Original Article

High fructose induced osteogenic differentiation of human valve interstitial cells via activating PI3K/AKT/mitochondria signaling

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ABSTRACT

Background: Aortic valve stenosis (AS) is a common, lethal cardiovascular disease. There is no cure except the valve replacement at last stage. Therefore, an understanding of the detail mechanism is imperative to prevent and intervene AS. Metabolic syndrome (MetS) is one of the major risk factors of AS whereas fructose overconsuming tops the list of MetS risk factors. However, whether the fructose under physiological level induces AS is currently unknown.

Methods: The human valve interstitial cells (hVICs), a crucial source to develop calcification, were co-incubated with fructose at 2 or 20 mM to mimic the serum fructose at fasting or post-fructose consumption, respectively, for 24 h. The cell proliferation was evaluated by WST-1 assays. The expressions of osteogenic and fibrotic proteins, PI3K/AKT signaling, oxidative phosphorylation and mitochondrial dynamics were detected by Western

Keywords:
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Aortic valve stenosis (AS), a status of aortic valve disease, is a common, lethal cardiovascular disease in the elderly. The development of AS is often subtle at first and progresses over time until months or years after the beginning. Once the progression of AS initiated, it is considered as irreversible.

Several risk factors are involved in the progression of AS including age, hypertension and dyslipidemia [2–4] which are the major criteria of metabolic syndrome [5]. Under physiological status, insulin binds to membrane-bonded insulin receptor to phosphorylate insulin receptor substrate (IRS)-1 for the activation of the phosphoinositide 3-kinase (PI3K)/phosphoinositide-dependent kinase 1 (PDK-1)/Akt pathway for glucose uptake [6] in response to the carbohydrate supply. Any disturbance in this cellular pathway, for instance, the upregulation of IRS-1 and PI3K p85, could initiate the progression of metabolic syndrome [7]. Notably, the PI3K/AKT signaling promotes osteogenic differentiation of mesenchymal stem cells [8,9] though the downstream signaling is inconclusive.

AKT signaling regulate mitochondrial oxidative phosphorylation [10,11]. Mitochondrion is the major source of cellular energy support by oxidative phosphorylation (OXPHOS). Intriguingly, the activation of OXPHOS drives calcium accumulation in mitochondria (Reviewed by Carafoli 2010 [12]). Recently, accumulating evidence indicated that active mitochondria positively regulate osteogenic differentiation of mesenchymal stem cells [13,14]. It is conceivable that the AKT-activated mitochondrial OXPHOS may play roles in the osteogenic differentiation in hVICs.

High activation results in large-scale impairment of mitochondria. To maintain the quality, mitochondria are highly dynamics and are strictly regulated by mitochondrial fission/fusion, and mitochondrial autophagy (mitophagy) in response to environmental nutrition [15,16]. Mitochondrial fission is controlled by dynamin-related protein 1 (DRP1), fission protein 1 (FIS1) and mitochondrial fission factor (Mff). Overnutrition may result in mitochondrial fission and the increased number of damaged mitochondria. The impaired mitochondria are divided by fission, engulfed by mitophagy [17,18]. In mammals, the best-studied proteins involved in mitochondrial fusion are the optic atrophy type 1 (OPA1), mitofusin 1 (Mfn1) and Mfn2. Mitophagy is responsible for the clearance of damaged mitochondria. The best-studied proteins involved in mitophagy are the PTEN-induced kinase 1 (PINK1) and Parkin [19]. Under physiological status, PINK1 is cleaved by presenilin-associated rhomboid-like serine protease (PARL) in mitochondria [20] while full length PINK1 recruits Parkin to initiate mitophagy [19]. However, rare study has been focused on the role of valve mitochondrial fission/fusion and mitophagy in response for the nutrient supply.

Fructose is a common sweetener in nature fruits and in our daily desserts. Overconsumption of fructose is a pressing worldwide health issue. A large body of evidence from both

At a glance of commentary

Scientific background on this subject

Aortic stenosis (AS) is a common, lethal cardiovascular disease. There is no cure except the valve replacement at the last stage. Metabolic syndrome (MetS) is one of the significant risk factors of AS whereas fructose over-consuming is a major risk factor of MetS. However, whether fructose under physiological level induces AS is currently unknown.

What this study adds to this field?

In this study, we demonstrated that high fructose promotes the osteogenic differentiation of human valve interstitial cells (hVICs) via the activation of PI3K/AKT/mitochondria signaling at the early stage. These results implied that high fructose at physiological level might have a direct, hazard effect on the progression of AS.

Results: hVICs proliferation was significantly suppressed by 20 mM fructose. The expressions of alkaline phosphatase (ALP) and osteocalcin were enhanced concurrent with the upregulated PI3K p85, AKT, phospho(p)S473-AKT, and pS636-insulin receptor substrate 1 (p-IRS-1) by high fructose. Moreover, ATP production capacity and maximal respiratory capacity were enhanced in the high fructose groups. Synchronously, the expressions of mitochondrial fission 1 and optic atrophy type 1 were increased.

Conclusions: These results suggested that high fructose stimulated the osteogenic differentiation of hVICs via the activation of PI3K/AKT/mitochondria signaling at the early stage. These results implied that high fructose at physiological level might have a direct, hazard effect on the progression of AS.
human and animals suggest that high fructose intake induces metabolic syndrome (MetS) [21–25] while metabolic syndrome is one of the major risk factor of valve stenosis [3]. It is conceivable that excessive fructose may contribute to the progression of valve stenosis. Moreover, high fructose diet-altered mitochondrial function has been documented in MetS [26]. In this study, we conducted the in vitro model to investigate the initiation of valve stenosis at different time points by using the human valve interstitial cells (hVICs) co-incubated with various concentrations of fructose. The levels of cell proliferation, cell fibrotic markers (e.g. a-smooth muscle actin, and collagen III), cell osteogenic markers (e.g. alkaline phosphatase, and osteocalcin), the PI3K/AKT signaling, mitochondrial OXPHOS, and mitochondrial dynamic proteins were detected by Western blot analysis. The level of calcium deposition was evaluated by Alizarin Red staining.

Materials and methods

Human valve interstitial cell culture

The human valve interstitial culture cells (hVICs) were purchased from Innoprot (Bizkaia Spain) and the hVICs specific medium (Fibroblast Medium II) were used. The cells from passage five were seeded into 6-cm culture dish coated with Poly-L-Lysine at a density of 1.0 × 10^5 cells/mL and grow for 4 h in the serum-free medium with FGF for further study. Cells were incubated in a humidified incubator at 37 °C in 5% CO_2_. After dripping fructose (0, 0.2, 2, or 20 mM) into cells, the cells were incubated at 37 °C in a CO_2_ incubator for 24, 48, 72, or 96 hours (h) prior test.

WST-1 cell proliferation assay

WST-1 cell proliferation assay kit (Takara Bio Inc., Shiga, Japan) was used to evaluate the cell proliferation following the guideline of the kit. 1 × 10^4 hVICs were seeded into a 96-well flat-bottomed plate for 24 h at 37 °C with 5% CO_2_ then subjected to various fructose (0, 0.2, 2, or 20 mM). At 24, 48, 72 and 96 h incubation, the cells were washed with PBS and replaced with 100 μL fresh medium. After washed by PBS, 100 μL of WST-1 Reagent were added to each well, and the plate was incubated for 2 h on an orbital shaker at room temperature. The Luminescence was detected by spectrophotometer. The luminescence of wells with no reagent were measured as Blank control. The value of Blank control were deducted from the values of the experimental wells. Values of proliferation of the treated-cells were expressed as a percentage of that from corresponding control cells. All experiments were repeated in triplicates.

Mitochondrial respiratory rate detection by the XF analyzer

XF24 Extracellular Flux Analyzer were used to perform all XF assays (Seahorse Bioscience; MA, USA). The sensor cartridge contains four reagent delivery chambers per well for injecting compounds, including inhibitors of mitochondrial respiratory complex I (rotenone), III (antimonycin A, AMA), and V ( oligomycin) as well as an uncoupling agent that disrupts the proton gradient (FCCP), into the wells during an assay to evaluate the rates of O_2_ consumption rate (OCR). 1 × 10^5 hVICs were seeded into 24-well XF24 plates for overnight attachment (except for background correction wells). After 24 h co-incubation with fructose (e.g. 0, 2, 20 mM), culture medium was washed out by PBS and substituted by 1X MAS buffer (Seahorse Bioscience) with substrate. The plate was then transferred to the XF24 instrument to initiate the measurement.

Total protein isolation

For Western blotting analysis, hVICs from each treatment were harvested after 3 times wash with PBS. Samples were homogenized with a Dounce grinder with a tight pestle in ice-cold lysis buffer (15 mM HEPES, pH 7.2, 60 mM KCl, 10 mM NaCl, 15 mM MgCl2, 250 mM Sucrose, 1 mM EGTA, 5 mM EDTA, 1 mM PMSF, 2 mM NaF, 4 mM Na3VO4). A protease inhibitor cocktail (Sigma–Aldrich) was included in the isolation buffer to prevent protein degradation. The lysate was stored at −80 °C for later use. The concentration of the total protein extracted was estimated by Micro Bicinchoninic acid (BCA) Protein Assay kit (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Western blotting

Samples from each group contain equivalent total protein concentration. Total proteins were separated by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (SDS-PAGE), using 8, 10 or 12% gels and a running buffer of 24 mM Tris–HCl, 0.19 Μ glycine, 0.5% SDS, pH 8.3. The electrophoretical proteins were transferred onto polyvinylidene difluoride (PVDF) membrane (Immobilon-P membrane; Millipore; Bedford, MA, USA) and probed with specific antibodies against a-SMA (1:1000, Abcam, Cambridge, UK), MMP9 (1:1000, Abcam), ALP (1:1000, Abcam), osteocalcin (1:1000, Abcam), IRh (1:1000, Abcam), phospho-IRS1 (S312) (1:1000, Abcam), P38X p85 (1:1000, Abcam), AKT (1:1000, Abcam), phospho-AKT (T308) (1:1000, Abcam) and phospho-AKT (S473) (1:1000, Abcam). Membranes were then incubated with appropriate horseradish peroxidase–conjugated secondary antibody. Specific antibody–antigen complex was detected using an enhanced chemiluminescence Western Blot detection system (Thermofisher Bioscience). The amounts of detected proteins were quantified by ImageJ software (NIH, MD, USA), and were normalized by b-actin protein.

The alizarin red staining

Human valve interstitial cells from different treatments were stained by alizarin red. The cells were fixed with 10% formaldehyde for 1 h. The cells was washed with PBS (pH 7.4) for 3 times followed by 3 times wash with PBS (pH 4.1). After incubation with alizarin red solution for 1 h, excessive dye was removed by washing with PBS (pH 4.1) then PBS (pH 7.4). The calcified nodules were observed by Olympus light microscope (IX51, Tokyo, Japan).
Statistical analysis

Data are expressed as means ± SEM. Nonparametric, Kruskal–Wallis test followed by the Dunn post hoc method was used for comparisons between groups. The differences were considered statistically significant when p < 0.05. Calculations were performed by GraphPad Prism (version 5) software (GraphPad Software, San Diego, CA).

Results

Fructose suppressed proliferation of human valve interstitial cells at high concentration

Fructose overconsuming is one of the major risk factors of metabolic syndrome [27,28], which plays an important role in the progression of aortic valve stenosis [3,29,30]. The valve interstitial cell (VIC) is the predominant cell type in the aortic valve [31,32]. As a fibroblast-like cell type, VICs are responsible for the valvular calcification [33,34]. However, whether high fructose provides direct effect to drive the human valve interstitial cells (hVICs) to calcification is unknown. According to previous study, the fasting serum fructose concentration is 1.9 ± 0.4 mM, and the peak of postprandial serum fructose concentration is 17.2 ± 1.1 mM in healthy volunteers [35]. Therefore, the fructose concentration used for in vitro study were ranged between 0 and 20 mM. To evaluate whether fructose impairs the cell proliferation of hVICs, the cells were co-incubated with various fructose concentration (e.g. 0, 0.2, 2, 20 mM) for 24, 48, 72 or 96 h for proliferation assays. The results indicated that the fructose concentration for 24, 48, 72 or 96 h for proliferation assays. The results indicated that the fructose concentration was significantly suppressed in these groups with extra added 20 mM fructose at 24, 48 and 96 h [Fig. 1]. At lower fructose dosage (e.g. 0.2 or 2 mM), hVICs proliferation showed the trends of decrement without statistical significant. Based on these results, the time point of 24-h was selected to further reveal the underlying mechanism of fructose (0.2, 20 mM)-induced impairment.

Fructose induced the expressions of alkaline phosphatase and osteocalcin in human valve interstitial cells at high concentration

The VICs can be differentiated as osteoblast or myofibroblasts. Valvular calcification is the primary cause of AS. Alkaline phosphatase (ALP) is an early calcification marker, and osteocalcin is an indicator of later-stage calcification [31,32]. We, therefore, examined the expressions of ALP and osteocalcin in hVICs co-incubated with various fructose concentration (0, 2, 20 mM) at 24-h. The results indicated that the expressions of ALP [Fig. 2A] and osteocalcin [Fig. 2B] were significantly increased at high fructose (20 mM) group. At lower fructose group (2 mM), the expressions of ALP or osteocalcin showed the trends of increment without statistically significant. On the contrary, the expressions of fibrotic markers, α-smooth muscle actin (α-SMA, Fig. 2C) and collagen III [Fig. 2D], showed no significant difference between groups. These results suggested that high fructose stimulation maybe dominate the progression of calcification in hVICs rather than fibrosis. Further, the results of the Alizarin-Red staining indicated that the mineral deposition was slightly increased in the high fructose-dose (20 mM) group when compared with the control (0 mM) and the low fructose-dose (2 mM) groups [Fig. 2E].

Fructose induced the expression of PI3K/AKT signaling and serine phosphorylation of insulin receptor substrate-1 in human valve interstitial cells at high concentration

The activation of PI3K/AKT signaling contributes to the osteogenic differentiation [8,9]. In addition, the increase of p85 subunit of phosphatidylinositol 3-kinase (PI3K) involves in the progression of MetS [7]. We detected the expressions of p85 subunit of PI3K, AKT and phospho(p)-AKT in hVICs by Western blotting. The results indicated that the expressions of PI3K p85 [Fig. 3A], AKT [Fig. 3B] and pS473-AKT [Fig. 3C] were significantly increased at high fructose group while no significant change was detected in the expression of pT308-AKT [Fig. 3D]. At lower fructose group, only the expressions of pS473-AKT [Fig. 3C] was significantly increased whereas PI3K p85 [Fig. 3A] and AKT [Fig. 3B] showed the trends of increment without statistically significant. These results suggested that PI3K/AKT cascade may mediate the high fructose-triggered progression of osteogenesis in hVICs at early stage.

The activated AKT exhibits the capability to phosphorylate insulin receptor substrate-1 (IRS-1) leading to the attenuation of T308-AKT phosphorylation [36]. Therefore, we examined the expressions of IRS-1 and p-IRS1 in hVICs by Western blotting. The expression of pS636-IRS1 [Fig. 3F] were significantly increased at high fructose group while no significant change was detected in the expression of IRS1 [Fig. 3E]. At lower fructose group, the expression of pS636-IRS1 [Fig. 3F] showed the trends of increment without statistically significant. These results provided the possibility that high fructose-
increased pS473-AKT may in turn activate IRS-1 to suppress the phosphorylation of T308-AKT in hVICs.

**Fructose enhanced mitochondrial oxygen consumption rate in human valve interstitial cells at high concentration**

The activation of AKT signaling promotes mitochondrial oxidative phosphorylation [10,11]. Intriguingly, the activation of mitochondrial respiration enhance calcium accumulation [12]. Oxygen consumption rate is an important index of mitochondrial aerobic respiration. To examine whether the mitochondrial respiration was altered by high fructose incubation, the oxygen consumption rate (OCR) of each group was measured by the XF24 Extracellular Flux Analyzer (Seahorse). The maximal respiratory capacity, basal respiratory capacity, and ATP production capacity of hVICs were further dissected from OCR curve.

The results indicated that OCR of hVICs was reduced to ~78% of baseline rates after oligomycin A (a mitochondrial ATP synthase inhibitor) in control group (common medium with 0 mM fructose) indicating that ~22% of oxygen consumption was related to ATP production. Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP) is a mitochondrial protonophoric uncoupler. FCCP application eliminated the proton gradient across mitochondrial inner membrane and enhanced OCR to the maximal of baseline rate.

Fig. 2 The expressions of alkaline phosphatase and osteocalcin as well as mineral deposition in human valve interstitial cells were increased by fructose at high concentration. Representative gels and densitometric analyses of (A) alkaline phosphatase (ALP) (B) osteocalcin (C) α-smooth muscle actin (α-SMA) and (D) collagen III detected at 24 h incubated with 0, 2, or 20 mM fructose (E) Mineral deposition detected by Alizarin Red staining. Values are mean ± SEM, n = 4–6 in each experimental group. The sample size of each group was noted on the bar. *p < 0.05 versus 0 mM fructose group using the nonparametric, Kruskal–Wallis test followed by the Dunn post hoc method. Arrow head: mineral deposition. Scale: 100 μm.
These results suggested that the maximal respiratory capacity of hVICs is about 1.2 times higher than the basal respiration [Fig. 3A and B]. In the high fructose (20 mM) group, the FCCP-treatment significantly decreased the OCR values when compared with the control group [Fig. 3B]. These results indicated that the capacity of maximal (~33.69%) oxygen consumption were enhanced by high fructose co-incubation [Fig. 3B].

To further evaluate the detail alteration in mitochondrial OXPHOS, the elements of oxygen consumption capacity were dissected from the area under curve of OCR as previous study described [37–39]. The area under curve before oligomycin A (Oligo.) treatment (area I) was defined as the basal respiration. The values between basal state and oligomycin A treatment of OCR curve and the time-period between oligomycin and FCCP treatment (area II) is regarded as the ATP production.

Fig. 3 The expressions of insulin receptor β and phospho-S636 insulin receptor substrate 1 in human valve interstitial cells were increased by fructose at high concentration. Representative gels and densitometric analyses of (A) PI3K p85 (B) total AKT (C) phospho(p)-S473-AKT (D) pT308-AKT (E) insulin receptor substrate 1 (IRS1), and (F) p-S636 IRS1 detected at 24 h incubated with 0, 2, or 20 mM fructose. Values are mean ± SEM, n = 4–6 in each experimental group. The sample size of each group was noted on the bar. *p < 0.05, **p < 0.01 versus 0 mM fructose group using the nonparametric, Kruskal–Wallis test followed by the Dunn post hoc method.
capacity of the cells. The area under curve between the oligomycin A and antinomycin A (AMA) treatment (area III) was defined as the maximal respiration capacity. The results indicated that ATP production capacity [Fig. 3D] and maximal respiratory capacity [Fig. 3E] were significantly increased in the high fructose group while the basal respiration [Fig. 3C] showed no significant difference between groups. These results suggested that high fructose treatment induced oxidative phosphorylation in the hVICs. Fig. 4.

Fructose induced the expression of mitochondrial fission 1 protein in human valve interstitial cells at high concentration

Mitochondria produce reactive oxygen species during the processes of ATP production [40–42]. Accumulated oxidative stress damages mitochondria in turn. Mitochondrial fission works to maintain the mitochondrial function by eliminating the damaged fraction of mitochondria [43]. Therefore, we
further examined the expressions of three key factors of mitochondrial fission, including FIS1, Mff, DRP-1 and p-DRP-1 in the hVICs by Western blotting. The results indicated that the expressions of FIS1 [Fig. 5A] was significantly increased at high fructose group while no significant change was detected in the expressions of Mff [Fig. 5B], DRP1 [Fig. 5C] or p-DRP1 [Fig. 5D]. At lower fructose group, FIS1 [Fig. 5A] only showed the trends of increment without statistical significant. These results suggested that high fructose may trigger the FIS1-associated mitochondrial fission in hVICs.

Fructose induced the expression of optic atrophy type 1 in human valve interstitial cells at high concentration

Mitochondrial fusion works to maintain the mitochondrial function by fusing the health and functional mitochondria [43]. Recently, optic atrophy type 1 (OPA1)-mediated mitochondrial fusion has been demonstrated to protects cells from calcium deposition [44]. Therefore, we examined the expressions of three key proteins of mitochondrial fusion, including OPA1, mitofusin (MFN1), and MFN2 in hVICs by Western blotting. The results indicated that the expressions of full length OPA1 and cleavage OPA1 [Fig. 6A and B] were significantly increased at high fructose group while no significant change was detected in the expressions of MFN1 [Fig. 6C], or MFN2 [Fig. 6D]. These results suggested the OPA1-associated mitochondrial fusion in hVICs with high fructose.

Fructose induced the expression of optic atrophy type 1 in human valve interstitial cells at high concentration

Mitochondrial autophagy is a protective mechanism for mitochondrial quality control [45]. Under physiological status, the process of mitophagy is strictly regulated. In this study, we examined the expressions of the key proteins of mitophagy, including phosphatase and tensin homologue-induced kinase 1 (PINK1), and Parkin in hVICs by Western blotting. The results indicated that the expression of cleaved PINK1 [Fig. 7B] was significantly increased at high fructose group while no significant change was detected in the expressions of full length PINK1 [Fig. 7A], full length Parkin [Fig. 7C], or short form Parkin...
Discussion

This study provided novel evidence to demonstrate that high fructose has direct effect on upregulating the calcification-related genes, ALP and osteocalcin, of hVICs after 24 h incubation. The underlying mechanisms involve the activation of PI3K/AKT signaling, the enhancement of mitochondrial OXPHOS, and the increase of mitochondrial fission/fusion concurrent with the limited proliferation ability. This finding suggested that the high fructose initiated an early onset of hVICs calcification via the activation of PI3K/AKT/mitochondrial OXPHOS pathway. This study provides new insights into the progression of calcified aortic valve disease. Therefore, we examined the adverse effect of fructose stimulation on the hVICs calcification at the levels of 2 and 20 mM to mimic the serum fructose levels of fasting (e.g. 2 mM) and of post-fructose consuming (20 mM) in human. The results indicated that high fructose triggered the progression of calcification in the hVICs by upregulation of ALP and osteocalcin as well as the mineral deposition. These data further implied that the repeated high fructose stimulation during daily life may initiate the development of calcified aortic valve disease.

Aortic valve stenosis is a common valvular heart disease. According to the conventional wisdom, the progression of valve stenosis is thought to be initiated from the valve fibrosis and gradually transformed into calcification. The progression of AS is strongly bounded to the differentiation of VICs to osteoblast [46]. In human study, high dietary fructose increased the concentration of serum ALP [47]. In line with previous evidence [47], our data further indicated that as early
after 24 h incubation, high fructose increased the expressions of ALP and osteocalcin, the initial and finalized markers of calcification, respectively. These results suggested that high fructose could induce an early onset of valve stenosis through the calcification of VICs. Consist with previous study [48], these results indicated that hVICs do not need to progress through a fibrotic stage before reaching an osteogenic stage. In other cell types, high fructose-induced cell proliferation and the fibrosis have been reported [49,50]. Different from those cell types, reduced proliferation of hVICs by high fructose at a series of time points was constantly detected in this study. The hVICs is a cell type prone to differentiate to osteoblasts or myofibroblasts in response for the metabolic stress. Our data further demonstrated the upregulation of ALP and osteocalcin, which are essential for the differentiation of osteoblast. Follow this line, we reason that the high fructose incubation might promote the switch from proliferation to osteogenic differentiation.

hVICs from healthy and calcified valves have various sensitivities when encounter the osteogenic stimuli. Cultured with common medium, hVICs from calcified valves appeared positive calcium deposits with Alizarin Red staining, but not significantly different from healthy hVICs. Stimulating with osteogenic medium, hVICs from calcified valves increased calcification [51]. The evidence suggests that hVICs may be more vulnerable for calcification under environmental stimulation. Therefore, we used a commercial primary hVICs to avoid the bias from the selected subject. The pathogenesis of aortic valve stenosis (AS) is a life-long progression. Repeated stimulation could be a risk factor to initiate AS. Fructose is a common sugar in beverages, desserts and even fresh fruits. Overconsumption of fructose has been demonstrated to induce metabolic syndrome which is a top risk factor of AS [2–5]. Valve interstitial cells (VICs) play important role in the development of calcific aortic valve disease. In this study, we investigated that whether high fructose (~20 mM, the post-prandial level of serum fructose [35]) might be a risk factor to initiate the progression of fibrosis and calcification of valve interstitial cells. In this study, the results indicated that high fructose triggered the expressions of ALP and osteocalcin, the downstream candidates of the calcification-related genes, instead of the fibrosis-related genes. These results further...
Mitochondrial fission and fusion are critical regulators for maintaining the function of mitochondria in response to the environmental nutrient support [16]. Overnutrition may enhance mitochondrial activity to accelerate mitochondrial oxidative damage [41,42,57]. Mitochondrial fission is essential for the elimination of damaged mitochondria. Although rare study has been conducted in the role of mitochondrial fission in the valve calcification, our results indicated that the high fructose-induced FIS1 was concurrent with the upregulation of ALP and osteocalcin implying the involvement of FIS1-mediated mitochondrial fission in the development of valvular calcification. Consist with this research, our results further indicated that the upregulation of OPA1 in hVICs might be a compromise results in response to the increased mitochondrial OXPHOS in the first place. Furthermore, OPA1 has been linked to the progression of calcification [44]. It is conceivable that the high fructose-increased mitochondrial dynamic signals could initiate a vicious cycle to develop valve stenosis. We, therefore, linked the increment of mitochondrial aerobic respiration and dynamics as the mediator of PI3K/AKT-induced ALP and osteocalcin upregulation. Follow these lines, it is possible that the activation of PI3K/AKT/mitochondria axis could be the key mechanisms to shift hVICs from healthy to the calcification-prone status.

Mitophagy strictly controls the mitochondrial quality [58]. In healthy mitochondria, PINK1, which translocates from cytosol to mitochondria is rapidly cleaved by PARL [20] while uncleaved PINK1 recruits Parkin to initiate mitophagy [19]. Previous study indicated that the suppression of mitophagy contributes to vascular calcification [59]. In this study, the PINK1 cleavage was increased in response to the increase fructose concentration. These results suggested that the mitochondria function properly under high fructose at 24 h incubation. These results implied that the mitophagy mechanism may not be altered by high fructose at the early stage.

Conclusions

By using a cell model to mimic the fasting and post fructose-consuming for the study of the progression of AS, we demonstrated that the proliferation of hVICs was reduced by high fructose concurrent with the activation of PI3K/AKT signaling, the increased capacity of mitochondrial respiration, and the enhancement of mitochondrial dynamics to initiate the calcification even at the physiological level of post-fructose consuming. Together, these results imply that PI3K/AKT/mitochondrial respiration of hVICs might be a feasible
therapeutic target of aortic valve stenosis as early at the initiation of AS progression.

Conflicts of interest

The authors declare no conflicts of interest.

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