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# Effects of β-carotene on glucose metabolism dysfunction in human subjects and type 2 diabetic rats

**Running title: Effect of β-carotene supplementation on glucose metabolism**

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**Declaration of conflict of interest**

None

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**Data and materials availability**

The authors declare that the data supporting the findings of this study will be freely available to any scientist from the authors upon request, without breaching participant confidentiality.

**Conflicts of Interest**

The authors have no conflict of interest to declare

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**Abstract**

**Background:**

Type 2 diabetes mellitus (T2DM) is a common chronic disease which is strongly associated with cardiovascular risk. Long-term high blood glucose level may induce cardiomyocytes apoptosis, cardiac dysfunction and fetal cardiomyocytes proliferation. Recent epidemiological studies have shown a link between antioxidant carotenoids and type 2 diabetes, but a comprehensive longitudinal study of this link has not yet been conducted.

**Methods:**

We included participants who had biological measurements for both serum cis-β-carotene and fasting glucose from NHANES (2001–2006). We divided participants into quartiles by the serum cis-β-carotene levels and supported this associations with glucose metabolism using multivariable regression models adjusted for confounding factors. The mechanism of β-carotene levels in regulating plasma glucose levels were further investigated in vivo and in vitro. In addtion, we have carried out a preliminary exploration of the effect of β-carotene on diabetic rat and primary cariomyocytes.

**Results**

We found that the higher cis-β-carotene (Q4) had a higher LDL-C level but with a lower fasting blood glucose. However, T2DM rats with β-carotene treatment showed decreased total triglycerides and LDL-c. β-carotene showed better cardiac function in DM+ group compared with diabetes groups ($P$<0.05). Our results also revealed that β-carotene to be an important protective factor of improving cardiac and mitochondrial function with diabetes exposure. At non-cytotoxic doses, β-carotene significantly increased glucose uptake in insulin-resistant cells. This potential effect is mediated by inducing the expression of GLUT4, the level of p-Akt and attenuating the phosphorylation of IRS-1. The analysis of gene expressions of PGC-1β and Nrf-1 showed a concordance between mitochondrial DNA content in PA-induced cardiomyocytes with or without β-carotene treatment, respectively.

**Conclusion**

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Our results indicate that β-carotene can treat metabolic disorders by inhibition of IR pathway in diabetes.

**Introduction**

Diabetes mellitus is one of the most common chronic metabolic disorders, which characterized by hyperglycemia due to lack of insulin or disturbances in insulin signaling[1, 2]. Diabetes has become a worldwide public health crisis. Owing to the global aging population, the prevalence of diabetes and pre-diabetes was estimated to be increased significantly in coming decades[3].

Type 1 and type 2 diabetes are both tough---and most of us do not meet health care targets[4]. Family history of diabetes was considered as a major risk factor for both T2DM and pre-diabetes[5, 6]. Currently, diabetes patients are advised to adopt an appropriate diet including a restrict energy in take or depending on hypoglycemic drug with lifelong time[7]. Needle free injection and an insulin pump give greater flexibility, but they do not make the problem go away. Diabetic patients are subjected to 2-4 times higher mortality rate of cardiovascular disease due to the incidence of heart failure[8]. Therefore, a better understanding of mechanism of diabetic cardiomyopathy will contribute to the development of novel strategies for DM patients.

Abnormal glycolipid metabolism is closely related to diabetes, atherosclerosis, coronary disease, hyper-triglyceridemia, low high-density lipoprotein (HDL) cholesterolemia, insulin resistance and other diseases. Free radicals including reactive oxygen species (ROS) and nitric oxide (NOS) are highly reactive by-products of oxidation and biochemical reactions in human cells. In the recent years, more attention has been paid to the antioxidant activity of naturally occurring substances in higher plants or cells. Carotenoids represent natural lipophilic pigments synthesized by plants, algae, fungi, and bacteria[9]. Most Carotenoids as same as insulin are lipophilic and with the ability to cross the blood-brain barrier[10]. It is well known for the high biological activity that provide various health advantages. Due to its chemical structure and interaction with biological membranes, Carotenoids have potential biological antioxidant properties [11]. More than 600 types of Carotenoids

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are identified in nature, however, according to its molecular structure, only α-carotene, β-carotene, lycopene and xanthophylls are usually present in the human diet[12].

β-carotene is highly abundant in Carotenoids and is an important food coloring agent[13]. The absorption or accumulation of β-carotene varies from species to species. So far, the effect of β-carotene on glycolipid metabolism remains unclear. Little known about metabolic turnover or pathway of β-carotene in human. Adequate nutritional intake is a non-pharmacological intervention and prevention strategy. Long-term high blood glucose level may induce cardiomyocytes apoptosis, cardiac dysfunction and fetal cardiomyocytes proliferation. Therefore, our study examines the hypoglycemic effects of β-carotene on the type 2 diabetes model and its protective effects on rat cardiomyocytes.

## Methods

### 2.1 Study participants

The data was obtained from NHANES public available files. It was conducted by the National Center for Health Statistics (NCHS) and used a stratified, complex multi-stage design to provide nationally representative sample of the non-institutionalized U.S. population. In our study, the publicly available files for the NHANES 2001 to 2006 cycles were merged using NCHS recommended methods[14]. The survey employs a multistage stratified probability sample. For our analysis, we included adults (ages 1 years and older) who had biological measurements for both serum cis-β-carotene and fasting glucose. The final sample size of 9281 individuals was tested for a the covariates included in the model . Excluded participants due to incomplete and invalid data. Missing values in the data can reduce the power of the model and leads to wrong predictions and classifications. Therefore, we used generalized mean imputation methods to replace missing values in our study. Meanwhile, a linear regression analysis was used to illustrate the correlation between serum level of cis-β-carotene and fasting glucose. This study is based on publicly available data from the NHANES website: https://wwwn.cdc.gov/nchs/nhanes/.

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**2.2 Laboratory assessments**

Blood analysis data were obtained from participants randomly assigned to be examined in morning after an overnight fast in this study. Results from low-density lipoprotein (LDL-cholesterol) cholesterol, triglycerides, C-peptide, C-reactive protein and fasting glucose were assessed under NHANES lab protocol .

**2.3 Animals, Diets, and Treatments**

An overview of the proposed workflow is shown in Figure 1. We used 6-7 weeks healthy male Sprague–Dawley (SD) rats (n=30, weighing 200 ± 30 g) and kept at the Key Laboratory of Molecular Imaging (Harbin, China). All rats were acclimated and housed under controlled humidity (10-30%) ,temperature (22-26˚C), and light cycle conditions and had free access to food and water. All experiments were performed at the same time in the morning to minimize differences in circadian rhythm. All research protocols were conducted in accordance with the guidelines for the Care and Use of Laboratory Animals. Rats were designed and randomly divided into control group (Con, n=10) with standard diet (overall calories of 3.5 kcal/g) and STZ-induced diabetes group (DM, n = 20). Mimicking the whole features of type 2 diabetes in humans, DM rats were injected intra-peritoneal with small dosage STZ at 20 mg/ kg body weight for 3 days. After an additional week, the rats showed fasting blood glucose (FBG > 16.7 mmol/L) were involved in the study as type 2 diabetes rats[15], which were fed with high fat diet (containing 20% protein, 60% fat, 20% carbohydrate, and 60% fat D12492 from Research Diets.Inc) for 8 weeks. DM rats were further divided into 4-week (DM and DM+ β-carotene) and 8-week (DM and DM+β-carotene) subgroups. β-carotene group was feed with with oral supplementation of β-carotene (50 mg/kg) by gavage

**2.4 Echocardiography measurement**

Cardiac functions were assessed using an echocardiography, which were treated on self-breathing rats under anesthesia with intraperitoneal injection of 10% chloral hydrate according to body weight) as reported previously[16]. LV end diastolic/systolic diameters (LVEDD, LVESD), and LV posterior wall thickness in diastole and systole (LVPWd, LVPWs) were measured on M-mode recordings. Reported values of all

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echocardiography parameters represent the means of at least three consecutive cardiac cycles.

Cardiac MBV and micro vascular blood flow velocity (MFV) were determined using contrast-enhanced ultrasound (CEU) before and at the end of the insulin infusion .Micro vascular blood flow (MBF) was the product of MBV and MFV. Once the blood micro bubble concentrations reached steady state (within 2-3 min), intermittent ultra-harmonic imaging was performed with the pulsing intervals of 1, 8, 12, 16, 20, and 32 cardiac cycles in the cardiac muscle. At least three images were captured digitally at each pulsing interval. Cardiac imaging included the apical two-, three-, and four-chamber views. Animals were were killed with overdose of sodium pentobarbital (100 mg/kg) and cardiac tissue were collected for biochemical and morphological studies.

**2.5 Ethics approval and consent**

The NHANES 1998–2012 protocol was approved by the Institutional Review Board of the Centers for Disease Control and Prevention (https://www.cdc.gov/nchs/nhanes/irba98.htm).Written informed consent were obtained from the parents of the children and from adult participants . Animal experiments were approved by the Ethical Committee for Animal Experiments from Second Affiliated Hospital of Harbin Medical University (Approval Number: SYDW2019-50).

**2.6 Chemicals and Reagents**

Streptozotocin (STZ, NO. S0130) and rat insulin Elisa kit (NO. RAB0904) were provided by Sigma (St. Louis Missouri, USA). A blood glucose meter and test strips (Roche Diagnostics, Indianapolis, IN) were used to measure the fasting blood glucose of each group once a week during the experiment.

**2.7 Induction the Model of Insulin-Resistant primary cardiomyocytes and Glucose Uptake Assay**

PA-induced insulin resistance is prepared by a stock solution of 100 mM PA with 100 mM NaOH at 70 ℃ with mixing 10% (w/v) BSA to obtain different concentrations of PA[17]. Different concentrations of PA (0, 150, 250, 350 μM) were added to primary

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cardiomyocytes incubated for 24 hours and then cellular glucose uptake was measured by 2-NBDG (Sigma-Aldrich) at excitation and emission wave lengths of 485 and 535 nm, respectively. Briefly, cells were incubated with PA in the presence of β-carotene or metformin (as positive control) for 24 h. Cells were seeded in glucose-free DMEM for 4 hours and then stimulated with insulin (500 nM) for 10 minutes. Following incubation with final concentration of 50 nM 2-NBDG in glucose-free DMEM for 30 min, cells were washed with cold PBS to remove 2-NBDG.

**2.8 Quantitative reverse transcription-polymerase chain reaction**

Total RNAs were extracted using TRIZOL (Life Technologies, Carlsbad, CA, USA) in accordance with the manufacturer's instructions, and reversed transcribed into cDNA using reverse transcriptase. The primer sequences were as follow: PGC-1β, Forward AACCCAACCAGTCTCACA, Reverse CTCCTAGGGGCCTTTGTT; TFAM Forward CCAAAAAGACCTCGTTCA; Reverse ATGTCTCCGGATCGTTTC; NRF1, Forward TATGGCGGAAGTAATGAA; Reverse CAACGTAAGCTCTGCC.

**2.10 Biochemical analysis**

The GLUT4 BioAssay ELISA Kit (US Biological, #383107), Total IRS-1 Cell-Based Colorimetric ELISA Kit (Immunoway, #KA4145C) and Akt (pS473) + total Akt ELISA kit (Abcam, #ab126433) were measured using commercially available ELISA kits, according to the manufacturer's instructions. The protocols of the Kits are all relatively simple. All prepared-samples are pipetted into the wells and indicated-sensitive assay kit present in a sample which is bound to the wells by the immobilized antibody. The stop solution changes the color from the originial blue to yellow, and read the absorbance under a micro-plate reader at 450nm with optional reference wavelength of 665 nm.

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**2.9 Statistical analysis**

Our data were given as the means ± the standard error of the mean (S.E.M.) for at least three independent experiments replicates. One-way ANOVA followed by a Tukey post hoc test was performed for assessing significance in three or more groups.

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Two-way ANOVA with Holm-sidak test for acquisition performance comparisons using Graph Pad Prism 7. Statistical analysis yielded significant different in P<0.05.

## Results

### *Demographic and baseline characteristic data*

In total, 31509 NHANES participants (2001–2006) were collected; 9281 participants had complete blood glucose and cis-β-carotene data. The participants categorized into four groups based on serum cis-β-carotene quartiles and shown in **Table 1**. A total of 9281 participants (4557 males and 4724 females) aged 38.82 ± 22.28 years (range, 0–85 years) with a BMI of 27.07 ± 6.72 kg/m$^2$ were included in this study. Compared to the other groups, Q4 group was older (49.0 ± 22.8 vs. 38.3 ± 22.6 vs. 33.6 ± 20.0, *P* < 0.001) and had a higher SBP (124.09 ± 23.20 vs. 119.33 ± 19.90 vs. 118.01 ± 17.74, *P* < 0.001) and a higher PIR. Participants with high level of cis-β-carotene have lower fasting blood glucose, fasting insulin, HOMA-IR and HOMA-B. It is noteworthy that cis-β-carotene have negative associated with fasting blood glucose and insulin level.

### *The relationship of variables used in the present study.*

We carried out a correlation analysis (Pearson correlation coefficient) to eliminate the strength of relationship between cis-β-carotene and blood biochemistry in 9281 participates (**Table 2)**. The FBG, triglyceride, insulin and HOMA-R were negative correlated with cis-β-carotene (*P*<0.001, r=-0.034; *P*<0.001, r=-0.041; *P*<0.001, r=--0.113; *P*<0.001, r=--0.088). SBP average was correlated positively with cis-β-carotene, fasting blood glucose, triglyceride, insulin and HOMA-R (*P* <0.001, r=0.109; *P* <0.001, r=0.220; *P*<0.001, r=0.145; *P*<0.001, r=0.331; *P*<0.001, r=0.094). HOMA-R showed a negative correlation with cis-β-carotene (*p*<0.001, r=-0.088), and with positive correlation with fasting blood glucose, triglyceride, insulin and SBP (*P*<0.001, r=0.481; *P*<0.001, r=0.198; *P*<0.001, r=0.895; *P*<0.001, r=0.094) according to Pearson relationship analysis.

### *Associations between fasting blood glucose and serum cis-β-carotene*

**Table 3** presents the association between serum cis-β-carotene and fasting blood glucose using the linear regression models. Multivariate logistic regression analysis

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showed that serum cis-β-carotene were significantly associated with fasting blood glucose after multivariate adjustment for age, gender, ratio of family income to poverty, BMI, diastolic blood pressure, systolic blood pressure, LDL-cholesterol, and C-reaction protein. Every 1 μmol/L increase in serum cis-β-carotene levels was associated with 0.013mmol/L (*P* <0.001, 95% CI = 0.009 to 0.017) and -8.01 (*P* <0.001, 95% CI = -12.64 to -3.56) decrease in fasting blood glucose and HOMA-IR.

***Subgroups analyses for the associations between cis-β-carotene and glucose metabolism in the participants***

According to Table 3, we summarized the results of subgroup analyses for adults as shown in Figure2. Female and HOMA-IR<2.5 groups were likely to have lower plasma glucose level with increasing insulin presenting in significant trend (Fig.3 A-C). Our analysis showed that positive effect of cis-β-carotene on glycemic control as well as measures of insulin sensitivity. These results inspired us to explore if the presence of cis-β-carotene might influence glucose metabolism in human body.

***Establishment of type 2 diabetic rat model***

Blood glucose levels in the 4 weeks DM and 8 weeks DM were significantly higher compared with the control group (**P*<0.05), indicating that type 2 DM model was successful established [15]. Although there were no statistically significant changes to FBG , food intake or water intake, the dynamic monitor of β-carotene treated diabetic rats showed that they had a tendency to decrease food intake during the 8 week treatment (**Figure**. 3A-B).

As shown in **Figure**. 4, SOD and GSH-Px, which are significant inhibitors of ROS, were used to balance the ROS content of injured hyperglycemia. The MDA, TG and LDL-C contents were significantly increased in DM groups versus control group (P<0.05), although there was significant decreased in DM+β-carotene group (*P*<0.05). The SOD, GSH-Px, TC and HDL-c contents were significantly decreased in DM groups versus control group (*P*<0.05), although there was significant increase in DM+β-carotene group (*P*<0.05).

***Cardiac functional recovery after β-carotene feeding***

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As shown in **Figure 5**, EF, FS and LVEDV were decreased in DM groups compared with the control group, whereas, both LVPWs and LVPWd increased at 4 weeks ($P$< 0.05). EF and FS were higher at 4 and 8 weeks in DM+β-carotene group compared with diabetes groups ($P$ < 0.05). There was no significant differences in LVESV between the 4 weeks diabetes and the DM+β-carotene group. In 8 weeks, groups, LVPWd was increased compared with control group, while LVPWs decreased compared with DM group ($P$< 0.05).

***Changes of cardiac muscle micro vascular parameters.***

At the end of experiments, rats were given insulin clamps with or without extra β-carotene for another 120 mins after fasting over 8 hours. As shown in Figure 6 A-C , insulin infusion significantly increased cardiac MBV but did not affect cardiac MFV in control group. β-carotene infusion caused a slight but significant increase in basal MBF without affecting MFV. MBV at 0 min DM groups showed a slight but no significantly lower during β-carotene infusion both in 4 weeks and 8 weeks .

***Effect of β-carotene on mitochondrial respiratory synthase and mitochondrial respiratory function of type 2 diabetic rats***

The Clark oxygen electrode method detects the respiratory function of myocardial mitochondria, including 3-state respiration, 4-state respiration, respiratory control rate (RCR) and phosphorus-oxygen ratio (ADP: O), meanwhile, and the electron transport chain complex I, II, III, IV enzyme activities were determined[18]. The results showed that after β-carotene treatment, it can significantly improve the activity of myocardial mitochondrial electron transport complexes I, III, and IV in diabetic rats (Table-4). Mitochondrial respiratory chain is extremely important for removing H+ from the mitochondrial matrix on or near the inner mitochondrial membrane. As shown in **Figure 7A** and **Figure 7B**, there was a decrease in the state 3 of respiration in cardiac mitochondria of diabetic rats and the state 4 of respiration was appeared to be normal in DM+β-carotene groups. ADP/O and RCR index both decreased in DM group, and these effects were restrained in the DM+β-carotene group, indicating that the respiratory chain was repaired by β-carotene (**Figure**. 7C&D).

***Effect of Different concentrations of β-carotene on primary cardiomyocytes***

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***viability with or without PA.***

The cytotoxicity of β-carotene on primary cardiomyocytes was evaluated using the MTT assay after 24 h incubation. The EC50 of β-carotene in primary cardiomyocytes at 24 h was 57.91±4.90 μM (**Figure. 8B**). 50μM β-carotene presented no significant cytotoxicity from 0 to 24 hours CCK-8 assay (**Figure. 8C**), indicating that the test concentration of β-carotene was not cytotoxic to primary cardiomyocytes.

Next, we evaluated the effect of PA on primary cardiomyocytes viability by MTT. Glucose uptake were conducted to identify the optimal concentration for inducing IR injured model. **Figure 8D** showed that cell proliferation was attenuated by concentration dependence of PA ($P$<0.05). **Figure 8E** shows that compared with the control groups, insulin (500 nM) treatment enhanced the 2-NBDG uptake in primary cardiomyocytes ($P$<0.05). Our results indicated that PA could evoke IR without obvious induction of cytotoxicity range from 150-350 μM. Therefore, 350 μM of PA and 500 nM of insulin were selected to establish insulin resistance model and its effect of increasing glucose uptake might be more efficiency.

Glucose uptake assay was performed to quantified whether β-carotene could inhibit IR-induced injury of cardiomyocytes. As shown in **Figure 8F**, there was a significant reductions in 2-NBDG uptake of cardiomyocytes by PA, while β-carotene (10-50 μM) and metformin (5 μg/mL, positive control) obviously modified the reduction in 2-NBDG uptake ($P$<0.05). Our result suggested that β-carotene could improve insulin sensitivity of PA-induced cardiomyocytes.

***Effect of β-carotene on Insulin Signaling Pathway***

To confirm that β-carotene can improve PA-induced insulin resistance, we examine the levels of insulin pathway-related proteins assessed by Elisa Kits in primary cardiomyocytes. As shown in Figure 9A, high glucose with or without PA treatment significantly reduced the level of GLUT4 and phosphorylation level of Akt (Ser473), and contributed to increase the phosphorylation of IRS-1 (Ser307) level. The results showed that β-carotene administration significantly block PA-induced changes in Akt and IRS-1 phosphorylation levels ($P$ < 0.05).

***β-carotene improve mitochondrial biogenesis in PA-induced cardiomyocytes***

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To further investigate the β-carotene effect on cardiomyocytes mitochondrial fuction, we conducted PCR-based assay allows for quantitative assessment of the mitochondrial mass number of mitochondrial by the mitochondrial DNA (mtDNA) copy number .As shown in **Figure 10**, mtDNA to nuclear DNA (nDNA) ratio was significantly decreased in PA-treated cardiomyocytes to control group. Thus, mitochondrial crista depletion and the mtDNA copy number decrease may be associated with partial respiratory inhibition resulting from insulin resistant insults. β-carotene significantly increased the mtDNA copy number and the activity was higher compared with metformin positive control ($P< 0.05$). PGC1 α, NRF-1, TFAM and AMPK genes were determined by RT-PCR. In the control group, there was a remarkable decrease in both PGC-1β and NRF1 mRNA, whereas, TFAM with a significant increase of mRNA level compared to high glucose alone and PA cultured group. Comparing the results in high glucose and PA group, there was an obviously increasing of PGC-1β and NRF1 mRNA in β-carotene treatment ($P<0.05$). These data suggested a novel pathway that β-carotene is associated with an increase in mitochondrial biogenesis under insulin-resistant cardiomyocytes.

**Discussion**

In recent decades, the prevalence of type 2 diabetes (T2DM) has increased and becomes a major health concern spread around the world[19]. Previous studies have limited evidence on the association between circulating vitamin C and carotenoids and the risk of T2DM, mainly due to the small number of participants in previous studies[20, 21]. The existence of our findings illustrates that: (i) Serum cis-β-carotene level has negative relationship with BMI index, blood glucose, and fasting insulin; (ii) β-carotene recover PA-induced cardiomyocytes mitochondria biogenesis via IR-related pathway; and (iii) β-carotene protected against diabetes via its recover glucose metabolism function.

Carotene and structurally related compounds are precursors to vitamin A, which play important roles in immune response, photosynthetic organisms, and cell differentiation. Therefore, carotenoids are widely used as dietary supplements in hopes of maintaining health and preventing diseases such as cardiovascular disease,

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inflammation, decline in physical performance and mortality. Unfortunately, large clinical trials have failed to prove that the tested formula is beneficial, and some people believe that taking high-dose supplements may be harmful[22]. Vitamins such as Vitamin A/D/K and E are considered relatively safety. Physiological level will be life-saving but dangerous at mega dose[23]. Higher doses of Vitamin D supplement did not decrease the risk of Vitamin deficiency, what's more, higher dose of Vitamin E may increase cardiovascular disease and all-cause mortality[24, 25]. Research into carotenoids and their application for diabetes mellitus has been growing, but the findings have been inconsistent. Little is known about the association of insulin resistance with serum carotenoids.

We used the NHANES 2001 to 2006 data, a total of 9281 participants were interviewed to explore the relationship between β-carotenoid and glucose metabolism. InTable1, participants in the highest quartile(Q4) of cis-β-carotenoid level had a lower BMI ($P$<0.01), blood glucose ($P$=0.046), and fasting insulin ($P$<0.01) than in the lowest quartile(Q1). Our study emphasize that high circulating levels of cis-β-carotenoid protect against blood glucose, level of insulin and HOMI-R. We also found that the higher cis-β-carotene (Q4) had a higher LDL-C level but had a lower TG (Table 1). Pearson analysis also demonstrated that the negative relationship between cis-β-carotene and TG(Table 2).However, T2DM rats experiments showed inconsistence results with Table 1. T2DM rats with β-carotene treatment showed decreased total triglycerides and LDL-cholesterol levels (LDL-c). This may be due to the relatively small number of animals recruited, and our findings do not appear to fully mimic the features in human. Moreover, these analyses could not take a number of patients taking statins or other medicine into count.

Insulin resistant (IR) begins before the onset of T2DM in vivo and it is the best predictor of onset of T2DM, and previous studies show that strategies to reduce IR limit the development of T2DM especially for ameliorating diabetic cardiomyopathy. Drugs such as resveratrol[26], trehalose[27], curcumin[28], and liraglutide[29] have been shown to promote autophagy in IR cardiomyocytes and diabetic animal models in

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various ways, thereby reducing cell death. Epidemiological studies have shown that diabetes is an independent risk factor to induce cardiac dysfunction, which is characterized by cardiac hypertrophy and diastolic dysfunction. We established type 2 diabetic model to demonstrate β-carotene treatment recover cardiac function and mitochondria biogenesis of cardiomyocytes in vivo and in vitro (Figure 5,Figure 6 & Figure 7).IR and vascular dysfunction are strongly associated with the development of cardiovascular complications, which are the major causes of mortality for T2DM patients. The peroxide and other ROS produced in vascular cells may play an important role in the pathogenesis of common vascular diseases[30, 31], especially diabetes. Long-term clinical observational studies on diabetes reveals that, in addition to metabolic toxicity factors, there may be also important protective factors which protect the function and survival of cells involved in microvascular diseases from the influence of blood glucose control[32-34]. Therefore, we also examine the cardiac microvascular recruitment by diastolic/systolic stress MBF ratio ,which regards as a novel diagnostic index with moderate diagnostic accuracy[35] .Similar to previous studies, our study observed that adding β-carotene increased MBF, which was also associated with significant increased mitochondrial respiratory function **(Figure 7).**

Some anti-diabetic drugs can decrease blood glucose level sufficiently, but do not improve the cardiac prognosis in patients with type 2 diabetes[36]. This may be due, at least in part, to a lack of understanding of cardiac insulin resistance. IR is implicated in the pathogenesis of diabetic cardiomyopathy[37], even if it occurs only locally in the heart. Myocardial insulin resistance not only interferes the metabolism of cardiomyocytes, but also leads to mitochondrial dysfunction and oxidative stress in cardiomyocytes. Mitochondrial function is further regulated by the transcription factors PGC-1α, NRF1, and TFAM, which are key mitochondrial enzymes and mtDNA synthesis[38]. Long term exposure to high glucose for cardiomyocytes could increase concentrations of superoxide (Figure 4 A-F) and simultaneously increased mitochondria damage (Table 4 &Figure7). Our results indicate that β-carotene can treat glucose metabolic disorders by inhibition of PA-induced mitochondria biogenesis in diabetic rat and cardiomyocytes (Figure 10).

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**Limitations**

First, our data were obtained from a cross-sectional sample from 2001 to 2006 NHANES to explore the relationship between β-carotene on glucose and lipid metabolism. We indirectly speculated that factors may affect blood glucose, insulin and HOMA-R, while the immune responses or protein levels of human should be interpreted with appropriate caution as prospective studies.

Second, some associations may have been missed due to limited statistical power, such as behavioral habits and lifestyle factors, ethnic differences , smoking, alcohol and coffee consumption were not adjusted.

Third, the sample size was reduced due to missing, incorrect, or incomplete on blood glucose and cis-β-carotene data missing in NHANES database.

Finally, due to genetic and socioeconomic differences between the US and other countries, the generalizability of our findings maybe affected by the study's exclusionary criteria and required consideration. Further longitudinal studies including additional factors that potentially could influence myocardial protection on the association between β-carotene and glucose/lipid metabolism are needed to replicate.

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