0.05). The intracellular oxidative states were also decreased, as indicated by the ratio of GSH to GSSG. DPP-4i treatment significantly reduced oxidative damage in cancer cells, as indicated by 8-oxo-dG staining. Treatment with
DPP-4i markedly increased protein levels of Nrf2, NQO1, HIF-1, Cox-2, APRIL, and Cortactin.

Conclusions: Treatment with DPP-4i significantly promotes the migration and invasion of cancer cells, and this effect may occur through the Nrf2/ROS pathway.

**O469**

**Tumour necrosis factor alpha-induced HepG2 liver cell lipid accumulation involves suppression of AMP-kinase signalling**

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**Background:** Clinical studies have found that inflammation may be involved in the early fat deposition process of nonalcoholic fatty liver disease, but its mechanism has not been elucidated. The aim of this study was to investigate the effect of Amp-activated protein kinase (AMPK) on lipid deposition in tumour necrosis factor-α (TNF-α)-induced HepG2 cells.

**Methods:** TNF-α was used to stimulate HepG2 cells to induce inflammatory stress. The levels of lipid accumulation were detected using triglyceride (TG) test kits. The expression of phosphorylated AMPK and ACC, phosphorylated mTOR and p70S6K, SREBP-1 and FAS were determined by Western blot analysis. The mRNA levels of SREBP-1 and FAS were determined by quantitative real-time polymerase chain reaction. AMPK agonist or antagonist was co-administered with TNF-α in HepG2 cells, and the aforementioned indexes were again examined.

**Results:** A significant increase in TG content in HepG2 cells was observed after TNF-α intervention. However, AMPK and ACC phosphorylation was suppressed, the phosphorylation of mTOR and p70S6K was subsequently enhanced, and the levels of FAS and SREBP-1 were increased significantly. Co-administration with metformin or AICAR significantly prevented the TNF-α-induced increase in intracellular TG content, accompanied by a significant enhancement of AMPK and ACC phosphorylation, suppressed mTOR and p70S6K phosphorylation, and reduced FAS and SREBP-1 expression. In contrast, when co-incubated with Compound C, AMPK and ACC phosphorylation was suppressed, and the inhibitory effect of metformin on lipid deposition in HepG2 cells was weakened.

**Conclusions:** These results demonstrate that TNF-α induces HepG2 cell lipid accumulation by suppressing AMPK phosphorylation, which is closely associated with the AMPK/mTOR/SREBP-1 pathway.

**O551**

**Relationship between serum osteocalcin and blood glucose by glucose metabolism status**

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**Objective:** This study was performed to explore the relationship between serum osteocalcin and blood glucose in a community-dwelling population with different glucose metabolism statuses.
Clinical Diabetes/Therapeutics

Methods: From May 2010 to June 2012, we conducted a questionnaire survey and measured body weight, height, and waist circumference in adults aged 45 years and above from the Shanghai Changfeng community. We also measured fasting blood glucose (FBG), 2 h glucose levels following a 75-g oral glucose challenge, [postprandial blood glucose (PPG)], haemoglobin A1c (HbA1c) and serum osteocalcin. A total of 3285 participants (male: 1137, 34.6%) with complete clinical data were included in the statistical analysis (SPSS 13.0).

Results: The median (inter-quartile range) was 19.59 (15.42, 24.77) ng/mL. Normal glucose tolerance was measured in 56.4% of the participants, and 21.3%, 11.9% and 10.3% had prediabetes, were newly diagnosed and had established diabetes, respectively. The level of serum osteocalcin decreased accordingly in the group with normal glucose tolerance, prediabetes, newly diagnosed and known diabetes (21.71, 20.93, 19.10 and 16.70, \( P_{\text{trend}} < 0.05 \)). In the population without a history of diabetes, serum osteocalcin correlated positively with age and negatively with waist circumference, body mass index, FBG, PPG and HbA1c. Stepwise regression analysis showed that HbA1c was an independent factor in the serum osteocalcin concentration (standardized partial regression coefficient was \(-0.107\)).

Conclusions: The level of serum osteocalcin decreased with the progress of diabetes, and HbA1c was an independent predictor of the serum osteocalcin concentration.

O687 Adrenal teratoma manifesting as specific types of diabetes

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Background: Corticomedullary mixed pathological changes (teratoma) originating from different germ layers rarely manifest as specific types of diabetes or hypertension in clinical settings. The diagnostic and treatment processes of a case of adrenal teratoma experienced in our department were analysed to pathologically present the correlation between the hierarchical structure and clinical manifestations of rare tumours as well as to explore the possible mechanism of pathogenesis.

Methods: After an adrenal teratoma was detected, endocrine function evaluation and laparoscopic surgery were performed after preoperative preparations. Postoperative pathological analyses and immunohistochemical staining were then conducted, followed by re-examinations of blood sugar level and blood pressure, hormone analysis, and imaging at 2, 12, 24, and 48 weeks after surgery.

Results: As abnormal cortisol levels were not suppressed by high/low dose dexamethasone suppression tests, the possibilities of hypercortisolism or right adrenal adenoma were considered in the preoperative diagnosis. On gross observation after surgery, the tumour diameter was approximately 5 cm and consisted of mainly the medullary substance; the remaining region revealed normal adrenal gland. Haematoxylin and eosin staining revealed the adrenal capsule, residual adrenal cortex, tumour capsule, cortical substance, and main medulla pheochromocytoma tumour with clear hierarchical structures. Immunohistochemical analysis showed that the tumour was positive for chromogranin A and S-100, with a Ki-67 index of <5%; therefore, the tumour was diagnosed as a corticomedullary mixed tumour. The tumour was positive for subcapsular inhibit, calretinin, and adrenocorticotropic hormone; staining for both CGA and steroidogenic factor-1 on the tumour surface indicated a double-mesodermal-originated adrenal teratoma containing partial cortical structures composed of pheochromocytes; and it had a cortical component showing mainly medullary lesions. Nestin and acetaldehyde dehydrogenase 1 staining were positive, suggesting that the double-mesodermal-originated tumour might be related to tumour stem cells. Although administration of medications for diabetes and hypertension was stopped until surgery was performed, the blood sugar level and blood pressure were maintained. Continuous glucose monitoring system results, cortisol rhythms, catecholamine metabolite levels, glycated haemoglobin A1c, C-peptide release, positron emission tomography-computed tomography results, and meto-iodobenzylguanidine scintigraphy results were normal.

Conclusions: Corticomedullary ‘teratoma’, which rarely manifests as specific types of diabetes and hypertension, was composed of pheochromocytes and cortical components with clear hierarchical structures showing neat arrangements as well as non-hybrid irregular arrangements. Therefore, the occurrence of corticomedullary ‘teratoma’ might be associated with tumour stem cells.

O613 Association of serum zinc with microvascular complications in type 2 diabetes

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Objectives: The relationship between diabetes, insulin and zinc is complex, with no clear cause and effect relationships. The aims of the present study were to identify various clinical and biochemical characteristics related to low levels of serum zinc, to investigate the association of serum zinc levels with microvascular complications in patients with type 2 diabetes mellitus (T2DM) and to compare the serum levels of zinc in diabetic patients with and without microvascular complications.

Methods: This was a non-interventional, cross-sectional study. The study involved 412 patients with T2DM: 271 with one or more microvascular complications and 141 without microvascular complications. We identified various clinical and biochemical characteristics and the prevalence of any form of microvascular complications related to different levels of serum zinc. In addition, we investigated the association of serum zinc levels with microvascular complications in patients with T2DM. We also used a logistic regression model to assess potential associations between serum levels of zinc and microvascular complications. Potential confounding risk factors were controlled for using multiple regression analysis.

Results: The distribution of serum zinc was not normal, so a logarithmic transformation was performed. The mean serum zinc level was significantly lower in diabetic patients with microvascular complications than in those without (mean: 5.71 vs. 5.92 μg/dL, \( P = 0.03 \)). The prevalence of microvascular complications was significantly higher in patients with serum zinc levels less than 6.0 μg/dL (OR: 2.31, 95% CI: 1.13-4.72, \( P = 0.02 \)). After adjusting for potential confounding risk factors, the odds ratio of having microvascular complications decreased slightly but remained significantly associated with lower serum zinc levels (OR: 1.70, 95% CI: 1.07-2.70, \( P = 0.02 \)).

Conclusions: Low serum zinc levels are associated with an increased risk of microvascular complications in patients with type 2 diabetes mellitus. Further studies are needed to clarify the mechanisms underlying this association.
factors included in all models were sex, age, duration of diabetes, prevalence of hypertension, body mass index, haemoglobin A1c and estimated glomerular filtration rate. Results: Compared with the normal level of serum zinc, subjects in the group with low serum zinc levels had a longer duration of diabetes, higher haemoglobin A1c, higher prevalence of hypertension, higher prevalence of any form of microvascular complication, lower C-P and lower 2-h C-P. The risk of each type of microvascular complication of type 2 diabetes evaluated was strongly associated with serum zinc levels. There was a significant decrease in serum zinc levels ($P < 0.01$) in the microvascular complications group. Low levels of serum zinc were a risk factor for the development of diabetic neuropathy (odds ratio 0.828; 95% confidence interval: 0.72, 0.951; $P < 0.01$). Conclusion: A low level of serum zinc is one of the characteristics of subjects with type 2 diabetes who also have a long duration of diabetes, poor glucose control, hypofunction of islet β cell mass and a high risk of microvascular complications. Patients with type 2 diabetes who also have low levels of serum zinc are more likely to develop microvascular complications.

**O730**

**Relationship between peripheral blood levels of methyl donor and folate and mild cognitive impairment in type 2 diabetic patients**

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Objective: To analyse and explore the relationship between peripheral blood levels of methyl donors, folate and mild cognitive impairment (MCI) in patients with type 2 diabetes mellitus (T2DM) by testing the blood levels of methyl metabolites and folate in MCI and normal cognition in elderly Chinese patients with T2DM. Methods: Fifty T2DM patients with MCI were detected using the Mini-Mental State Examination and a short memory questionnaire, and 50 age-matched ($±$3 years), sex-matched and education-matched diabetic subjects with normal cognition were randomly selected as controls. In all subjects, plasma levels of S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH) and homocysteine (Hcy) were analysed by high-performance liquid chromatography, and serum folate concentrations were determined using a chemiluminescence assay. Logistic regression was used to estimate the association between folate, methyl donors and MCI. Pearson’s correlation analysis was used to assess associations between folate and methyl donors. Results: Patients with MCI had significantly lower levels of SAM, folate and SAM/SAH ratios and higher levels of Hcy and SAH (all $P < 0.05$). Furthermore, logistic regression analysis indicated that plasma SAM, SAM/SAH ratio and serum folate (OR, 0.96, 0.698 and 0.72, respectively; $P < 0.05$) were negatively associated with the risk of MCI, even after adjusting for the duration of diabetes, GHBA1c, diabetic retinopathy and cardiovascular disease risk factors. Pearson’s correlation analysis showed that the level of folate was significantly positively correlated with that of SAM ($r = 0.12, P < 0.05$) and the SAM/SAH ratio ($r = 0.30, P < 0.01$) and negatively correlated with that of SAH ($r = −0.37, P < 0.01$), only among the subjects within the middle tertile of folate levels (6.3–9.1 μg/L). In contrast, there was no significant correlation among those in the lower (<6.3 μg/L) ($P > 0.05$) or upper (≥9.1 μg/L) tertile of folate levels ($P > 0.05$). Conclusions: Folate deficiency, a lower level of SAM and weakened methylation potential were associated with MCI in elderly patients with T2DM. Plasma SAM and methylation potential may be predicted by serum folate within a suitable range of folate concentrations in patients with T2DM. An appropriate folic acid supplement might be helpful to protect against cognitive impairment in T2DM patients.

**O785**

**Effect of different glucose metabolic states on the occurrence and severity of breast cancer**

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Background: Some studies have found that diabetes is closely related to breast cancer. The incidence of breast cancer in diabetic patients is higher than in those of the same age with normal blood glucose. Type 2 diabetes is an independent risk factor of recurrence and metastasis in breast cancer patients. However, between the state of normal glucose tolerance (NGT) and diabetes mellitus (DM), there is an intermediate state named impaired glucose regulation (IGR, also known as pre-diabetes mellitus). Relevant data concerning the effect of IGR on the occurrence of breast cancer are needed. In addition, few studies have been conducted to compare the impact of different glucose metabolic states on breast cancer. Therefore, we compared the incidence, metabolic characteristics, and pathological features, among others, of breast cancer in different glucose metabolic states. Here, we discuss the effect of different glucose metabolic states on the occurrence and severity of breast cancer so as to provide a theoretical basis for prevention and treatment.

Methods: According to inclusion criteria and exclusion criteria, we included 131 female patients with breast cancer from the breast surgery ward of Beijing Hospital to the breast cancer group, and 72 healthy women from the physical examination centre of Beijing Hospital as a control group. In all of the enrolled women, we performed oral glucose tolerance test (OGTT) for those who were not previously diagnosed with diabetes and judged their glucose metabolic state based on the OGTT result. Next, we divided them into the NGT, IGR, and DM groups. Women with a previous diagnosis of diabetes were automatically included in the DM group. IGR and DM were collectively referred to as abnormal glucose metabolism (AGM). We created a database that included information about age, menopausal status, body mass index, blood pressure, glucose metabolic, liver and renal function, and blood lipids for all of the enrolled individuals. For the breast cancer group, we also recorded pathology results. All of the statistical analyses were processed using SPSS 17.0. We compared the effect of different glucose metabolic statuses on the occurrence of breast cancer and its characteristics such as demographics, metabolism, and pathology.
Results: AGM, IGR, and DM were independently associated with the occurrence of breast cancer. Among them, IGR had the greatest impact. In IGR, the risk of breast cancer was 4.357 times higher than that in NGT ($P = 0.004$). Compared with the control group, the breast cancer group had a significantly increased age, BMI, and systolic blood pressure (SBP), decreased glucose tolerance, elevated glucose metabolic parameter values, increased partial liver enzyme parameters, and an increased prevalence of metabolic syndrome, and all of these differences were significant. Among patients with breast cancer, glucose tolerance gradually decreased; mean age, age at menopause, and prevalence of metabolic syndrome gradually increased; and SBP, body mass index, and partial liver enzyme parameters increased, and all of these differences were significant. The pathological features of breast cancer patients between different glucose metabolic statuses were not significantly different. Both IGR and DM can increase the prevalence of breast cancer and affect related demographics and metabolic parameters of breast cancer. In terms of increasing breast cancer prevalence and blood pressure, among others, the role of IGT was more significant than DM.

Conclusions: AGM, IGR, and DM were independently associated with the occurrence of breast cancer. Compared with the control group, patients with breast cancer had a significantly increased age and liver enzyme parameters and a significantly increased prevalence of metabolic syndrome and related parameters. Among patients with breast cancer, glucose tolerance gradually decreased; mean age, age at menopause, and prevalence of metabolic syndrome gradually increased; and liver enzyme parameters increased. In terms of monitoring the occurrence of breast cancer and related parameters, IGR should be given equal importance to DM.

O1301
Risk factors of ischemic cardiovascular diseases in a population-based study in Beijing
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Objective: To identify other risk factors not included in the Framingham risk score and China risk score for 10-year ischemic cardiovascular risk.

Methods: From July 2010 to March 2011, a population-based survey of chronic diseases and health survey was conducted in a population of 1.66 million persons in the Changping District of Beijing. A total of 5490 cases of patients were investigated in this sub-study. The Framingham risk score and China risk score predicting the incidence risk of ischemic cardiovascular disease over 10 years were calculated.

Results: The Framingham risk score and China risk score for ischemic cardiovascular risk were inconsistent in the Chinese population (Kappa 0.246, $P < 0.05$). The 10-year incidence risk of ischemic cardiovascular disease based on the China risk score revealed subjects with low, moderate or high risks in 4852 cases (88.4%), 383 cases (7.0%) and 255 cases (4.6%), respectively. Logistic regression analysis indicated that the waist-to-height ratio and non-high-density lipoprotein (HDL) were risk factors for ischemic cardiovascular diseases. Using the area under the curve (receiver operating characteristic) to assess the predictive value of risk factors, the waist height ratio was 63.4% for males and 81.8% for females (both $P < 0.01$). The non-HDL cholesterol was 59.9% for males and 72.7% for females (both $P < 0.01$).

Conclusions: Waist-to-height ratio and non-HDL are independent predictors of ischemic cardiovascular diseases in a population.

O1406
Prevalence of metabolic syndrome in children and adolescents according to three different diagnostic criteria
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Objectives: To calculate prevalence of metabolic syndrome (MS) in children and adolescents, respectively according to the Pediatric Academy of Chinese Medical Association in 2012 (Chinese criterion), International Diabetes Federation (IDF) in 2007 (IDF criterion) and Cook according to the National Cholesterol Education Program—Adult Treatment Panel in 2003 (Cook criterion), and to explore the characteristic of prevalence for MS and test consistency check by Kappa test.

Methods: A total of 920 students were selected from 11- to 16-year-old students in Liaoyang City and were asked to complete a questionnaire and undergo a complete physical examination, like body height, weight, waist, and blood pressure. Blood samples were collected to test fasting plasma glucose, serum total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglyceride (TG) and so on.

Results: The prevalence of metabolic syndrome in children and adolescents was 10.98%, 7.93% and 16.20% according to the Chinese criterion, IDF criterion and Cook criterion, respectively. Cook criterion has the highest detection rate of metabolic syndrome in children and adolescents. In the patients of metabolic syndrome in children and adolescents, abdominal obesity was accounted for 100%, 100% and 76.51%, respectively; hypertension was accounted for 73.23%, 63.01% and 87.25%, respectively; hyperglycemia was accounted for 17.82%, 20.55% and 8.05%, respectively; abnormal cholesterol metabolism was accounted for 93.07%, 87.67% and 79.19%, respectively; and hypertriglyceridemia was accounted for 51.49%, 45.58% and 76.51%, respectively, according to the Chinese criterion, IDF criterion and Cook criterion. The concordance between Chinese criterion and IDF criterion, Kappa value was 0.789. Comparing Cook criterion and Chinese criterion, Kappa value was 0.682. Comparing IDF criterion and Cook criterion, Kappa value was 0.682.

Conclusions: The prevalence of MS is higher in children and adolescents of Liaoyang urban in northeast China than in other areas. Cook criterion has the highest detection rate of metabolic syndrome in children and adolescents. The concordance between Chinese criterion and IDF criterion for diagnosing metabolic syndrome was the best.
O1485
Investigating the heterogeneity of metabolic syndrome and its clinical significance
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Background: Metabolic syndrome (MS) is a pathological state with various metabolic abnormal components, representing a complex metabolic disorder syndrome that has serious effects on human health. There are many different metabolic disorder combinations of MS; however, the constituent ratio of the different combinations and their clinical consequences are unclear. The purpose of this study was to explore the constituent ratio of different MS combinations and their cardiovascular complications in patients hospitalized in the Department of Endocrinology, which offers evidence for risk stratification of MS.

Methods: Clinical data for 608 MS patients hospitalized in the Department of Endocrinology at the second Xiangya Hospital of Central South University from April 2013 to March 2014 were collected. Among them, there are 13 patients in combination 1 (central obesity + dyslipidemia + abnormal blood pressure), 112 patients in combination 2 (central obesity + dyslipidemia + dysglycemia), 65 patients in combination 3 (central obesity + abnormal blood pressure + dysglycemia), 76 patients in combination 4 (dysglycemia + abnormal blood pressure + dyslipidemia) and 342 patients in combination 5 (central obesity + dysglycemia + abnormal blood pressure + dyslipidemia). Blood pressure, glucose, lipids and other biochemical indexes and microvascular complications were analysed in different combinations of MS.

Results: Among the MS patients hospitalized in the Department of Endocrinology at the Second Xiangya Hospital of Central South University from April 2013 to March 2014, the prevalence of central obesity, dyslipidemia and abnormal blood pressure had the lowest proportion. The risk of suffering microvascular or macrovascular complications increased in combinations containing dysglycemia, abnormal blood pressure and dyslipidemia.

P289
Metformin inhibits NF-κB activation and inflammatory cytokine expression induced by high glucose via AMP-activated protein kinase activation in rat glomerular mesangial cells in vitro
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Background: The renoprotective mechanisms of the AMP-activated protein kinase (AMPK) agonist metformin have not been clearly described. We hypothesized that metformin may ameliorate inflammation via AMPK interaction with critical inflammatory cytokines. The aim of this study was to observe the effects of metformin on the expression levels of nuclear factor-κB (NF-κB), monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1) and transforming growth factor-beta 1 (TGF-β1) induced by high glucose (HG) in cultured rat glomerular mesangial cells (MCs).

Methods: MCs were cultured in media with normal glucose (5.6 mmol/L; group NG) or with high glucose (25 mmol/L; group HG) and with different concentrations of metformin (groups M1, M2 and M3). After 48 h of exposure, the supernatants and MCs were collected. The expression levels of NF-κB, MCP-1, ICAM-1 and TGF-β1 mRNA were analysed by real-time polymerase chain reaction. Western blotting was used to detect the expression of AMPK, phospho-Thr-172 AMPK (p-AMPK), NF-κBp65, MCP-1, ICAM-1 and TGF-β1 proteins.

Results: After stimulation by HG, the expression of NF-κB, MCP-1, ICAM-1, TGF-β1 at the mRNA and protein levels in MCs in the HG group increased significantly compared with the NG group (P<0.05). Both mRNA and protein expression levels of NF-κB, MCP-1, ICAM-1 and TGF-β1 in MCs induced by high glucose were markedly reduced in a dose-dependent manner after metformin treatment (P<0.05). The expression of p-AMPK increased with increased metformin concentration, showing the opposite trend, while the total AMPK protein level was unchanged with exposure to HG or metformin.

Conclusions: Metformin can suppress the expression of NF-κB, MCP-1, ICAM-1 and TGF-β1 in glomerular MCs induced by high glucose via AMPK activation, which may partly contribute to its renoprotection.
Neutrophil-to-lymphocyte ratio is a predictive and reliable marker for early-stage diagnosis of diabetic nephropathy

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Background: Diabetic nephropathy (DN) is one of the most common complications in diabetic patients. Recent evidence suggests that the neutrophil-to-lymphocyte ratio (NLR) plays a role in the development and acceleration of some diabetic complications. However, no studies have investigated the relationship between DN and NLR. Thus, we aimed in this study to evaluate the relationship between DN and NLR and to determine whether NLR can be used as a reliable marker for the early stage of DN.

Methods: The study included 259 patients diagnosed with type 2 diabetes mellitus, 115 of whom were also in the early stage of DN. The control group was composed of 210 age-matched and sex-matched healthy subjects.

Results: NLR values were significantly higher in diabetic patients than in the healthy control group (P < 0.001), and the NLR values of patients with early-stage DN were higher than those of patients without DN (P < 0.001). Risk factors associated with DN were higher total cholesterol [P = 0.045, EXP(B) = 1.248, 95% CI = 1.005–1.549] and higher NLR [P = 0.008, EXP(B) = 1.814, 95% CI = 1.170–2.811].

Conclusions: Our data suggest that increased NLR is significantly associated with DN and that higher NLR values may be a predictive and reliable marker of early-stage DN.

Visfatin causes apoptosis of endothelial progenitor cells by inducing proinflammatory mediators through the NF-kB pathway

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Objectives: To explore the effect of visfatin on endothelial progenitor cell (EPC) apoptosis and to study its underlying mechanisms. Methods: Cultured EPCs pretreated with visfatin (50, 100 and 150 ng/mL), FK866 (an inhibitor of visfatin; 10 μM) and BAY11 [an inhibitor of nuclear factor (NF)-κB; 5 μM] for 48 h were used to study the relationship between visfatin and EPC apoptosis. Apoptosis was evaluated by flow cytometry. The expression of apoptosis-related proteins and genes (caspase 3, BAX and Bcl-2) was measured by quantitative real-time polymerase chain reaction and Western blot analysis. The protein levels of proinflammatory mediators [ICAM1 and interleukin-6 (IL-6)] and of nuclear factor-κB (NF-κB) were evaluated by Western blot analysis.

Results: After treatment with various concentrations of visfatin for 48 h, apoptosis of EPCs increased in a dose-dependent manner; included 7309 participants. Serum GGT, lipids, blood pressure, and glucose were measured.

P395
Serum gamma-glutamyltransferase is associated with impaired fasting glucose in Chinese adults: the Cardiometabolic Risk in Chinese Study

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Objectives: To investigate the relationship between gamma-glutamyltransferase (GGT) and impaired fasting glucose (IFG) and to evaluate the modification effects of age, body mass index (BMI), prehypertension, and lipids in a large sample of Chinese adults.

Methods: The study samples are from a community-based health examination survey in China. The sample for our analysis included 7309 participants. Serum GGT, lipids, blood pressure, and glucose were measured.

Results: Individuals with higher GGT were older and had higher fasting plasma glucose, fasting insulin, HOMA-IR, BMI, total cholesterol, triglyceride, WBCs, ALT, and low-density lipoprotein cholesterol (LDL-C) levels, but had lower high-density lipoprotein cholesterol (HDL-C) levels, which indicates that GGT tends to be higher as people grow older; the test has also showed higher fasting plasma glucose, fasting insulin, HOMA-IR, BMI, total cholesterol, triglyceride, WBCs, ALT, and LDL-C with the increase of age, but lower HDL-C levels. In models adjusted for age and gender, GGT was significantly related to IFG (P < 0.0001). When further adjusted for BMI, total cholesterol, triglyceride, HDL-C, and LDL-C, the association between GGT and IFG remained significant (P < 0.0001). We further performed stratified analyses on the associations between GGT and IFG with other risk factors, including age, BMI, lipids, and blood pressure. We found significant interactions between GGT and age (P for interaction < 0.0001) and between GGT and BMI (P for interaction = 0.0015) in relation to IFG risk. The associations were significant in individuals of 39–59 and >60 years (P < 0.05) and also in patients of <39 years (P < 0.05). In addition, the associations between GGT and IFG were more evident in groups with high and median triglyceride (TG) levels than in those with low TG levels. The interaction tests for blood pressure, total cholesterol, HDL-C, and LDL-C were not significant (P for interaction > 0.05).

Conclusions: Our data indicate that serum GGT concentration is associated with the risk of IFG, and the association was modified by TG levels.

P444
the expression levels of Bax and caspase 3 were up-regulated at both mRNA and protein levels, but the expression of Bcl-2 was decreased. Compared with the control group, significant increases in EPC apoptosis, along with increased mRNA and protein expression of Bax and caspase 3, were detected at a visfatin concentration of 150 ng/mL, but this effect was suppressed by pretreatment with FK866. Various concentrations of visfatin resulted in a dose-dependent increase of IL-6 and ICAM1 protein expression by EPCs, an effect that was significant at a concentration of 150 ng/mL and that could be suppressed by FK866. Visfatin up-regulated the expression of NF-κB at both the mRNA and protein levels. EPCs were preincubated with BAY11 for 1 h and then treated with visfatin (150 ng/mL) for 48 h. Compared with the 150 ng/mL visfatin-treated group, the visfatin-induced apoptosis, the increased expression of caspase 3, Bax, ICAM1 and IL-6, and the decreased expression of Bcl-2 were all abolished in the BAY11-treated group.

Conclusions: Our findings suggest that visfatin causes EPC apoptosis by increasing the expression of proinflammatory mediators, at least partially through up-regulation of NF-κB expression.

P521
Beraprost sodium inhibits inflammation and ameliorates diabetic nephropathy via p38 mitogen-activated protein kinase in diabetic rats
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Background: P38 mitogen-activated protein kinase (MAPK) is known to play a regulatory role in inflammatory processes in disease. Inflammation has been linked to the development of diabetic nephropathy. This study aimed to examine whether the prostaglandin I2 analog beraprost sodium (BPS) ameliorates diabetic nephropathy by inhibiting inflammation via p38 MAPK in diabetic rats.

Methods: Forty male Sprague Dawley rats were randomly divided into the normal control group (N = 10) and the diabetic group (N = 30). The diabetic group was fed with a high-fat diet (HFD, 40% of calories from fat) for 4 weeks to induce insulin resistance followed by a single intraperitoneal injection of streptozotocin (STZ, 30 mg/kg) to develop a type 2 diabetic rat model. After the successful establishment of the model, diabetic rats were randomly divided into the type 2 diabetes mellitus group (T2DM group) and the BPS treatment group (BPS group, BPS 0.6 mg/kg/day). At the end of the 8-week experiment, kidney weight, renal function, 24-h urine protein, lipid profiles and blood glucose level were examined. Renal pathological changes, activation of the p38 MAPK signalling pathway and inflammation were also measured.

Result: In the T2DM group, HFD/STZ-induced type 2 diabetic mice exhibited severe pathological changes in the kidney, renal dysfunction and inflammation, which were increased by activation of the p38 MAPK signalling pathway. In the BPS group, renal function, 24-h urine protein, lipid profiles and blood glucose level were significantly improved, while inflammation and expression of the p38 MAPK signalling pathway in the diabetic kidney were attenuated.

Conclusions: These results suggest that BPS significantly prevented type 2 diabetes-induced kidney injury characterized by renal dysfunction and pathological changes. The protective mechanisms are complicated but may be mainly attributed to inhibition of the p38 MAPK signalling pathway and inflammation in the diabetic kidney.

P792
Oxidized low-density lipid protein impairs the survival of bone marrow stem cells partially through membrane damage independent of reactive oxygen species production in vitro
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Aims: Cell therapy with bone marrow stem cells (MSCs) remains a viable option for tissue repair and regeneration. One of the major challenges for cell-based therapy is the limited survival of cells after in vivo administration. The exact mechanism(s) for impaired in vivo survival of the implanted MSCs remains to be defined. Oxidized low-density lipid protein (ox-LDL) is a natural product in human blood and the major contributor to the development of atherosclerosis. The present study was designed to investigate the effect of ox-LDL on the survival of bone marrow stem cells and the associated mechanisms in vitro.

Methods and Results: Rat bone marrow multipotent adult progenitor cells (MAPCs) were treated with ox-LDL (final concentration of 10 and 20 μg/mL) for up to 48 h. Exposure to ox-LDL resulted in significant cell death and apoptosis of MAPCs in association with a significant increase in LDH release in the conditioned medium in a dose-dependent and time-dependent manner, indicating significant cell membrane damage. The membrane damage was further confirmed by the rapid entry of the small fluorescent dye FM1–43, as detected using confocal microscopy. Ox-LDL generated a significant amount of reactive oxygen species (ROS) in the culture system, as measured using electron paramagnetic resonance spectroscopy. The antioxidant N-acetylcysteine (NAC, 0.1 mM) completely inhibited the production of ROS by ox-LDL. However, it did not prevent ox-LDL-induced cell death or apoptosis. However, pre-treatment of the cells with the specific membrane protective recombinant human MG53 protein (rhMG53) (66 μg/mL, final concentration)
significantly reduced LDH release and entry of FM1-43 dye into cells exposed to ox-LDL.

Conclusion: ox-LDL enhanced cell death and apoptosis of MAPCs via a mechanism independent of ROS generation in vitro. ox-LDL impaired the survival of MAPCs partially through cell membrane damage in vitro.

P802
Hydrogen peroxide inhibits the proliferation and endothelial differentiation of bone marrow stem cells partially through reactive oxygen species generation

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Aims: The aim of the present study was to investigate the effect of hydrogen peroxide (H2O2) on bone marrow stem cells and their endothelial differentiation and underlying mechanisms in vitro. Methods and results: Rat bone marrow multipotent adult progenitor cells (MAPCs) were used as the source of bone marrow stem cells and treated with H2O2 (final concentration from 0 to 50 μM) with or without N-acetylcysteine (NAC, 0.1 mM). H2O2 generated a significant amount of intracellular and extracellular reactive oxygen species (ROS) in the culture system, substantially inhibited the proliferation of MAPCs and expression of Oct-4, and induced apoptosis in a dose-dependent manner. Exposure to H2O2 also significantly attenuated the endothelial differentiation of MAPCs, with reduced expression of the endothelial markers CD31 and FLK-1 as well as impaired in vitro vascular structure formation. Both intracellular and extracellular ROS production in response to H2O2 was completely blocked by NAC. NAC treatment completely prevented H2O2-induced reduction of Oct-4 expression in cells. However, NAC treatment only partially prevented H2O2-induced apoptosis, inhibition of cell proliferation and endothelial differentiation in MAPCs. Conclusions: H2O2 exposure suppressed Oct-4 expression in MAPCs through a ROS-dependent mechanism, while it increased MAPC apoptosis and inhibited their proliferation and endothelial differentiation via a mechanism due in part to ROS generation in vitro.

P993
Is serum thyroid stimulating hormone associated with the prevalence of metabolic syndrome in East China?

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Objective: To evaluate the association between serum thyroid stimulating hormone (TSH) and the prevalence of metabolic syndrome (MetS) in East China.

Methods: Data were collected from a cross-sectional study focusing on the health and metabolic status of adults (≥18 years old) living in East China. Additionally, only subjects with TSH in the normal reference range (0.3–4.8 mU/L) were enrolled. All of the participants were asked to complete the questionnaires, and physical examinations including height, weight, waist circumference and blood pressure were performed. TSH, triglycerides, high-density lipoprotein cholesterol and fasting plasma glucose were also tested. MetS was defined according to International Diabetes Federation criteria (2005). In the subgroup analysis, the subjects were divided according to their gender, age and smoking status.

Results: The prevalence of MetS in the cohort living in East China was approximately 15.54% (743/4781). TSH was significantly higher in MetS patients than in non-MetS patients among middle-aged female nonsmokers (2.60 ± 1.01 vs. 2.40 ± 0.98 mU/L, P < 0.05). There were no differences in the other subgroups (P > 0.05). Among middle-aged female nonsmokers, subjects with the highest TSH levels (2.82–4.80 mU/L) had a significantly increased prevalence of MetS compared with subjects with the lowest TSH levels (0.30–1.88); the odds ratio was 1.64, and the 95% confidence interval was 1.13–2.36 (P < 0.05).

Conclusions: Even within the normal range, a slight increase in serum TSH may be a risk factor for MetS in middle-aged female nonsmokers.

P1363
Dissecting the association between plasma ghrelin and components of metabolic syndrome among children and adolescents in Northeast China

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Objective: To evaluate the association between plasma ghrelin and components of metabolic syndrome (MS) among children and adolescents in Northeast China.

Methods: A population-based cross-sectional study was conducted among 764 children and adolescents aged 11 to 16 years (mean age of 13.54 ± 1.42 years) from junior and senior high schools in Liaoyang city of Northeast China. They were asked to complete a questionnaire and received a complete physical examination, including height, weight, waist circumference, hip circumference and blood pressure. Fasting blood samples were collected to determine concentrations of plasma ghrelin, fasting plasma glucose, fasting insulin, serum total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol,

triglycerides (TGs), serum concentrations of uric acid (uric acid) and haemoglobin A1c. Plasma ghrelin was detected by enzyme-linked immunosorbent assay. The 2007 International Diabetes Federation in children and adolescents with metabolic syndrome unified diagnostic criteria were adopted. Based on the aforementioned criteria, the 764 subjects were divided into the MS group and non-MS group. The levels of plasma ghrelin were compared in the MS and non-MS groups, and the association between ghrelin and the prevalence of MS and its components was analysed.

Results: Fifty-three subjects were diagnosed with MS (male 77.35%), and the prevalence of metabolic syndrome was 6.94%. Among children and adolescents in Northeast China, the plasma level of ghrelin was higher in girls than in boys [41.71 (30.72, 55.37) vs 39.56 (28.88, 52.94) μg/L, P = 0.026]. After adjustment for sex and age, no significant differences were observed in the plasma levels of ghrelin between the MS and non-MS groups [38.88 (30.65, 51.67) vs 40.53 (29.53, 53.59) μg/L, P = 0.536]. In the multivariate linear regression model, the plasma ghrelin concentration was positively correlated to age (β = 0.153, P = 0.001) and gender (β = 0.1, P = 0.022), but there was no significant correlation with other metabolic parameters. Pearson’s correlation analysis showed that the plasma ghrelin concentration correlated negatively with the TG level (r = −0.018, P = 0.017). However, a partial correlation analysis showed that there was a poor association between plasma ghrelin and TG after adjustment for age and gender.

Conclusion: In a population of children and adolescents in Northeast China, there was no obvious correlation between the plasma level of ghrelin and metabolic syndrome.

P1402
Increased nucleotide-binding oligomerization domain 1 activity in subcutaneous adipose tissue from patients with metabolic syndrome
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Background: Insulin resistance (IR) and low-grade inflammation are typical features of metabolic syndrome (MetS). Nucleotide-binding oligomerization domain 1 (NOD1) protein, as a cytoplasmic pattern recognition receptor of the innate immune response, plays important roles in adipose inflammation and insulin resistance in diet-induced obesity. However, there are scant data examining the expression and activity of NOD1 in adipocytes from MetS patients. The aim of this study was to determine NOD1 expression in adipocytes from MetS patients and to investigate its association with MetS features.

Methods: Fourteen nascent MetS subjects and 16 age-matched controls were recruited. Fasting blood was collected, and abdominal subcutaneous adipose tissue was obtained by biopsy to study NOD1 expression and activity.

Results: MetS subjects showed significantly increased NOD1 gene expression in adipose depots compared with controls, after adjustment for waist circumference. In addition to increased expression of the downstream signalling mediator RIPK2 and nuclear factor-κB (NF-κB) p65 nuclear translocation, there was a remarkably higher release of monocyte chemoattractant protein-1 (MCP-1), interleukin (IL)-6, and IL-8 in MetS versus control subjects following priming of the isolated adipocytes with the NOD1-specific ligand iE-DAP. Circulatory levels of proinflammatory cytokine and high-sensitivity C-reactive protein were highly elevated in MetS compared with control subjects, following adjustment for waist circumference. Increased expression of NOD1 positively correlated with waist circumference (r = 0.31, P < 0.05). NOD1 expression was also correlated with haemoglobin A1c (r = 0.28; P < 0.05) and HOMA-IR (r = 0.42, P = 0.006). NOD1 expression positively correlated with serum levels of IL-6, MCP-1, and NF-κB activity.

Conclusions: NOD1 expression and activity are increased in the adipocytes of patients with MetS. Activation of the innate immune pathway via NOD1 may be partially responsible for increased systemic inflammation and increased risk of IR and diabetes in MetS subjects.
Epidemiology/Genetics

O273
Comparison of hyperuricemia prevalence in normal glucose tolerance subjects and type 2 diabetes patients in a Chinese population
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Background: The prevalence of hyperuricemia has been reported to be high in patients with type 2 diabetes (T2D) and varies across different populations. In the current study, we aimed to assess the prevalence of hyperuricemia in a Chinese population.

Methods: We recruited 4221 participants, comprising 1892 T2D patients and 2329 subjects with normal glucose tolerance (NGT). Uric acid-related traits were extensively measured for all participants.

Results: The overall prevalence of hyperuricemia was 11.16% in NGT subjects and 17.02% in T2D patients. In females, the prevalence of hyperuricemia was significantly higher in T2D patients than in those with NGT (20.39% vs 7.63%, P < 0.001). In contrast, in males, the prevalence of hyperuricemia showed a tendency to be higher in NGT subjects than in T2D patients (17.05% vs 13.88%, P = 0.059). Next, we assessed the prevalence of hyperuricemia stratified by age in both NGT subjects and T2D patients. The results showed that in females aged <55 years, the prevalence of hyperuricemia was significantly higher in T2D patients than in those with NGT (P < 0.0001 for age <45 years and P = 0.004 for 45 ≤ age < 55 years). In males, there was a clear decrease in the prevalence of T2D patients compared with those with NGT (P < 0.0001 for 45 ≤ age < 55 years and P = 0.017 for 55 ≤ age < 65 years).

Conclusion: Hyperuricemia is prevalent in Chinese T2D patients, and the age-associated prevalence of hyperuricemia was significantly higher in females than in males. Therefore, strategies to prevent and treat hyperuricemia in T2D patients are urgently needed to curtail this national pandemic.

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O534
Cardio-metabolic effects of weight change among Chinese women with prior gestational diabetes mellitus from pre-pregnancy to postpartum years 1–5
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Objective: To evaluate the association of weight change from pre-pregnancy to postpartum years 1–5 with cardio-metabolic implications among Chinese women with prior gestational diabetes mellitus (GDM).

Methods: We performed a retrospective cohort study in 1263 women with a history of GDM at 1–5 years after delivery using data from participants of the Tianjin Gestational Diabetes Mellitus Prevention Program. At the postpartum baseline survey, all of the study participants filled in a questionnaire about their pre-pregnancy weight, weight gain during pregnancy, dietary habits and physical activity. Body weight, height, waist circumference (WC) and blood pressure were measured concomitantly. According to the definition from the International Diabetes Federation and the American Diabetes Association, the prevalence of metabolic syndrome (MeS) was diagnosed as the presence of any three of five risk factors: WC ≥ 90 cm (35 in.) in men and ≥ 80 cm (31 in.) in women for Asians; triglyceride (TG) ≥ 150 mg/dL (1.7 mmol/L) or using a drug treatment for elevated TG; high-density lipoprotein cholesterol (HDL-C) ≥ 40 mg/dL (1.03 mmol/L) in men and ≥ 50 mg/dL (1.3 mmol/L) in women or using a drug treatment for reduced HDL-C; fasting glucose ≥ 100 mg/dL (5.6 mmol/L), 2-h glucose ≥ 140 mg/dL.
(7.8 mmol/L) or using a drug treatment for elevated glucose; or systolic blood pressure ≥130 mmHg, diastolic blood pressure ≥85 mmHg or using an antihypertensive drug treatment.

**Results:** Participants were divided into four groups based on their weight change from pre-pregnancy to postpartum years 1–5: loss of ≥3 kg, ≥3 kg, gain of 3–7 kg and gain of ≥7 kg. MeS was 12.1% (16/132), 16.5% (77/479) and 44.2% (110/259) (P < 0.001) among women with weight loss ≥3 kg, stable weight (±3 kg), and weight gain of 3–7 kg and ≥7 kg from pre-pregnancy to postpartum years 1–5, respectively. When stratified by pre-pregnancy BMI (<24, 24–27.9 and ≥28 kg/m²), positive associations of weight change from pre-pregnancy to postpartum years 1–5 with overall MeS and its individual components were observed among women with a pre-pregnancy normal weight, overweight and obesity. MeS was 0.0% (0/54), 7.9% (34/428), 16.5% (32/194) and 33.3% (49/147) (P < 0.001) among women with a pre-pregnancy normal weight BMI <24 kg/m²; 8.2% (4/49), 37.5% (48/128), 41.8% (33/79) and 54.4% (43/79) (P < 0.001) among women with a pre-pregnancy overweight BMI of 24–27.9 kg/m²; and 41.4% (12/29), 45.5% (15/33), 60.0% (12/20) and 78.3% (18/23) (P = 0.035) among women with a pre-pregnancy obesity BMI ≥28 kg/m². The prevalence of MeS was similar among pre-pregnancy normal weight women with a weight gain ≥7 kg, pre-pregnancy overweight women with a stable weight (±3 kg) and pre-pregnancy obese women with a weight loss ≥3 kg from pre-pregnancy to postpartum.

**Conclusions:** Women with GDM who experience a large weight gain from pre-pregnancy to postpartum years 1–5 were more likely to develop cardio-metabolic implications. Women with GDM with a higher pre-pregnancy BMI require additional weight control after delivery.

**O544**

**Association of the MnSOD-rs4880 polymorphism with cognitive performance and the development of type 2 diabetes mellitus**

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**Objective:** To explore the association of the MnSOD-rs4880 polymorphism with type 2 diabetes as well as cognitive function in patients with type 2 diabetes.

**Methods:** Two age-matched and sex-matched groups were compared: 450 patients with type 2 diabetes and 514 healthy controls. All of the subjects were from the Han population of China. Diagnoses of type 2 diabetes were according to WHO 1999 diagnostic criteria for type 2 diabetes. Using RBANS to assess the cognitive function of all of the subjects, the MnSOD-rs4880 polymorphism was detected using PCR-RFLP technology.

**Results:** Hardy–Weinberg equilibrium test: The chi-square (χ²) goodness-of-fit test showed that the genotype distribution of MnSOD-rs4880 did not deviate from Hardy–Weinberg equilibrium both in the patient and in the control group. Single locus analysis: In female samples, the rs4880 polymorphism was associated with type 2 diabetes mellitus (T2DM) (odds ratio = 1.926, 95% confidence interval: 1.310–2.830). Quantitative trait analysis: The quantitative trait analysis was performed using the UNPHASED program (version 3.1.4). The MnSOD-rs4880 polymorphism was associated with a delayed memory score in T2DM patients (χ² = 6.213, P = 0.024), as well as changes in HOMA-IR, low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) (χ² = 5.346, P = 0.012; χ² = 4.354, P = 0.028; χ² = 4.826, P = 0.037).

**Conclusions:** The MnSOD-rs4880 polymorphism may be a susceptibility gene site in female patients with type 2 diabetes. The allele C was a risk factor for the development of type 2 diabetes in Chinese Han women. The MnSOD-rs4880 polymorphism was associated with changes in HOMA-IR, LDL-C and TC and delayed memory performance of T2DM patients.

**O642**

**A follow-up study of glucose, lipid metabolism and β cell function at postpartum years 5–6 in patients with prior gestational abnormal glucose metabolism**

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**Objectives:** To investigate glucose metabolism at postpartum years 5–6 in patients with prior gestational abnormal glucose metabolism and to determine associated factors. To investigate changes in β cell function and insulin resistance in patients with prior gestational abnormal glucose metabolism and their relationships with present glucose metabolism status. To explore lipid metabolism at postpartum years 5–6 in patients with prior gestational abnormal glucose metabolism and how the values changed over time.

**Methods:** In total, 85 women who were diagnosed with hyperglycemia during pregnancy and delivered in our hospital from February 2007 to December 2009 were included in the study. Serum lipids and the oral glucose tolerance test (OGTT) were performed at 6–12 weeks and 5–6 years after delivery, respectively. HOMA-IR, HOMA-β and insulin sensitivity index (ISI) were calculated. The women were divided into two groups based on the OGTT results performed 5–6 years postpartum. Their pregestational, gestational and postpartum data were analysed to evaluate factors affecting glucose metabolism.

**Results:** Among the 85 women, 42 (49.41%) women had abnormal glucose metabolism, including 10 (11.76%) with type 2 diabetes mellitus (T2DM) and 32 (37.65%) with impaired glucose tolerance. A logistic regression model analysis showed that the 3-h plasma glucose level during pregnancy was a risk factor for 5–6-year postpartum abnormal glucose metabolism, while weight loss after delivery was a protective factor (odds ratio = 1.449 [95% confidence interval (CI) 1.067–1.968], 0.876 [95% CI 0.791–0.969]), respectively. Fasting insulin, 2-h postprandial insulin and HOMA-IR were significantly higher, and ISI was significantly lower in the 85 women at postpartum years 5–6 than postpartum weeks 6–12, regardless of whether
they had normal glucose metabolism. Among the 85 women, 44.71% had abnormal lipid metabolism, including 16 (18.82%) with hypertriglyceridemia, 18 (21.18%) with hypercholesterolemia, 8 (9.41%) with low levels of high-density lipoprotein cholesterol and 26 (30.59%) with high levels of low-density lipoprotein cholesterol. Hypertriglyceridemia was the most common form of abnormal lipid metabolism during late pregnancy, while high levels of low-density lipoprotein cholesterol were the most common form 5–6 years after delivery. The triglyceride and total cholesterol levels and the incidence of hypertriglyceridemia and hypercholesterolemia gradually declined from late pregnancy to 5–6 years after delivery.

Conclusions: A high prevalence of abnormal glucose and lipid metabolism was found at postpartum years 5–6 in women with hyperglycemia during pregnancy. The 3-h plasma glucose level during pregnancy was a risk factor for abnormal glucose metabolism at years 5–6 postpartum, while weight loss after delivery was a protective factor. Insulin resistance increased after delivery in women with hyperglycemia during pregnancy regardless of whether they had normal glucose metabolism. Hypertriglyceridemia was the most common form of abnormal lipid metabolism during late pregnancy, while high levels of low-density lipoprotein cholesterol were the most common form at 5–6 years after delivery. The triglyceride and total cholesterol levels and the incidence of hypertriglyceridemia and hypercholesterolemia gradually declined from late pregnancy to years 5–6 after delivery.

O885
Epidemiological investigation of hyperuricemia and analysis of risk factors in the Gansu province of China

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Objective: To examine the epidemiological characteristics and risk factors of adult hyperuricemia in the Gansu province of China.

Methods: Using a stratified and cluster random sampling design, we conducted a population-based, cross-sectional survey in the Gansu province. A representative sample of a resident population (five and more than 5 years) aged 20–74 years from eight regions, including a provincial capital city, participated in the study. A total of 25 807 people (12 041 males, 13 766 females) were invited to participate in and completed the study. The questionnaire survey was conducted for each participant. The overall response rate was 94.5%. Physical examinations (weight, height, body mass index, waist and hip circumference, waist-to-hip ratio, blood pressure and heart rate) were included in the study variables. Cholesterol and uric acid were measured after an overnight fast. Fasting plasma glucose and a 2-h oral glucose tolerance test were conducted in participants without a self-reported history of diagnosed diabetes to identify undiagnosed diabetes and prediabetes (impaired glucose regulation, impaired fasting glucose and/or impaired glucose tolerance). All of the data were entered using Epidata by independent double entry with SPSS19.0 software for statistical analysis.

Results: The prevalence of hyperuricemia was 16.8% (19.1% among men and 10.9% among women). The age-standardized prevalence of hyperuricemia was 16.3%. The prevalence of diagnosed hyperuricemia and new onset hyperuricemia were 5.5% and 11.3%, respectively. The average age of male and female patients was 43.62 ± 9.28 and 46.35 ± 9.66 years old,
respectively. The uric acid level of male and female patients was 463.20 ± 46.95 and 408.79 ± 47.26 mmol/L, respectively. The prevalence of hyperuricemia among urban residents was higher than in rural residents (19.2% vs 13.9%). The prevalence of hyperuricemia in Han, Hui, Tibetan and other minorities was 15.8%, 17.6%, 14.2% and 10.8%, respectively. The prevalence of hyperuricemia in patients with type 2 diabetes was 15.3% (odds ratio (OR) = 2.69, 95% confidence interval (CI): 1.82–5.06); with obesity, the prevalence was 41.9% (OR = 2.35, 95% CI: 1.67–3.12); with hypertension, the prevalence was 24.6% (OR = 3.15, 95% CI: 1.57–4.89); and with hypertriglyceridemia, the prevalence was 29.8% (OR = 2.84, 95% CI: 1.62–4.83). Binary logistic regression analysis showed that male sex, overweight or obesity, hypertension, diabetes, drinking and hypertriglyceridemia were significantly associated with hyperuricemia.

Conclusions: The prevalence of hyperuricemia among a sample of adults in the Gansu province was significantly higher than those reported previously. The prevalence was higher in economically developed regions than in less developed areas. The prevalence was higher in males than in females and in urban compared with rural areas. The prevalence was higher in Hui than Han and higher in Han than among other minorities. Male sex, overweight or obesity, hypertension, diabetes, drinking and hypertriglyceridemia were significantly associated with hyperuricemia.

O895
Prevalence and risk factors for diabetes in the Gansu province of China

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Aims: To examine the prevalence and risk factors for diabetes and prediabetes [impaired glucose regulation (IGR)] among adults in the Gansu province of China.

Methods: Using a stratified and cluster random sampling design, we conducted a population-based, cross-sectional survey in Gansu province. A representative sample of a resident population (five and more than 5 years) aged 20–74 years from 14 cities in the Gansu province participated in the study. Physical examinations (weight, height, body mass index, waist and hip circumference, waist-to-hip ratio, blood pressure and heart rate) were included in the study variables. Plasma lipids and uric acid were measured after an overnight fast among all study participants. Fasting plasma glucose and a 2-h oral glucose tolerance test were conducted in participants without a self-reported history of diagnosed diabetes to identify undiagnosed diabetes and prediabetes (IGR, impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT)). All of the data were entered using Epidata by independent double entry. SPSS19.0 software was used for the statistical analysis. Diabetes was diagnosed and classified in accord with the 1999 WHO criteria. The diagnosis of diabetes was based on blood sugar. Diabetes was confirmed according to a self-reported or those who had a fasting glucose ≥7.0 mmol/L and/or a 2-h post-glucose level ≥11.1 mmol/L; isolated impaired fasting glucose was defined as fasting glucose level ≥6.1 and <7.0 mmol/L, and a 2-h post-glucose value <7.8 mmol/L; IGT was defined as a 2-h post-glucose ≥7.8 but <11.1 mmol/L and a fasting value <7.0 mmol/L; and prediabetes was defined as individuals with IGF or IGT or both.

Results: A total of 34 792 people were invited to participate in the study, and 31 417 people (14 083 males, 17 334 females) completed the study. The questionnaire survey was conducted for each participant, and the overall response rate was 90.3%. The prevalence of diabetes was 10.6% (12.2% among men and 9.1% among women). The prevalence of previously diagnosed diabetes and new onset diabetes was 5.4% and 5.2%, respectively. The prevalence of prediabetes was estimated to be 15.2% (15.7% in males and 14.8% in females). The prevalence of new onset prediabetes was 15.1%. The prevalence rates of isolated IFG and isolated IGT were 3.5% and 11.7%, respectively. The prevalence of diabetes was higher among urban residents than among rural residents (11.4% vs 9.4%). The prevalence of diabetes was 10.5%, 11.3%, 3.3% and 5.2% among Han, Hui, Tibetan and other minorities, respectively. Binary logistic regression analysis showed that risk factors of diabetes and prediabetes were significantly associated with older age, male sex, overweight or obesity, urban residents, smoking, family history of diabetes, hypertension, dyslipidemia and a high level of economic development.

Conclusions: The estimated prevalence of diabetes and prediabetes among a representative sample of adults in Gansu province was significantly higher than reported previously. The prevalence was higher in economically developed regions than in less developed areas and in males compared with females. The prevalence in urban areas was higher than that in rural areas. The prevalence in Hui was higher than that in Han, but the prevalence in Han was higher than that in other minorities. Risk factors of diabetes and pre-diabetes were old age, male gender, overweight or obesity, urban resident, smoking, family history of diabetes, hypertension, dyslipidemia and a high level of economic development.

O956

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DOI: 10.1002/dmrr
Background: The incidence of diabetes and cardiovascular diseases (CVDs) has rapidly increased in China in recent years, and data regarding the gender disparity of these conditions among the middle-aged population in China are lacking.

Methods: Data were obtained from the China National Diabetes and Metabolic Disorders Study in 2007–2008, which included a nationally representative sample of 46 239 men and women (aged ≥20 years). We compared the prevalence rates of diabetes, CVDs, and some CVD risk factors between men and women in the middle-aged population (30–50 years) and in individuals older than 50 years.

Results: The prevalence rates of diabetes and CVDs were significantly greater in middle-aged men than in middle-aged women (8.07% vs 5.06% for diabetes, \(P < 0.001\): 0.64% vs 0.22% for CVDs, \(P < 0.001\)). Specifically, men showed higher prevalence rates of central obesity (29.6% [95% confidence interval (CI) 28.1–31.1%] vs 18.7% [95% CI 17.7–19.7%]); hypertension [24.35% (95% CI 23.0–25.8%) vs 14.77% (95% CI 13.9–15.7%)]; and dyslipidemia [triglycerides, 1.87 mmol/L (95% CI 1.82–1.92 mmol/L) vs 1.31 mmol/L (95% CI 1.28–1.33 mmol/L); low-density lipoprotein cholesterol, 2.66 mmol/L (95% CI 2.63–2.69 mmol/L) vs 2.53 mmol/L (95% CI 2.50–2.55 mmol/L), all \(P < 0.01\)]. Alcoholic beverage intake and smoking were also more common in men than women, and men were less likely to be under diet control than women. The sex-specific differences in impaired glucose tolerance, CVD, and CVD risk factors between men and women were diminished or even reversed in the population aged 50 years and older. In both populations, there were no sex-specific differences in the prevalence of a family history of diabetes, myocardial infarction, coronary heart disease, or hypertension.

Conclusions: The prevalence of diabetes, CVD, and CVD risk factors that could be controlled by lifestyle intervention was higher in men than in women in a middle-aged Chinese population. Specific strategies for diabetes and CVD prevention and control should be designed for this population.

O1086
Diabetic microangiopathy in subjects with diabetes mellitus in Beijing communities

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Objective: The aim of this study was to assess the prevalence and risk factors of diabetic microangiopathy in a diabetic population in Beijing communities.

Methods: A prevalence survey of diabetic microangiopathy in 1179 adults with diabetes from 30 Beijing communities was performed. Questionnaire, demographic information, and personal and family histories of disease were collected. Physical examination and laboratory tests were performed. Digital non-mydriatic fundus photography was performed for each eye in all subjects. Microalbuminuria was determined by measuring the urinary albumin-to-creatinine ratio, and the glomerular filtration rate (GFR) was estimated from fasting serum creatinine. Peripheral neuropathy (PN) was diagnosed based on the Michigan Neuropathy Screening Instrument.

Results: The prevalence of diabetic retinopathy was 20.2% in the diabetic population, and the prevalence of mild, moderate and severe non-proliferative retinopathy, and proliferative...
retinopathy was 11.9%, 5.3%, 2.4% and 0.6%, respectively. Logistic regression analysis revealed that haemoglobin A1c level and diabetes duration were both independently associated with diabetic retinopathy. The prevalence of microalbuminuria was 21.8% in subjects with diabetes. The prevalence of an estimated GFR (eGFR) $\geq 60$ and $<90$, $\geq 30$ and $<60$, and $<30$ mL min$^{-1}$ (1.73 m$^{-2}$) was 42.4%, 38.6% and 1.6%, respectively, in patients with diabetes. The prevalence of impaired renal function was 39.3%. Systolic blood pressure, body mass index, fasting plasma glucose and history of hypertension were all independently associated with hyperglycemic microalbuminuria; serum creatinine, age and systolic blood pressure were independently associated with a diabetic eGFR $<60$ mL$^{-1}$ min$^{-1}$ 1.73 m$^{-2}$. The incidence of PN was 42.3% in diabetes mellitus subjects. Logistic regression revealed that age, duration of diabetes, body mass index and haemoglobin A1c were independent risk factors for PN in subjects with diabetes.

Conclusions: There is a high prevalence of diabetic microangiopathy in Beijing communities. Intensive glycemic control, blood pressure control and body weight reduction may be important for the prevention of diabetic microangiopathy.

O1212

Influence of standard glycated haemoglobin values on glycemic control in type 2 diabetic patients in China

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Methods: A cross-sectional study was conducted in outpatients with T2DM receiving oral anti-diabetic drugs (OADs), insulin or OAD combined with insulin treatment from selected hospitals all over China in 2009 and 2012. A questionnaire interview was used by trained surveyors to collect data on general characteristics, therapy, complications and blood glucose.

Results: In this study, 30 853 and 48 232 patients were enrolled in 2009 and 2012, respectively. The distribution of haemoglobin A1c $\leq 5.5$, 6.5 to $\leq 7$, $>7.0$ to $\leq 8$, $>8.0$ to $\leq 9$, $>9.0$ to $\leq 10$, and $>10$ was, respectively, 20.35%, 12.59%, 35.50%, 18.94%, 6.46% and 6.16% (in 2012), and 14.81%, 27.72%, 14.55%, 6.55% and 8.36% (in 2009). The top three OAD were biguanides, sulfonylureas and thiazolidine. For the combined therapy, the most common treatment options were metformin combined with sulfonylurea in both 2009 and 2012.

Conclusions: There was an increase in the proportion of patients with good and general blood glucose control in 2012; however, there was a decline in the proportion of patients with poor glycemic control. Generalization of the Chinese Diabetes Prevention Guide permitted the presentation of steady blood glucose control.

O1396

Clinical features and gene analysis of 14 suspected maturity-onset diabetes in the young pedigrees

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Background: In China, the population of diabetes has increased gradually, especially in younger onset patients in recent years. Increasing attention is paid to these patients, including maturity-onset diabetes in the young (MODY), which is mainly characterized by an early onset of diabetes and a positive family history of diabetes with an autosomal dominant mode of inheritance. MODY patients are similar to those with type 2 diabetes mellitus in the clinic, with non-essential insulin treatment for at least 2 years. Until now, 13 genotypes of MODY (MODY 1–13) have been discovered worldwide. However, 80%–90% of MODY patients in Asia represent other genotypes, which were called MODY-X.

Methods: One hundred and fifty-eight young diabetic patients (age of onset <35 years) diagnosed from January 2012 to May 2014 in the Shanghai Tenth People's Hospital were analysed. Fourteen probands that matched the diagnosis criteria were identified. The clinical characteristics of the probands and their pedigrees were researched. Additionally, these patients were analysed by direct sequencing for MODY 1–13 and related genes (HNF4A, GCK, HNF1a, PDX1, NEUROD1, KLF11, CEL, PAX4, INS, BLK, ABC8, and KCNJ11). The related literature was searched in the PubMed and Embase Libraries, and the associated mutations were analysed.

Results: No exon mutation was found among the 14 probands that were diagnosed as clinically suspected MODY. However, some polymorphisms and frequencies were found, including I27l (9/14, 64.3%) and S487N (7/14, 50%) in HNF1a (MODY3), which was the most frequently detected proband; T412I (4/14) and D505H (1/14) in CEL (MODY8); H321P (4/14) in PAx4 (MODY9); A1369S (3/14) in ABC8 (MODY12); and V337I (5/14) and K23E (2/14) in KCNJ11 (MODY13).

Conclusions: Non-mutation in clinical MODY patients in the present study may be due to differences in the genes associated with MODY-X in Asian populations. Functional gene polymorphisms may play a role in MODY. Different polymorphisms were associated with different clinical manifestations of MODY, and multiple polymorphisms could be responsible for the cumulative effect of MODY.

O1471

Pregnancy losses in women with type 1 diabetes mellitus in Guangdong: a retrospective study

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Objective: To investigate pregnancy losses in women with type 1 diabetes mellitus in Guangdong Province.

Method: In this retrospective study, pregnancy outcomes between 1993 and 2013 in female participants with type 1 diabetes were identified in the Guangdong Type 1 Diabetes Translational Study. Pregnancy losses were identified from medical records, and telephone surveys were conducted among these patients to analyse the causes of pregnancy loss. Student's t-test and χ² test were employed to compare differences of age, diabetic duration and the awareness rate of planned pregnancy for type 1 diabetes mellitus, respectively.

Results: Among 210 pregnancies identified in women with type 1 diabetes, 76 of them had one or more gestational episode(s) after diabetes diagnosis. Among these, 35.56% (27/76) had a history of pregnancy losses, among which 30.26% (23/76) were spontaneous and 5.26% (4/76) were induced. The proportion of spontaneous losses in women with type 1 diabetes mellitus was higher than in the general population (15%). Maternal age was significantly higher (28.54 ± 4.22 years vs 26.08 ± 3.74 years, P < 0.05), and diabetes duration at the time of pregnancy was longer (5.54 ± 3.77 years vs 5.22 ± 4.04 years, P < 0.05) in patients with spontaneous pregnancy losses than those without. Additionally, a lower proportion of planned pregnancy was observed in patients with spontaneous pregnancy losses compared with those without (12% vs 26.5%, P < 0.05).

Conclusion: The proportions of spontaneous losses in women with type 1 diabetes were higher than in the general population. Higher maternal age, longer diabetes duration at the time of pregnancy and lack of awareness of planned pregnancy may worsen the situation.

P215
Serum gamma-glutamyltransferase and ferritin are associated with risk of chronic kidney disease in a population from a Chinese minority
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Background: The incidence of chronic kidney disease (CKD) is increasing, but the underlying pathogenesis is not yet fully elucidated. Both gamma-glutamyltransferase (GGT) and ferritin have effects on oxidative stress, a common mechanism for many chronic diseases, including CKD. Several but not all studies have shown that GGT is associated with an increased risk of CKD. Few studies have analysed the association between ferritin and CKD, especially the interaction between GGT and ferritin on the risk of CKD. The present study aimed to investigate the interactive effects of GGT and ferritin on the risk of CKD.

Methods: All the participants included in this study were of the Yi nationality, a longtime minority nationality in Southwest China. A total of 1024 participants (436 men and 588 women) were included in the analysis. They were first divided into quartiles by their serum levels of GGT and ferritin and then were reconstituted into three groups: Group 1 (neither GGT nor ferritin in the fourth quartile), Group 2 (only GGT or ferritin in the fourth quartile), and Group 3 (both GGT and ferritin in the fourth quartile). Chronic kidney disease was defined as microalbuminuria or estimated glomerular filtration rate less than 60 mL/min per 1.73 m², as described in previous studies. The risks of CKD in Groups 2 and 3 compared with Group 1 were analysed by multiple logistic regression.

Results: The incidence of CKD, especially microalbuminuria, increased across the three groups. Correspondingly, MDA levels gradually increased from Group 1 to Group 3. The risk of CKD was higher in Groups 2 and 3 than in Group 1. In Group 3, the increased risk was independent of age, body mass index, alcohol drinking, diabetes mellitus, hypertension, hypertriglyceridemia and metabolic syndrome (odds ratios from 1.887 to 2.293, P < 0.05).

Conclusions: GGT and ferritin can interactively influence the risk of CKD. The mechanism might be related to enhanced oxidative stress.

P602
Increased leptin levels were positively associated with homeostasis model assessment of beta-cell functions in Tibetan men
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Background: Adipose tissue is involved in energy and metabolic adaptations during hypoxia exposure through the release of adipokins including adiponectin and leptin. This study was designed to compare the levels of leptin, adiponectin and C-reactive protein (CRP) and their correlations with some metabolic parameters between highlanders (Tibetan men) and lowlanders (Han males).

Methods: Three hundred and fourteen subjects were involved in this study, including 150 Han males and 164 Tibetan men. Homeostasis model assessment parameters (HOMA) including HOMA-IR and HOMA-B% were used to estimate insulin resistance and beta-cell functions, respectively. Adiponectin, leptin and CRP were measured using enzyme-linked immunosorbent assay kits.

Results: Our data showed that Tibetan men had a higher body mass index, total cholesterol, low-density lipoprotein cholesterol levels and lower high-density lipoprotein cholesterol levels when compared with Han males. Tibetan men with diabetes appeared to have higher fasting insulin levels and were more insulin resistant as determined by HOMA-IR compared with Han males with diabetes. No differences in adiponectin and CRP levels were found between highlanders and lowlanders. Leptin levels were significantly increased in Tibetan men, and the increase was blunted to some degree in Tibetan men with diabetes. Stepwise linear regression analysis revealed that leptin was independently and positively associated with HOMA-B% in Tibetan men.

Conclusions: This study revealed different characteristics of glucose and lipid metabolism between Tibetan and Han males. Among Tibetan men, the blunted elevation in leptin levels in diabetic subjects may in part explain their worsened beta-cell function.
P1151
Complete mitochondrial DNA genomes reveal similar penetrance of maternally inherited type 2 diabetes in two Chinese families
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Background: Previous work has revealed that mitochondrial DNA (mtDNA) derived from the maternal genome has a close affinity with type 2 diabetes mellitus. This finding would suggest a familial pattern for type 2 diabetes. However, little is known about the penetrance and familial patterning in Eastern populations.

Methods: We analysed the complete mtDNA genomes of two families (A and B) from Southwest China that demonstrated maternally inherited type 2 diabetes.

Results: Our data showed that the mtDNA lineages in families A and B belonged to haplogroups A4 and D4h1, respectively. The findings suggested that maternally inherited T2DM with similar penetrance may arise in Chinese individuals with strikingly different maternal genetic backgrounds. One mutation, G13759A, in the MT-ND5 gene was detected in family B; this mutation occurs at the first base pair of the codon and causes an amino acid change from alanine to threonine.

Conclusions: Further evolutionary and phylogenetic analyses demonstrate that G13759A has multiple origins and is unlikely to be a disease-causing mutation.

P1400
Comparison of clinical characteristics and medical costs of patients with diabetic foot ulcer between 2004 and 2012 in China
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Objective: To compare and analyse the clinical characteristics, prognosis and medical cost in patients with diabetic foot ulcer in 2004 and 2012 in China.

Methods: Diabetic foot disease data from 14 teaching hospitals in 2004 and from 15 teaching hospitals in 2012 in China were collected and analysed. The data included demographic characteristics, medical history, physical and biochemical examinations, prognosis and medical cost.

Results: A total of 386 cases in 2004 and 682 cases in 2012 were studied. No significant differences in age, educational level, duration of diabetes, haemoglobin A1c, triglyceride, high-density lipoprotein cholesterol, uric acid, prevalence of dyslipidemia, cerebrovascular and peripheral artery disease, peripheral neuropathy, the percentage of neuropathic or ischemic foot ulcer, or medical cost were found between the two groups. In 2012, compared with 2004, duration of diabetic foot was shorter, there were more men, there were more patients with smoking and/or drinking, and there was lower fasting and post-meal glucose, total cholesterol and low-density lipoprotein cholesterol. In 2012, there was a higher prevalence of hypertension, coronary heart disease, diabetic kidney disease and diabetic retinopathy, and there were more patients with infectious foot ulcer and more patients with severe foot disease whose foot ulcer classified as Wagner 3 and above or Texas D (76.6% vs 68.7%, 52.4% vs 29.5%, 46.7% vs 34.3%, respectively; all P < 0.05). There were significantly lower major amputation rates, higher ulcer healing rate and shorter hospital stays in 2012 (2.3% vs 5.9%, 52.3% vs 18.2%, 18 (12–32) vs 21 (15–32) days, respectively, all P < 0.05), but there was a higher total amputation rate (17.2% vs 10.2%, P < 0.05). The medical cost for these patients in 2012 and 2004 was 17 183 (9535–3599) versus 12 364 (7985–18 725) Yuan, while the average day cost was 955 versus 589 Yuan, an insignificant difference after correction for the consumer price index.

Conclusions: Compared with patients in 2004, patients with diabetic foot disease in 2012 had more concomitant diseases and complications, with more severe foot ulcers and infections and a higher total amputation rate, but with a lower major amputation rate, a higher ulcer healing rate and shorter hospital stays.

P1442
The effect of single nucleotide polymorphisms in miR-27a, miR-124a and miR-146a on susceptibility to type 2 diabetes mellitus in Chinese Han people
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Background: Polymorphisms in miRNAs represent a recently discovered disease mechanism that is closely associated with disease; they operate by interfering with miRNA function. A previous study demonstrated a direct connection between miRNA single nucleotide polymorphisms (SNPs) and type 2 diabetes mellitus (T2DM), but the effect of SNPs in microRNAs on T2DM in the Chinese population has not been clarified. The aim of this study was to analyse the association of common SNPs in miR-27a, miR-146a and miR-124a with T2DM in China and to analyse the diabetic pathological mechanism and environmental factors.

Methods: An effective technology known as SNP scan was used to genotype 995 newly diagnosed T2DM patients and 967 healthy controls. A logistic regression analysis was conducted to compare the mutation frequencies in the two groups.

Results: We found that there was no significant connection between any genotype of the studied miRNAs and T2DM in our research; however, in stratification analysis, we observed a lower risk of T2DM in younger (age < 45 years) persons who carried the rs531564GC genotype [adjusted odds ratio (OR) = 0.73; 95% confidence interval (CI) = 0.54–0.99]. Moreover, the rs895819CC genotype in overweight persons (24 ≤ BMI < 28) was significantly associated with T2DM risk (adjusted
OR = 1.73; 95% CI = 1.02–2.94). Meanwhile, rs2910164 in miR-146a was not significantly correlated with T2DM. The GRS was calculated according to the number of risk alleles of the three SNPs and was necessarily related to total cholesterol (adjusted \( P = 0.021 \)).

Conclusions: The rs531564GC genotype acts as a protective factor that decreases the risk of T2DM in younger persons (age < 45 years). However, the presence of the rs895819CC genotype was a risk factor for the development of illness among overweight individuals (24 ≤ body mass index < 28). Thus, gene mutations in miRNAs most likely play a dominant role in Chinese individuals with T2DM. The presence of SNPs in miRNAs might lead to disease by affecting miRNA expression level and gene function. Thus, miRNA mimics or inhibitors are novel and promising therapeutic compounds to directly regulate miRNA expression.

**P1469**

**A practical way to estimate insulin sensitivity in adult patients with type 1 diabetes**

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**Background:** Increasing evidence has shown the important role of insulin resistance in type 1 diabetes (T1D). However, it is a challenge to assess insulin sensitivity in large-scale T1D studies. In adult patients with T1D, although an insulin sensitivity prediction model has been developed in the Pittsburgh Epidemiology of Diabetes Complications (EDC) study, this model includes a variable of haemoglobin A1c (HbA1c), which is not currently recommended in clinical use. Thus, the aim of this study was to develop an appropriate estimated formula for insulin sensitivity with routine clinical factors in adult patients with T1D.

**Methods:** Adult patients with T1D (n = 36) were recruited from the Guangdong Type 1 Diabetes Translational Study. A euglycemic–hyperinsulinemic clamp was performed to measure glucose disposal rate (GDR; mg·kg\(^{-1}\)·min\(^{-1}\)). Demographic factors (age, sex and diabetes duration), anthropometric measurements (body mass index and waist to hip ratio) and metabolic characteristics (blood pressure, HbA1c and lipids) were collected to estimate GDR by stepwise linear regression.

**Results:** The best formula for estimating insulin sensitivity in adult patients with T1D was as follows: Loge estimated GDR (eGDR) = 4.964 – 0.121 × HbA1c (%) − 0.012 × diastolic blood pressure (mm Hg) − 1.409 × waist–hip ratio (adjusted \( R^2 = 0.616 \)), among which HbA1c was the strongest predictor (partial \( R^2 = 0.412 \)). In these patients, when compared with GDR from the clamp, the performance of our formula was superior to the modified EDC formula.

**Conclusion:** Insulin sensitivity can be estimated with routine clinical factors in adult patients with T1D. This formula may be a potential tool for estimating insulin sensitivity in large-scale epidemiological and clinical studies in the future.

**P1511**

**A new investigation of three single nucleotide polymorphisms associated with type 2 diabetes in a Chinese population**

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**Background:** Recently, Richa Saxena et al. found a new locus in SGCG (rs9552991) that was associated with type 2 diabetes in Punjabi Sikhs from India and two suggestive loci at 7q32 near PLXNA4 (rs1593304) and at 3p21 in SCAP (rs4858889) in a combined South Asian population. The aim of this study was to validate this finding in a Chinese population.

**Methods:** We genotyped three single nucleotide polymorphisms (SNPs), rs9552991, rs1593304 and rs4858889, in a group of 1972 Chinese individuals comprising 966 type 2 diabetic patients and 976 controls. Anthropometric variables and biochemical traits were measured in both the cases and the controls.

**Results:** The SNP rs1593304 was associated with beta cell function as estimated by HOMA-B (\( P = 0.041 \)). In addition, the SNP rs9552991 was associated with weight (\( P = 0.033 \)), total cholesterol (\( P = 0.006 \)) and low-density lipoprotein cholesterol (\( P = 0.007 \)). The genotype frequency differed statistically between the cases and controls as shown by \( \chi^2 \) tests (\( P = 0.017 \)). A difference could also be observed between the cases and controls with \( \chi^2 \) tests in male participants (\( P = 0.023 \)) and non-obese participants (\( P = 0.023 \)).

**Conclusions:** In this study, we found that the SNP rs9552991 variant was associated with body mass index and lipid metabolism. The SNP rs1593304 was associated with beta cell function.

**P1513**

**Association of common genetic variants in MAP4K4 with type 2 diabetes mellitus in the North Chinese population**

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**Background:** A study has identified several novel type 2 diabetes susceptibility variants of the MAP4K4 gene within the German population. We aimed to evaluate whether common single nucleotide polymorphisms (SNPs) in the MAP4K4 gene were associated with type 2 diabetes and metabolic traits in the population of China.

**Methods:** According to the previous study, five candidate single-nucleotide polymorphisms were genotyped in 1000 individuals with type 2 diabetes and 1000 normal controls using the SNP scan method.

**Results:** Of the five variants, the SNP rs2236935 was significantly associated with type 2 diabetes in our study population (odds ratio = 0.773, \( P = 0.025 \)). In addition, we found that genotype CT of SNP rs2236935 was associated with type 2 diabetes mellitus in all female subjects (\( P = 0.023 \)), and two SNPs, namely rs1003376 and rs2236935, were associated with type 2 diabetes in non-obese subjects in our study. Furthermore, among the controls, rs1003376 was significantly associated with an increased
body mass index (BMI) \( (P = 0.045) \) and HOMA-IR \( (P = 0.037) \). In contrast, the SNPs rs1003376 and rs11674694 were significantly associated with an increased BMI \( (P = 0.01 \) and 0.047, respectively), and rs2236935 was associated with high-density lipoprotein cholesterol \( (P = 0.005) \) in the case subjects.

Conclusions: The MAP4K4 gene variants may contribute to protection against the development of type 2 diabetes in the North Chinese population.

### Acute and Chronic Complications

**O113**

**SUDOSCAN technique to screen cardiac autonomic neuropathy in patients with type 2 diabetes mellitus**

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**Objective:** To attempt a non-invasive and easy way to screen diabetic cardiovascular autonomic neuropathy (CAN) and observe related factors in the occurrence of disease, which will provide new insight regarding the early screening and prevention of diabetic autonomic neuropathy complications.

**Methods:** A cohort of 75 patients with type 2 diabetes at Qilu hospital and 45 cases without diabetes, coronary heart disease or other related diseases were randomly enrolled in this study. All of the patients provided signed informed consent and completed both the traditional evaluation criteria cardiovascular reflex tests (CART) and SUDOSCAN screening. According to the CART diagnostic criteria, the type 2 diabetes group was divided into the normal group, early group and diagnosis group. Among the multiple groups, we used one-way analysis of variance. Binary logistic regression analysis was used to predict relative factors, and the receiver operating characteristic (ROC) curve was used to evaluate the sensitivity and specificity of the diagnostic evaluation methods. Significance was defined as a two-tailed \( P < 0.05 \).

**Results:** Compared with the normal control group using the CART test, the prevalence of autonomic neuropathy in type 2 diabetes was significantly higher (40% vs 13.3%). SUDOSCAN screening revealed differences \( (P = 0.046 \) and \( P = 0.025 \) in hand and foot sweat conductivity (ESC) between the two groups. Comparison of the normal CAN group, the early group and the diagnosis group showed that SUDOSCAN screening can measure ESC differences in the hands and feet among the three groups \( (P = 0.010 \) and \(< 0.001 \). Among all of the factors, the average hand sweat conductivity [odds ratio (OR) = 0.943 (0.766–1.147), \( P = 0.033 \)] and foot sweat conductivity [OR = 0.930 (0.884–0.979), \( P = 0.006 \)] measured by binary logistic regression analysis were relatively good factors in CAN. The respective areas under the curve of the ROC curve were 0.750 and 0.747. The corresponding cutoff points were 75.76% and 66.67% \( \mu \text{Si} \). To screen CAN, the average hand sweat conductivity was much better than the other methods, with a sensitivity of 76.7%, specificity of 75.6% and Youden index of 0.522.

The operation and analysis process of the traditional CART test to diagnose CAN are complicated, semi-quantitative and time-consuming (nearly 40 min), which greatly impacts the results based on the cooperation of patients. However, the SUDOSCAN technique is a simple, quantitative and fast (only 2 min) method with good compliance.

**Conclusions:** Compared with the normal control population, the prevalence of cardiovascular autonomic neuropathy (DCAN) was significantly elevated, hand and foot sweat conductivity was significantly decreased, autonomic symptoms were elevated and the severity of symptoms was relatively increased. The SUDOSCAN technique is a feasible, noninvasive and quantitative method used to screen for diabetic cardiac autonomic neuropathy; it is faster and easier to use than other diagnostic methods and is suitable for large-scale epidemiological or clinical screening surveys. The average sweat conductivity of the hands detected by the SUDOSCAN technique was the best and most relatively independent index of DCAN. The cut-off point to diagnose DCAN was 75.76 \( \mu \text{Si} \).

**O221**

**The treatment of patients with diabetic peripheral neuropathic pain in China: a double-blind randomized trial of duloxetine versus placebo**

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**Objective:** Duloxetine has been approved for the treatment of diabetic peripheral neuropathic pain (DPNP) in more than 100 countries or regions. This study aimed to assess the efficacy and safety of duloxetine (60 mg once daily) by comparing it with placebo in patients with DPNP in China.

**Methods:** This was a phase 3, multicentre, randomized, double-blind, parallel, placebo-controlled, 12-week trial of the treatment of DPNP with duloxetine. Patients who met entry criteria were randomized 1:1 to treatment with duloxetine 60 mg once daily or placebo. The primary efficacy measure was the reduction in pain severity from baseline to 12 weeks, as measured by the weekly mean of the daily pain ratings recorded in the patient’s diary using repeated measures analysis. The secondary efficacy measures included the weekly means of night pain and worst daily pain from the patient’s diary, Brief Pain Inventory—Modified Short Form Severity (BPI-S) average pain, and Patient Global Impression of Improvement (PGI-I). Response rates were defined as 30% and 50% pain reduction of the 24-h average pain recorded in the patient’s diary and of the BPI-S average pain. Mean changes from baseline in efficacy measures were
analysed using a restricted maximum likelihood-based, mixed-effects model with repeated measures approach and by analysis of covariance. The Cochrane–Mantel–Haenszel and Fisher’s exact tests were applied to compare the treatment groups in the response rate analysis and incidences of AEs, respectively.

Results: A total of 405 patients were randomized to duloxetine 60 mg QD (n = 203) or placebo (n = 202). One hundred seventy-three (85.2%) patients in the duloxetine group and 176 (87.1%) patients in the placebo group completed the 12-week treatment phase. Duloxetine-treated patients experienced significantly reduced pain as the primary efficacy measure (24-h average pain rating) beginning at the first week after randomization, and this effect was sustained through 12 weeks of the treatment phase (LS mean changes at the endpoint, duloxetine vs placebo: −2.40 vs −1.97, P = 0.030). A significant treatment difference between the duloxetine and placebo groups was observed for almost all of the secondary efficacy measures, including 24-h night pain rating (−2.65 vs −2.11, P = 0.008) and worst pain rating (−2.80 vs −2.25, P = 0.017), as determined by the patients’ diary recordings: response rate of reduction on 24-h average pain by patient diary [≥30%: (61.5% vs 49.0%, P = 0.014) and ≥50%: (42.0% vs 28.8%, P = 0.006)] and BPI-S [≥30%: (63.0% vs 46.7%, P = 0.003) and ≥50%: (46.0% vs 29.4%, P = 0.001)], BPI-S [items of average pain (−2.50 vs −2.00, P = 0.016), worst pain (−2.86 vs −2.36, P = 0.032), and least pain (−1.97 vs −1.41, P = 0.004)], PGI-I (2.44 vs 2.65, P = 0.034), and SF-MPQ total score (−6.45 vs −5.33, P = 0.022). The overall incidence of AEs in duloxetine-treated and placebo-treated patients was 50.0% and 38.6%, respectively (P = 0.027). Duloxetine-treated patients experienced higher rates of nausea (10.4% vs 3.5%, P = 0.010), somnolence (8.4% vs 0.5%, P < 0.001), and asthenia (5.0 vs 0.0, P = 0.002) compared with placebo.

Conclusions: Duloxetine at 60 mg QD demonstrated evidence of efficacy across multiple measures in the treatment of DPNP compared with placebo in Chinese patients. This study also confirmed that duloxetine was well tolerated in patients with DPNP.

O354
Mannose-binding-associated serine protease 2 may participate in diabetic nephropathy in type 2 diabetes patients
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Background: To date, the pathogenesis and prediction of the progress of diabetic nephropathy (DN) remain unclear. Here, clinical proteomics was applied to explore molecules involved in the pathogenesis of DN and biomarkers to predict the progress of DN.

Methods: Four groups of age-matched and gender-matched subjects were enrolled in the study. Group A was the normal control. Group B, C, and D were type 2 diabetes patients whose urine albumin excretion rate was less than 30 mg/g creatinine, 30 mg/g creatinine to 300 mg/g creatinine, and more than 300 mg/g creatinine, respectively. The fasting plasma was collected, and a plasma pool was formed in each group. After removal of albumin, four groups of plasma with equivalent protein were separated by two-dimensional electrophoresis (2DE) and silver-stained. The experiment was repeated three times, and the data were analysed with specified software in ImageMaster 2D Platinum (GE company). Proteins with significantly different expression among the four groups were determined and then detected by mass spectrometry (MS).

Results: A total of 2119 protein spots were detected and matched after separation by 2DE among the four groups. Fourteen spots were successfully assessed by MS. The expression of mannann-binding lectin serine protease 2 was significantly elevated in group B. Prothrombin had a much higher expression level in group C. Meanwhile, the expression of haemoglobin subunit alpha, Ig alpha-1 chain C region, and Ig lambda chain C region was significantly increased in group D.

Conclusions: Proteomics is a valuable and efficient tool in clinical practice and research. Mannann-binding lectin serine protease 2 may participate into the pathogenesis or progress of diabetic nephropathy in type 2 diabetic patients.

O361
Effectiveness and safety of a multi-parameter bolus advisor in insulin-pump therapy among hospitalized patients with diabetes
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Objectives: To assess the clinical safety and efficacy of a multi-parameter bolus advisor in insulin pump therapy and to explore the clinical application value of the multi-parameter bolus advisor.

Methods: A total of 158 diabetic patients from the endocrine departments of 10 tertiary comprehensive hospitals were randomly (2:1:1) assigned to three groups from May 2012 to July 2013, including therapy with insulin in the form of continuous subcutaneous insulin infusion (CSII) with a multi-parameter bolus advisor (BA-CSII group), common CSII system (CO-CSII group), and multiple daily injection (MDI group). The insulin dosage, blood glucose, and incidence of hyperglycemia or hypoglycemia episodes during the hospitalization were compared and analysed among the three groups using the chi-square test, Mann–Whitney U-test and Kruskal–Wallis H test.

Results: After treatment, the ratio of postprandial blood glucose reaching the individualized target in the BA-CSII group was significantly higher than in the CO-CSII group (78.1% vs 52.3%,
$\chi^2 = 7.955$, $P = 0.007$) and MDI group (78.1% vs 50.0%, $\chi^2 = 8.375$, $P = 0.007$), and the postprandial blood glucose level in the BA-CSII group was remarkably lower than that in the CO-CSI group (7.8 ± 2.0 vs 9.1 ± 2.8 mmol/L, $Z = -2.301$, $P = 0.021$) and MDI group (7.8 ± 2.0 vs 9.1 ± 2.2 mmol/L, $Z = -2.920$, $P = 0.004$). The incidence of hypoglycaemia (blood glucose ≥ 3.9 mmol/L) and the per capita frequency of hyperglycaemia (blood glucose ≥ 10 mmol/L) in the BA-CSII group were lower than those in the CO-CSI group and MDI group, but no significant differences were observed. Moreover, during hospitalization, the average daily insulin dosage in the BA-CSII group was lower than that in the MDI group (44.4 ± 17.0 vs 55.7 ± 27.3 U, $P > 0.05$) and that in the CO-CSI group (39.3 ± 11.6 vs 55.7 ± 27.3 U, $Z = -2.690$, $P = 0.007$) was significantly lower than that in the MDI group, as well. Compared with the day of hospital admission, the insulin dosage in the BA-CSII group (44.0 ± 22.4 vs 43.0 ± 18.4 U) decreased more than that in the CO-CSI group (37.3 ± 10.1 vs 37.2 ± 12.2 U) on the day of discharge, whereas the insulin dosage in the MDI group (33.8 ± 10.2 vs 37.3 ± 13.3 U) increased. There were no obvious differences among the three groups.

Conclusions: The application of a multi-parameter bolus advisor can improve postprandial glucose control in intensively treated diabetic patients, with a reduced risk of hypoglycaemia and a lower insulin dosage. Accordingly, an automated bolus advisor was proven to be a safe and effective feature of the insulin-pump system.

O363 Poly (ADP-ribose) polymerase and diabetic peripheral neuropathy

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Objectives: To detect the levels of poly (ADP-ribose) polymerase (PARP) in diabetic patients with or without peripheral neuropathy and in healthy subjects. The aim of this study was to investigate changes in serum levels of PARP in all of the groups, the factors influencing these changes and their relationships with diabetic peripheral neuropathy (DPN). To analyse risk factors for diabetic peripheral neuropathy.

Methods: We screened 101 in-ward patients with type 2 diabetes mellitus (T2DM) who were hospitalized in the department of endocrinology of the second artillery force general hospital from June 2013 to August 2013. All of the patients were diagnosed according to the Word Health Organization 1999 criteria. The patients with type 2 diabetes were divided into two groups: the DM group (T2DM, n = 45) and diabetic peripheral neuropathy group (DPN, n = 51). Healthy subjects were assessed as the control group (NC, n = 48), with gender-matching and age-matching. Conventional measurement indexes of body parameters, biochemical analyses and glycosylated haemoglobin, among others, and the age of patients and duration of diabetes were recorded. Enzyme-linked immunosorbent assay was used to determine the degree of PARP in all subjects. We determined serum PARP levels and analysed changes associated with diabetic peripheral neuropathy.

Results: Compared with the NC group, higher serum levels of PARP were detected in the T2DM and DPN groups. The patients in the DPN group had higher serum levels of PARP than those in the T2DM group ($P < 0.01$). Compared with the NC group, the levels of triglyceride, haemoglobin A1c (HbA1c) and fasting plasma glucose (FPG) were higher in the T2DM and DPN groups. Compared with the T2DM group, the duration of diabetes was higher in the DPN group, and the difference was statistically significant. Multiple regression analysis showed that age, disease course, and levels of HbA1c and FPG were positively correlated with serum levels of PARP. Logistic regression analysis showed that duration of disease and serum levels of PARP were two independent risk factors for DPN.

Conclusions: Subjects in the DPN group had a high concentration of PARP. The concentration of PARP was positively associated with age, the duration of diabetes mellitus, and the levels of HbA1c, and FPG. A longer duration of disease and toxicity related to high blood glucose eventually resulted in increased serum PARP levels. The duration of disease and the serum levels of PARP were two independent risk factors for DPN.

O373 Relationship between blood glucose excursions and vascular endothelial cell function and carotid intima-media thickness

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Objective: The aim of the present study was to assess the relationships of blood glucose excursions, vascular endothelial cell function and carotid intima-media thickness (CIMT) by a continuous glucose monitor system to monitor 72 h blood glucose. A high-resolution Doppler ultrasound diagnostic instrument was used to measure vascular endothelial cell function and CIMT.

Methods: One hundred seventeen subjects were recruited in this study, including 69 normal glucose tolerance (NGT) and 48 impaired glucose regulation (IGR) subjects. Basic information, oral glucose tolerance test (OGTT), haemoglobin A1c (HbA1c), INS, routine blood lipid, urine, liver and kidney function tests and UA were assessed after 10 h of fasting. Seventy-two-hour continuous glucose monitoring was conducted using the Medtronic MiniMed dynamic glucose monitoring system. CIMT and endothelial function were tested using the PHILIPS IU22 high-resolution colour Doppler ultrasound diagnostic instrument.

Results: Compared with the NGT group, the rate of family history of diabetes, hypertension, lipid disorders, smoking and alcohol consumption were higher in the IGR group. In addition, patients in the IGR group were older (NGT: 40.17 ± 15.021, IGR: 55.50 ± 12.65) and had a greater body mass index (BMI) (NGT: 21.64 ± 1.82, IGR: 23.45 ± 2.48), waist circumference (WC) (NGT: 76.59 ± 7.60, IGR: 83.49 ± 7.89), waist-to-hip circumference ratio (NGT: 0.81 ± 0.06, IGR: 0.86 ± 0.06), systolic blood
pressure (SBP) (NGT: 111.00 ± 12.60, IGR: 122.25 ± 16.78), diastolic blood pressure (NGT: 74.55 ± 7.99, IGR: 78.06 ± 9.51), OGTT0 (NGT: 5.09 ± 0.48, IGR: 5.87 ± 0.65), OGTT2 (NGT: 5.84 ± 0.98, IGR: 8.46 ± 1.01), Hba1c (NGT: 5.66 ± 0.39, IGR: 6.18 ± 0.55), INS2 (NGT: 44.37 ± 28.03, IGR: 65.96 ± 39.45), triglyceride (TG) (NGT: 0.99 ± 0.38, IGR: 1.23 ± 0.53) and total cholesterol (TC) (NGT: 4.48 ± 0.75, IGR: 4.80 ± 0.74) (P < 0.05 or 0.01). Compared with the NGT group, the IGR group was associated with significantly higher mean blood glucose (MBG) (NGT: 5.56 ± 0.48, IGR: 6.18 ± 0.78), mean of daily differences (MODD) (NGT: 0.71 ± 0.25, IGR: 1.90 ± 0.71) and CIMT (NGT: 0.48 ± 0.09, IGR: 0.55 ± 0.09) (P < 0.01) but had a lower EDD (NGT: 10.22 ± 4.65, IGR: 7.95 ± 4.92, P < 0.01). Pearson and Spearman’s correlation analysis showed that CIMT was positively correlated with age, BMI, WC, SBP, MBG and MAGE (P < 0.05 or 0.01); EDD was positively correlated with high-density lipoprotein cholesterol (HDL-c) and EID and was negatively correlated with age, BMI, WC, SBP, MBG and MAGE (P < 0.05 or 0.01); EID was positively correlated with HDL-c and EDD and was negatively correlated with age, BMI, WC, SBP, Hba1c, UA and mean amplitude of glycemic excursions (MAGE) (P < 0.05 or 0.01). Multiple linear stepwise regression analysis showed that age and WC were important risk factors for CIMT thickening (P < 0.01). BMI and MAGE were important predictive factors of EDD reduction (P < 0.05 or 0.01), and EID was an important risk factor for EID reduction (P < 0.01).

Conclusions: Intra-day glucose variability was an independent risk factor for decreased endothelial function. Daily differences in glucose excursions were associated with thickening of CIMT. Age and WC were independent predictors of CIMT thickening.

O425  
Pancreatic kallikrein protects renal function in db/db diabetic mice  
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Background: Diabetic nephopathy is one major complication of diabetes and the leading cause of end-stage renal disease worldwide. Clinically, pancreatic kallikrein (PKK) has been shown to decrease albuminuria in diabetic patients with diabetic nephropathy. However, the specific mechanism by which PKK reduces proteinuria and protects against renal disease remains unknown.

Methods: Four-week-old db/db mice were randomly divided into two groups. One group was treated with PKK (DM-PKK group, n = 10), and the other was given saline (DM-NS group, n = 10). In addition, littermate db/mice (NC group, n = 8) were given saline as the non-diabetic control group. Blood glucose and body weight were measured weekly, 24-h urine was collected monthly, and both systolic and diastolic blood pressure were monitored monthly. After treatment for 16 weeks, the mice were sacrificed, and kidney tissues and blood plasma were collected. Enzyme-linked immunosorbent assay was performed to determine urinary protein levels. Light microscopy and transmission electron microscopy were used to examine renal pathological changes and ultrastructure, respectively. Immunohistochemistry was performed to detect the expression of transforming growth factor-beta 1 (TGF-β1), CD68 and collagen I. Real-time polymerase chain reaction (PCR) was applied to determine the gene expression levels of interleukin-1β (IL-1β), TGF-β1 and fibronectin in the whole kidney.

Results: Both blood glucose and body weight were significantly higher, and the kidney weight/body mass index was lower in the DM-NS and DM-PKK groups compared with the NC group (P < 0.05). However, there were no differences between the DM-NS and DM-PKK groups. Neither systolic nor diastolic blood pressures differed between any two groups. The results of HE, PAS and Masson staining implied that PKK could reduce the clear mesangial matrix deposition in the DM-NS group. The transmission electron microscopy results showed that PKK could decrease the thickness of the glomerular basement membrane and protect the foot process and fenestrae of endothelial cells in db/db mice. Meanwhile, PKK could significantly improve cellular mitochondrial swelling, which occurred in both proximal and distal tubular epithelial cells in the DM-NS group. In addition, PKK also increased the number of mitochondria and ameliorated lipid metabolism disorders in proximal tubular cells compared with the DM-NS group. Compared with the NC group, CD68 and TGF-β1 protein expression was significantly increased in the DM-NS group, while PKK decreased their expression. Quantitative PCR showed that PKK could dramatically decrease the gene expression of IL-1β, TGF-β1 and fibronectin compared with the DM-NS group. Finally, our results showed that PKK could significantly reduce urinary albumin excretion.

Conclusions: PKK had renal protective effects in db/db mice that were independent of improvements in blood pressure and blood glucose. This effect of PKK might be mediated by a reduction of inflammation and fibrosis.

O529  
Expression of MMP-1, TIMP-1 and collagen in the myocardium of type 2 diabetes mellitus rats  
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Objectives: To establish a type 2 diabetic rat model and investigate the change in the expression of MMP-1 and TIMP-1 and the content of collagen (CL) in the myocardial tissue of experimental animals and the significance of these changes.

Methods: In this study, a type 2 diabetes mellitus (T2DM) rat model was established by giving Sprague–Dawley rats a long-term high-fat diet and a low dose of streptozotocin (STZ). We detected the protein expression and activity of MMP-1 and TIMP-1 and CL in the myocardial tissue of experimental animals to evaluate the association with changes in blood glucose and blood
lipids with the goal of determining the mechanism underlying diabetic cardiomyopathy (DC).

Results: The blood glucose and insulin levels in the model rats were significantly higher than that in the normal group (P < 0.01). The expression of MMP-1 in the rat myocardium in group B was significantly higher than that in group A (P < 0.01). TIMP-1 expression in the rat myocardium in group B was significantly higher than that in group A (P < 0.01). The ratio of TIMP-1/MMP-1 in group B was significantly higher than that in group A (P < 0.01).

Conclusions: The type 2 diabetic rat model that was induced by a high-sugar, high-fat diet, and a low dose of STZ displayed some characteristics similar to human T2DM. The protein expression of the high-sugar, high-fat diet, and a low dose of STZ displayed some characteristics similar to human T2DM. The expression of MMP-1 and TIMP-1 were increased in T2DM rat myocardium. These changes resulted in collagen hyperplasia and ECM remodelling and indicated that the TIMP-1/MMP-1 balance is an important factor in the occurrence and development of DC.

**O775**

Analysis of an optimal protocol for lower extremity MR angiography using single-dose contrast agent in patients with diabetic foot

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Objective: To explore the optimal protocol for lower-extremity MR angiography using single-dose contrast agent.

Methods: Twenty-eight healthy volunteers were scanned using CE-MRA in crus twice with parallel imaging factor (PIF) 3 or 4. The signal intensity, signal-to-noise ratio (SNR), contrast-to-noise ratio (CNR) and image quality of the popliteal artery, posterior tibial artery, anterior tibial artery and peroneal artery were compared. Twenty patients with diabetic foot underwent CE-MRA by both Protocols I and II of the leg, crus and foot. The difference between the two protocols in venous aliasing and in the display of the femoral artery, popliteal artery, posterior tibial artery, anterior tibial artery, peroneal artery, dorsalis pedis artery, medial planter artery and lateral planter artery were compared. The subtraction effects of two substructural methods were compared in the leg, crus and foot.

Results: Compared with images obtained using PIF = 4, SNR and CNR of the popliteal artery, posterior tibial artery and peroneal artery, and the SNR of the anterior tibial artery were higher using PIF = 3 (P < 0.05). The venous aliasing and display of the popliteal artery, posterior tibial artery, anterior tibial artery, peroneal artery, dorsalis pedis artery and medial planter artery in Protocol I were superior to those in Protocol II (P < 0.05). Compared with vessel mask acquisition before the test bolus, the background suppression of subtraction CE-MRA in the leg, crus and foot were better than those obtained using the mask after acquisition (P < 0.05).

Conclusions: Proper use of a higher PIF, setting the personalized k-space centre filling time and acquisition of the vessel mask after the test bolus can improve the image quality of whole lower-extremity MRA in patients with diabetic foot.

**O820**

Acarbose treatment attenuated postprandial hypotension in elderly patients with diabetes mellitus

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Background: Postprandial hypotension is a common disease among elderly adults, especially elderly patients with diabetes mellitus. This study aimed to assess the effectiveness of acarbose treatment in postprandial hypotension in elderly diabetic patients.

Methods: In this randomized, placebo-controlled trial, 40 elderly diabetic patients with PPH between 60 and 80 years of age were enrolled from Beijing Hospital in China. Diabetic patients with postprandial hypotension were randomly divided into placebo or acarbose 100 mg treatment before standard meals. Primary endpoints were to establish whether acarbose was effective in attenuating postprandial hypotension in patients with diabetes mellitus. Secondary endpoints were to observe the change in blood glucose, insulin, c-peptide, glucagon-like peptide-1 (GLP-1), catecholamine, and heart rate variability (HRV), and to discuss the mechanism underlying postprandial hypotension in patients with diabetes mellitus.

Results: Forty PPH patients were found among 53 elderly diabetic patients. The morbidity was 76.9%. In the placebo group, there was a negative correlation between the maximum falling value of MAP and postprandial E (P < 0.05), the maximum falling value of systolic blood pressure (SBP) and rMSSD, PNN50, BB50, and HF in HRV (P < 0.05), and the maximum falling value of SBP and postprandial INS (P < 0.05). There was also a positive correlation between the maximum falling value of SBP and BGdiff2 and mean amplitude of glycemic excursions in CGSM (P < 0.05). In the acarbose group, the falling magnitude of SBP and diastolic blood pressure (DBP) was reduced (P < 0.05), the falling magnitude of MAP was reduced (P < 0.01), the persistence of SBP, DBP, and MAP was shortened (P < 0.05), the CV, SD of SBP, DAP, and CV of MAP were shortened (P < 0.05), the SD of MAP was shortened (P < 0.01), and the SD and CV of HR were smaller (P < 0.05).

In the acarbose group, the postprandial blood glucose peak time was delayed (P < 0.01), and MBG2 and BGdiff2 were smaller (P < 0.01) compared with the placebo group. The postprandial GLP-1 was higher, and the INS and CP were lower in the acarbose group.

Conclusions: We found 40 PPH patients among 53 elderly patients with diabetes. The morbidity was 76.9%. Acarbose attenuated the fall in postprandial blood pressure and reduced fluctuations in blood pressure after meals in elderly patients with diabetes mellitus. Postprandial hypotension in elderly diabetic patients might be related to impaired autonomic nerve function, fluctuations in blood glucose, and insulin resistance.
**O919**

**External validation of the ADVANCE cardiovascular evaluation model to predict cardiovascular events in patients with type 2 diabetes in China**

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**Objective:** To assess the discrimination and calibration of the ADVANCE cardiovascular risk score to predict 4-year cardiovascular risk in patients with type 2 diabetes in the Shanghai area.

**Methods:** We retrospectively analysed 420 patients with diagnosed type 2 diabetes based on the diabetes management database at Dahua Hospital in Shanghai Xuhui District. During a median follow-up period of 3.8 years, the incidence of cardiovascular events was the study endpoint. Discrimination was examined using the c-statistic and calibration using the Hosmer–Lemeshow (HL) $\chi^2$ statistic. All statistical analyses were performed using R software.

**Results:** Of 108 patients diagnosed with cardiovascular events, there were 55 cases of coronary heart disease and 53 cases of stroke. The incidence rates of 4-year cardiovascular events were observed, and the ADVANCE predictive risk score was 25.7% and 2.17%, respectively. The ADVANCE risk score showed moderate discrimination for cardiovascular events (c-statistic of 0.66, 95% confidence interval: 0.67–0.79), and the calibration was poor (HL $\chi^2$ statistics were 14.41, $P = 0.025$).

**Conclusions:** ADVANCE risk scores underestimated the prediction rates of cardiovascular disease in type 2 diabetes patients in Chinese populations.

**O1147**

**Association of type 2 diabetes susceptibility loci with peripheral nerve function in a Chinese population with diabetes: results from nerve conduction studies**

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**Aims:** To investigate whether there was an association between type 2 diabetes mellitus (T2DM) susceptibility genes and peripheral nerve function in a Chinese population with T2DM.

**Methods:** A total of 1900 T2DM patients were recruited in the study. We selected 10 single nucleotide polymorphisms (SNPs) from 10 T2DM susceptibility genes that were previously confirmed in the Chinese population. Genotyping was conducted using a MassARRAY Compact Analyzer. Peripheral nerve function was evaluated by nerve conduction studies in all subjects. The composite Z-scores for the nerve conduction parameters, including the conduction velocity, amplitude and latency, were calculated. A total Z-score combining all three composite Z-scores was also calculated.

**Results:** In an additive genetic model, rs5219 of KCNJ11 (E23K) was identified to be associated with all of the parameters obtained in nerve conduction studies (Z-score of conduction velocity: $P = 0.01$; Z-score of amplitude: $P = 0.01$; Z-score of latency: $P = 0.01$; total Z-score: $P = 0.036$) after adjustment for confounding factors including age, duration and haemoglobin A1c levels. Specifically, each copy of the K allele was associated with a significant improvement in nerve conduction parameters. We found that CDKAL1 rs7756992 and TCF7L2 rs7903146 were related to the composite Z-score for amplitude ($P = 0.028$ and
compared with the HG group (17.36 ± 1.13 vs 17.28 ± 1.23, respectively). There was no significant association between the other 7 SNPs and peripheral nerve functions. Additionally, in a recessive model, KK carriers had better composite Z-scores for amplitude (P = 0.046), latency (P = 0.012) and conduction velocity (P = 0.027) than EE/EK carriers. In a dominant model, KK/EK carriers had better composite Z-scores for amplitude (P = 0.029) and conduction velocity (P = 0.044) than EE carriers.

Conclusions: The rs 5219 at KCNJ11 (E23K) was associated with peripheral nerve function in a Chinese population with type 2 diabetes in adult patients.

Objective: To investigate the effect and mechanism of forkhead transcription factor O1 overexpression on collagen synthesis in rat mesangial cells under high glucose conditions.

Methods: The coding sequences of constitutively active FoxO1 were constructed, followed by packaging and purifying of lentiviral vectors. After MCs were transfected with either constitutively active FoxO1 lentiviral vectors (LV-CA-FoxO1) or empty vectors (LV-NC-GFP), flow cytometry was performed to assess the transfection efficiency. The MCs cultured under normal glucose (5.6 mmol/L) conditions served as the normal control group (NG group), while those cultured under high glucose (16.7 mmol/L) conditions were high glucose group (HG group), high glucose plus LV-CA-FoxO1 group (CA group) and high glucose plus empty lentiviral vector (LV-NC-GFP) group (NC group). After 72 h of culture, the mRNA levels of FoxO1, Col I, Col IV, transforming growth factor-β1 (TGF-β1), and TGF-β type I and type II receptor (TGF-βRI/II) were measured by real-time polymerase chain reaction. The protein levels of FoxO1, phosphorylated FoxO1 (p-FoxO1), TGF-β1 and TGF-βRI/II were detected by Western blot analysis. Immunofluorescence was applied to investigate the distributions of TGF-βRI/II in MCs.

Results: The transfection efficiencies of MCs in the CA and NC groups were 78.38 ± 1.36% and 80.30 ± 2.20%, respectively, with no significant differences (t = −1.489, P = 0.28). No significant differences were observed in either mRNA or protein levels of FoxO1 in the HG group compared with the NG group (P > 0.05), whereas the protein levels of p-FoxO1 and the ratio of p-FoxO1/FoxO1 were markedly increased (all P < 0.05). Both FoxO1 mRNA and protein levels were increased in the CA group compared with the HG group (17.36 ± 1.13 vs 17.28 ± 1.23, respectively). There was no significant difference in the prevalence of carotid plaques between the two groups. The areas under the receiver operating characteristic curves based on the DN and DR models were larger in LADA than in patients with type 2 diabetes (0.72 vs 0.61, P = 0.013; 0.76 vs 0.68, P = 0.056).

Conclusions: A lower prevalence of microvascular complications was observed in patients with LADA when the disease duration was <5 years. Clinical features together could predict microvascular complications in both groups.
O1441
Association of serum ferritin level with carotid artery lesions in patients with abnormal glucose metabolism: a prospective cohort study
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Background: Epidemiological studies have shown that increased serum ferritin levels were positively correlated with carotid artery lesions; however, the study on the relationship between serum ferritin levels and carotid artery lesions in patients with abnormal glucose metabolism showed a reduced effect. In this work, we focused on investigating the association between serum ferritin and carotid artery lesion in a population with abnormal glucose metabolism.

Methods: Seventy participants with abnormal glucose metabolism (fasting plasma glucose ≥6.1 mmol/L or 2-h postprandial plasma glucose ≥7.8 mmol/L) and 70 participants with normal glucose tolerance aged ≥20 years were included between 2007 and 2008. Serum ferritin levels were measured at baseline. Carotid intima-media thickness (IMT) and carotid plaques were evaluated using carotid artery colour Doppler ultrasound during the follow-up in 2012.

Results: Among the participants, 20 patients displayed intima-media proliferation and 29 patients had carotid artery plaques at the study endpoint. Compared with the participants with normal glucose metabolism, serum ferritin levels increased significantly in participants with metabolic abnormalities in glucose (p < 0.01). In the subgroup analysis of patients with abnormal glucose metabolism, we further divided the patients with abnormal glucose metabolism into intima-media proliferation and non-intima-media proliferation subgroups and found that serum ferritin levels at baseline were higher in patients with intima-media proliferation compared with those without intima-media proliferation (157.6 ± 102.4 vs 109.4 ± 78.4 ng/mL, P = 0.037). Pearson’s correlation analysis also indicated that serum ferritin levels were positively and significantly related to IMT (P = 0.029) in participants with intima-media proliferation. Furthermore, age (p < 0.001), waist circumference (P = 0.048), levels of serum ferritin (P = 0.006), 2-h postprandial plasma glucose (P < 0.001), and total cholesterol (P = 0.026) were significantly different between participants in the subgroups with and without carotid artery plaques. The aforementioned data were used to generate a logistic regression model, and the results showed that the P value for age, serum ferritin, and 2-h postprandial plasma glucose were 0.004, 0.032, and 0.011, respectively.

Conclusions: In a Chinese population, serum ferritin levels increased significantly in patients with abnormal glucose metabolism. The carotid IMT might be closely related to increased serum ferritin levels in patients with abnormal glucose metabolism. Moreover, the serum ferritin level is an important risk factor for carotid atherosclerosis in patients with abnormal glucose metabolism.

O1489
Research on the consistency of ankle-brachial indexes of diabetic patients measured by automated oscillometric measurements and traditional eco-Doppler methods
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Objective: To discuss the consistency of ankle-brachial index (ABI) results in diabetic patients, as measured by automated oscillometric measurements and traditional eco-Doppler methods, respectively.

Methods: Two hundred and thirty diabetic patients who were admitted to the Department of Endocrinology and Metabolism at West China Hospital from May 2013 to June 2014 were enrolled in this study. The ankle-brachial indexes were simultaneously by the same nurse measured using both automated oscillometric measurements and eco-Doppler methods. The results were analysed using statistical analysis techniques for the diagnostic test.

Results: The total number of subjects was 230. Eco-Doppler ABI of the right and left limb was 1.003 ± 0.2856 (0.21–1.39) and 0.99 ± 0.287 (0.00–1.000), respectively. The automated oscillometric measurement ABI of the right and left limb was 1.002 ± 0.3315 (0.00–1.90) and 0.9934 ± 0.3190 (0.00–1.39), respectively. There were no significant differences in the results (right, P = 0.930; left, P = 0.727). Eco-Doppler is considered the gold standard [defining peripheral arterial disease (PAD) as an ABI ≤0.9]. For PAD diagnosis of the right limb, the accuracy of automated oscillometric measurement of ABI was 95.22%, with sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, coefficient of contingency and Kappa values of 94.34%, 95.48%, 20.8726, 0.0593, 0.657 and 0.869, respectively. For the left limb, the accuracy, sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, coefficient of contingency and Kappa values were 96.94%, 97.11%, 33.3643, 0.0363, 0.677 and 0.919, respectively. The time required for the two methods was 16.98 ± 3.20 (10.00–30.00) and 8.60 ± 1.38 (7.00–14.00), respectively, and significant differences were detected (P < 0.001).

Conclusions: A good consistency of ankle-brachial indexes of diabetic patients was measured using the automated oscillometric measurement and traditional eco-Doppler method. The time needed for the former measurement was shorter, and this method was also simpler and easier to operate.

O1517
Effect of hedysari or membranaceus on the treatment of patients with diabetic nephropathy
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Objective: To observe the effect of hedysari or membranaceus on the effect of therapy in patients with diabetic nephropathy stages III and IV.

Methods: Six hundred patients with diabetic nephropathy stages III and IV were divided into three groups stochastically. A total of 80 patients with diabetic nephropathy stage III and 116 patients with diabetic nephropathy stage IV were treated with benazepril hydrochloride 10 mg/day (group A). A total of 116 patients with diabetic nephropathy stage III and 82 patients with diabetic nephropathy stage IV were treated with hedysari or membranaceus 60 g/day (group B). A total of 86 patients with diabetic nephropathy stage III and 120 patients with diabetic nephropathy stage IV were treated with hedysari or membranaceus together with benazepril hydrochloride (group C). The course of treatment lasted for 12 weeks. The urinary albumin excretion rate (UAER), glomerular filtration rate (GFR), blood urea nitrogen (BUN), creatinine (Cr), urinary protein (24-h UTP), fasting plasma glucose (FBG), postprandial blood glucose (2-h PG), glycated haemoglobin (HbA1c), total cholesterol (TC), triglycerides (TG), urine beta 2-microglobulin (β2-M), urine alpha 1-microglobulin (α1-M), and urinary albumin/uric creatinine (Up/Ugcr) were determined before and after treatment.

Results: Compared with before treatment, the proportion of patients with diabetic nephropathy stage III and UAER decreased significantly in each group (P < 0.05). This change was more significant in group C compared with group A or B (P < 0.05), while no significant decrease in Cr or BUN was found in each group during the follow-up (P > 0.05). In patients with diabetic nephropathy stage IV, 24-h UTP, GFR, and Up/Ugcr improved significantly in each group after treatment (P < 0.05), with a more significant change detected in group C compared with group A or B (P < 0.05). After treatment for 12 weeks, α1-M and β2-M decreased significantly in each group (P < 0.05), with more significant changes in group C than in group A or B (P < 0.05), which suggests that hedysari or membranaceus and benazepril hydrochloride played a key role in improving renal tubule function. There were no significant differences in the detection of each index (FBG, 2-h PG, HbA1c, TC, and TG) between the pre-treatment and post-treatment in each group. The effect rate was 79% in group C, which was significantly superior to that in group A (60%) and group B (59%).

Conclusions: Use of hedysari or membranaceus as treatment may effectively improve the renal function and provide protection against the progression of diabetic nephropathy.

P576
CKD vital indicators: estimated glomerular filtration rate and albuminuria predict incidence of macrovascular diseases among a Chinese diabetic population

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Background: Chronic kidney disease (CKD) patients show high risk for cardiovascular disease (CVD), stroke and peripheral arterial disease (PAD). We aimed to find a simple way to predict macrovascular complications using CKD parameters in diabetes and to initiate early prevention.

Methods: A total of 838 hospitalized adult patients with type 2 diabetes were screened for at-risk foot. Neural and vascular disorders were evaluated by assessing vibration perception thresholds and ankle brachial indexes (ABIs). After excluding 12 patients with abnormally high ABIs, the remaining individuals with neural and/or vascular disorders were identified as patients with at-risk foot and were further classified into three subtypes: isolated neural disorder, isolated vascular disorder and mixed disorder. Potential associated factors were examined using step-wise multivariate logistic regression models.

Results: In the final sample of 826 individuals, the prevalence of diabetic at-risk foot was 30.6%. Among all at-risk patients, isolated neural disorder (69.6%) was more common than mixed disorder (16.2%) or isolated vascular disorder (14.2%). Isolated neural and vascular disorders shared specific risk factors, including age per 20-year increment [odds ratio (95% confidence interval) 3.73 (2.59–5.37) and 4.01 (1.98–8.11), respectively], diabetic duration ≥ 10 years [1.69 (1.13–2.54) and 3.29 (1.49–7.24), respectively] and systolic blood pressure > 140 mmHg [1.96 (1.31–2.93) and 2.90 (1.38–6.10), respectively]. In addition, isolated neural disorder was associated with heavy smoking history [2.69 (1.15–6.31)] and increased high-sensitivity C-reactive protein levels [1.30 (1.04–1.62)]. Isolated vascular disorder was linked with decreased high-density lipoprotein cholesterol level [3.42 (1.31–8.96)] and increased triglyceride level [2.74 (1.26–5.97)].

Conclusions: Diabetic at-risk foot is epidemic among hospitalized patients with type 2 diabetes. Ageing, long-term diabetes, hypertension, smoking, inflammatory response and dyslipidemia may be associated with the prevalence of diabetic at-risk foot.

P541
Identifying at-risk foot among hospitalized patients with type 2 diabetes: a cross-sectional study in a Chinese tertiary hospital

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Objective: To investigate the prevalence of diabetic at-risk foot and its associated factors.
dysfunction (assessed by eGFR and UACR) and macrovascular disease, including CVD, stroke and PAD.

Results: The deteriorated macroalbuminuria and kidney dysfunction are correlated with increased morbidity and risk of incident PAD, CVD and stroke. We found that the risks of stroke, peripheral arterial stenosis and peripheral arterial occlusion for patients with eGFR of 60–75 mL/min per 1.73 m² were 1.488, 2.024 and 2.438 times greater, respectively, than those of patients with eGFR ≥ 90 mL/min per 1.73 m². The risk of peripheral arterial plaques increased much earlier, ranking 1.797 at the stage of eGFR of 75–90 mL/min per 1.73 m². The risk of PAD increased as kidney function declined (as assessed by eGFR and UACR), and the odds ratios were 1.456, 1.511 and 1.948 for PAD (from mild to severe). In contrast, the risk of CVD was ranked differently and was highest at the stage of eGFR of 60–75 mL/min per 1.73 m² or UACR of 30–300 mg/g.

Conclusions: Our study indicates that the risk of PAD can be identified by microalbuminuria or serum creatinine examination. Taking action for early prevention of macrovascular complications is warranted once UACR is greater than 30 mg/g or eGFR², and it is also warranted much earlier for PAD when eGFR².

P900
Pathological characteristics of the neuromuscular junction of the gastrocnemius in a rat model of diabetes
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Background: In the present study, we established a rate model of DM and measured the sciatic nerve motor nerve conduc-
tion velocity (MNCV), tibial repetitive nerve stimulation (RNS) and the amount of NMJ AChE in gastrocnemius muscles (GOM) at different periods. In addition, we observed ultrastructural changes in the NMJ of GOM under the trans-
mission electron microscope (TEM). Thus, we investigated the effect of a high sugar environment on the morphology and function of NMJ. We provide a theoretical basis for clinical effective prevention and treatment of diabetic nerve and muscle joint disease.

Methods: Male Sprague-Dawley rats (220–240 g) were intraper-
itoneally injected via chain urea with cephalosporins (streptozotocin). Seventy-two hours after injection, rat tails were acupunctured to acquire blood, and fasting blood glucose (FBG) was tested using a glucose metre. We used an FBG level exceed-
ing 11.1 mmol/L as a standard of successful establishment of the diabetic rat model. The GOM organization profile was assessed in frozen 10-µm-thick sections, and histochemical staining and immunofluorescence were used to observe the levels of AChE and AChR. For electron microscopy, 70-nm-thick slices were pre-
pared, and TEM was used to observe the ultrastructure of NMJ in gastrocnemius muscle.

Results: After 12 weeks of diabetes, compared with the NC group, the level of MNCV in the sciatic nerve had slowed significantly (P < 0.01). The RNS of the tibial nerve from 4 to 20 weeks displayed no obvious change. Compared with the NC group, the number of NMJ was clearly reduced in the DM4, DM8, DM12, DM16 and DM20 groups (P < 0.05). The decline occurred in a duration-dependent manner (P < 0.05). The expression of AChE decreased from DM8 weeks, and the change was significant compared with the NC group (P < 0.05). The change also occurred in a time-dependent manner compared with DM8 weeks, and the level of AChE decreased remarkably at DM20 weeks (P < 0.05). Compared with the NC group, the pixel den-
sity of anti-alpha 1 AChR/FITC staining fluorescence from 4 to 20 weeks displayed no obvious changes. The TEM results showed that the presynaptic structure of the NMJ in gastrocnemius muscle was damaged in the DM4, DM8, DM12, DM16 and DM20 groups, which showed organelle fusion, structure disturbances and corpuscle atrophy. It was difficult to identify the interior structure, and the degree of damage had no relationship with the duration of diabetes.

Conclusion: Over short durations of diabetes, diabetic model rats showed changes in the structure of gastrocnemius nerve muscle joints, with fewer AChE and NMJ, in a duration-dependent manner. In diabetic model rats, the ultrastructural changes in neuromuscular connections of gastrocnemius muscle were characterized by body fusion in synaptic small organelles, which differed from neuromuscular joint pathological changes observed in myasthenia gravis. The diabetic rat model of neuromuscular joints displayed few synaptic structural changes and reduced AChE content, and the reduced number of NMJ did not cause the change in repeated electrical stimulation of the tibia.

P1034
Regulation of glucagon-like peptide-1 analogues in the hippocampus and its metabolites in type 2 diabetic patients with mild cognitive impairment
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Background: Based on the analysis and MoCA scale on diabe-
tes patients, hippocampal volume and its metabolites were compared in type 2 diabetic patients with mild cognitive impairment (MCI) and normal cognition (NC). Moreover, subjects with NGT and NC were collected as the control group and compared with the two aforementioned groups. Type 2 diabetic patients with MCI were administered glucagon-like peptide-1 (GLP-1) analogs for 3 months. Hippocam-
pal volume and its metabolites were compared before treatment to investigate the mechanism by which GLP-1 ana-
logues regulate cognitive function.

Methods: All of the subjects had type 2 diabetes mellitus (T2DM). Subjects were collected by oral glucose tolerance test and MoCA. Twenty subjects with NGT and 30 subjects with MoCA comprised the control group (the NGT-NC group). Twenty subjects with T2DM and 26 < MoCA ≤ 30 comprised the T2DM-NC group. Twenty subjects with T2DM and 23 < MoCA ≤ 26 comprised the
T2DM-MCI group. Using Siemens 3.0 T magnetic resonance, the hippocampus of the subjects in three groups was scanned, and the relative volume of the hippocampus was calculated. Next, we standardized the data and obtained the absolute volume of the hippocampus. The NAA, Cho, Cr and MI were determined based on the magnetic resonance spectrum (MRS). The T2DM-MCI group was administered GLP-1 analogs for 3 months. Use of liraglutide: first week, 0.6 mg per day, subcutaneous injection; after 1 week, 1.2 mg per day, subcutaneous injection. MRI and MRS analyses were repeated to calculate the volume of the hippocampus and determine the levels of NAA, Cho, Cr and MI.

Results: Compared with the NGT-NC and T2DM-NC groups, the bilateral hippocampal volume of the T2DM-MCI group was significantly reduced (P < 0.01). Compared with the NGT-NC and T2DM-NC groups, the level of NAA in the hippocampus of the T2DM-MCI group declined dramatically (P < 0.01) and the level of MI in the hippocampus of the T2DM-MCI group increased significantly (P < 0.01). After GLP-1 analog treatment in the T2DM-MCI group, the bilateral hippocampal volume did not change markedly (P > 0.05). After GLP-1 analog treatment in the T2DM-MCI group, the level of NAA in the hippocampus increased significantly compared with pre-treatment (P < 0.05). It did not change much compared with the T2DM-NC group (P > 0.05) and declined sharply compared with the NGT-NC group (P < 0.01).

MoCA scores and bilateral hippocampal volume were positively correlated in type 2 diabetic patients with MCI.

Conclusions: MRI showing hippocampal structure and the technology permitting spectrum analysis of material in the hippocampus made it possible to study cognitive dysfunction in type 2 diabetic patients. The bilateral hippocampal volume was reduced in type 2 diabetic patients with MCI, and this phenomenon was associated with cognitive decline. The reduction of hippocampal volume might be the structural basis of MCI in patients with type 2 diabetes. Changes in NAA and MI in the hippocampus might be the material basis for the reduction of hippocampal volume. After short-term treatment with GLP-1 analogs, the level of NAA in the hippocampus rose significantly in type 2 diabetic patients with MCI, indicating that GLP-1 analogues might regulate metabolites in the hippocampus.

**P1039**
**Transplantation of bone marrow-derived endothelial progenitor cells to treat peripheral neuropathy in diabetic rats**

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Background: We used bone marrow-derived endothelial progenitor cells (EPCs) from rats to treat sciatic neuropathy in diabetic rats by measuring the sciatic nerve motor nerve conduction velocity (MNCV), observing the expression of pathologic changes in the sciatic nerve, nuclear transcription factor-kappa B (NF-kB) and myelin basic protein (MBP) before and after transplantation to evaluate the therapeutic effect of EPCs on diabetic peripheral neuropathy (DPN) and the underlying mechanism.

Methods: EPCs were identified by double fluorescence staining and microvascular formation. H&E staining and electron microscopy specimens were prepared, and pathological changes in the sciatic nerve were observed using an optical microscope and electron microscope. The expression levels of NF-κB and MBP were measured in the sciatic nerve.

Results: We successfully obtained sufficient numbers of EPCs using density-gradient centrifugation in vitro. Compared with normal rats, the 12-week sciatic nerve MNCV of diabetic rats was remarkably slower, and this difference was statistically significant (p < 0.01). Compared with group NC, groups A and B had a slower sciatic nerve MNCV (p < 0.01). In group A, the number of nerve fibres was reduced, myelin became uneven and degenerated, and axons became thin. In group B, the number of nerve fibres was seriously decreased, myelin became more uneven, degeneration was aggravated and axons became rather thin and even disappeared. In group C, there was more relief compared with group B. In group A, myelin became uneven, part of the myelin layer separated, some myelin grew into axons, axons became thin, Schwann cell organelles were damaged, chromatic agglutination was observed and the nuclear membrane was incomplete. In group B, myelin became more uneven, the scope of disappearance of the plate layer increased, axons became thin and even displayed atresia. Schwann cell organelles were badly damaged and even disappeared, chromatic agglutination and even karyopyknosis were observed, and the nuclear membrane was incomplete. In group C, the unevenness of the myelin was not serious, most of the plate layers were present, Schwann cell organelles were normal and the nuclear membrane was complete; however, chromatic agglutination was observed. Compared with group NC, the expression of NF-κB was increased in the sciatic nerve of group A (p < 0.01). The expression of NF-κB in the sciatic nerve of group C was lower than that in group A (p < 0.05). Compared with group NC, the expression of MBP was reduced in the sciatic nerve of group A (p < 0.01), and compared with group B, it was higher in group C (p < 0.01).

Conclusions: After 12 weeks of feeding, diabetic rats could progress to DPN rats. Transplantation of EPCs could improve MNCV, demyelination and axonal degeneration associated with pathological changes in the sciatic nerve of DPN rats. Transplantation of EPCs could increase the expression of NF-κB and inhibit the expression of MBP, which might contribute to the repair of nerve injury.

**P1509**
**The novel CXCR4 antagonist SDF-1βP2G enhances ischemic angiogenesis via endothelial progenitor cell mobilization, infiltration and incorporation**

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Background: Stromal cell-derived factor 1 (SDF-1) and the CXCR4 axis play a critical role in vascular injury recovery and
angiogenesis. However, the multi-functional characteristics of native SDF-1 limit its potential application. We developed a novel CXCR4 antagonist, SDF-1P2G (P2G), derived from human native SDF-1β to block CXCR4 and promote mobilization of endothelial progenitor cells (EPCs) necessary for vascular injury recovery and angiogenesis.

Methods: A mouse and rat hind limb ischemia model was introduced to evaluate the therapeutic efficacy of P2G on ischemia angiogenesis and to dissect the underlying cellular and molecular mechanisms.

Results: As opposed to native SDF-1, P2G exerted antagonistic effects against CXCR4 by promoting CXCR4 internalization and competitively inhibiting downstream signalling events in vitro. In addition, P2G dose-dependently and time-dependently stimulated the mobilization of EPCs (CD31+/c-Kit+) from bone marrow into the peripheral circulation in vivo. Moreover, intravenous administration of P2G significantly stimulated ischemic angiogenesis, blood reperfusion and skeletal muscle regeneration in an acute hind limb ischemia model. A mechanistic study showed that P2G significantly stimulated EPC mobilization in the peripheral blood and promoted their infiltration into ischemic skeletal muscle tissues and incorporation into the newly formed blood vessel. In addition, P2G enhanced the activation and/or expression of angiogenesis and progenitor cell chemotaxis-related factors including Akt, ERK, mTOR, MMP-9, SDF-1/CXCR4 and vascular endothelial growth factor (VEGF). Furthermore, neutralization of VEGF with its specific antibody abolished P2G-induced blood reperfusion and angiogenesis. More importantly, no obvious inflammatory and apoptotic effects were observed in multiple organs after P2G administration. These data suggest that the novel antagonist of CXCR4, P2G, can be successfully utilized to stimulate ischemic angiogenesis and muscle regeneration through mobilization of EPCs in a VEGF-dependent manner.

Conclusions: Our work has demonstrated for the first time that P2G is a non-toxic, specific CXCR4 antagonist with great potential for clinical application for the treatment of ischemic vascular diseases.

Diabetes and Obesity/Surgery for diabetes

O326
Loss of aldose reductase activates LKB1-AMPK and insulin signalling in liver cells and ameliorates Agouti signalling peptide-induced glucose intolerance, insulin resistance, inflammation, hepatosteatosis and obesity in mice

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Background: It has been proposed that aldose reductase (Ar) and the polyol pathway might participate in the regulation of cellular glucose-lipid homeostasis. However, few data are available regarding the metabolic regulatory roles of Ar before the onset of overt hyperglycemia or under normoglycemic conditions. Here, we aimed to investigate how changes in the activity of Ar might affect LKB1-AMPK and insulin signalling.

Methods: For in vitro studies, changes in Ar activity in AML12 cells were achieved by plasmid-mediated Ar overexpression or lentivirus-mediated Ar knockdown. For in vivo studies, an Ar null allele (Ar<sup>−/−</sup>) was introduced into Agouti (A<sup>y</sup>/a) yellow obese mice to generate C57BL/6 double mutant mice, namely a/a: Ar<sup>+/+</sup>, a/a: Ar<sup>−/−</sup>, A<sup>y</sup>/a: Ar<sup>+/+</sup> and A<sup>y</sup>/a: Ar<sup>−/−</sup>.

Results: Our results showed that phosphorylated LKB1, AMPKα and ACC were reduced from the control by 47%, 46% and 56%, respectively, in mouse AML12 hepatocytes overexpressing Ar. Conversely, Ar knockdown greatly elevated the levels of phosphorylated LKB1, AMPKα and ACC. Additionally, overexpression of Ar reduced the mRNA and protein expression of IRS-1, whereas knockdown of Ar greatly elevated IRS-1 mRNA and protein expression. In animal models, body weight, the weights of epididymal fat pads, and serum levels of triglycerides and insulin but not cholesterol were significantly reduced in A<sup>y</sup>/a: Ar<sup>−/−</sup> mice compared with A<sup>y</sup>/a: Ar<sup>+/+</sup> mice. The Ar knockout also greatly improved Agouti-induced glucose intolerance, insulin insensitivity and inflammation. Oil Red O staining further indicated that loss of Ar greatly ameliorated hepatosteatosis in the livers of A<sup>y</sup>/a: Ar<sup>−/−</sup> mice.

Conclusions: Ar is capable of regulating insulin and LKB1-AMPK signalling, which contribute significantly to overall energy homeostasis. Importantly, our results suggest that Ar might be a potential target for the development of drugs that prevent or treat metabolic disorders.
Changes in the gut microbiota in response to berberine in obese rats fed a high-fat diet

**Objective:** To study changes in the gut microbiota in response to berberine in obese rats fed a high-fat diet and the ability of berberine to protect the function of the intestinal mucosal barrier.

**Methods:** Eight-week-old male Sprague-Dawley rats (n = 18) were randomly divided into the normal chow diet group (NCD, n = 6), high-fat diet group (HFD, n = 6), and high-fat diet + berberine group (HFD + BBR, n = 6). After 16 weeks of normal and high-fat diets, the HFD + BBR group was treated with berberine (150 mg/kg) by gavage. An equal volume of distilled water was given to the NCD and HFD controls. Fresh stool samples were collected after the treatments for 18 weeks. The 16S rRNA gene sequence analysis was conducted to investigate changes in the gut microbiota. Subsequently, the rats were sacrificed. The ileum tissue of rats was stained with HE, and changes in ileum tissue morphology in the three groups were examined using light microscopy.

**Results:** Three groups of gut microbiota in stool were distributed mainly in the Firmicutes and Bacteroidetes. NCD group: the percentages of Bacteroidetes and Firmicutes were 60.75% and 37.25%, respectively. HFD group: the percentages of Bacteroidetes and Firmicutes were 45.14% and 53.45%, respectively. HFD + BBR group: the percentages of Bacteroidetes and Firmicutes were 66.67% and 23.22%, respectively. At the genus level, the percentage of beneficial *Lactobacillus* bacteria in the HFD + BBR group was greater than the percentage in the HFD group. In the NCD group, the mucosal villus and structural integrity of the ileum surface was tightly and neatly arranged. In the HFD group, the mucosal villus was broken, loose, and disorganized, and the gap in the intervillus space was widened. In the HFD + BBR group, the mucosal villus of the ileum was relatively neatly arranged, and the mucosal villus displayed reduced breakage, looseness, and disorganization compared with the HFD group.

**Conclusions:** Berberine can change the proportion of Firmicutes and Bacteroidetes in obese rats and increase the proportion of beneficial bacteria of the genus *Lactobacillus*. Berberine has a certain protective effect on the damage to the intestinal mucosa caused by a high-fat diet.
respectively. The BARD score and AST/ALT provided no diagnostic value for G2–3.

Conclusion: The NAFLD liver fat score was the best predictor of G2–3 and advanced fibrosis in patients with abnormal glucose metabolism. The NAFLD fibrosis score was the second best score for the prediction of advanced fibrosis.

P401
The effect of PARP1 on insulin sensitivity in lipotoxicity
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Background: High serum (plasma) free fatty acid levels are associated with the development of insulin resistance in type 2 diabetes. However, the precise mechanisms of lipid toxicity remain unclear.

Methods: HepG2 hepatocytes were cultured with 500 μM oleic acid for 48 h. The PARP1 inhibitor PJ34 and PARP1 small interfering RNA (siRNA) transfection were used to identify the downstream effects of PARP1 activation.

Results: Oleic acid-treated cells exhibited more reactive oxygen species generation and lipid accumulation than control cells. The inhibition of PARP1 by PJ34 prevented the oleic acid-induced impairment of insulin signalling pathway; similar results were observed using PARP1 siRNA. Meanwhile, treatment with PJ34 reversed the decrease in the intracellular NAD content of oleic acid-treated HepG2 cells. Exogenously added NAD preserved the downregulated phosphorylation of insulin signalling proteins in response to insulin stimulation under oleic acid treatment conditions.

Conclusions: These data suggest that NAD depletion by PARP1 activation is essential to the modulation of insulin sensitivity in oleic acid-induced lipotoxicity.

P705
Identification of miR-199a as an essential regulator of adipocyte thermogenesis
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Objectives: To investigate the role and mechanisms of miR-199a in brown adipogenesis and thermogenesis.

Methods: Brown preadipocytes (bfc), primary brown adipocytes and primary white adipocytes were cultured and stimulated to differentiate. We transfected bfc with a miR-199a mimic or inhibitor or with their respective nonspecific controls. RT-PCR was used to detect the expression profiles of miR-199a and related genes during differentiation. In silico analysis using online programs was used to search for potential targets of miR-199a, which were then verified using a dual luciferase reporter system. We checked the tissue expression pattern of miR-199a and analysed the levels of the miRNA in the fat tissues of either high-fat-diet mice or ob/ob mice. A cold stress test (4 °C for 24 h or 7 days) was performed on C67BL6 mice, and the miRNA expression was analysed.

Results: The expression of miR-199a showed tissue specificity, with the lowest expression in BAT compared with sWAT, vWAT and muscle. The expression of miR-199a decreased during the differentiation processes of bfc, primary brown and subcutaneous white adipocytes. Thus, we hypothesized that miR-199a might play a negative role in brown adipogenesis and thermogenesis. The expression of miR-199a in fat tissues was dramatically reduced in response to cold stress and was negatively correlated with the levels of thermogenic genes, including PGC1α, UCP1 and PRDM16. The expression of miR-199a was significantly increased in the fat tissues of high-fat diet mice or ob/ob mice. Over-expression or inhibition of miR-199a in bfc significantly reduced and increased, respectively, the levels of thermogenic genes, including PGC1α, UCP1 and PRDM16; therefore, miR-199a might be a negative regulator of thermogenesis. In silico analysis showed that PGC1α and PRDM16 may be potential targets of miR-199a. Dual luciferase reporter assays showed that miR-199a directed targets PGC1α and PRDM16.

Conclusion: Our data suggest that miR-199a plays a negative role in brown adipocyte thermogenesis by directly targeting PGC1α and PRDM16.

Islet Biology/Insulin Secretion

O441
Effect of transcription factor TIP27 overexpression on glucose homeostasis and insulin sensitivity in mice
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Background: TIP27 (juxtaposed with another zinc finger protein 1) is a 27-kDa transcription factor containing three putative zinc finger motifs, and its expression is associated with diabetes mellitus and prostate cancer. However, little is known about its role in regulating metabolism. In this study, we investigated the effects of TIP27 overexpression on glucose homeostasis and the insulin signalling pathway in high-fat diet (HFD)-fed TIP27 transgenic (Tg) mice.

Methods: We mainly used TIP27-Tg mice in our research. Additionally, we established a gain-of-function model of TIP27 in db/db mice using an adenovirus-mediated cDNA. Using these models, we assessed the effects of TIP27 overexpression in TIP27-Tg mice on glucose metabolism and changes in insulin sensitivity during glucose tolerance tests and insulin tolerance tests. Hyperinsulinemic-euglycemic clamp experiments were...
performed in Tip27-Tg mice. Glucose rates of appearance (GRa) were determined with 3-[3H] glucose. Whole body GRa and glucose uptake (GRu) were calculated using the non-steady-state equation. The mRNA and protein expression levels were measured by quantitative real-time polymerase chain reaction and Western blot analysis, respectively.

Results: We showed that TIP27 overexpression in TIP27-Tg mice led to reduced total cholesterol and fasting plasma insulin levels and enhanced glucose tolerance and insulin sensitivity. Hyperinsulinemic–euglycemic clamp experiments also demonstrated that TIP27 overexpression in TIP27-Tg mice enhanced insulin sensitivity. In addition, the expression levels of PEPCK and glucose-6-phosphatase mRNA and protein were significantly decreased in TIP27-Tg mice, whereas the phosphorylation of insulin-receptor, insulin receptor substrate-1, adenosine monophosphate-activated protein kinase and Akt were significantly increased in insulin target tissues.

Conclusions: TIP27 plays an important role in glucose homeostasis by regulating hepatic glucose metabolism and insulin sensitivity. TIP27 overexpression enhances the insulin signalling cascade.

O443
Intracerebroventricular administration of vaspin triggers a brain-liver circuit to improve hepatic glucose homeostasis via the hepatic branch of the vagus

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Background: Visceral adipose tissue-derived serpin (vaspin) is a serpin A12 member of the serine protease inhibitor family, which plays an important role in modulating glucose metabolism and insulin sensitivity. Increasing findings raise the possibility that vaspin regulates glucose metabolism and energy balance by acting on the central (hypothalamic) site. However, the regulatory role of vaspin in the brain in the control of liver glucose fluxes is unknown. In this study, we investigated the effects of the vaspin signal conveyed by the hypothalamus on liver glucose fluxes in normal-chow-diet or high-fat diet (HFD)-fed male rats with or without hepatic branch vagotomy. Here, we report a novel role for central vaspin in triggering a brain-liver molecular signalling pathway and neuronal network to control glucose production in vivo.

Methods: We established a model of central vaspin administration. Hyperinsulinemic–euglycemic clamp and hepatic branch vagotomy were used to assess the effects of central vaspin on glucose metabolism and changes in liver signalling pathways. The [3-1H] glucose radioactivity was determined using a scintillation counter. The specific activities of hepatic [14C] phosphoenolpyruvate (PEP), [3H] diphosphoglucose (UDP)-glucose and [14C] UDP-glucose were measured by high-performance liquid chromatography. The mRNA and protein expression levels were measured by quantitative real-time polymerase chain reaction and Western blot analysis, respectively.

Results: We showed that central infusion of vaspin in HFD-fed animals significantly increased glucose uptake in peripheral tissues and decreased HGP. These changes were accompanied by a significant decrease in hepatic glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK) expression. In agreement with this finding, we found that central vaspin in HFD rats activated the insulin receptor → insulin receptor substrate-1 (IRS-1) → Akt kinase (Akt) → forkhead box-containing protein of the O subfamily 1 (FoxO1) signalling cascade in the liver, leading to increased insulin sensitivity and improved glucose metabolism. In this study, we surgically transected the hepatic branch of the vagus nerve and found that selective hepatic vagotomy significantly abolished the effect of central vaspin on glucose production.

Conclusions: We demonstrated herein for the first time the important role of central vaspin in the regulation of insulin signalling via activation of the Akt/FoxO1 pathway in the liver. Importantly, our results provide evidence that the hepatic branch of the vagus nerve is required for central vaspin action. Moreover, we identified a novel pathway in which the N-methyl-d-aspartic acid receptor in the dorsal vagal complex from the hepatic vagal branch to the liver for brain-liver crosstalk couples central vaspin signalling in the hypothalamus to liver glucose homeostasis.

O573
Cholesterol activates autophagy in pancreatic beta-cells

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Background: In our previous study, we found that cholesterol induced apoptosis in beta cells. The present study was undertaken to investigate whether autophagy is activated by cholesterol and its role in cholesterol-induced apoptosis of the mouse pancreatic β-cell line βTC-6.

Methods: First, cells were cultured in 5 mmol/L cholesterol for 1, 3, 6 and 12 h. Second, cells were pre-incubated with 10 μg/mL (2S,3S)-trans-epoxysuccinyl-l-leucylamido-3-methylbutane ethyl ester (E64-d) and 10 μg/mL Pepstatin A or with 100 nmol/L bafilomycin A1 for 1 h, followed by incubation with cholesterol for 6 h. Cleaved caspase 3 and LC3II were detected by Western blot analysis, while apoptotic cells were quantified by DAPI staining. The LC3II dots were detected by immunofluorescence, while autophagic vacuoles were detected by transmission electron microscopy.

Results: Compared with the control, cholesterol treatment significantly increased the level of LC3II in a time-dependent manner, as assessed by Western blot analysis, which suggested that autophagy was activated by cholesterol. This result was confirmed by immunofluorescence with anti-LC3, which showed that LC3II dots significantly increased in cholesterol-treated cells. Furthermore, many autophagic vacuoles were detected by transmission electron microscopy in cells treated with...
cholesterol for 6 h. To further investigate the role of autophagy, cells were pre-incubated with 10 µg/mL E64-d and 10 µg/mL Pepstatin A or with 100 nmol/L bafilomycin A1 for 1 h, which blocked the process of autophagy, followed by an incubation with cholesterol for 6 h. Pretreatment of the cells with E64-d and Pepstatin A or with bafilomycin A1 induced a greater increase in LC3II levels and the LC3II dots than that observed following cholesterol treatment alone, indicating that the process of autophagy was interrupted. Concomitantly, the percentage of apoptotic cells induced by cholesterol increased approximately 15%, as determined by DAPI staining, and increased levels of cleaved caspase 3 were detected.

Conclusions: These data suggest that autophagy attenuates cholesterol-induced apoptosis in βTC-6 cells.

O586
Role of PGC-1α in INS-1 cell proliferation, apoptosis and function induced by palmitic acid
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Background: The peroxisome proliferator-activated receptor-γ co-activator-1α (PGC-1α) plays a critical role in regulating adaptive thermogenesis, energy metabolism, fat and glucose metabolism. It has been demonstrated that PGC-1α displays a high tissue-specific expression level; however, little is known about its role in the changing β cell function. To explore the role of PGC-1α in the change in β cell function induced by free fatty acids, we studied the expression of PGC-1α in INS-1 cells treated with different concentrations of palmitic acid and its relationship with cell proliferation, apoptosis and function.

Methods: INS-1 cells were cultured in RPMI1640 containing 50, 100 and 400 µmol/L palmitic acid for 4, 8, 12, 24 and 48 h, and the expression of PGC-1α mRNA and protein and MTT, Bcl-2 and GKO protein, TUNEL staining, and basal and glucose-stimulated insulin concentrations were examined.

Results: Compared with the control group, in the 50 µM palmitic acid group, MTT decreased at 8 and 12 h but did not differ at 24 and 48 h; basal and glucose-stimulated insulin secretion increased; PGC-1α mRNA showed a trend toward an increase up to 48 h but without statistical significance; TUNEL staining and protein expression levels of Bcl-2 and GK revealed no differences. In the 100 µM palmitic acid group, MTT decreased at 8, 12 and 24 h but did not differ at 48 h; basal and glucose-stimulated insulin secretion increased; PGC-1α mRNA increased from 24 h, and PGC-1α protein increased up to 48 h; TUNEL staining and protein expression levels of Bcl-2 and GK revealed no differences. In the 400 µM palmitic acid group, MTT decreased from 8 h, both basal and glucose-stimulated insulin secretion decreased and the expression of PGC-1α mRNA significantly increased from 12 h and that of PGC-1α protein increased up to 24 h. Bcl-2 protein decreased from 24 h, GK protein decreased from 12 h and TUNEL staining increased up to 48 h.

Conclusions: The expression of PGC-1α in the 50 and 100 µM palmitic acid groups displayed a mild increase and had a close relationship with changes in proliferative vitality and increased insulin secretion. Incubation with 400 µM palmitic acid significantly increased the expression of PGC-1α and was associated with damaged cell proliferative vitality and insulin secretion function. Our study indicated that PGC-1α played an important role in changes in cell proliferation, apoptosis and function induced by palmitic acid.

O755
Effect on glucagon secretion of Alpha TC1 cells after knocking down IA-2 and IA-2β expression by RNA interference
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Background: IA-2 and IA-2β belong to the transmembrane protein tyrosine phosphatase family, with physiological functions that remain unclear. This study was designed to investigate whether IA-2 and IA-2β participate in the secretion of glucagon.

Methods: Alpha TC1 cells (CRL-2350, ATCC) were cultured with DMEM containing 16.7% glucose in a 24-pore plate with 1×10⁵ cells in each pore. The IA-2 siRNA and IA-2β siRNA sequences were designed and manufactured by Invitrogen. The target gene sequence of IA-2 Stealth siRNA was 5-AAU CUC UGC AGA CUC AUC AUU GGU U-3. The target gene sequence of IA-2β Stealth siRNA was 5-AAG GUU AUC AGG AUA CUU GGC CGG C-3. A 90 nmol/L Stealth siRNA was transfected into AlphaTC1 cells with Lipofectamine 2000 for 72 h. The expression levels of IA-2 and IA-2β were detected with Western blot analysis. Arginine was applied to stimulate glucagon secretion by AlphaTC1 cells, and the glucagon levels were measured by radioimmunoassay.

Results: The expression levels of IA-2 and IA-2β decreased 64.5% and 57.7%, respectively, after RNA interference (RNAi). Glucagon levels in the supernatant decreased from 698.43 ± 6.17 to 371.34 ± 52.78 (P<0.01) in the IA-2 RNAi group, while the glucagon levels did not change significantly in the negative RNAi group (698.43 ± 6.17 vs 649.62 ± 51.25). After arginine stimulation, the glucagon levels also decreased significantly in the IA-2 RNAi group (897.23 ± 35.18 vs 506.63 ± 138.52, P<0.01), but no significant differences were detected in the negative RNAi group (897.23 ± 35.18 vs 837.52 ± 150.05). The glucagon levels did not change significantly in the IA-2β RNAi group (698.43 ± 6.17 vs 590.67 ± 48.34) or in the RNAi group (698.43 ± 6.17 vs 706.33 ± 109.14). However, after arginine stimulation, the glucagon levels decreased significantly in the IA-2β RNAi group (897.23 ± 35.18 vs 401.23 ± 64.93, P<0.01), but no significant difference was detected in the negative RNAi group (897.23 ± 35.18 vs 826.65 ± 42.39).

Conclusions: IA-2 and IA-2β participated into the secretion of glucagon in AlphaTC1 cells.
O841
Effects of microRNA-126 on glucose metabolism in normal human liver cells
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Objective: To investigate the roles of microRNA-126 (miR-126) on hepatic glucose metabolism in diabetes mellitus by observing the effects of its overexpression in normal human liver cells on hepatocyte glucose utilization, glycogen synthesis, gluconeogenesis and glycolysis.

Methods: Normal human liver cells were cultured in vitro. Cells were divided into three groups: NC-FAM group, inhibitor NC-FAM group and normal control group. Cells in each group were transfected with the respective sequence at different concentrations (10, 20, 40 and 80 nM) via lip2000, and fluorescence microscopy was used to observe the transfection efficiency to determine the optimal transfection conditions. Cells were divided into six groups: miR-126 mimics group, miR-126 inhibitor group, negative control (NC) group, microRNA inhibitor NC group, lip2000 group and normal control cell group, and every group included six samples. Under the most effective transfection conditions ascertained earlier, the artificial synthetic miR-126 mimic, miR-126 inhibitor, negative control and microRNA inhibitor NC were transferred via lip2000, and the transfection efficiency was tested by quantitative real-time polymerase chain reaction (qRT-PCR). After 6 h, the original cell culture medium was replaced with complete medium. Cells were cultured for 48 h and then stimulated with synthetic Insulin (100 nM) for 12 h. The GOD-POD method, BCA protein assay kit, anthrone method, lactate assay kit and pyruvate kinase kit were used to assess the glucose concentration in the supernatant, the protein concentration in cells in the lower plates, glycogen content, lactic production and pyruvate kinase activity, respectively. The ratio of glycogen to protein content (µmol/mg) showed the account of glycogen synthesis; in response to lactic acid, the ratio of the glucose concentration in the supernatant to the protein concentration (mmol/mg) represented the account of gluconeogenesis. In the presence of high glucose levels, the ratio of the lactic acid concentration to the protein concentration (mmol/mg) and pyruvate kinase activity to protein concentration (U/gprot) indicated the account of glycolysis. Through these indicators, the effect of miR-126 at different concentrations on hepatic glucose metabolism was observed.

Results: Under serum-free condition without antibiotics, 6 h after transfection, NC-FAM and inhibitor NC-FAM were effectively transfected into normal human liver cell in a dose-dependent manner via lip2000, as observed by fluorescence microscopy. The most effective transfection condition was a concentration of 80 nM, and the original cell culture medium was replaced with complete medium after 6 h. The miR-126 expression level was measured by qRT-PCR, and the results showed that the level of miR-126 in the miR-126 mimic transfection group was higher than that in the other groups, and the difference was statistically significant (P < 0.05). Using the most effective conditions ascertained earlier, the results showed that the miR-126 mimic group had significantly decreased glucose utilization (P < 0.05), reduced glycogen synthesis (P < 0.05), effectively increased gluconeogenesis (P < 0.05), reduced lactate production (P < 0.05) and decreased pyruvate kinase activity (P < 0.05).

Conclusions: Increased expression of miR-126 in normal human liver cells revealed that miR-126 regulated glucose metabolism by decreasing glycogen synthesis, lactate production and pyruvate kinase activity, and increasing the amount of gluconeogenesis. The research showed that over-expression of miR-126 in hepatocytes reduced glucose utilization and sensitivity to insulin and enhanced insulin resistance in hepatocytes.

O1157
ACE2/Ang-(1–7) improves insulin resistance in hepatic cells
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Background: The renin angiotensin system (RAS) is involved in glucose metabolism. ACE2 is expressed in the liver, which is one of the primary organs in the pathogenesis of insulin resistance. This study examines the effect of the new RAS, ACE2/Ang-(1–7) pathway on glucose metabolism in hepatic cells.

Methods: HepG2 cells were treated with Ang-(1–7) or A779, or transfected with ACE2 overexpression plasmid DNA, and then the cellular glycogen content and glucose uptake were analysed. Expression of the glucose metabolism gene was detected by real-time polymerase chain reaction and Western blot analysis. Reactive oxygen species (ROS) was measured by DHE in HepG2 cells and ACE2 knockout (KO) mice, and Western blot was used to study NADPH in ACE2 KO mice. Western blot was used to study the JNK pathway in HepG2 cells and ACE2 KO mice and the PI3K/AKT pathway in HepG2 cells and ACE2 KO mice.

Results: Activation of the ACE2/Ang-(1–7) axis resulted in increased glucose uptake and glycogen synthesis in HepG2 cells. The mRNA expression levels of GLUT2 and IRS-2 were increased in Ang-(1–7)-treated cells, whereas glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) mRNA levels decreased under the same conditions. In ACE2-overexpressing HepG2 cells, the expression of G6Pase and PEPCK decreased. In ACE2 KO mice, GLUT2 in the membrane and total protein decreased. Ang-(1–7) reduced ROS production in HepG2 cells, and ROS and total-p47phox, p22phox and p67phox expression decreased significantly in the livers of ACE2 KO mice. The phosphorylation of JNK at residue Ser307 in IRS-1 was stimulated in ACE2 KO mice but inhibited following ACE2 overexpression. The phosphorylation levels of AKT at residues Thr308 and Ser473 and of PI3K at residue Tyr458 were significantly inhibited in ACE2 KO mice but markedly increased in ACE2-overexpressing cells compared with control cells.

Conclusions: Activation of the ACE2/Ang-(1–7)/Mas axis led to improved hepatic insulin resistance through the Akt/PI3K/IRS-1/JNK insulin signalling pathway. Our current findings reinforce the idea that the ACE2/Ang-(1–7)/Mas axis plays a role in metabolic processes, and they provide new insights into the potential insulin-sensitization mechanisms of the ACE2/Ang-(1–7)/Mas axis.
**O1270**

**CFTR channel and insulin secretion**

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**Objective:** To explore the expression and activity of the CFTR channel on pancreatic beta cells.  
**Methods:** We used reverse transcription-polymerase chain reaction amplification, Western blotting and immunofluorescence techniques to identify whether CFTR channels are expressed in beta cells. To observe the functions of the CFTR, we applied the CFTR inhibitor to MIN6 cells and injected C57 mice with A-inhibitor (0, 10, 50 and 250 μM) intraperitoneally twice weekly. One month later, weight, fasting blood glucose, postprandial blood glucose and insulin concentration were measured in the mice.  
**Results:** CFTR mRNA and protein were expressed in Min-6 cells. The secretion of insulin gradually increased with progressive elevations in the dose of CFTR inhibitor. A significant elevation of insulin secretion was detected after treatment with CFTR inhibitor compared with the control group (P < 0.01). CFTR inhibitor reduced fasting and postprandial blood glucose levels dose-dependently in C57 mice.  
**Conclusions:** Our data indicate that the application of CFTR inhibitor could reduce fasting and postprandial blood glucose and insulin concentration were measured in the mice.

**P497**

**Effects of high glucose and hypoxia on autophagy and the biological function of MIN6 cells**

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**Objective:** To investigate changes in autophagy and insulin secretion of MIN6 cells treated with high glucose and hypoxia.  
**Methods:** (1) MIN6 cells pre-treated with euglycemia and hyperglycemia (5.5 and 33.3 mmol/L, respectively) were further divided into two groups: a normoxia group and a hypoxia group. Expression levels of HIF-1α and Beclin-1 were detected by real-time polymerase chain reaction. (2) MIN6 cells pre-treated with euglycemia and hyperglycemia (5.5 and 33.3 mmol/L) were further divided into five groups with different durations of anoxia: 0, 1, 2, 3, and 4 days. The concentration of insulin in the supernatant in each group was detected by enzyme-linked immunosorbent assay.  
**Results:** The expression levels of HIF-1α and Beclin-1 were upregulated under hypoxia and were related (r = 0.965, P < 0.05). Under hypoxic conditions with the same duration of anoxia, the insulin level of the hyperglycemia group was lower than that of the euglycemia group (P < 0.01). Insulin levels decreased gradually under hypoxia in a time-dependent manner, both in the euglycemia group and the hypoglycemia group (euglycemia group r = −0.997, P < 0.01; hyperglycemia group r = −0.999, P < 0.01).  
**Conclusions:** Hypoxia induces autophagy by HIF-1α. Autophagy leads to a decrease in cell number and to disorder of islet beta cells, which results in decreased insulin production.

**P562**

**Effects of RNA interference selective blocking of the renin–angiotensin system on glucagon secretory function**

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**Objective:** To investigate the effects of intra-islet inhibition of angiotensin II type 1 receptor (AT1R) expression using a RNA interference strategy to assess the glucagon secretory function of islets in vitro in db/db mice.  
**Methods** Islets of db/db and db/m mice were isolated using digestion by ductal injection of collagenase. The expression of AT1R in islets was detected by real-time polymerase chain reaction and Western blot analysis. To further evaluate the role of AT1R in α cell function, we constructed a recombinant adenovirus containing a small interfering RNA (siRNA) specific to AT1R (Ad-siAT1R). Isolated islets of db/db mice were divided into three groups: (1) Ad-siAT1R group, islets were transduced with Ad-siAT1R for 2 h; (2) Ad-siControl group, islets were transduced with Ad-siControl; (3) Mock group, islets were not exposed to virus. Islets were cultured for 48 h. AT1R mRNA and protein levels were measured for each condition, and islet perfusion was performed to evaluate the kinetics of insulin and glucagon release in vitro.

**Results** The expression level of AT1R (both mRNA and protein) in isolated islets of db/db mice was nearly three times higher than that in db/m mice (P < 0.05). After viral transduction, Ad-siAT1R treatment resulted in a 75% decrease in AT1R mRNA levels and a 65% decrease in AT1R protein compared with islets treated with Ad-siControl (P < 0.05). In addition, insulin secretion in the Ad-siAT1R group immediately increased to the peak level of 140 μmol/L at 1–2 min after administration of 16.7 mmol/L glucose. Furthermore, glucagon secretion immediately decreased to 14 pmol/L, which was less than 13 pmol/L lower than the basal level. There was also a small rise in insulin secretion in the Ad-siAT1R group at 1–2 min after administration of 16.7 mmol/L glucose. The peak was only 1.8 times that of the basal level. In addition, glucagon secretion slowly decreased to 35 pmol/L, which was only 5 pmol/L lower than the basal level.  
**Conclusions:** The siRNA specific to AT1R can suppress AT1R expression and ameliorate glucagon secretory function in islets of db/db mice.

**P838**

**PGC-1α expression is suppressed by p38MAPK activation in the skeletal muscle of high-fat-diet-fed rats**

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**Objective:** To explore the expression and activity of the CFTR channel on pancreatic beta cells.  
**Methods:** We used reverse transcriptase-polymerase chain reaction, Western blotting and immunofluorescence techniques to identify whether CFTR channels are expressed in beta cells. To observe the functions of the CFTR, we applied the CFTR inhibitor to MIN6 cells and injected C57 mice with A-inhibitor (0, 10, 50 and 250 μM) intraperitoneally twice weekly. One month later, weight, fasting blood glucose, postprandial blood glucose and insulin concentration were measured in the mice.  
**Results:** CFTR mRNA and protein were expressed in Min-6 cells. The secretion of insulin gradually increased with progressive elevations in the dose of CFTR inhibitor. A significant elevation of insulin secretion was detected after treatment with CFTR inhibitor compared with the control group (P < 0.01). CFTR inhibitor reduced fasting and postprandial blood glucose levels dose-dependently in C57 mice.  
**Conclusions:** Our data indicate that the application of CFTR inhibitor could promote insulin secretion and improve insulin sensitivity. CFTR inhibitor is a potential therapeutic drug for the treatment of diabetes.
Objectives: To investigate the expression of PGC-1α in rats with insulin resistance fed a high-fat diet and to explore the regulating effect of the MAPK pathway.

Methods: Male Wistar rats were divided into two groups: normal control group (NC, n = 15) and high-fat group (HF, n = 15). At the end of week 8, blood samples were collected to measure fasting blood glucose, insulin, triglycerides (TGs) and free fatty acids (FFAs). Five rats were randomly selected from each group to be administered a hyperinsulinemic euglycemic clamp to evaluate insulin sensitivity. The expression levels of muscle PGC-1α mRNA and protein were measured. The skeletal muscle cell line C2C12 was incubated with palmitic acid for different times, with or without P38 inhibitor (SB203580), and the expression levels of PGC-1α, ERK, p-ERK, JNK, p-JNK, P38, and p-P38 were assayed by polymerase chain reaction and Western blot analysis.

Results: (1) Fasting plasma insulin (15.4 ± 2.1 vs 9.7 ± 3.0 mIU/L), fasting plasma glucose (6.5 ± 0.2 vs 4.7 ± 0.4 mmol/L), FFAs (1.53 ± 0.40 vs 0.68 ± 0.18 mmol/L), and skeletal muscle TGs (2.07 ± 0.30 vs 1.09 ± 0.17 mmol/L) were higher in the HF group compared with the NC group (P < 0.05 or P < 0.01). The glucose infusion rate decreased in the HF group (20.9 ± 2.2 mg kg⁻¹ min⁻¹) compared with the NC group (30.4 ± 4.2 mg kg⁻¹ min⁻¹) at week 8 (P < 0.01). (2) Compared with the NC group, the expression of muscle PGC-1α decreased in the HF group, and this difference was significant (P < 0.01). (3) The expression of ERK, p-ERK, JNK, and p-JNK did not differ in C2C12 cells incubated with palmitic acid. (4) The expression of P38 did not differ in C2C12 cells incubated with palmitic acid (P > 0.05). However, the expression of p-P38 increased gradually at 1, 6, 12, and 24 h (all P < 0.05). (5) The expression of PGC-1α increased in the palmitic acid group with P38 inhibitor compared with the palmitic acid group without P38 inhibitor (2.0 ± 0.24 vs 0.33 ± 0.16, P < 0.05).

Conclusions: Plasma FFA and skeletal muscle TG increased and insulin sensitivity decreased in rats fed a high-fat diet. The expression of muscle PGC-1α decreased in rats fed a high-fat diet. The expression of PGC-1α was suppressed by p38MAPK activation in muscle cells cultured with palmitic acid.

O553

Phase II study of adding Retagliptin to metformin therapy in patients with inadequately controlled type 2 diabetes

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Objective: To evaluate the efficacy and safety of Retagliptin (SP2086), a new dipeptidyl peptidase-4 inhibitor, as add-on treatment in type 2 diabetes mellitus (T2DM) patients with inadequate glycemic control by metformin alone.

Methods: T2DM patients with inadequate glycemic control [7.5% ≤ haemoglobin A1c (HbA1c) ≤ 11.0%] on a stable dose of metformin (1500 mg/day ± 10 weeks) were randomized 1:1:1 into three arms: SP2086 100 mg (n = 39), SP2086 50 mg (n = 40) and placebo (n = 38) after a 2-week single-blind placebo plus metformin run-in period. The primary end point was the change in HbA1c from baseline to week 12.

Results: The mean age in each group (placebo, SP2086 50 mg and SP2086 100 mg) was 52.74, 52.70 and 54.97 years, respectively. The mean duration of diabetes in each group was 26.5, 43.0 and 26.0 months, respectively. The average HbA1c was 7.99%, 8.25% and 7.69%, respectively. The groups were generally well balanced in terms of demographics at baseline. At week 12, Retagliptin 50 and 100 mg significantly (P < 0.05) reduced HbA1c compared with placebo (differences between groups were −0.74% and −0.68%, respectively), while there was no significant difference between the two Retagliptin treatment groups. HOMA-β improved from baseline with Retagliptin 50 and 100 mg versus placebo (17.59, 11.50 and −7.90, respectively), but there were no statistically significant effects. The incidence of overall clinical adverse events was similar in all three groups (P > 0.05, SP2086 50 mg 34.21%, SP2086 100 mg 35.00%, placebo 33.33%). None of the patients experienced serious adverse events or hypoglycemia. Common adverse events were observed, including abdominal pain, diarrhoea, erythra, upper respiratory tract infection and urinary infection, among others.

Conclusions: The addition of Retagliptin 50 or 100 mg to ongoing metformin treatment in patients with type 2 diabetes was well tolerated and resulted in significant and clinically relevant improvements in glycemic control and β-cell function.

O693

Erythropoietin alleviates insulin resistance in hepatocytes via the PPARγ-dependent PI3K/AKT pathway

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Background: Erythropoietin (EPO) has been shown to have beneficial effects on glucose metabolism and insulin resistance. Peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear
receptor that plays important roles in the regulation of glucose and in lipid metabolism in the liver. This study aimed to investigate whether PPARγ mediates the effect of EPO on regulating glucose metabolism and alleviating insulin resistance in hepatocytes.

**Methods:** HepG2 cells were treated with EPO in normal or palmitate-induced insulin-resistant conditions. Erythropoietin receptor (EPOR) expression in HepG2 cells was inhibited using an adenosine virus vector expressing an EPOR shRNA sequence. To silence SIRT1 or PPARγ gene expression, small interfering RNA (siRNA) against PPARγ or SIRT1 was transfected into HepG2 cells. The transcriptional activity of gene promoters was determined with a luciferase reporter assay system.

**Results:** EPO administration promoted the activation of the PI3K/AKT pathway and increased phosphorylation of FOXO1 and GSK3β; these effects were blocked by down-regulation of EPOR both in normal and PA-treated HepG2 cells. Moreover, PPARγ was found to mediate the beneficial effects of EPO on PI3K/AKT pathway, as both a PPARγ antagonist and PPARγ siRNA abrogated the EPO-induced increases in the protein levels of PI3K-p85α and p-AKT. Furthermore, the transcriptional activity of the human PI3K-p85α promoter was stimulated by EPO administration or by PPARγ overexpression in HepG2 cells. In addition, we found that SIRT1 expression was also sharply elevated by EPO, while SIRT1 knockdown attenuated the PPARγ-dependent activation of PI3K/AKT upon EPO exposure.

**Conclusions:** Collectively, these findings provide the first evidence that EPO stimulates the PI3K/AKT pathway through the up-regulation of PPARγ expression in liver cells, suggesting a role for EPO in regulating hepatic glucose metabolism and insulin sensitivity.

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**O819**  
**Effect of Mfn2 in palmitate-induced insulin resistance and lipid deposition in skeletal muscle cells**  
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**Objectives:** To establish an insulin resistance model in skeletal muscle cells induced by palmitic acid and to explore the effects of Jinlida on ameliorating insulin resistance and lipid deposition by examining the adenosine monophosphate-activated protein kinase (AMPK) signal pathway and mitochondrial function in skeletal muscle cells. 

**Methods:** Rat L6 myoblasts were cultured and induced in monolayers to the stage of myotubes. The cells were then randomly divided into two groups: normal control (NC) group and palmitic acid (PA) group, as a model of insulin resistance. The PA group was further divided into five subgroups: PA group, Jinlida low, middle, and high (0.75, 1.5 and 3.0 mg/mL) groups and pioglitazone (4.5 × 10⁻⁴ mg/mL) group. After 48 h of drug intervention, glucose uptake in each group was performed using the glucose oxidase–peroxidase method to evaluate insulin sensitivity; the concentrations of triglycerides (TG) and total cholesterol (TC) in cells were measured with commercial enzyme assay kits. The mRNA expression of AMPK, ACC, GLUT4, CPT1, NRF1, COXIV, ACADM, PPARα, PPARγ and PGC-1α in each group was detected using real-time polymerase chain reaction. The protein expression levels of AMPK, P-AMPK, ACC and P-ACC were assayed by Western blot.

**Results:** Glucose uptake decreased significantly in the PA group compared with the NC group, and glucose uptake increased significantly after intervention with Jinlida and pioglitazone. The concentrations of TG and TC in the PA group were significantly higher than in the NC group after the treatment with Jinlida and pioglitazone, and the concentrations of TG and TC were significantly lower than in the PA group. Protein and gene expression in the AMPK signalling pathway: the protein expression of P-AMPK, P-ACC, CPT1 and GLUT4 were down-regulated in the PA group in comparison with the NC group. Jinlida and pioglitazone significantly up-regulated the expression of P-AMPK, P-ACC, CPT1 and GLUT4. There were no significant differences among the groups in protein expression of AMPK and ACC. The mRNA expression of AMPK, ACC, GLUT4 and CPT1 were consistent with the protein expression. Mitochondrial gene and protein expression to assess mitochondrial function and fatty acid oxidation: the mRNA expressions of NRF1, COXIV, ACADM, PPARα, PPARγ and PGC-1α were notably decreased. Jinlida significantly increased the mRNA expression of COXIV, ACADM, PPARα, PPARγ and PGC-1α. The mRNA expressions of COXIV and NRF1 were not affected by pioglitazone, and the mRNA expression of NRF1 was not affected by pioglitazone. The protein expression of PGC-1α was consistent with the gene expression.

**Conclusions:** Jinlida can improve insulin resistance and lipid deposition in palmitate-cultured skeletal muscle cells. This phenomenon was most likely a result of activation of the AMPK pathway and improved mitochondrial function.

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**P147**  
**Effects of metformin on serum serotonin level and the relationship between serum serotonin level and diarrhoea after taking metformin**  
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**Objective:** To examine the effect of metformin on serotonin (5-HT) by observing serum 5-HT levels before and after taking metformin and by analysing the relationship between serum 5-HT levels and gastrointestinal reaction (diarrhoea) after taking metformin.

**Methods:** The sample group consisted of 41 healthy adults. After taking metformin, 19 adults presented diarrhoea (diarrhoea group), while the other 22 did not (non-diarrhoea group). Serum 5-HT levels before and after taking metformin were examined by high-performance liquid chromatography with UV detection.

**Results:** Serum 5-HT levels [mean (minimum, maximum); ng/mL] were 76 (8, 217), 102 (38, 218), and 96 (36, 248) at 0, 30, and 60 min before taking metformin, respectively. The 5-HT levels reached 68 (12, 218), 90 (10, 170), and 114 (44, 276) at 0, 30, and 60 min after taking metformin. Serum 5-HT levels reached a peak 30 min after a meal before taking metformin, and they showed a rising trend from 0 to 60 min after taking metformin while falling 30 min after taking metformin (P < 0.01). The serum 5-HT levels in the diarrhoea group were higher than those in the...
non-diarrhoea group 0 min after taking metformin ($P < 0.05$), and the increase in serum 5-HT levels in the diarrhoea group was also higher than in the non-diarrhoea group 0 and 30 min after taking metformin ($P < 0.05$). Logistic regression analysis showed that the decrease in 5-HT levels may be a protective factor in the non-diarrhoea group ($P < 0.05$).

Conclusion: Changes in 5-HT levels may lead to diarrhoea, and a decrease in 5-HT levels may be a protective factor against diarrhoea after taking metformin.

**P382**

Up-regulation of Sirtuin 5 contributes to the protection of mouse pancreatic beta cells against apoptosis induced by high fat and glucose

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**Background:** Sirtuin 5 (Sirt5), located in mitochondria, serves as a crucial regulatory factor in cell metabolism and intracellular signalling. Nevertheless, its function in pancreatic beta cells and in the cellular apoptosis process induced by fatty acid and glucose has not yet been systematically investigated.

**Methods:** Mouse pancreatic NIT-1 cells were transfected with GFP-Sirt5-Ad plasmid, and cell apoptosis was induced by a 13-h treatment with high fatty acid (palmitic acid, 0.05 mM) and high glucose (25 mM). Apoptosis was verified by TUNEL-DAPI double staining and a DNA ladder assay after optical microscopy. Glucose-stimulated insulin secretion was detected to evaluate the basic insulin-secretion functions of the treated and control groups, and cell lysates were collected for Western blotting, enzyme-linked immunosorbent assay (ELISA) and Caspase 3/9 activity assays to clarify the roles of mitochondrial Sirt5 in pancreatic beta cells and in their apoptosis signalling pathway.

**Results:** TUNEL-DAPI double staining and the DNA ladder assay revealed that Sirt5 overexpression could significantly reduce NIT-1 cell apoptosis in response to hyper-glucose and hyper-fatty acid. In accord with this result, cellular caspase activity in Sirt5-overexpressing cells was lower than in the control group. In addition to classic apoptotic protein factors, such as Bad and p53/phospho-p53, multi-ELISA analysis also revealed correlations in NIT-1 cells between Sirt5 and certain inflammatory factors, especially IκB-α, a result confirmed by Western blotting. Meanwhile, an insulin secretion assay demonstrated that Sirt5 overexpression could improve the capacity of beta cells to react against glucose fluctuations, suggesting a protective role of Sirt5 in beta cells in the presence of high fatty acid and glucose and suggesting its involvement in energy metabolism.

**Conclusions:** These primary findings reveal a novel role for Sirt5 in the regulation of NIT-1 cell apoptosis induced by high fatty acid and glucose. Up-regulation of Sirt5 expression might contribute to beta cell protection and functional improvement against abnormal glucose and fatty acid levels.

**P938**

Geniposide ameliorates hyperglycemia in obese diabetic mice by promoting β-cell survival and regeneration

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**Background:** The main goal of diabetes therapy is to prevent the loss and dysfunction of existing β-cells and to promote new β-cell formation. In our previous studies, TCF7L2, an important transcription factor of Wnt signalling, was demonstrated to have a role in β-cell turnover and regeneration. Geniposide, a natural active compound, was found to up-regulate TCF7L2 mRNA levels in a primary screen. In this study, the glucose-lowering activity of geniposide and its impacts on β-cell survival and regeneration were evaluated.

**Methods:** The metabolic benefits of geniposide were examined in high-fat diet (HFD)-induced diabetic mice and db/db mice. Mouse pancreatic sections were used to investigate β-cell regeneration by immunostaining. Isolated mouse islets and Min6 cells were used to explore the effects of geniposide on β-cell survival and elucidate its underlying mechanisms.

**Results:** Geniposide prevented diabetes progression and normalized blood glucose in HFD mice and db/db mice while also increasing serum insulin content and regulating cholesterol and adipokine levels. Increased β-cell proliferation and small islet-like cell clusters originating from ductal epithelium differentiation in vivo were observed in geniposide-treated diabetic mice. In cultured mouse islets, geniposide was found to enhance insulin secretion, increase β-cell proliferation and decrease β-cell apoptosis induced by diabetic stimuli. Furthermore, studies in islets and Min6 cells demonstrated that geniposide activated Wnt signalling by enhancing the expression of TCF7L2, inhibiting GSK-3β and promoting β-catenin nuclear translocation. Remarkably, the β-cell-protecting effects of geniposide could be blocked by ICG001, a β-catenin/TCF-mediated transcription inhibitor.

**Conclusions:** Our findings reveal a novel role for geniposide in promoting β-cell survival and regeneration by activating β-catenin/TCF7L2 signalling to maintain glucose homeostasis.

**P1464**

Vitamin B12 status in metformin treated patients: a systematic review

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**Background:** Randomized controlled trials and observational studies have yielded inconsistent results about the effects of metformin on vitamin B12 reduction. We therefore performed a systematic review to analyse the effects of metformin on vitamin B12 concentration.

**Methods:** PubMed, Medline, Embase, and the Cochrane central registry of controlled trials were searched to identify randomized controlled trials and observational studies exploring the association between metformin and vitamin B12 concentration in patients with type 2 diabetes mellitus or polycystic ovary
syndrome. The main outcome measure was changes in serum vitamin B12 concentration after 6–208 weeks of treatment with metformin, compared with placebo or other anti-hyperglycemic therapy.

Results: Six randomized controlled trials met the inclusion criteria. Serum vitamin B12 concentrations were significantly lower in patients treated with metformin than in those who received placebo or rosiglitazone [mean difference (MD), 2000 mg/day]. In two studies in which patients received a higher dose (>2000 mg/day), the corresponding MDs in vitamin B12 concentration after metformin treatment was 0.00001.

Conclusions: Reductions in serum vitamin B12 may be induced by metformin in a dose-dependent manner.

Basic and Clinical Studies on GLP-1

O296
Effects of incretin on artery lesions in a model of atherosclerosis in apolipoprotein E-deficient mice
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Objective: To investigate the effects of internal and exogenous incretin (DPP-4 inhibitor sitagliptin and glucagon-like peptide-1 receptor agonist exenatide) on aortic lesions in a model of atherosclerosis in apoE/C0 mice, a preliminary exploration of the incretin pathway mechanism was conducted using different drug interventions in vascular lesions.

Methods: ApoE/C0 mice were bred in an SPF environment. The mice were randomly divided into the normal diet group and the high-fat diet group. After 12 weeks, some of the mice were analysed randomly for HE staining of the aortic arch and to validate the model of atherosclerosis. After establishing a successful model, the mice were administered different drug (atorvastatin, exenatide and sitagliptin) interventions for 6 weeks. Next, we collected blood samples from the retro-orbital venous plexus of the mice after overnight fasting and blood glucose detection, and then the sampled mice were killed. Fasting serum insulin (FINS) and hypersensitive C reactive protein (hs-CRP) were identified by enzyme-linked immunosorbent assay, and the level of low-density lipoprotein cholesterol (LDL-C) was identified using a selective precipitation assay.

Results: Feeding with a high-fat diet for 12 weeks in the high-fat diet group revealed early plaque formation of AS but without the lipid core and thickening of the intima-media, as compared with the normal diet group. This result suggested that the model of AS was successful. After the drug interventions for 6 weeks, FINS levels in the exenatide high-dose group were significantly decreased compared with the other groups (P = 0.008, 0.005, 0.032, 0.021, 0.047, 0.017, all P < 0.05). The FINS levels in the exenatide low-dose group were decreased compared with the non-drug group (P = 0.046, P < 0.05). However, the insulin resistance index and insulin sensitivity index were not significantly different (P > 0.05). The levels of hs-CRP in the exenatide low/high-dose groups were significantly decreased compared with the sitagliptin low/high-dose groups, the normal diet group and the non-drug intervention group (P = 0.050, 0.011, 0.003, 0.004, P = 0.042, 0.003, 0.001, 0.001, all P < 0.05). The hs-CRP levels in the sitagliptin low/high-dose group showed a much greater decrease than those in the normal diet group and the non-drug intervention group, but this change was not significantly different (P > 0.05). The levels of LDL-C in the exenatide and sitagliptin groups and the normal diet group were lower than those in the non-drug intervention group and the atorvastatin group (all P < 0.05).

Conclusions: Under conditions with a similar degree of insulin functions, exenatide and sitagliptin could down-regulate the levels of hs-CRP and LDL-C in a model of atherosclerosis in apoE/C0 mice. Furthermore, exenatide was a much better option than sitagliptin because it decreased cardiovascular disease risk factors and reduced endothelial damage to protect the cardiovascular system.

O1348
Glucagon-like peptide-1 promotes adipocyte differentiation via the Wnt/β-catenin signalling pathway
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Background: The incretin hormone glucagon-like peptide-1 (GLP-1) has been previously found to regulate adipogenesis, while the relative importance of its many downstream branches has not been defined. This study aimed to investigate the involvement of the Wnt signalling pathway on the effect of GLP-1 on adipocyte differentiation.

Methods: 3T3-L1 preadipocytes were stimulated to differentiate in the presence or absence of GLP-1. The expression levels of adipogenic transcription factors and Wnts were measured by quantitative polymerase chain reaction using gene-specific primers and by Western blotting. Preadipocyte cell growth was evaluated using MTT and BrdU staining. Subcellular fractionation and immunofluorescence staining of β-catenin were performed to determine the translocation activity of β-catenin.

Results: Our study shows that GLP-1 stimulated adipocyte differentiation and lipid accumulation, which were accompanied by the expression of adipocyte marker genes. The expression level of Wnt4 increased after differentiation induction, and GLP-1 further enhanced this increment. To further explore the involvement of the Wnt pathway, the important mediator β-catenin was measured. Interestingly, induction of differentiation immediately increased the stability of β-catenin and thereafter mediated its translocation from cytoplasm to nucleus. In the presence of GLP-1, however, β-catenin was redirected to the cell membrane, leading to decreased accumulation in the nucleus.

Conclusion: Collectively, these findings demonstrate that GLP-1 acts on Wnt4 to specifically trigger β-catenin relocalization to the cytoplasmic membrane, thereby resulting in increased adipogenesis.
GLP-1 inhibits GSK-3β secretion in the process of adipogenic differentiation

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Background: Glucagon-like peptide-1 (GLP-1) has effects that include weight loss, inhibition of the central nervous system and altered gastric emptying rate, and it has almost no risk of hypoglycemia. Previous work showed that there is no GLP-1 receptor on the surface of adipocytes. However, it remains less clear whether GLP-1 plays a role in human adipogenesis. Glycogen synthase kinase3β (GSK-3β) is a key component of Wnt/β-catenin signalling, which can inhibit adipogenesis. In this study, we compared the effects of GLP-1 and human insulin on GSK-3β secretion, lipid synthesis and lipolysis during adipogenic differentiation.

Methods: We established cell models of primary cultured adipose-derived stem cells (ASCs) from human adipose tissue and then differentiated them into multiple cell-type lineages, including adipocytes, chondroblasts and osteoblasts. Adipogenic differentiation of the ASCs was induced in the presence of GLP-1 or human insulin at different concentrations (0, 0.1, 1, 10 and 100 nmol/L). Cell supernatants and lysates were collected before and 3, 9, 15 or 21 days after differentiation. We then separately measured GSK-3β secretion, glycerol and triglyceride concentrations in cell supernatants or lysates using an enzyme-linked immunosorbent assay kit.

Results: GLP-1 reduced the secretion of GSK-3β while promoting glycerol levels in a dose-dependent manner during human adipogenic differentiation. In contrast, insulin promoted the secretion of GSK-3β but reduced glycerol levels. GLP-1 did not influence the level of triglycerides during human adipogenic differentiation, while insulin could promote lipid synthesis.

Conclusions: GLP-1 and insulin have opposite effects on GSK-3β secretion and lipolysis during differentiation.

The effects of incretin on artery lesions in a model of atherosclerosis in apolipoprotein E-deficient mice

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Background: To investigate the effects of exenatide and sitagliptin on aortic lesions in a model of atherosclerosis in apoE−/− mice, and to conduct a preliminary exploration of their direct action and possible mechanisms.

Methods: ApoE−/− mice were randomized into groups, fed for 12 weeks and randomly tested with HE staining and oil red O staining. Next, they were administered different drug interventions for 6 weeks. Fasting serum insulin and the levels of hypersensitive C-reactive protein (hsCRP) and low-density lipoprotein cholesterol (LDL-C) were detected by enzyme-linked immunosorbent assay and selective precipitation assays. The protein expression levels of VCAM-1 and ICAM-1 in the aorta were studied by Western blot.

Results: Fed with a high-fat diet for 12 weeks, the high-fat diet group showed early plaque formation of atherosclerosis but had not yet formed the lipid core or the thickening intima-media thickness. Oil red O staining showed that the high-fat diet group had more plaques of atherosclerosis. The insulin and hsCRP levels of the exenatide high-dose group were significantly decreased (P < 0.05). LDL-C levels in both the exenatide and sitagliptin groups were lower than the non-drug intervention group and the atorvastatin group (P < 0.05). HE staining showed that the drug intervention groups widely showed pathological endometrium thickening. Western blots showed that high-dose exenatide could reduce VCAM-1 and ICAM-1 expression and that sitagliptin significantly down-regulated VCAM-1 expression and tended to down-regulate ICAM-1 expression (P < 0.05).

Conclusions: Exenatide and sitagliptin could inhibit the formation and development of atherosclerotic lesions in apoE−/− mice. This effect may partly be attributed to mitigation of the inflammatory response of atherosclerosis and may also be a result of comprehensive effects, such as reducing insulin resistance and lowering hsCRP and LDL-C levels.
A decrease in p-NF-κB (p-Ser276, p-Ser536) was observed in cells treated with exenatide or exenatide + BAY11-7082.

Conclusions: We show for the first time that exenatide can inhibit human VSMC calcification through NF-κB/RANKL signalling. This finding may elucidate new therapeutic targets in arterial calcification for GLP-1RA, which is currently being evaluated in clinical trials for the treatment of type 2 diabetes.

P1058
Protective effect of liraglutide against endoplasmic reticulum stress in the liver of high-fat diet-induced insulin-resistant rats
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Aim: To investigate whether the glucagon-like peptide-1 (GLP-1) analog liraglutide can alleviate endoplasmic reticulum (ER) stress and insulin resistance (IR) in the liver of high-fat diet-induced insulin-resistant rats.

Methods: Eighty-five male Sprague–Dawley rats were fed with normal chow or a high-fat (HF) diet for 12 weeks. The IR was evaluated using the hyperinsulinemic–euglycemic clamp technique. The rats in the HF group were further divided into four groups (HF, LG, MG and HG) and treated with or without liraglutide (0, 50, 100 and 200 μg/kg) by subcutaneous injection twice daily. Body weight (BW), fasting blood glucose (FBG), fasting insulin (FIN), insulin sensitivity were measured. Expression of the ER stress marker GRP78 and its signalling mediators, such as IRE1α, PERK and ATF6, in the liver were examined. The ultrastructure of the ER in the liver was examined by transmission electron microscopy. The expression levels of chemerin in the liver and serum were also measured.

Results: Fasting blood glucose, postprandial blood glucose or HbA1c fell dramatically from baseline to exenatide and insulin treatment (p < 0.05). There was no significant difference in fasting glucose, postprandial blood glucose or HbA1c between the two treatment groups. The mRNA expression levels of tumour necrosis factor-α, interleukin (IL)-1β and IL-6 fell to 45.6 ± 14.2%, 24.3 ± 18.4% and 48.8 ± 12.5% of baseline, respectively, after exenatide treatment (p < 0.05), and fell to 29.4 ± 17.6%, 18.6 ± 15.4% and 46.3 ± 11.8% after insulin treatment, respectively (p < 0.05 for all). The expression levels of TLR-2, JNK-1 and IKK were suppressed by 72.1 ± 8.7%, 60.1 ± 15.0% and 59.3 ± 15.6% (p < 0.05), respectively, and the expression of p47phox fell by 45 ± 20.6% (p < 0.05). There was a reduction of 64.5 ± 19.9% in TLR-2, and in IKKβ were suppressed by 72.1 ± 8.7%, 60.1 ± 15.0% and 59.3 ± 15.6% (p < 0.05), respectively, and the expression of p47phox fell by 45 ± 20.6% (p < 0.05) in the EXE group. There was a reduction of 64.5 ± 19.9% in TLR-2, 38.3 ± 10.9% in JNK-1, 61.4 ± 19.2% in IKKβ and 53.4 ± 10.1% in p47phox expression in the INS group (p < 0.05). There was no significant difference in the expression of cytokines, TLR-2, JNK-1 and IKKβ between the two groups after treatment.

Conclusions: Exenatide therapy has similar anti-inflammation effects as insulin in PBMCs of newly diagnosed and drug-naïve T2D patients.

Translational Medicine

O1286
Maternal and post-weaning high-fat diet modulates glucose homeostasis and hypothalamic POMC gene methylation in murine offspring
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Objective: To investigate the anti-inflammatory effects of exenatide versus insulin in the peripheral blood mononuclear cells (PBMCs) of newly diagnosed and drug-naïve type 2 diabetic (T2D) patients.

Methods: A total of 18 newly diagnosed and drug-naïve T2D patients were randomized to be treated with exenatide twice daily (EXE group, 5 μg × 4 weeks followed by 10 μg × 8 weeks; n = 9; mean age 45.1 ± 6.1 years; mean body mass index (BMI) 27.1 ± 3.7 kg/m²; mean glycosylated haemoglobin (HbA1c) 8.5 ± 0.9%) or a premixed insulin analogue twice daily (INS group; n = 9; mean age 48.8 ± 11.0 years; mean BMI 25.9 ± 3.0 kg/m²; mean HbA1c 8.3 ± 1.2%) for 12 weeks. PBMCs were collected, and the expression levels of cytokines, toll-like receptor (TLR)-2, c-Jun N-terminal kinase (JNK)-1, IκB kinase (IKK) β and p47 phox were investigated before and after treatment.

Results: Fasting and postprandial blood glucose and HbA1c fell dramatically from baseline to exenatide and insulin treatment (p < 0.05). There was no significant difference in fasting blood glucose, postprandial blood glucose or HbA1c between the two treatment groups. The mRNA expression levels of tumour necrosis factor-α, interleukin (IL)-1β and IL-6 fell to 45.6 ± 14.2%, 24.3 ± 18.4% and 48.8 ± 12.5% of baseline, respectively, after exenatide treatment (p < 0.05), and fell to 29.4 ± 17.6%, 18.6 ± 15.4% and 46.3 ± 11.8% after insulin treatment, respectively (p < 0.05 for all). The expression levels of TLR-2, JNK-1 and IKKβ were suppressed by 72.1 ± 8.7%, 60.1 ± 15.0% and 59.3 ± 15.6% (p < 0.05), respectively, and the expression of p47phox fell by 45 ± 20.6% (p < 0.05) in the EXE group. There was a reduction of 64.5 ± 19.9% in TLR-2, 38.3 ± 10.9% in JNK-1, 61.4 ± 19.2% in IKKβ and 53.4 ± 10.1% in p47phox expression in the INS group (p < 0.05). There was no significant difference in the expression of cytokines, TLR-2, JNK-1 and IKKβ between the two groups after treatment.

Conclusions: Exenatide therapy has similar anti-inflammation effects as insulin in PBMCs of newly diagnosed and drug-naïve T2D patients.
**Background:** Many epidemiological studies and animal experiments have demonstrated that imbalances in maternal nutrition can significantly increase susceptibility to developing metabolic disorders in adult offspring, and this effect can be further exacerbated by postweaning imbalanced nutrition. However, the underlying mechanism is not clearly understood. Furthermore, investigations of the effects of the interaction of maternal and postweaning diets on epigenetic modifications of hypothalamic genes during the later life of offspring are limited.

**Methods:** Using C57BL/6J mice, we examined the effects on male offspring of dams fed a high-fat (HF) diet (58 kcal/100 kcal) during pregnancy and lactation and weaned to a HF diet for 29 weeks, as compared with those fed a control (C) diet. Some metabolic parameters were detected. Next, DNA methylation and gene expression of the hypothalamic proopiomelanocortin (POMC) and melanocortin receptor 4 (Mc4r) genes were determined.

**Results:** There was no significant difference in body weight between C–C and HF–HF offspring. However, from 16 weeks of age, the body weight of HF–HF offspring was significantly higher until 32 weeks of age compared with the C–C offspring ($P < 0.001$). The blood glucose levels of the male offspring of HF dams weaned to a HF diet were significantly higher at 30 ($P < 0.001$), 60 ($P < 0.001$) and 120 min ($P < 0.001$) after intraperitoneal glucose administration compared with those in the C–C group. Furthermore, the area under the curve in the HF–HF group was significantly larger compared with the C–C group ($P < 0.001$). To evaluate the insulin sensitivity of the offspring, serum levels of fasting blood glucose and insulin were determined. There was no significant difference in serum insulin levels between the two groups. However, fasting blood glucose and HOMA-IR were significantly higher in offspring in the HF–HF group than in the C–C group at 32 weeks of age ($P < 0.05$). Serum leptin levels were also significantly higher in HF–HF offspring. We observed that serum triacylglycerol and total cholesterol were significantly elevated in the offspring of dams in the HF–HF group ($P < 0.001$). Histological examination of the liver revealed that offspring in the control group had a normal liver structure at weaning. However, lipid vacuoles of various sizes were observed within hepatocytes of HF–HF offspring at 32 weeks of age. Both POMC and Mc4r were significantly up-regulated in offspring exposed to a HF diet during gestation, lactation and at 32 weeks of age ($P < 0.05$). Hypomethylation of the POMC promoter in the hypothalamus occurred in HF diet offspring ($P < 0.05$). However, no change was detected in the Mc4r promoter. Furthermore, POMC-specific methylation was negatively associated with the response to glucose load ($r = -0.273$, $P = 0.03$).

**Conclusions:** A maternal and post-weaning high-fat diet predisposes offspring to obesity, glucose intolerance and insulin resistance in later life. It also induces hypomethylation and increased expression of the POMC gene in the hypothalamus. This work is novel because it demonstrates the epigenetic plasticity of the hypothalamus between a maternal and post-weaning imbalanced diet and glucose homeostasis.

**O1477**

**Insulin regimes and impact on glycemic control in type 1 diabetes in the Guangdong province**

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**Background:** Insulin regimens are believed to affect glycemic control, but the relationship is controversial. The aim of this study was to describe how insulin regimens impact glycemic control and to explore factors associated with intensive insulin therapy.

**Methods:** This was a cross-sectional study of type 1 diabetes (T1D) patients recruited from 16 centres in Guangdong Province. Demographic and clinical data were collected. The patients were grouped according to insulin regimens: insulin pump (R1), basal insulin plus regular insulin or short-acting insulin (R2), premix insulin 2–3 times per day (R3), and other therapies (included basal insulin plus premix insulin or preprandial insulin 1–3 times per day or basal insulin 1–2 times per day; R4). The study had three aspects: (1) distribution of insulin regimens, (2) relationship between insulin regimens and haemoglobin A1c (HbA1c), and (3) logistic regression of factors identified to be associated with intensive insulin therapy.

**Results:** T1D patients ($N = 1529$) were recruited [704 males; median age of 27.0 (extreme value 0.27–86.4) years; median duration of 2.9 (extreme value of 0–41.6) years]. R1 (206 cases, 13.5%), R2 (609 cases, 39.8%), R3 (686 cases, 44.9%), and R4 (28 cases, 1.8%) were assessed. Median (quartile range) HbA1c were 8.1% (6.8, 9.9), 8.8% (7.3, 11.8), 9.4% (7.8, 11.8), and 8.9% (7.1, 12.6) in R1, 2, 3, and 4, respectively. After adjusting for age, sex, duration, self-monitoring of blood glucose, and household income, HbA1c was associated with insulin regimens ($p = 0.00$). The HbA1c target rate (2014 American Diabetes Association HbA1c targets: <7.5% for less than 18 years, <7.0% for adults) was 13.6% for age 18 years (354 cases) and 17.8% in adults (1175 cases). The HbA1c target rate was 43.3%, 31.5%, 15.0%, and 19.2% in R1, 2, 3, and 4, respectively ($p = 0.00$). Mother's education level (college or above) was a protective factor for intensive insulin therapy for T1D patients aged <18 years [odds ratio (OR) 3.22, $p = 0.00$]. Female gender (OR 1.36, $p = 0.01$), older age (OR 1.02, $p = 0.00$), and education level (college or above) were protective factors for intensive insulin therapy in adults.

**Conclusions:** The results suggested that intensive insulin therapy was insufficient in T1D patients and was associated with a reduced HbA1c. The increased percentage of intensive insulin therapy and enhanced diabetes education may facilitate glycemic control.
Aims: To observe the effect of caloric restriction (CR) on C57/BL mice and to identify the molecular mechanism underlying the improved spatial learning ability of C57/BL mice on a CR diet.

Methods: Male seven-week-old C57/BL mice were randomly divided into three groups: normal control group (NC group), high-energy group and low-energy group (CR group). We measured the expression of brain insulin signalling pathway-related proteins in the brain after 30 weeks.

Results: Compared with the NC group, body weight and serum glucose decreased in the CR group and increased in the high-energy group at all time points assessed in the study. Average escape latency and swimming distance were lower in the CR group than that in the control group after 30 weeks. The expression levels of IGF-1 (13.00 ± 5.78 vs 24.98 ± 7.92), PI3K (11.30 ± 4.37 vs 24.97 ± 7.20), Akt/PKB (11.94 ± 22.37 vs 22.59 ± 7.61) and p-CREB protein (11.30 ± 3.91 vs 22.59 ± 8.17) in the CR group were significantly lower, and the expression levels of IRS-1 (12.33 ± 2.77 vs 24.98 ± 7.92), PI3K (11.30 ± 4.37 vs 24.97 ± 7.20), Akt/PKB (11.94 ± 22.37 vs 22.59 ± 7.61) and p-CREB protein (11.30 ± 3.91 vs 22.59 ± 8.17) in the CR group were significantly lower, and the expression levels of P53 (16.75 ± 4.21 vs 24.97 ± 7.20) and Akt/PKB (17.33 ± 3.36 vs 22.37 ± 7.61) protein in the high-energy group were significantly lower than those in the control group after 30 weeks.

Conclusions: Our findings demonstrate that the CR diet improves learning and memory ability in C57/BL mice, possibly by regulating the insulin-PI3K/Akt signalling pathway.

Serological profiling of autoantibodies against Chromogranin A in type 1 diabetes mellitus patients by peptide microarray

Background: Early studies have identified type 1 diabetes mellitus (T1DM) as an autoimmune disease caused by autoimmune destruction of pancreatic β-cells. The seroreactivity of T1DM patients to islet cells has revealed the existence of autoantigen and autoantibodies in serum samples of T1DM patients. Until now, autoantibodies against GAD65, insulin, IA-2 and ZnT8 in T1DM have been used as biomarkers to identify T1DM risk and diagnosis of T1DM. A previous study has identified Chromogranin A (ChgA) as an autoantigen in a non-obese diabetic mouse model of T1DM. Peptide WE14 from ChgA (ChgA358–371) has also been identified as the antigen for highly diabeticogenic CD4+ T-cell clones. ChgA is a neuroendocrine secretory protein in the granin family. To our knowledge, no studies have profiled the autoantibodies against ChgA in T1DM patients. Here, we report the results of ChgA autoantibody profiling and B-cell antigenic epitopes of ChgA identified in T1DM patients.

Methods: Autoantibodies against ChgA were profiled by peptide microarray. The peptide microarray was printed on SJ membrane, which has excellent anti-NPA capabilities. The peptide microarray containing peptides from the ChgA sequence was screened using 180 serum samples from T1DM patients and 260 serum samples from healthy controls for the first-round screening. After peptides containing epitope were defined, a second round of screening was performed to identify the precise epitope sequence.

Results: Eight linear B-cell epitopes of ChgA were identified in T1DM patients, including K46-F52, K88-K96 and R331-M345. These epitopes were consistent with the theoretical predicted epitope by IEDB and a previous study that was conducted using polyclonal antibodies against ChgA. Among these eight linear B-cell epitopes identified using our platform, only two epitopes were identified in T1DM patients but not with polyclonal antibodies against ChgA. These two epitopes might be related to the pathogenesis in T1DM in which ChgA is recognized as an autoantigen and presented, thus playing an important role in the immune response in T1DM.

Conclusions: We profiled autoantibodies against ChgA and identified for the first time eight linear B-cell epitopes of ChgA in T1DM patients. These eight linear B-cell epitopes of ChgA can be used as biomarkers to diagnose T1DM. Two epitopes that were only found in T1DM patients also can provide new insight into the pathophysiology of T1DM.
Diabetes Education and Management, Behavioral Medicine and Exercise

O236

The effect and function of SMS follow-up in the cognitive-behavioural management of the treatment of type 2 diabetes

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Objectives: To evaluate the effect of SMS follow-up on patients undergoing diabetes treatment and the effect of cognitive–behaviour management of patients by SMS.

Methods: Section 1: Divide 100 type 2 diabetic patients into a mobile message follow-up group (50 cases) and normal outpatient follow-up group (50 cases). Communication by SMS was conducted once a week and was continued in the normal outpatient group for 12 weeks once a month. The SMS content is basic knowledge of diabetes and DSME proper exercise, diet, regular continuation of medication, hypoglycemia prevention and emergency treatment. The changes in indicators such as haemoglobin A1c (HbA1c), blood glucose, blood pressure, serum lipid profile and body weight were compared. Section 2: Divide 120 patients with type 2 diabetes into a mobile message follow-up group (60 cases) and normal outpatient follow-up group (60 cases). The research doctor or nurse contacted the patient by SMS weekly and another group once every month for 12 weeks. In addition, a questionnaire was used to survey knowledge of diabetestes, psychological conditions, cognitive behaviour and life satisfaction before and after the study.

Results: Section 1: There was a significant decline post-treatment in HbA1c, fasting and postprandial blood glucose, blood pressure, the serum lipid profile and body weight, among which the changes in haemoglobin A1c and fasting and postprandial blood glucose were comparable between the SMS and routine groups (P = 0.052). The changes in blood pressure and body weight were significantly higher in the SMS group compared with the routine group (p = 0.030 and 0.032, respectively). Section 2: After the 12-week follow-up, HbA1c decreased by 1.9% in the SMS group and by 1.2% in the control group (P = 0.016). Weight was lower in the SMS group than in the control group (71.2 kg ± 6.7 vs 73.1 kg ± 7.3, P = 0.030). ADDQoL and treatment satisfaction scores were all improved compared with baseline in the SMS group (−45.3 ± 14.6 vs −59.9 ± 12.1; 17.9 ± 2.9 vs 29.9 ± 1.7, respectively, P < 0.05). Similarly, diabetes knowledge and psychological conditions improved (P < 0.05). In addition, the increase in exercise per week (4.6 h ± 1.3 vs 3.8 h ± 1.9, P = 0.025) and reduced smoking (8.0 ± 4.7 vs 10 ± 5.4, P = 0.037) were all improved compared with the control group.

Conclusions: Diabetes management by SMS is effective not only for blood glucose and HbA1c levels but also for blood pressure and body weight control. The cognitive–behaviour intervention management of diabetes patients by SMS follow-up could improve knowledge, promote behaviour change, enhance self-management abilities and behavioural strategies to cope with negative emotions, and contribute to the improvement of treatment satisfaction and quality of life.

O672

Lipodystrophy prevalence and effects in diabetic patients with subcutaneous insulin injections in an outpatient department

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Objective: To discover the prevalence and effects of lipohyperplasia and lipoatrophy in diabetic patients receiving subcutaneous insulin injections in the outpatient department at Peking University.

Methods: Individuals aged between 18 and 85 years and diagnosed with diabetes (type 1 or type 2) for at least 1 year were enrolled in the study. A number of background questions were completed for each subject. Background questions include demographic feature and diabetes history. Additionally, height and weight will be measured for each subject. Each subject will undergo a physical examination at the body site commonly used for subcutaneous insulin injection.

Results: In total, 283 subjects were enrolled in the current study. The percentage of males was 45.0%. The mean age was 62.3 ± 11.5 years, the body mass index was 24.9 ± 3.2 kg/m², and the course of diabetes was 12.6 ± 7.4 years. The percentage of patients who reused needles was 90.0%. The percentage of lipohyperplasia among all patients was 14.5%, and the percentage of lipoatrophy was 0%. The frequency of needle use and years of insulin use were important effect factors in lipohyperplasia, as shown in Tables 1 and 2.

Conclusions: The percentage of needle reuse was high in an outpatient department. When the frequency of needle use was higher or the years of insulin use was longer, the risk of lipohyperplasia increased.
Clinical Diabetes/Therapeutics

Table 1. Frequency of needle use affects the lipohyperplasia risk

<table>
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Table 2. Years of insulin use affects the lipohyperplasia risk

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O962
Influence of exercise intervention on body mass index, blood glucose and blood lipid levels in obesity and newly diagnosed patients with type 2 diabetes mellitus
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Objective: To investigate the influence of exercise intervention on body mass index (BMI), blood glucose and blood lipid levels in obesity and newly diagnosed patients with type 2 diabetes mellitus (T2DM).

Methods: Fifty-six obesity and newly diagnosed patients with T2DM were randomly divided into two groups: 30 cases in the observation group and 26 cases in the control group, after 12 weeks of treatment to evaluate BMI, blood glucose, haemoglobin A1c (HbA1c) and blood lipids.

Results: Compared with baseline, BMI, blood glucose, HbA1c, and blood lipid levels were significantly improved in the observation group after 12 weeks of treatment, while in the control group, only a decrease in BMI and blood glucose was observed (P < 0.05). The BMI, blood glucose, HbA1c and blood lipid levels were significantly improved in the observation group compared with the control group (P < 0.05).

Conclusions: Exercise intervention can effectively control BMI, blood glucose and blood lipid levels in obesity and newly diagnosed patients with T2DM.

O1002
Effect of peer support intervention on diabetes distress: a pilot for a randomized controlled trial
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Background: In China, 113.9 million people suffer from diabetes. Previous studies have revealed that a high percentage of diabetic patients are affected by distress. The current focus of most diabetes education is improvement of the metabolic index. Research investigating the impact of psychological factors on the treatment of diabetes is lacking. This study aimed to compare the...
effectiveness of a diabetes peer support programme with conventional education in reducing diabetes distress and improving glycemic control among Chinese patients with type 2 diabetes.

Methods: This study is a cluster randomized controlled trial. Four hundred patients with type 2 diabetes from eight communities were randomized to receive conventional education or conventional education and peer support based on each community in Xuanwu distict Nanjing. Patients who received conventional education spent 2 h on diabetes education every 2 months. We chose the peers based on residence, demographics and other characteristics. Every 5 to 15 patients formed a group with 1–2 peer leader(s). Peer leaders conducted activities with the help of a community health centre or medical volunteers. The activities included theme and non-theme activities. The activities covered diabetes education and skills in medical care at least once a month. Non-theme activities included inter-peer communication through home visits, telephone and E-mail, among others. Diabetic distress survey and biomedical measures of all participants were collected at baseline, 6 and 12 months.

Results: All 400 diabetic patients participated fully in our research programme. Total scores of diabetes distress and in each domain were lower in the peer support group at 6 and 12 months compared with the control group. Differences in regimen-related distress and total scores between the two groups were statistically significant at 6 months. Differences in Emotional burden, Physician-related distress and total scores between the two groups were statistically significant at 12 months. Emotional burden, Physician-related distress and total scores in the peer support group continuously decreased in the three surveys. The levels of fasting blood glucose and postprandial blood glucose in the peer support group were lower than in the control group at 6 and 12 months.

Conclusions: Compared with conventional education, peer support is superior for reducing diabetes distress, improving glycemic control and providing long-term health education support.

O1011
Wishes and needs for diabetes care in employees with type 2 diabetes in Chinese corporations: a questionnaire survey
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Objective: To assess the wishes and needs regarding diabetes care in patients with type 2 diabetes.

Methods: A total of 120 patients with type 2 diabetes from the employees of different Chinese corporations who were admitted to the Department of Endocrinology of Zhongda Hospital between February and May 2014 were enrolled in this survey. They were asked to complete the questionnaire of their wishes and needs for diabetes care regarding education, self-care and follow-up.

Results: All of the participants returned the questionnaire forms. Up to 81.67% of these patients showed a great desire for diabetes care. Among the wishes and needs, the most required diabetes care was diet education (85.70%), monitoring frequency and optimal goals of some markers related to diabetes (87.5%), diabetes self-care (86.67%) and exercise education (81.67%). In addition, 85.83% wished that diabetes educators were specialized physicians. The most widely accepted tools for follow-up were telephone (37.50%) and Internet (23.33%), while the best time for doing this was the night of workdays or the daytime of weekends (65.83%).

Conclusions: The survey suggested that employees with type 2 diabetes primarily preferred to receive diabetes education from specialized physicians. Healthcare providers need to consider these needs when providing diabetes care.

Blood Glucose Monitoring
O514
Changes in infants of diabetic mothers and healthy mothers in macrosomia blood glucose, insulin and cortisol levels
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Background: Gestational diabetes mellitus (GDM) refers to glucose intolerance that is first discovered during pregnancy. Pre-pregnancy overweight or obese status and excessive weight gain during pregnancy are risk factors for the occurrence of GDM. Newborns of mothers with GDM have an elevated risk of hypoglycemia, macrosomia, birth trauma, hyperbilirubinemia, respiratory distress syndrome, congenital malformation and a series of other complications. The live birth weight of newborns with macrosomia is >4000 g after birth, and these newborns also present hypoglycemia, birth trauma, asphyxia and neonatal complications like GDM. By detecting blood glucose, serum insulin, and cortisol levels and macrosomia upon delivery of newborns of GDM and healthy pregnant women after birth, changes in the endocrine system of the two groups will be determined to prevent the occurrence of GDM, reduce the future development of hypoglycemia and hyperinsulinemia, and provide clinical data.

Methods: Fifty-two cases of pregnant women with GDM with hospitalized neonates born at the same time as the newborns of 41 healthy pregnant women with fetal macrosomia (birth weight >4000 g), termed the macrosomia group, and 48 healthy newborns of healthy parturient women (birth weight ≥2500 g) and <000 g) as the control group. Age, height, body weight before pregnancy, birth weight, gestational weeks, and pre-pregnancy body mass index (BMI) were recorded in the three groups. All of the subjects were measured immediately after birth for birth weight and within 30 min after birth for peripheral blood glucose measured by the micro method. Within 2–48 h after the collection of upper limb or scalp vein blood (2 mL, collected at 7:30–8:00), centrifuged serum was assessed for insulin and cortisol content using the electrochemical luminescence method.

Results: Compared with the control group, the GDM and macrosomia newborn blood glucose were significantly decreased, and insulin and cortisol serum levels were significantly

DOI: 10.1002/dmrr
increased ($P < 0.05$). Compared with the macrosomia group, trace blood glucose in the GDM group was significantly decreased, and the serum levels of glucose and insulin were significantly increased ($P < 0.01$). Compared with the non-macrosomia GDM group, trace blood glucose and serum insulin levels were significantly increased in the macrosomia group ($P < 0.05$).

Group 4. In the GDM and macrosomia newborn groups, blood glucose and birth weight were negatively correlated ($r_1 = –0.413, r_2 = –0.564, P < 0.01$) and birth weight and insulin levels were positively correlated ($r_3 = 0.413, r_4 = 0.708, P < 0.05$).

Conclusions: The GDM and macrosomia groups showed a greater proportion of increased maternal pre-pregnancy BMI and weight gain during pregnancy. Newborns of mothers with GDM and those with a large size were susceptible to hypoglycemia and elevated insulin and cortisol serum levels. Newborns of mothers with GDM were prone to hypoglycemia and hyperinsulinemia, and newborns of mothers with GDM and those of healthy mothers with macrosomia had higher serum insulin levels and birth weights, lower blood glucose levels and were more susceptible to hypoglycemia. Thus, maintaining a normal body weight before pregnancy and during pregnancy prevents the occurrence of GDM and future neonatal hypoglycemia and hyperinsulinemia.

**O1487**

Glycemic variability predicts cardiovascular complications in acute myocardial infarction patients with type 2 diabetes mellitus in the coronary care unit care unit: results of a 1-year follow-up

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**Objectives:** To describe the results of a continuous glucose monitor system (CGMS) for type 2 diabetic patients with acute myocardial infarction (AMI) and to investigate the relationship between glucose variability and major adverse cardiac events or some predictive biomarkers.

**Methods:** All of the patients in the CCU at Zhongshan hospital affiliated with Fudan University from Oct 2011 to Apr 2012 were enrolled. Inclusion criteria were as follows: (1) meeting the diagnostic criteria for AMI with a time of symptom onset within 24 h regardless of whether revascularization was performed and (2) having been diagnosed with DM before admission or with a haemoglobin A1c (HbA1c) greater than 6.5% without a known diabetes history. All of the patients were evaluated with a retrospective CGMS. General conditions, laboratory results, adverse events during the CCU stay and 1 year after the attack, ultrasonic cardiogram examination results, medicine taken and coronary artery angiography were recorded. The relationship between data from the CGMS results and adverse events or predictive biomarkers were investigated. A survival curve was used to analyse the relationship between major adverse cardiac events and the mean amplitude of glycemic excursions (MAGE).

**Results:** Thirty-four patients with type 2 diabetes were enrolled in the study (5 females and 29 males) aged 64 ± 12 years. Twenty-eight patients had a known history of diabetes, and 6 patients were diagnosed based on a HbA1c greater than 6.5% without a known diabetes history. During the CCU stay, one patient had acute heart failure, two patients had hypotension, and one patient had aggravated chest pain. At the 1-year follow-up, there were four deaths, one reinfarction and one acute discompensation heart failure. The mean glucose level during the CCU stay was 9.99 ± 2.5 mmol/L, the total time with a glucose level greater than 10.0 mmol/L in 24 h was 11.1 h (47.05%), and the total time with a glucose level lower than 3.9 mmol/L was 0.267 h (1.23%). The MAGE was 6.49 ± 2.48 mmol/L. Grouped by MAGE with cut-off points at 6.05 mmol/L, there were no significant differences between a low MAGE (less than 6.05 mmol/L) and a high MAGE (equal to or more than 6.05 mmol/L) in terms of age, height, weight, C reactive protein (CRP) level at onset, glycated albumin level, cardiac troponin level, b-type natriuretic peptide or left ventricular ejection fraction. However, there were significant differences between the two groups in terms of the change in CRP over 72 h [high MAGE group vs low MAGE group, 23.47 (−4.205 – 38.924) vs −8.12 (−35.38 – 13.150)]. During the CCU stay, there was an increasing trend toward the risk of major adverse cardiac events but with no significant differences (high MAGE group vs low MAGE group, 1/19 vs 0/15). At the 1-year follow-up, the risk of MACE in the high MAGE group was significantly higher than that in the low MAGE group (6/19 vs 0/15, $P < 0.05$ by Kaplan–Meier analysis).

**Conclusions:** Acute infarction patients with type 2 diabetes during CCU commonly have poor glycemic control and high MAGE. MAGE is associated with major adverse cardiac events in one year.

**P528**

High prevalence of gestational diabetes in polycystic ovary syndrome-assisted reproduction

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**Background:** Polycystic ovary syndrome (PCOS) is associated with an increased risk for recurrent spontaneous abortions and for gestational diabetes (GDM). It is also associated with hyperinsulinemia, insulin resistance and pregnancy rates as low as 8%. Recent studies have shown that in addition to the aforementioned risk factors, singleton and twin pregnancies resulting from assisted reproductive techniques (ART) have been associated with increased risk of GDM. Up-to-date studies that describe the frequency of GD or impaired glucose tolerance in women undergoing assisted reproduction techniques (ART), especially those who suffer from PCOS, are lacking. To avoid miscarriage due to pathological glucose tolerance in women at risk for GD, we conducted oral glucose tolerance test as soon as pregnancy was confirmed in ART patients.

**Methods:** This cross-sectional study evaluated the medical records of 230 women who conceived spontaneously and...
258 women who conceived following ART. For better comparison of the incidence of GDM, the ART group was further subdivided into (i) an in-vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) group (n = 200) and (ii) an intra-uterine insemination (IUI) group (n = 58). The diagnosis of GDM was based on the criteria of the American Diabetes Association.

Results: The incidence of GDM was significantly higher in the IVF/ICSI and IUI groups (43% and 26%, respectively) than in the spontaneous pregnancy group (10%). Age, pre-pregnancy body mass index (BMI) and weight gain in pregnancy were similar among women with GDM in all three groups. Logistic regression analysis demonstrated four strong risk factors for GDM: age, BMI, mode of ART and progesterone use during pregnancy.

Conclusions: This study indicates that the risk of GDM is twofold higher in women with singleton pregnancies conceived following ART compared with women who conceived spontaneously. In addition, progesterone use during pregnancy was found to be an important risk factor for GDM. This subject warrants further study.

P1232
The longitudinal trend of fasting plasma glucose is associated with retinal microvasculature in persons without established diabetes

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Background: Retinal vascular abnormalities are considered as early signs of systemic vascular damage. We examined the associations of the 5-year mean level, longitudinal trend and fluctuation in fasting plasma glucose (FPG) with retinal vascular calibre in persons without established diabetes.

Methods: A prospective study was conducted in a cohort of Chinese persons aged ≥40 years in Guangzhou, southern China. FPG was measured at baseline in 2008 and annually until 2012. In 2012, retinal vascular calibre was assessed using standard fundus photographs and validated software. Baseline non-diabetic participants with FPG measured at baseline and follow-up data on FPG for three or more annual visits were included for statistical analysis. The associations of retinal vascular calibre with 5-year mean FPG level, the longitudinal FPG trend (slope of linear regression-FPG) and fluctuation (standard deviation and root mean square error of FPG) were analysed using multivariable linear regression analyses.

Results: A total of 3645 non-diabetic participants were included in the analysis. Multivariable linear regression models adjusted for baseline FPG and other potential confounders showed that a 10% annual increase in FPG was independently associated with a 1.92-μm narrowing in retinal arterioles (P = 0.022) and a 3.27-μm widening in venules (P = 0.001). Associations with mean FPG level and fluctuation were not statistically significant.

Conclusion: An annual rising trend in FPG, but not its mean level or fluctuation, is associated with altered retinal vasculature, even in persons without established diabetes.

Primary Care

O216
The effect of PKC on the mechanism underlying calcium signal-regulated GLUT4 translocation in skeletal muscle cells

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Background: The Ca2+ ionophore ionomycin increases the intracellular Ca2+ concentration of skeletal muscle cells. The elevation of Ca2+ mediates exercise/contraction-stimulated glucose uptake in muscle cells. To explore the signalling mechanism, we investigated the role of conventional PKC (cPKC) and novel PKC (nPKC) in the mechanism of Ca2+ signal-regulated translocation of glucose transporter 4 (GLUT4) in rat L6 muscle cells stably expressing GLUT4 myc.

Methods: L6-GLUT4 myc myoblasts were seeded in 24-well plates and divided into basal + DMSO, basal + Gö6983, basal + Gö6976, basal + Gö69976, ionomycin + DMSO, ionomycin + Gö6983, and ionomycin + Gö6976 groups. The cell surface GLUT4 myc levels and endocytosis and exocytosis of GLUT4 myc were measured by enzyme-linked immunosorbent assay. The phosphorylation of PKC substrate and Akt was detected by Western blot analysis to explore the roles of cPKCs and nPKCs in ionomycin-stimulated GLUT4 myc translocation.

Results: Ionomycin markedly increased cell surface GLUT4 myc levels (P < 0.001) and phosphorylation of PKC substrate but not Akt in L6-GLUT4 myc myoblasts. Both the conventional and novel PKC inhibitor Gö6983 and conventional PKC inhibitor Gö6976 reduced the ionomycin-induced gain of GLUT4 myc on the cell surface (P < 0.01) and lowered ionomycin-stimulated phosphorylation of PKC substrate. Ionomycin stimulated GLUT4 myc exocytosis and inhibited its endocytosis in live cells. Both Gö6983 and Gö6976 prevented ionomycin-reduced GLUT4 myc endocytosis (P < 0.01, P < 0.05, respectively) and reversed ionomycin-enhanced GLUT4 myc exocytosis (P < 0.001, P < 0.05, respectively).

Conclusions: Ionomycin stimulates GLUT4 exocytosis and inhibits its endocytosis to contribute to the ionomycin-induced gain of GLUT4 on the muscle cell surface. cPKC mediates ionomycin-regulated GLUT4 endocytosis and exocytosis in L6-GLUT4 myc myoblasts.

P405
Serum 25-hydroxyvitamin D levels are associated with carotid atherosclerosis in normotensive and euglycemic Chinese postmenopausal women: the Shanghai Changfeng Study

HUI MA1, HUANDONG LIN1, YU HU1, XIAOMING LI1, WANYUAN HE1, XUEJUAN JIN1, JIAN GAO2, NAIQING ZHAO2, ZHENQI LIU3, XIN GAO1

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DOI: 10.1002/dmrr
Background: The role of serum 25-hydroxyvitamin D (25(OH)D) in atherogenesis is unclear. We investigated whether 25(OH)D is independently associated with carotid intima-media thickness (CIMT) and carotid plaque in normotensive and euglycemic postmenopausal women.

Methods: A total of 671 normotensive and euglycemic postmenopausal women (mean age, 58.8 years) were enrolled from the Changfeng Study. A standard interview, anthropometric measurements and laboratory analyses were performed for each participant. Bilateral CIMTs were measured using ultrasonography, and the presence of carotid plaques was assessed. Serum 25(OH)D was measured using an electrochemiluminescence immunoassay.

Results: Serum 25(OH)D was 43.6 ± 18.2 nmol/L in the postmenopausal women. Multivariate linear stepwise regression analysis demonstrated that parathyroid hormone (PTH) (standardized β = -0.334, P < 0.001), body mass index (BMI) (standardized β = -0.141, P = 0.001) and 2-h glucose levels following a 75-g oral glucose challenge (postprandial blood glucose) (standardized β = -0.053, P = 0.003) were independently associated with serum 25(OH)D. Compared with subjects whose 25(OH)D levels fell into the first, second and third quartiles, subjects with 25(OH)D in the fourth quartile had decreased CIMT and prevalence of carotid plaque. After adjusting for conventional CVD risk factors, PTH, liver and renal function, postmenopausal women with 25(OH)D levels in the fourth quartile had a 0.421-fold decreased risk of carotid plaques relative to those in the lowest quartile.

Conclusions: These results suggest that 25(OH)D has a relatively strong negative correlation with carotid atherosclerosis, even after adjusting for conventional CVD risk factors, PTH, liver and renal function in postmenopausal women with normal blood glucose levels and normal blood pressure. Our findings suggest that individuals with decreased 25(OH)D require aggressive management of CVD risk factors.

Public Health/Health Economics

O1338
Metformin affects the characteristics of HepG2 cells by regulating macrophage polarization in a microenvironment
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Background: There is a close link between diabetes and cancer, and inflammation is one of the key associated pathological processes. In addition to its hypoglycemic activity, metformin has been known to have anti-cancer effects; however, the mechanism is not clear. This study investigated whether metformin would regulate the microenvironment of macrophage polarization to inhibit HepG2 cell proliferation and migration, and the possible role of the Notch signalling pathway.

Methods: First, RAW264.7 macrophages were treated with metformin, and the expression of M1/M2 signature genes and Notch1–4 receptor mRNA was analysed by quantitative polymerase chain reaction (qPCR) to determine whether metformin could regulate macrophage polarization and affect activation of the Notch signalling pathway. Next, metformin-treated RAW264.7 macrophages were co-cultured with HepG2 cells. The polarization of RAW264.7 macrophages under co-culture conditions with/without metformin was analysed by qPCR. HepG2 cell proliferation and migration were detected by MTS and cell scratch assay, respectively.

Results: In the single RAW264.7 macrophage culture, metformin decreased the expression of M1 signature genes (IL-1β and IL-6) but greatly increased the expression of M2 signature genes (Arg1, IL-10 and IL-4). In addition, the levels of Notch 1 and Notch 4 were significantly decreased. Co-culture with RAW264.7 macrophages promoted the proliferation of HepG2 cells, but this effect was suppressed in metformin-treated RAW264.7 macrophages in a time-dependent manner (with the most significant effects observed at 72 h). The cell scratch assay showed that HepG2 cell migration was enhanced in the co-culture group compared with single culture. At 60 h, the scratch of the co-culture group healed completely, demonstrating faster healing compared with the co-culture group with metformin and single culture group with/without metformin. There were significant changes in cytokine expression in RAW264.7 macrophages in the co-culture microenvironment. Compared with the co-culture group without metformin, metformin significantly up-regulated M1 signature gene expression [interleukin (IL)-1β, IL-6, nuclear factor-κB and tumour necrosis factor-α] but greatly down-regulated M2 signature gene expression (IL-10, IL-4, Mgl1 and Mrc1) in RAW264.7 macrophages co-cultured with HepG2 cells.

Conclusions: Metformin skews single-culture RAW264.7 macrophages toward an M2 phenotype and down-regulates activation of the Notch signalling pathway. Metformin can reverse the M2 phenotype of RAW264.7 macrophages co-cultured with HepG2 cells. In the co-culture microenvironment, metformin suppresses HepG2 cell proliferation and migration in a M1 polarization-dependent manner that promotes RAW264.7 macrophage secretion of Th1 cytokines.

Diabetes Standard Care

O920
Decision-support software to improve the standardization of metformin treatment in type 2 diabetes
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Objective: To develop a decision-support software according to Chinese diabetes society guidelines to improve the implementation of the standard care in type 2 diabetes.

Methods: Three parts were included in our study as follows. (1) Development of the decision-support software: For healthcare professionals, a decision-support software was developed. It was an independent software based on pad to record data for patients and treatments. In addition to recording the data, the other major function of the software was to remind doctors when and how they should implement the standard care (metformin as a first line anti-hyperglycemic drug, blood pressure control, statin use and aspirin treatment) to their patients as recommended by the Chinese Diabetes Society in their guideline published in 2010. (2) Comparisons between baseline data for metformin usage with data from a previous study: We compared the baseline data collected using our software during the first quarter of the study with data from tertiary level hospitals in the ‘3B study’ to assess whether there was an improvement in the standardization of metformin treatment in type 2 diabetes. (3) Data analysis and comparison among four quarters: We further compared the data for metformin usage during the four quarters of the first year to evaluate whether there was a continuous improvement of standardized prescription of metformin. Statistical methods: SPSS18.0 was used to analyse the data. Variables with a normal distribution are presented as the mean ± standard deviation. Variables with an abnormal distribution were logarithmically transformed prior to analysis. An independent sample T-test was used to compare means between two groups. The chi-square test was performed to compare rates among groups.

Results: (1) 27 594 cases were collected with complete information. Among all of these patients, 15 156 (54.9%) were male. The mean age was 56 ± 13 years. The median duration of diabetes was 40 months. The mean body mass index (BMI) was 24.8 ± 3.6 kg/m², and the mean haemoglobin A1c (HbA1c) was 8.3 ± 2.2%. There were 16 644 (60.3%) patients receiving metformin treatment. In the previous 3B study, we selected hospitals at a similar level to the hospital included in our study and found 9351 cases with complete information. Among these patients, 4894 (52.4%) were males. The mean BMI of the patients was 24.9 ± 3.6 kg/m², with a mean HbA1c of 7.8 ± 2.0%. There were 3352 (35.9%) patients in this population receiving metformin. Comparison of the characteristics between populations revealed no significant difference in terms of BMI (p = 0.171). The baseline HbA1c in our study was significantly higher than that in the 3B study (p < 0.001). In our study, the percentage of patients who were on metformin treatment was significantly higher than that in the 3B study (60.3% vs 35.9%, p < 0.001). (2) During the first year of our study, we recorded 132 761 cases. We defined two circumstances followed as substandard usage of metformin: first, no metformin treatment in patients who were already on medications without any specific reason; second, patients on only lifestyle modification but with a HbA1c > 7%. During the first year, the percentage of substandard usage of metformin was 11.5% in the first quarter, 8.2% in the second quarter, 8.7% in the third quarter and 5.0% in the fourth quarter. There was a significant difference among quarters, with the highest values observed in the first quarter and the lowest in the last quarter (p < 0.001).

Conclusions: Our decision-support software was shown to be effective for improving the standardization of metformin treatment in type 2 diabetes.