Immunomodulator FTY720 Rejuvenates β-cell and Ameliorates Cardiorenal Complications in Nonhuman Primate Model of Diabetes

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Abstract

Inadequate β-cell mass is essential for the pathogenesis of type 2 diabetes (T2D). Previous report showed that an immunomodulator FTY720, a sphingosine 1-phosphate (S1P) receptor modulator sustainably normalized hyperglycemia by stimulating β-cell in vivo regeneration in db/db mice. To further evaluate the therapeutic potential, we examined the effects of FTY720 on glucose homeostasis in a translational nonhuman primate (NHP) model of spontaneous diabetes. Daily administration of FTY720 (5 mg/kg) effectively lowered HbA1c, blood concentrations of fasting glucose (FBG) and insulin, hence, decreased homeostatic model assessment of insulin resistance (HOMA-IR); ameliorated glucose intolerance and restored glucose-stimulated insulin release, which was largely diminished in the vehicle-treated diabetic NHPs. Importantly, after discontinuation of FTY720, FBG and HbA1c remained at the reduced levels in washout period for 8 weeks. Accompanied by the glucose lowering effects, echocardiography revealed that FTY720 significantly improved cardiac left ventricular systolic function measured by increase in ejection fraction and fractional shortening, which was compromised in the diabetic NHPs. Finally, flow cytometry analysis detected that FTY720 significantly reduced CD4+ and increased DC cells. These data strongly suggest that immunomodulator FTY720 may be a novel immunotherapy to reverse T2D progression via rejuvenation of β-cell function with benefit to improve the cardiac function.

Introduction

Type 2 diabetes (T2D) is characterized by insulin resistance and reduction of functional pancreatic b-cell mass [1,2]. Although there is an initial compensatory increase of b-cell mass, hence, elevated fasting insulin concentration and enhanced glucose-stimulated insulin response to insulin resistance in early stage of the disease progression, diabetes occurs when the functional b-cell mass fails to expand sufficiently [3,4]. Current treatments for T2D are only able to ameliorate diabetes symptoms by decreasing hyperglycemia without halting the causes of the disease. Development of a strategy to restore the mass of functional b-cells in diabetic patients is therefore a key step for the cure/reverse of T2D in humans [2,5-8].

Increasing evidence suggests that immune system plays an essential role in the progression of T2D, thus, it is proposed that immunotherapy may be a novel strategy for treatment of T2D [9,10]. It was reported previously that administration of FTY720 (Fingolimod), a sphingosine 1-phosphate (S1P) receptor modulator, to *db/db* mice led to sustained normalization of hyperglycemia by stimulating *in vivo* regeneration of β-cells [11]. Importantly, normalized blood glucose can be maintained even after withdrawal of FTY720 treatment, indicating that immunotherapy could be a curative approach for the treatment of T2D [11]. The mechanism of FTY720-induced β-cell regeneration identified in the islets from *db/db* mice includes: 1) increasing β-cell proliferation through a PI3K-dependent downregulation of $p57^{KIP2}$ and upregulation cyclin D3; 2) increasing the neogenesis of β-cells from pancreatic duct region through upregulation of PDX-1 expression; and 3) increasing β-cell survival by upregulation of Bcl-2 and BcL-xL [11,12].

Immunomodulator FTY720 is a derivative of ISP-1 (myriocin), a fungal metabolite of the Chinese herb Iscaria sinclarii as well as a structural analog of sphingosine, which was approved as a new treatment for multiple sclerosis (MS) [13,14]. FTY720 becomes active *in vivo* following phosphorylation by sphingosine kinase 2 (SphK2) to form FTY720 (S)-phosphate (FTY720-P), which binds to four of the five S1P receptors ($S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$ but not $S1P_2$) [15,16], thus, inhibits the egress of lymphocytes from lymph nodes leading to modulation of MS pathology [17,18].

The present study aimed to evaluate the therapeutic benefits of FTY720 in the treatment of T2D using a nonhuman primate (NHP) model with spontaneously developed diabetes, which has been shown to have all the characteristics of T2D in human patients at different stages of the progressive disease, and is widely used in academia and pharmaceutical industry as by far the most predictive animal model for both the basic and preclinical research in testing novel therapeutics for human metabolic disease, including diabetes [19-25].

Results

Pharmacokinetics (PK) and pharmacodynamics (PD) characterization of FTY720 (Fig. 1)

The PK and cell trafficking dynamics of FTY720 in normal cynomolgus monkeys has been reported previously [26]. In the present study, the PK and PD characteristics of FTY720 was examined in the spontaneous diabetic NHPs.

Pharmacokinetics (PK)

Following the first oral dose of FTY720 (5 mg/kg), plasma concentration of FTY720 gradually elevated, reached to peak concentration (Cmax) 35 ng/mL at ~ 8 hours (Tmax) with a terminal half-life ($t_{1/2}$) ~17 hours (Top). Thereafter, the blood drug concentrations accumulatively increased following repeated daily dose of FTY720; and then quickly diminished to 7 and 3 ng/mL on the 1^{st} and 2^{nd} week, respectively; and to below detectable levels in the remaining washout period when the drug administration was stopped. The stability of FTY720 in the dosing solution (5 mg/mL) was measured at day 3, 7, 10 and 14 with a recovery rate ranging from 95-104% (Bottom).

Pharmacodynamics (PD)

The counts of blood cell (CBC), used as a PD biomarker to measure the systemic functional kinetics of FTY720, showed that lymphocytes were lowered significantly by about ~50%, while total white blood cells and neutrophils were moderately reduced (Table 1).

FTY720 reduced glycemia and insulin resistance (Fig. 2)

Apparently, FTY720 treatment for 10 weeks had no significant effects on body weight, BMI, blood concentrations of lipids and C-reactive protein (Table I), however, both FBG and HbA1c were significantly

decreased, and remained at the reduced levels with very slow recovery during washout period after termination of FTY720 treatment (Fig. 2). In contrast, both FBG and HbA1c were gradually drifting up over the experiment period in the original control group of diabetic NHPs treated vehicle, which, however, significantly decreased during the crossover period when the treatment changed from the vehicle to FTY720 at week 10.

Accompanied by the anti-glycemic effects of FTY720, blood concentrations of insulin following overnight fasting also decreased in both the original FTY720 group as well as in the original control group after switching to FTY720 at week 10. As a result, the calculated Homeostatic Model Assessment of Insulin Resistance index (HOMA-IR) by FBG and insulin significantly decreased by FTY720.

FTY720 ameliorated glucose intolerance and restored glucose-stimulated insulin response (Fig. 3)

It was reported previously that administration of FTY720 to *db/db* mice led to sustained normalization of hyperglycemia by stimulating β-cell *in vivo* regeneration [11]. To verify if this is the case in the diabetic NHPs with glucose intolerance, glucose tolerance tests (GTT) were performed in the diabetic NHPs with treatment of vehicle or FTY720.

Intravenous glucose tolerance test in anesthetized NHPs (ivGTT, Top)

Following intravenous administration of glucose in the diabetic NHPs with overnight fasting (~16 hours), the blood concentrations of glucose significantly elevated, but both glucose-stimulated insulin and C-peptide responses were flat in the control diabetic NHPs treated with vehicle. In contrast in the diabetic NHPs with daily administration of FTY720 (5 mg/kg) for 10 weeks, the glycemic response was significantly reduced measured by both the time course of glucose concentration curve (Left) and integration of the area under the glucose response curve (AUC, Right), while glucose-stimulated insulin and c-peptide responses were enhanced compared with the responses in the vehicle group, indicating functional restoration of pancreatic β-cells.

Oral glucose tolerance test in conscious NHPs (oGTT, Bottom)

The oGTT was performed in all 12 NHPs before (baseline) and after FTY720 treatment for 8 weeks. On the experiment day, the NHPs were fasted for 16 hours. Following oral glucose loading, blood glucose concentrations gradually elevated, however, the glycemic response, measured by both glucose concentration time course (Left) and AUC (Right), was significantly reduced in the second oGTT after FTY720 treatment for 8 weeks compared to the baseline before the treatment, indicating that FTY720 ameliorated glucose intolerance in the diabetic NHPs.

FTY720 enhanced cardiac left ventricular (LV) systolic functions (Fig. 4)

Previous reports showed that the diabetic NHPs had the compromised cardiac functions, particularly, the LV systolic functions measured by reduced ejection fraction (EF) and fractional shortening (FS) [20,27]. In the present study, the standard echocardiography was performed in the anesthetized diabetic NHPs

before (baseline) and 9 weeks after (post-dose) administration of vehicle or FTY720 (5 mg/kg, PO, QD). Comparing to the baseline measurements, FTY720 treatment significantly enhanced the LV systolic function measured by elevation of EF and FS, and moderately reduced the end systolic volume (ESV), which was slightly increased in the diabetic NHPs [20]. In addition, FTY720 treatment showed no significant effects on the LV diastolic function, including the E/A ratio, end diastolic volume (EDV) and LV inner diameter at diastole (LVIDd), nor on general cardiac function, including the cardiac output (CO), heart rate (HR) and mean blood pressure (MBP). These results suggested that FTY720 treatment significantly improved cardiac systolic function, which is compromised in the diabetic NHPs.

FTY720 diminished glucosuria and proteinuria (Fig. 5)

Daily urinary excretion of glucose (Uglucose), protein (Uprotein), albumin (Ualbumin), albumin to creatinine ratio (ACR), urine volume (Uvolume) and water intake were measured before (Week -2, baseline) and after once daily oral administration of vehicle (n=5) or FTY720 (5 mg/kg, n=7) in the diabetic NHPs. Over the experiment period, the urinary excretion of glucose, albumin and ACR, as well as the water intake gradually elevated, reaching significance from the baseline levels (week -2) at week 8 for ACR and week 2 for water intake in the diabetic NHPs treated with vehicle only, while in the FTY720 group, most of the urine parameters gradually decreased, reaching statistical significance for glucose at all time points, urine volume and ACR at weeks 6 and 8, suggesting that FTY720 treatment improved renal functions in the diabetic NHPs.

FTY720 decreased food intake without significantly impacting body weight (Fig. 6)

Body weight and daily food intake were measured before and after once daily oral administration of vehicle (n=5) or FTY720 at 5 mg/kg (n=7) in the diabetic NHPs. Both the body weight and food intake were stable in the vehicle group. However, treatment of FTY720 significantly decreased food intake without affecting body weight. After stop dosing, the food intake slowly came back. Interestingly, the crossover of the treatment switching from the vehicle to FTY720 in the original control group reproducibly resulted in a similar decrease in food intake without apparent change in body weight.

FTY720 modulated peripheral immune-cell profiles (Fig. 7)

To analyze the peripheral immune cell profiles, the flow cytometry analysis was performed in the diabetic NHPs with once daily oral treatment of vehicle and FTY720 (5 mg/kg) for 10 weeks as well as a group of normal NHPs treated with the vehicle only. There were no significant differences for all analyzed cell lineages including CD3, CD4 and CD8 T lymphocytes, as well as NKT, NK and DC cells between the diabetic and normal control NHPs treated with the vehicle. However, FTY720 treatment in the diabetic NHPs significantly increased the ratio of DCs and lowered the ratio of T lymphocytes, including CD3+ (total), CD4+ (effector), CD127+ (memory), and CCR6+ (antigen experienced memory) in the circulation, with no significant effects on CD8 T lymphocytes and NKT and NK cells compared to the diabetic NHPs treated with vehicle, indicating that FTY720 selective modulated peripheral immune-cell profiling.

Discussion

We report here that oral administration of an immunomodulator FTY720 in spontaneous diabetic NHPs effectively lowered the FBG and HbA1c, which, intriguingly, remained at lower levels even after withdrawal of FTY720. This observation is consistent with the previous finding in *db/db* mice that FTY720 led to sustained normalization of hyperglycemia, which was also remained for life even after withdrawal of FTY20 [11]. The animal model used in the present study is well-characterized naturally occurred diabetic NHPs with all the T2D characteristics in human patients at different stages of the disease progression and is by far the most predictive translational animal model for human metabolic syndrome, including diabetes [19-25]. To our knowledge, FTY720 is the only compound with a sustained anti-glycemic effect even after cease of the treatment in both the diabetic mice and NHPs. These results suggest that treatment of T2D with FTY720 may be a novel immunotherapy that can potentially reverse T2D progression.. Furthermore, we demonstrated in this diabetic NHP model that FTY720 treatment can rejuvenate the b-cell function evidenced by the secretion of insulin c-peptide in response to glucose stimulation in ivGTT, while the glucose-stimulated insulin response was almost flat in the control NHPs without FTY720-treatment. T2D is characterized by insulin resistance and diminished functional pancreatic b-cell mass [1,2]. Although there is an initial compensatory increase of b-cell mass in response to insulin resistance in early stage, diabetes occurs when the functional b-cell mass fails to expand sufficiently in late stage of the disease [3,4]. Therefore, it is proposed that development of a therapeutic strategy to increase the mass of functional b-cells in diabetic patients may lead to the cure/reverse of T2D in humans [2,5-8]. The present data from NHPs along with previous finding from db/db mice [11] strongly supports this hypothesis.

Adult pancreatic b-cells have been found to be mainly expanded by self-duplication [28]. Interestingly, it was reported that FTY720 was able to stimulate functional β-cell expansion in *db/db* mice mainly by increasing β-cell proliferation through a PI3K-dependent downregulation of the cell cycle inhibitor $p57^{KIP2}$ and upregulation of cyclin D3 [11]. $p57^{KIP2}$ is an imprinted gene that is conserved between rodents and humans and required for normal development and differentiation [29]. It has been reported that $p57^{KIP2}$ is paternally imprinted and highly expressed in human pancreatic b-cells [30], which controls both the self-renewal and the exit from the cell cycle of pancreatic progenitors during pancreatic development [31]. Targeting $p57^{KIP2}$ promotes adult human b-cell replication [32]. Similarly, downregulation of $p57^{KIP2}$ by FTY720 may promote the differentiated adult human b-cell proliferation *in vivo*. S1P signaling plays a key role in adiponectin-mediated survival of pancreatic b-cells, nutrient uptake, nutrient utilization, and mitochondrial proliferation [33,34]. It is apparent that FTY720, a S1P analog, when administered to the diabetic NHPs, acts on an intrinsic pathway that is physiologically important for b-cell survival and regeneration under metabolic stress.

Following the FTY20 treatment, functional b-cell expansion occurs to against the increased insulin resistance in the diabetic NHPs. Our date shown that the FBG and HbA1c still remained in the lowered levels even after withdrawal of FTY720, suggesting that FTY720 may have improved conditions of T2D

patents that favor functional b-cell survival. In human T2D patients, b-cell mass is decreased due to increased apoptosis [3]. In the studies in *db/db* mice, it was found that FTY720 treatment increased the expression of anti-apoptotic gene products Bcl2 and Bcl-xL in the islets isolated from the db/db mice treated with FTY720 [11,12], indicating newly regenerated pancreatic b-cells are more resistant to apoptotic induction. In addition, Obesity is one of the major risk factors for T2D. it has been reported that FTY720 reverses high-fat diet-induced weight gain, insulin resistance and adipose tissue inflammation in C57BL/6 mice [35]. In consistence with this report, the present data showed that the body weight of the diabetic NHPs during the period of FTY720 treatment and of withdrawal slightly lower than the vehicle treated NHPs although not significantly different (Fig. 6) and insulin resistance is improved (Fig. 2). These changes following the treatment of FTY720 may favor the survival of b-cells.

T2D is often associated with chronic inflammation that may affect the survival of b-cells [36]. The main mechanisms and molecular signaling of the induction of inflammation in T2D are still unknown. Some evidence showed that stimulated T cells produce cytokines that cooperate with saturated free fatty acids in b-cell destruction in diabetes pathogenesis. [37]. FTY720 inhibits the egress of lymphocytes from LNs and preferentially traps CD4+ T cells in lymph nodes in MS patients [17]. The T cells trapped in LNs by FTY720 contain the pro-inflammatory CD4+ TH17 subset that produce IL-17 and IL-22, thereby inducing a massive tissue reaction owing to the broad distribution of the IL-17 and IL-22 receptors [17]. Interestingly, it was reported that IL-17 stimulates inducible nitric oxide synthase-dependent toxicity in mouse b-cells [38]. The present finding that FTY720 treatment in the diabetic NHPs significantly decreased the CD4+ counts (Fig.7) may contribute to the prevention of IL-17 stimulated b-cell injury.

Another important finding in this study is that FTY720 treatment improves the cardiac function of the diabetic NHPs. Cardiovascular disease remains one of the leading causes of death in the United States and about 52 % T2D patients have a higher risk of mortality at comparable levels of coronary artery disease to those without T2D [39]. It is expected that anti-diabetes drug also can improve the cardiovascular function associated with diabetes [40]. Diabetes is a major risk factor for heart failure with preserved ejection fraction (EF), and is highly associated with LV diastolic dysfunction in human [41]. Using noninvasive echocardiography, it has been shown that the diabetic NHPs used here are associated with LV diastolic dysfunction similar to that in humans [20]. It appears that FTY720 treatment can reverse the decreased EF and FS in the diabetic NHPs to relative normal levels. Indeed, other research suggests that signaling through the S1P receptor by FTY720 may play a role in the treatment of cardiac microvascular dysfunction in diabetes [42]. It has been proposed that FTY720 might be capable to serve as a potential therapeutic approach for diabetic heart disease through ameliorating cardiac microvascular barrier impairment and pathologic angiogenesis [43]. Although it is notable in clinical that the first dose of FTY720 may cause asymptomatic bradycardia in some patients [44], the present data along with the others indicate that FTY720 may improve heart function in diabetes patients.

Diabetic nephropathy is a leading cause of end-stage renal failure worldwide. Inhibition of angiotensin II has been used clinically to reduce proteinuria, an early biomarker of diabetic nephropathy [45]. We

previously reported that the diabetic NHP model accompanied with proteinuria [24], which can also be significantly reduced by Losartan, an angiotensin II receptor blocker (ARB) [25]. Diabetic nephropathy is closely linked to inflammatory cell infiltrations in the kidney [46], and the number of CD4+ and CD20+ cells correlated with the amount of proteinuria in T2D patient [47], indicating that inflammation plays an important role. Indeed, the present data also demonstrated that FTY720 treatment prevented the progression of proteinuria in the diabetic NHPs. Although the exact mechanism of this effect remains to be determined, it may attribute to the immunomodulatory effects of FTY720 to decrease circulating lymphocytes, hence reducing the aberrant recruitment and activation of T cells in the kidney.

In summary, the present study demonstrated that FTY720 effectively rejuvenates β-cell function, improves cardiac function, and reduces proteinuria in the translational NHP model of diabetes with complication of cardiorenal dysfunctions. These results strongly suggest that FTY720 as an immunomodulator has a great therapeutic promise for the treatment of T2D with benefit to improve the cardiorenal functions. Because the drug is already approved by the FDA, it could be tested in clinical trial for its ability to reverse or prevent the progression of T2D.

Methods

Materials

FTY720 (2-amino-2-[4-octylphenyl] ethyl)-1,3-propanediol was purchased from Nanjing Chemipioneer Pharma & Tech Co., Ltd., (China). Dosing solution was prepared weekly in purified water at a concentration of 5 mg/mL and stored at 4^0C for daily oral administration at a volume of 1 mL/kg and 5 mg/kg.

Animals and procedures

The male cynomolgus macaques (Macaca fascicularis) were individually housed in species appropriate cages at temperature-controlled rooms (20 ± 3°C) on a 12-hour light–dark (6:00 – 18:00) cycle, were fed normal primate chow containing 19% protein, 5% fat and 3.6% fiber (Shanghai Shilin Biotechnology Inc., Shanghai, China) twice daily and had free access to tap water. All animal procedures used in this study were in accordance with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), and in compliance with the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines; as well as approved by the Crown Bioscience institutional animal care and use committee (IACUC).

After 2-week acclimation and training, the baseline blood chemistries, including fasting blood glucose (FBG), hemoglobin A1c (HbA1c), blood cell count (CBC), etc. were measured, based on which, qualified NHPs were enrolled and randomly divided into 2 groups: Vehicle (n=5) and FTY720 (n=7) with once daily oral gavage of purified water (1 mL/kg) or FTY720 (5 mg/kg), respectively. After 10-week initial treatment, two original groups were switched each other, namely, the original control group treated with vehicle was

changed to FTY720 and the original FTY720 group was changed to the purified water observed as a washout period for another 10 weeks. All NHPs were subjected to continuously monitoring food intake (FI), body weight (BW), the blood concentrations of fasting glucose and insulin, HbA1c, etc. every 2 weeks. The systemic insulin resistance index was calculated based on homeostatic model assessment for insulin resistance (HOMA-IR): HOMA-IR = Insulin (mIU/L) x glucose (mmol/L) / 22.5 [48].

Glucose tolerance tests (GTT)

Intravenous GTT (ivGTT) in anesthetized NHPs

After treatment of either vehicle (n=5) or FTY720 (n=7) for 10 weeks, the diabetic NHPs were fasted for 16 hours and then anesthetized with intramuscular injection of ketamine (16 mg/kg), supplemented with 0.16 mg/kg as needed. Blood samples were collected prior (0 min) and post (3, 5, 7, 10, 15, 20 and 30 min) iv administration of Dextrose (50%, 250 mg/kg) via saphenous vein for measurements of blood concentrations of glucose, insulin, and c-peptide by SIEMENS ADVIA-2400 [49,50].

Oral GTT (oGTT) in conscious NHPs

In the same 12 diabetic NHPs, oGTT was performed before (baseline) and after treatment of FTY720 for 8 weeks. On the experiment day, following 16 hour fasting, blood glucose concentrations were measured by a Glucometer (Roche, Accu-chek) prior (0 min) and post (30, 60, 90, 120, and 180 min) oral glucose loading via gavage (35%, 1.75 g or 5 mL/kg body weight,) [51].

Florescence activating cell sorter (FACS) analysis

About 24 hours after the last dosing, circulating blood samples were collected from 2 groups of diabetic (DM) NHPs treated with once daily oral dose of vehicle (purified water, n=6) or FTY720 (5 mg/kg, n=7) and a group of normal NHPs treated with vehicle as control (Normal + Veh, n=7) for 8 weeks. The blood samples were lysed by RBC lysis and stained according to the user manual (BD Bioscience) for all antibodies against CD3 PerCP-cy5.5, CD4 BV605, CD8 PE, CD11c BV650, CD56 PE-cy7, CD127 AF647 and CCR6 FITC, respectively. The immune cells were analyzed by flow cytometry (Fortessa) with the gating strategy described in the Supplement section.

Pharmacokinetics (PK) and pharmacodynamics (PD) of FTY720 in the diabetic NHPs

Blood samples were taken in 7 conscious diabetic NHPs immediate before (0 hour), and at 0.5, 1, 2, 4, 6, 24 and 48 hours, 7 and 14 days, after the first oral dose and every 2 weeks followed by daily repeated dose of FTY720 (5 mg/kg) for 10 weeks, as well as in the washout period for 10 more weeks after drug administration.

Pharmacokinetics (PK)

LC-MS/MS technology was used to measure blood concentrations of FTY720 with detailed analytic methodology described in the Supplement section. The plasma concentrations of FTY720 at each time point in 0-24 hours after the 1st dose of FTY720 from each individual NHP were used for calculation of the PK parameters by linear trapezoidal interpolation and Lambda Z best fit under non-compartmental model (WinNonlin 8.2). When less than 3 points were available after peak plasma concentration (Cmax) was established, the best fit model cannot calculate the terminal half-life ($t_{1/2}$) value.

Pharmacodynamics (PD)

The counts of blood cell (CBC) was used as a PD biomarker to measure the systemic functional kinetics of FTY720.

Statistical analysis

Data were expressed as mean value ± standard error of the mean (SEM). Significant differences among groups were evaluated by one-way ANOVA and Turkey's multiple comparison test or by unpaired two-tailed Student's *t* test using a statistic software (PRISM). Significant levels of the difference among the comparison groups are set as p value < 0.05.

Abbreviations

AUC: Area under the curve

Cmax: Maximum concentration

CO: Cardiac output

E/A ratio: Ratio of the left ventricular early over late trans-mitral Doppler inflow velocity

EDV: Left ventricular end diastolic volume

EF/FS: Left ventricular ejection fraction/Fractional shortening

ESV: Left ventricular end systolic volume

FACS: Florescence activating cell sorter

FBG: Fasting blood glucose concentration

FTY720: (2-amino-2-[4-octylphenyl] ethyl)-1,3-propanediol; S1P, sphingosine 1-phosphate. BrdU: 5-bromo-2'deoxyuridine.

HDL: High density lipoprotein concentration

HOMA-IR: Homeostatic Model Assessment of Insulin Resistance

LDL: Low density lipoprotein concentration

LN: Lymph note

LVIDd: Left ventricular inner diameter at diastole

o/ivGTT: Oral/Intravenous glucose tolerance test

NHP: Nonhuman primate

T2D/DM: Type 2 diabetes/Diabetes Miletus

$T_{1/2}$: Terminal half life

TC: Total cholesterol concentration

TG: Total triglyceride concentration

Tmax: The time to reach maximal concentration

Vz: The apparent volume of distribution during the terminal phase

Declarations

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Author contributions

YW, XW, YX, GY, and ZM involved in study design, data interpretation and discussion; XW and YX managed experimental execution; AA, LX and MF ran the FACS analysis; KG ran the pharmacokinetic analysis; YW, XW and ZM wrote the manuscript

References

1. Porte, D., Jr. Banting lecture 1990. Beta-cells in type II diabetes mellitus. *Diabetes* **40**, 166-180 (1991).
2. Weir, G.C., Gaglia, J. & Bonner-Weir, S. Inadequate beta-cell mass is essential for the pathogenesis of type 2 diabetes. *Lancet Diabetes Endocrinol* **8**, 249-256 (2020).
3. Butler, A.E.*, et al.* Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* **52**, 102-110 (2003).
4. Rhodes, C.J. Type 2 diabetes-a matter of beta-cell life and death? *Science* **307**, 380-384 (2005).

5. Leahy, J.L., Hirsch, I.B., Peterson, K.A. & Schneider, D. Targeting beta-cell function early in the course of therapy for type 2 diabetes mellitus. *The Journal of clinical endocrinology and metabolism* **95**, 4206-4216 (2010).
6. Bonner-Weir, S.*, et al.* Beta-cell growth and regeneration: replication is only part of the story. *Diabetes* **59**, 2340-2348 (2010).
7. Yagihashi, S., Inaba, W. & Mizukami, H. Dynamic pathology of islet endocrine cells in type 2 diabetes: beta-Cell growth, death, regeneration and their clinical implications. *J Diabetes Investig* **7**, 155-165 (2016).
8. Aguayo-Mazzucato, C. & Bonner-Weir, S. Pancreatic beta Cell Regeneration as a Possible Therapy for Diabetes. *Cell metabolism* **27**, 57-67 (2018).
9. Zhou, T.*, et al.* Role of Adaptive and Innate Immunity in Type 2 Diabetes Mellitus. *J Diabetes Res* **2018**, 7457269 (2018).
10. Fujii, M., Sonoda, N., Takayanagi, R. & Inoguchi, T. Targeting obesity, insulin resistance and Type 2 diabetes with immunotherapy: the challenges ahead. *Immunotherapy* **6**, 5-7 (2014).
11. Zhao, Z., Choi, J., Zhao, C. & Ma, Z.A. FTY720 normalizes hyperglycemia by stimulating beta-cell in vivo regeneration in db/db mice through regulation of cyclin D3 and p57(KIP2). *The Journal of biological chemistry* **287**, 5562-5573 (2012).
12. Moon, H.*, et al.* FTY720 preserved islet beta-cell mass by inhibiting apoptosis and increasing survival of beta-cells in db/db mice. *Diabetes/metabolism research and reviews* **29**, 19-24 (2013).
13. Brinkmann, V.*, et al.* Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. *Nature reviews. Drug discovery* **9**, 883-897 (2010).
14. Ontaneda, D., Hara-Cleaver, C., Rudick, R.A., Cohen, J.A. & Bermel, R.A. Early tolerability and safety of fingolimod in clinical practice. *Journal of the neurological sciences* **323**, 167-172 (2012).
15. Brinkmann, V.*, et al.* The immune modulator FTY720 targets sphingosine 1-phosphate receptors. *The Journal of biological chemistry* **277**, 21453-21457 (2002).
16. Verzijl, D., Peters, S.L. & Alewijnse, A.E. Sphingosine-1-phosphate receptors: zooming in on ligand-induced intracellular trafficking and its functional implications. *Molecules and cells* **29**, 99-104 (2010).
17. Brinkmann, V. FTY720 (fingolimod) in Multiple Sclerosis: therapeutic effects in the immune and the central nervous system. *British journal of pharmacology* **158**, 1173-1182 (2009).
18. Brown, B.A., Kantesaria, P.P. & McDevitt, L.M. Fingolimod: a novel immunosuppressant for multiple sclerosis. *The Annals of pharmacotherapy* **41**, 1660-1668 (2007).
19. Wang, X.*, et al.* Quantification of beta-cell insulin secretory function using a graded glucose infusion with C-peptide deconvolution in dysmetabolic, and diabetic cynomolgus monkeys. *Diabetol Metab Syndr* **5**, 40 (2013).
20. Gu, H.*, et al.* Left ventricular diastolic dysfunction in nonhuman primate model of dysmetabolism and diabetes. *BMC Cardiovasc Disord* **15**, 141 (2015).

21. Wang, B.H.*, et al.* Complete mitochondrial genome of the Nigeria-Cameroon chimpanzee, Pan troglodytes ellioti (Primates: Hominidae). *Mitochondrial DNA A DNA Mapp Seq Anal* **27**, 2018-2019 (2016).
22. Wikstrom, J.*, et al.* Diastolic dysfunction and impaired cardiac output reserve in dysmetabolic nonhuman primate with proteinuria. *J Diabetes Complications*, 107881 (2021).
23. Ruggenenti, P.*, et al.* Measurable urinary albumin predicts cardiovascular risk among normoalbuminuric patients with type 2 diabetes. *J Am Soc Nephrol* **23**, 1717-1724 (2012).
24. Guo S, Q.W., D Fu, F, Wang B, Wang X, Fang, Y, Chen, X, Benzinou, M, Gregoire FM, Staup, M, Chng K, Chen, Y, Xiao, Y, Wang, YX. Proteinuria in Cynomolgus macaques (Macaca fascicularis) with Spontaneously Developed Metabolic Disorder and Diabetes: Transcriptome Analysis of Biopsy Kidney. *Journal of Diabetes and Metabolism* **5**(2014).
25. Gao J, W.X., Song W, Wang YX, Xiao YF. Dysglycemia and Dyslipidemia MModels in nonhuman Primates: Part V. Diabetic Nephrophathy and Effects of Angiotensin II Receptor Antagonist Losartan *J Diabetes Metabolism* **12**(2021).
26. Li, H.*, et al.* Pharmacokinetics and cell trafficking dynamics of 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride (FTY720) in cynomolgus monkeys after single oral and intravenous doses. *The Journal of pharmacology and experimental therapeutics* **301**, 519-526 (2002).
27. Wikstrom, J. Diastolic dysfunction and impaired cardiac output reserve in dysmetabolic nonhuman primate with proteinuria. *Journal of Diabetes and its complications* (2021).
28. Dor, Y., Brown, J., Martinez, O.I. & Melton, D.A. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* **429**, 41-46 (2004).
29. Pateras, I.S., Apostolopoulou, K., Niforou, K., Kotsinas, A. & Gorgoulis, V.G. p57KIP2: "Kip"ing the cell under control. *Mol Cancer Res* **7**, 1902-1919 (2009).
30. Kassem, S.A.*, et al.* p57(KIP2) expression in normal islet cells and in hyperinsulinism of infancy. *Diabetes* **50**, 2763-2769 (2001).
31. Georgia, S., Soliz, R., Li, M., Zhang, P. & Bhushan, A. p57 and Hes1 coordinate cell cycle exit with self-renewal of pancreatic progenitors. *Dev Biol* **298**, 22-31 (2006).
32. Avrahami, D.*, et al.* Targeting the cell cycle inhibitor p57Kip2 promotes adult human beta cell replication. *The Journal of clinical investigation* **124**, 670-674 (2014).
33. Holland, W.L.*, et al.* Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. *Nature medicine* **17**, 55-63 (2011).
34. Maceyka, M., Harikumar, K.B., Milstien, S. & Spiegel, S. Sphingosine-1-phosphate signaling and its role in disease. *Trends Cell Biol* **22**, 50-60 (2012).
35. Kendall, M.R. & Hupfeld, C.J. FTY720, a sphingosine-1-phosphate receptor modulator, reverses high-fat diet-induced weight gain, insulin resistance and adipose tissue inflammation in C57BL/6 mice. *Diabetes, obesity & metabolism* **10**, 802-805 (2008).

36. Donath, M.Y., *et al.* Islet inflammation in type 2 diabetes: from metabolic stress to therapy. *Diabetes care* **31 Suppl 2**, S161-164 (2008).
37. Cvjeticanin, T., Stojanovic, I., Timotijevic, G., Stosic-Grujicic, S. & Miljkovic, D. T cells cooperate with palmitic acid in induction of beta cell apoptosis. *BMC Immunol* **10**, 29 (2009).
38. Miljkovic, D., *et al.* Interleukin-17 stimulates inducible nitric oxide synthase-dependent toxicity in mouse beta cells. *Cellular and molecular life sciences : CMLS* **62**, 2658-2668 (2005).
39. Morrish, N.J., Wang, S.L., Stevens, L.K., Fuller, J.H. & Keen, H. Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes. *Diabetologia* **44 Suppl 2**, S14-21 (2001).
40. Drucker, D.J. & Goldfine, A.B. Cardiovascular safety and diabetes drug development. *Lancet* **377**, 977-979 (2011).
41. Mogelvang, R., *et al.* Tissue Doppler echocardiography in persons with hypertension, diabetes, or ischaemic heart disease: the Copenhagen City Heart Study. *Eur Heart J* **30**, 731-739 (2009).
42. Xu, H., Jin, Y., Ni, H., Hu, S. & Zhang, Q. Sphingosine-1-phosphate receptor agonist, FTY720, restores coronary flow reserve in diabetic rats. *Circ J* **78**, 2979-2986 (2014).
43. Yin, Z., *et al.* FTY720 Protects Cardiac Microvessels of Diabetes: A Critical Role of S1P1/3 in Diabetic Heart Disease. *PloS one* **7**, e42900 (2012).
44. Budde, K., *et al.* First human trial of FTY720, a novel immunomodulator, in stable renal transplant patients. *J Am Soc Nephrol* **13**, 1073-1083 (2002).
45. De Cosmo, S., *et al.* Impact of the PPAR-gamma2 Pro12Ala polymorphism and ACE inhibitor therapy on new-onset microalbuminuria in type 2 diabetes: evidence from BENEDICT. *Diabetes* **58**, 2920-2929 (2009).
46. Duran-Salgado, M.B. & Rubio-Guerra, A.F. Diabetic nephropathy and inflammation. *World J Diabetes* **5**, 393-398 (2014).
47. Moon, J.Y., *et al.* Aberrant recruitment and activation of T cells in diabetic nephropathy. *Am J Nephrol* **35**, 164-174 (2012).
48. Matthews, D.R., *et al.* Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**, 412-419 (1985).
49. Zhao, Z., *et al.* Advanced glycation end products inhibit glucose-stimulated insulin secretion through nitric oxide-dependent inhibition of cytochrome c oxidase and adenosine triphosphate synthesis. *Endocrinology* **150**, 2569-2576 (2009).
50. Zhao, Z., *et al.* Protection of pancreatic beta-cells by group VIA phospholipase A(2)-mediated repair of mitochondrial membrane peroxidation. *Endocrinology* **151**, 3038-3048 (2010).
51. Song, K., Zhang, X., Zhao, C., Ang, N.T. & Ma, Z.A. Inhibition of Ca2+-independent phospholipase A2 results in insufficient insulin secretion and impaired glucose tolerance. *Mol Endocrinol* **19**, 504-515 (2005).

Table

Table 1. General characteristics of the diabetic NHPs following once daily oral administration of vehicle (purified water, 1 mL/kg) or FTY720 (5 mg/kg) for 10 weeks.

Group	Vehicle	FTY720	Ttest
Number of Animals	6	7	p value
General			
Age (years)	18 ± 1.2	19 ± 0.9	0.79
Body weight (kg)	8.1 ± 1.3	8.0 ± 0.9	0.97
BMI (kg/m^2)	13 ± 1.4	13 ± 0.9	0.74
Blood Chemistry			
TC (mmol/L)	3.9 ± 0.4	3.4 ± 0.3	0.30
TG (mmol/L)	2.9 ± 0.5	3.4 ± 0.8	0.69
HDL (mmol/L)	1.1 ± 0.1	0.8 ± 0.1	0.16
LDL (mmol/L)	1.7 ± 0.3	1.3 ± 0.1	0.26
C-Reactive Protein (mg/L)	2.4 ± 0.3	2.6 ± 0.3	0.61
Blood cells			
White blood Cells ($10^9/L$)	11 ± 1.0	7.9 ± 1.1	0.06
Neutrophil ($10^9/L$)	4.8 ± 0.8	4.2 ± 0.6	0.56
Lymphocyte ($10^9/L$)	5.4 ± 0.5	2.7 ± 0.5	**0.00**

P value is calculated with t-test between the 2 groups and the statistical significant difference was highlighted in bold when p value is less than 0.05.

Figures

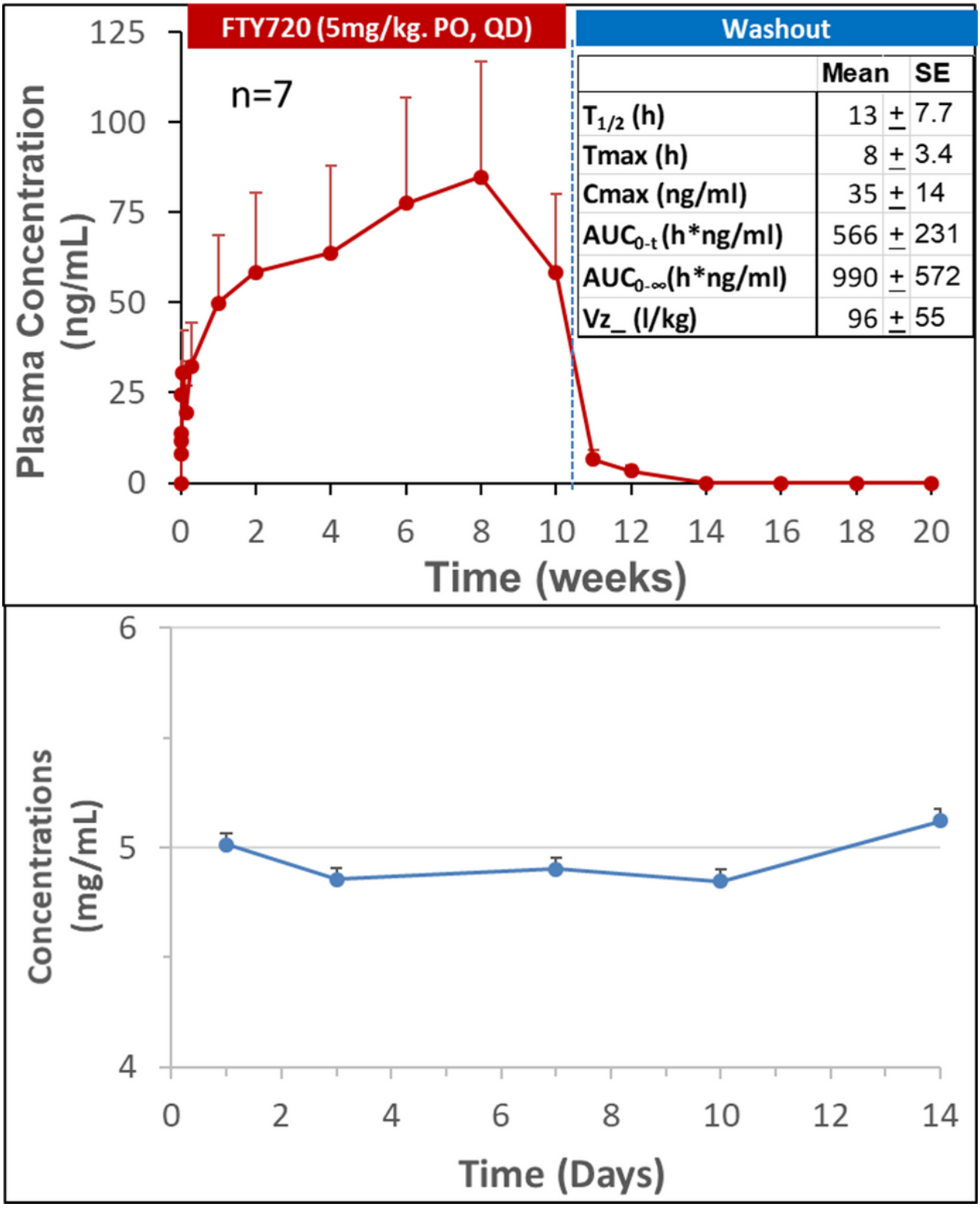

Figure 1

Pharmacokinetics (PK) and solution stability of FTY720 in the diabetic NHPs Top: Plasma concentrations of FTY720 after daily oral administration (5 mg/kg) for 10 weeks followed by 10-week washout, which were measured at time 0 (Baseline), and 0.5, 1, 2, 4, 8, and 24 hours after the 1st dose to calculate PK profile, and at day 2, 7 and 14, every 2 weeks thereafter following repeated daily dose, and then,

termination of FTY720 administration, respectively. Bottom: Stability of FTY720 in dosing solution at 4oC for 2 weeks.

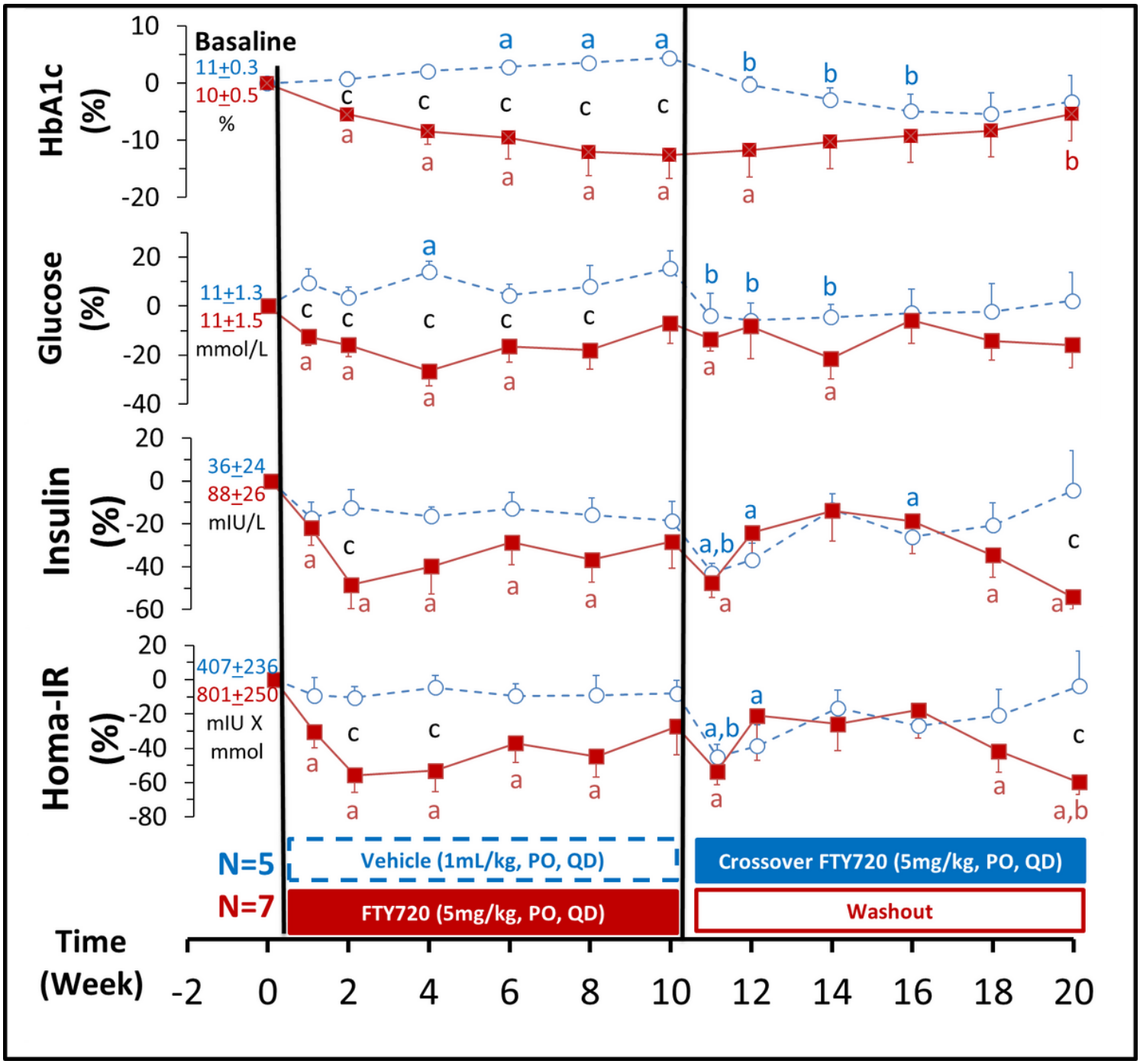

Figure 2

FTY720 decreased glycemia and insulin resistance From top to bottom: Percent changes of blood hemoglobin A1c (HbA1c), glucose and insulin concentrations, and calculated Homeostatic Model Assessment of Insulin Resistance Index (Homa-IR) from baseline (week 0) and after once daily oral administration of vehicle (purified water, 1 mL/kg, n=5) or FTY720 (5 mg/kg, n=7) for 10 weeks in the diabetic NHPs. The actual baseline values are presented next to the first time point colored blue as vehicle and red as FTY720 group, respectively. After week 10, the treatments were crossover, the original

control group started with vehicle administration (blue) was changed to FTY720 (5 mg/kg, PO. QD); while the drug administration in the original FTY720 group (red) was stopped for continue observation as a washout period in the next 10 weeks. Statistical significance: p value < 0.05: Paired t-test for a. Post- vs. pre-dosing value (baseline) at week 0; b. Post vs. pre-crossover value at week 10; Unpaired t-test c. Compared the values between the vehicle and FTY720 group at each time points.

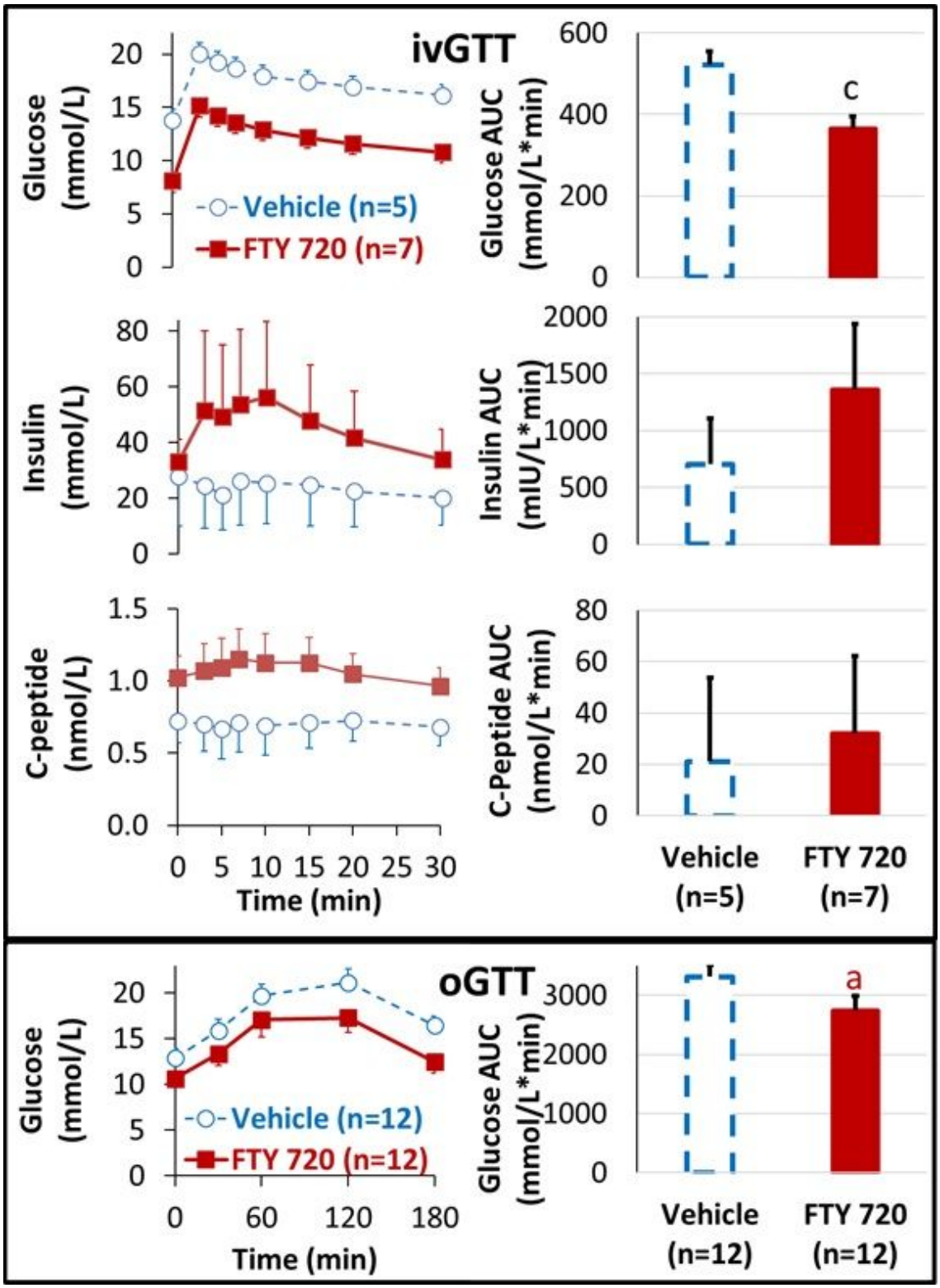

Figure 3

FTY720 ameliorated glucose intolerance and restored glucose-stimulated insulin response Top: Intravenous glucose tolerance test (ivGTT) in anesthetized diabetic NHPs after once daily oral administration of vehicle (blue, purified water 1 mL/kg, n=5) or FTY720 (red, 5 mg/kg, n=7) for 10 weeks. Left: Concentrations and Right: integration of the area under the concentration curve (AUC) of blood glucose, insulin and C-peptide. “c”: $p < 0.05$, unpaired t-test, Vehicle vs. FTY720 group at week 10 after respective treatment. Bottom: Oral glucose tolerance test (oGTT) in the same conscious diabetic NHPs (n=12) before (blue, Vehicle) and after once daily oral administration of FTY720 (5 mg/kg) for 8 weeks (red, FTY720). “a”: $p < 0.05$, paired t-test, baseline at week 0 vs. (Vehicle) vs. post treatment at week 8 (FTY720) in the same 12 NHPs.

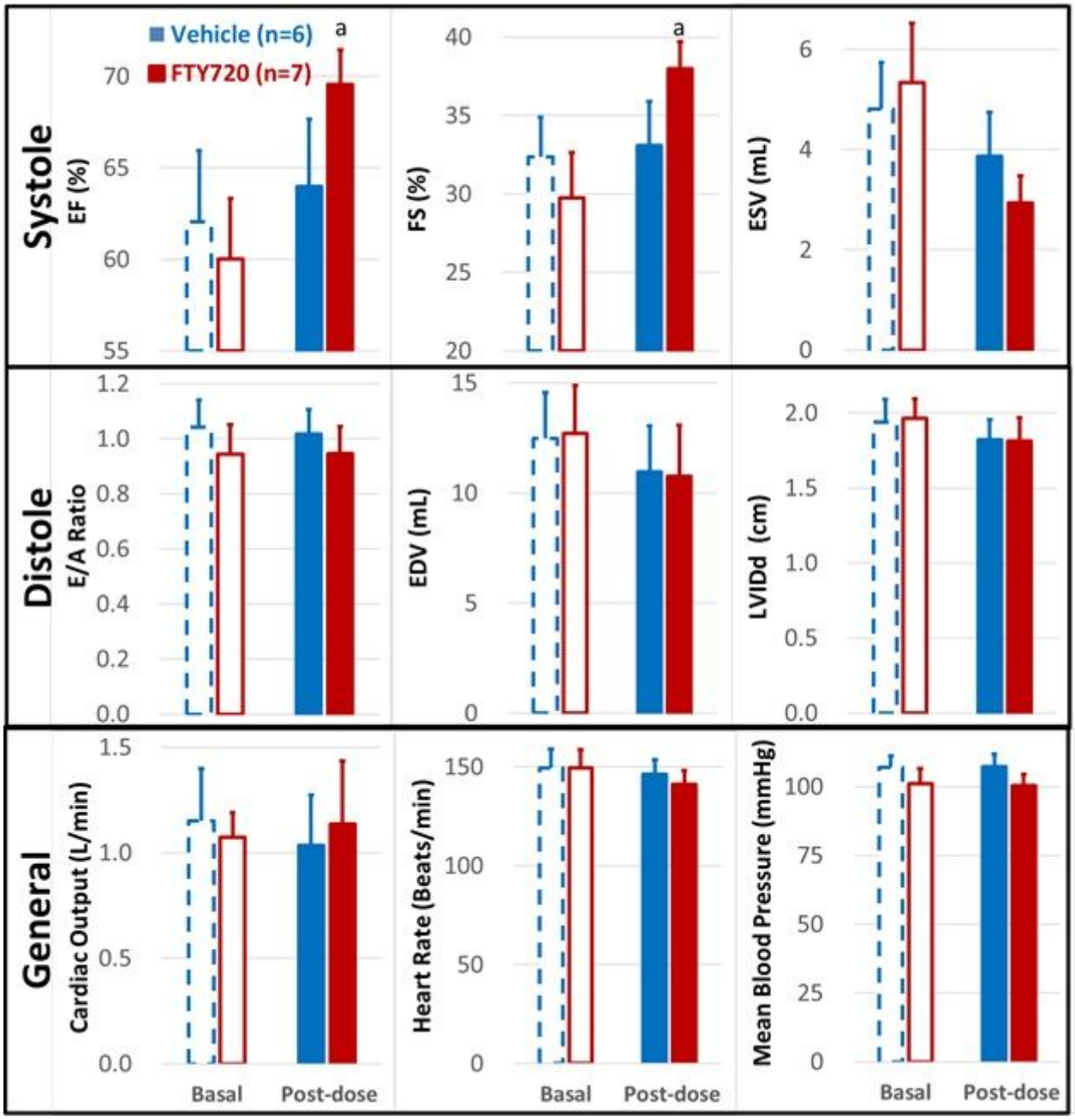

Figure 4

FTY720 enhanced systolic function of the heart Cardiac functions measured noninvasively by echocardiography in anesthetized diabetic NHPs before (basal) and 10 weeks after (post-dose) once daily oral administration of vehicle (blue, n=5) or FTY720 (red, 5mg/kg, n=7), which are grouped from top to bottom below: Systole: Left ventricular ejection fraction (EF); fractional shortening (FS) and end systolic volume (ESV). Diastole: Ratio of left ventricular early over late trans-mitral Doppler inflow velocity (E/A), end diastolic volume (EDV); and inter diameter at diastole (LVIDd) General: Cardiac output (CO); heart rate (HR); and mean arterial blood pressure (MBP). Statistical significance: p value < 0.05, a. paired t-test, baseline vs, post-dose treatment of FTY720 for 10 weeks in the same NHPs.

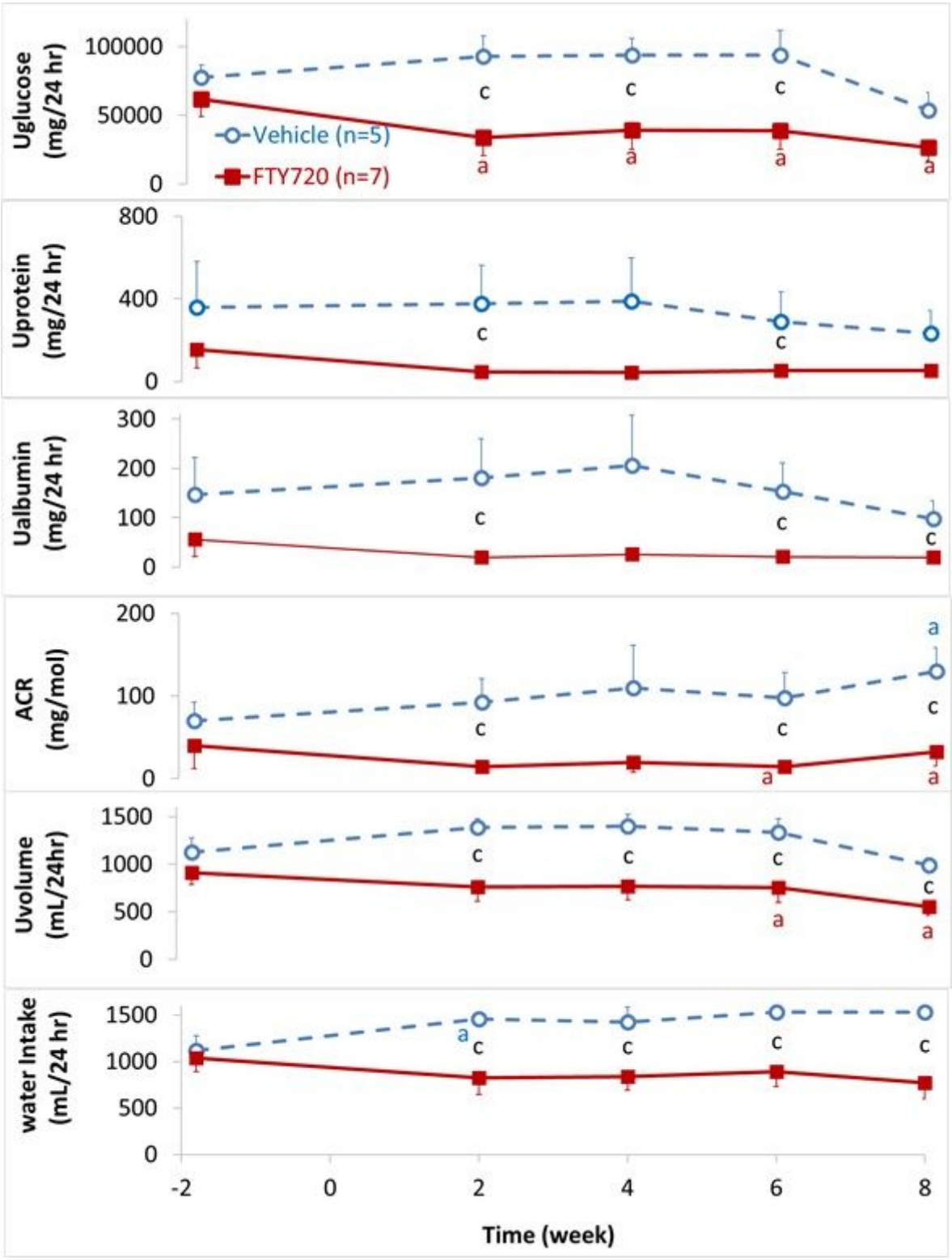

Figure 5

FTY720 diminished glucosuria and urinary albumin/creatine ratio (ACR) From top to bottom: Daily urinary excretion of glucose (Uglucose), protein (Uprotein), albumin (Ualbumin), albumin to creatinine ratio (ACR), volume (Uvolume) and water intake before (Week -2) and after once daily oral administration of vehicle (blue, purified water 1 mL/kg, n=5) or FTY720 (red, 5 mg/kg, n=7) in the diabetic NHPs.

Statistical significance: p value < 0.05: a. Paired t-test, Post- vs. pre-dosing value (baseline) at week -2; c. Unpaired t-test, comparison of the values between the vehicle and FTY720 group at each time points.

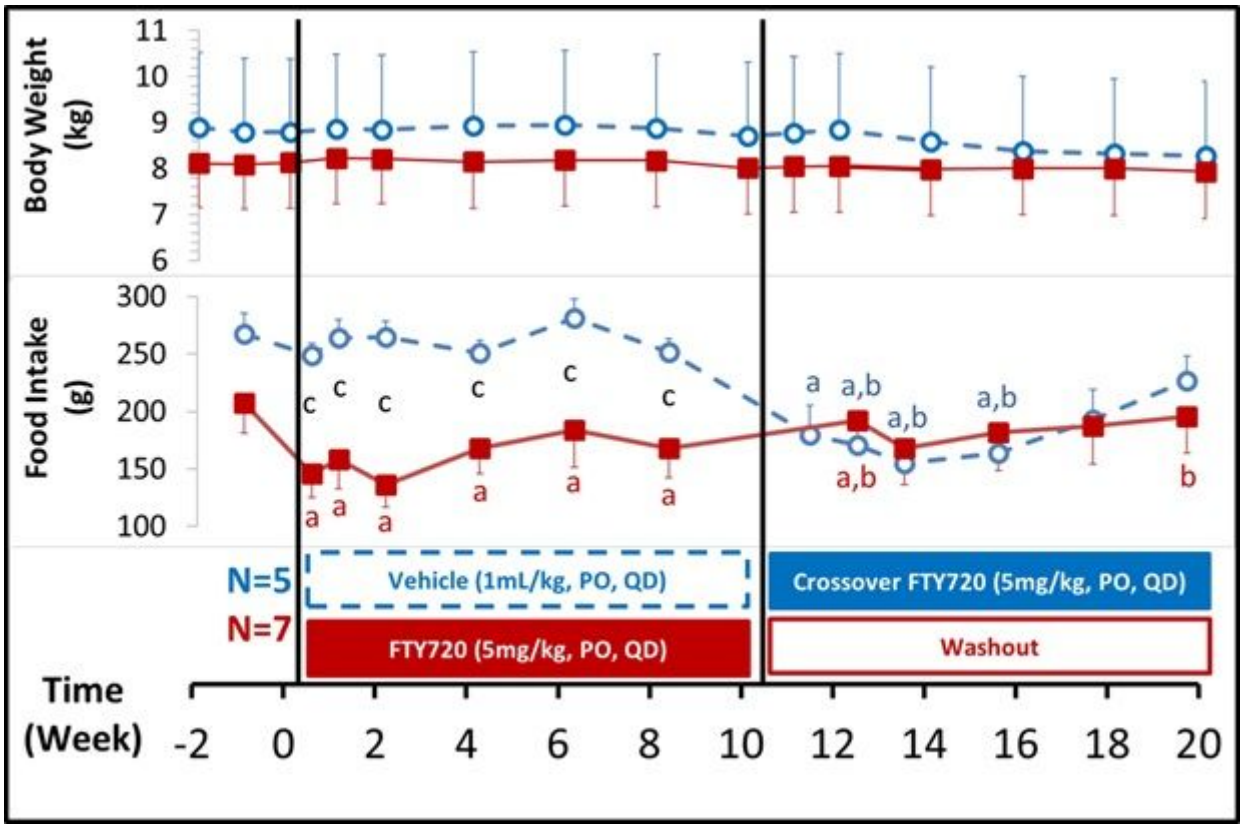

Figure 6

FTY720 decreased food intake without significantly impacting body weight Body weight (top) and daily food intake before (Week -1) and after once daily oral administration of vehicle (blue, purified water 1 mL/kg, n=5) or FTY720 (red, 5 mg/kg, n=7) in the diabetic NHPs. Statistical significance: p value < 0.05: Paired t-test for a. Post- vs. pre-dosing value (baseline) at week 0; b. Post vs. pre-crossover value at week 10; Unpaired t-test c. Compared the values between the vehicle and FTY720 group at each time points.

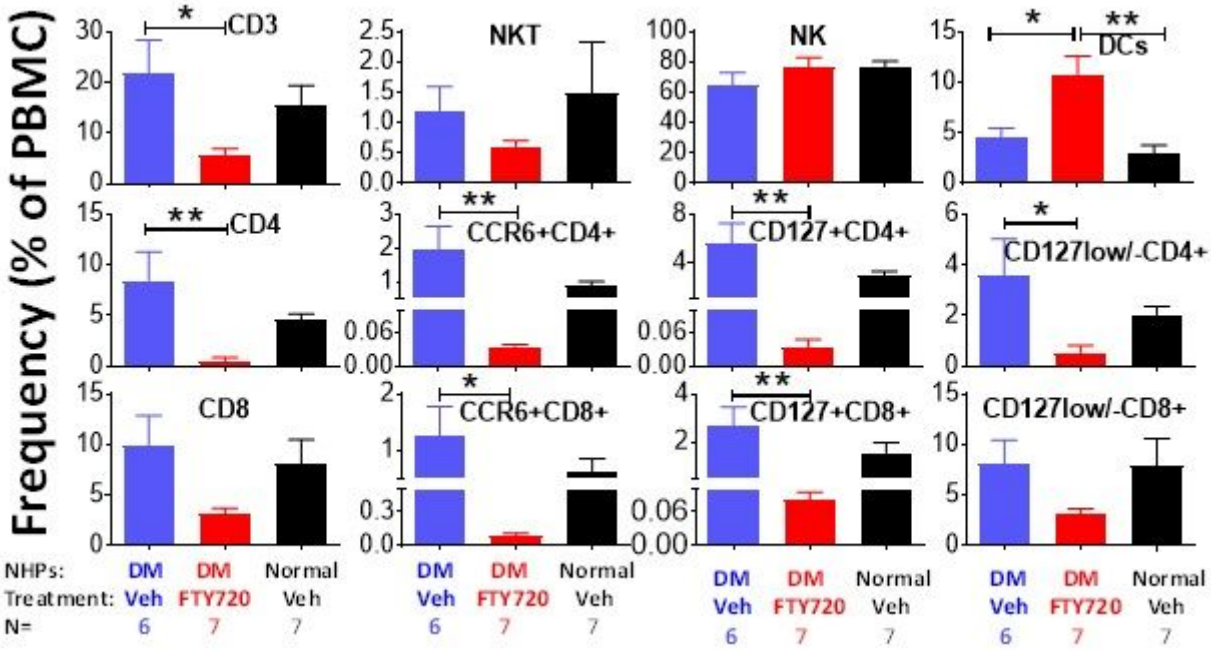

Figure 7

FTY720 modulated peripheral immune-cell profiles Percentage of individual positively stained live cells over total circulating peripheral blood mononuclear cells (PBMC), including T lymphocytes of CD3+ (total), CD4+ (effector), CD127+ (memory), CCR6+ (antigen experienced memory), CD8+, Natural Killer (NK), and dendritic cells (DC) in the diabetic NHPs treated with once daily oral dose of the vehicle (Blue, purified water 1 mL/kg, DM + Veh, n=6) or FTY720 (Red, 5 mg/kg, DM + FTY720, n=7) and non-diabetic NHPs treated with the vehicle (Black, Normal + Veh, n=7) for 8 weeks. Blood samples were taken 24 hours after the last dose for flow cytometry analysis.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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