EFFECTS OF FRUCTOSE-1,6-DIPHOSPHATE ON THE GLYCOLIPID METABOLISM OF ADIPOCYTES AND ANGIOGENESIS IN THE GRAFT

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ABSTRACT

Objective: *To investigate the effects of 1,6-fructose diphosphate on glycolipid metabolism of adipocytes and angiogenesis in the graft.*

Methods: *Free fat was obtained and treated. FBP was dissolved in normal saline and divided into 4 mg / g, 2.5 mg/g,1 mg/g,0.5 mg/g FBP injection group and normal saline group was selected as control. There were 5 mice in each group at each time point. Mice were given intraabdominal injection 2 hours before operation and continuous intraperitoneal administration for 6 days after operation. HE staining, eight-factor immunofluorescence staining and real-time fluorescence quantitative PCR were used to analyze the effects of FBP on lipid metabolism of adipocytes and angiogenesis in the graft.*

Results: *For 2 weeks, 12 weeks after transplantation, the number of survival adipocytes in 2.5 mg/g, 4mg/g FBP injection group was significantly higher than that in control group ($P<0.05$). The intercellular profile was clear and full in the FBP injection group, but the cells in control group had more damage. From 2 weeks after transplantation, GPDH in 4mg/g FBP injection group was significantly higher than that in control group ($P<0.05$). At 2 weeks after transplantation, the level of PPAP-γ in 4 mg / g FBP injection group was significantly higher than that in control group ($P<0.05$), but there was no significant difference between the two groups after 8 weeks ($P>0.05$). At 2 weeks after transplantation, the number of blood vessels in 4 mg / g FBP injection group was remarkably higher than that in control group ($P<0.05$), but there was no significant difference in the number of blood vessels among the groups at 12 weeks after transplantation ($P>0.05$). At 2 weeks after transplantation, the expression of vascular endothelial cells VEGF in 4 mg / g FBP injection group was markedly higher than that in control group ($P<0.05$).*

Conclusion: *FBP can protect the key enzymes of glycolipid metabolism of adipocytes in grafts, maintain the normal function of cells, and promote the formation of blood vessels.*

Keywords: *FBP, adipocytes in grafts, AFP, glycolipid metabolism, angiogenesis.*

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Introduction

Autologous fat transplantation (AFP) can be used to suck the fat from the fat-rich part of the human body by a negative-pressure liposuction method, and can be transplanted to the part where the soft tissue filling needs to be performed by injection or the like, so as to improve the shape of the recipient area[(1)]. AFP plays an important role in the treatment of facial atrophy, superficial wrinkles and post-traumatic morphological repair[(2)]. Compared with some artificial prostheses and allogenic soft tissue fillers, autologous adipose tissue is taken from the body itself, which will not lead to immune rejection, has good biocompatibility, less adverse reactions, less limited, with a wide range of sources, easy to obtain materials, low cost and other advantages[(3)].

Clinical studies show that autologous fat granules contain abundant adipose-derived mesenchymal stem cells (ADMSCs), which are powerful in assisting tissue regeneration, can promote the reconstruction of peripheral tissue by means of proliferation, differentiation or paracrine, thus promoting the angiogenesis and the maturation of adipocytes in the

recipient area, so autologous adipose tissue becomes an ideal soft tissue filling material[4]. However, there are few studies on AFP follow-up, and the long-term effect and the applicable population are unknown.

The difference of operation level can cause the curative effect of AFP to be different, some of the transplanted fat particles will show necrosis and apoptosis, and eventually lead to absorption by the body or fibrosis[5]. In addition, it can also lead to pain, bleeding, numbness, fever, skin necrosis, even fester and a series of complications[6].

The occurrence of these problems limits the wide application of AFP in reconstruction and plastic surgery. In order to solve these problems, the clinical researchers have conducted a number of studies, such as mixing the vascular endothelial growth factor with the fat particles, and injected subcutaneously into nude mice to improve the survival rate of the fat graft. In the process of AFP transplantation, most of the tissues and cells were in a bad extracellular environment due to the destruction of blood vessels and the limited osmotic ability of interstitial fluid, which led to necrosis and apoptosis[7].

Therefore, before the effective blood supply is formed, free fat necrosis can be reduced by alleviating the oxygen and energy supply of tissues and cells. The related literature and research show that fructose-1,6-diphosphate (FBP) can effectively protect the organ, improve the phenomenon of tissue ischemia and hypoxia, protect myocardial tissue in a variety of operations, and the curative effect is significant in organ transplantation, hepatotoxicity and so on[8]. Therefore, this study will further explore the effects of FBP on glycolipid metabolism and angiogenesis of adipocytes.

Main experimental materials and equipment

Sample source

Human free fat particles (Orthopaedic Hospital of Peking Union Medical College of Chinese Academy of Medical Sciences); Clean grade SD female mice (Shanghai Academy of Chinese Sciences), with body weight of 20 to 22 g.

Inclusion criteria:

All patients signed informed consent forms; The study is approved by the Ethics Committee of the Hospital.

Main instruments

Vacuum suction device (Shanghai Boyu Pump Co., Ltd.); Super clean bench (Beijing YataiKelong Co., Ltd.); Biosafety cabinet (Su Jie Medical Devices Co., Ltd.); Electric heating air-blowing drier (Suzhou Kaimiao electric heating equipment Co., Ltd.); Water-Jacket Thermostatic Constant Incubator (Shanghai Hetian Scientific instrument Co., Ltd.); Inverted phase contrast microscope (WeiteShijie Technology Co., Ltd.); Ice machine (Wuxi JieRuiAn instrument and equipment Co., Ltd.); Desk centrifuge (Guangzhou JiDi Instrument Co., Ltd.); Optical microscope (Tianjin Laike Optical instrument Co., Ltd.); Precision electronic balance (Shanghai Tianmei balance instrument Co., Ltd.); Calibrated free running pipette (Beijing Jiahua Zhongxin Technology Co., Ltd.).

Method

The free fat was obtained and treated, and the natural layered free adipose tissue was obtained, which was preserved at 4 °C for further use.

FBP was dissolved in normal saline and divided into 4 mg/g FBP injection group, 2.5 mg/g FBP injection group and normal saline group was selected as control. There were 5 mice in each group at each time point.

Mice were given intraabdominal injection 2 hours before operation and continuous intraperitoneal administration for 6 days after operation. 0.5ml free fat particles were absorbed by syringe, and injected subcutaneously into each mouse. At 2 weeks, 8 weeks and 12 weeks after operation, half of the grafts were used for histologic examination and half for RNA extraction and enzyme detection. The specimens were fixed with 10% neutral formalin, dehydrated routinely, embedded in paraffin, sliced continuously by 5 μm and stained with HE. 3-phosphate glycerol dehydrogenase (GPDH) was used to detect the activity of the samples.

The blood vessels were stained by eight-factor Immunohistofluorescence staining method. The RNA of sample tissues was extracted by real-time fluorescence quantitative PCR to detect the key transcription factor of adipose differentiation, peroxidosome proliferation and activated receptor γ (PPAR-γ).

Statistical method

Graphpad Prism 5.0 software is used for data processing, and each quantitative data is represented by (Mean ± SEA) or %. The t test was used for inter-group comparison, and One-way ANOVE was used for inter-group comparison of quantitative data. $P<0.05$ indicates that the difference between groups has statistical significance.

Results

Staining results of adipose cell biopsies in adipose grafts

For 2 weeks, 12 weeks after transplantation, the number of survival adipocytes in 2.5 mg/g, 4mg/g FBP injection group was significantly higher than that in control group (P<0.05). The intercellular profile was clear and full in the FBP injection group, but the cells in control group had more damage. The results were shown in figure 1.

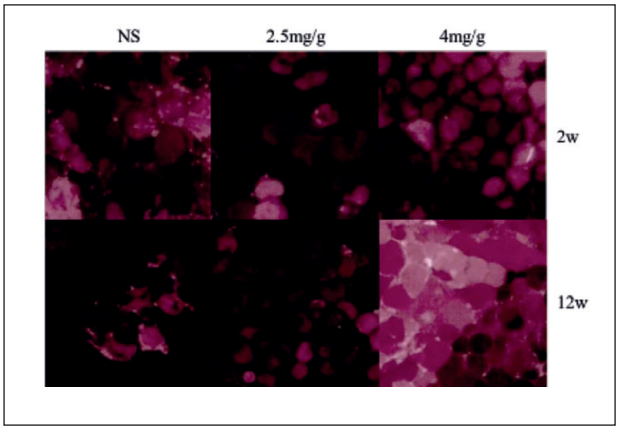

Figure 1: Staining of adipose cell biopsies in adipose grafts.

Detection results of GPDH activity of surviving adipocytes in adipose grafts

From 2 weeks after transplantation, GPDH in 4mg/g FBP injection group was significantly higher than that in control group (P<0.05). The results were shown in figure 2.

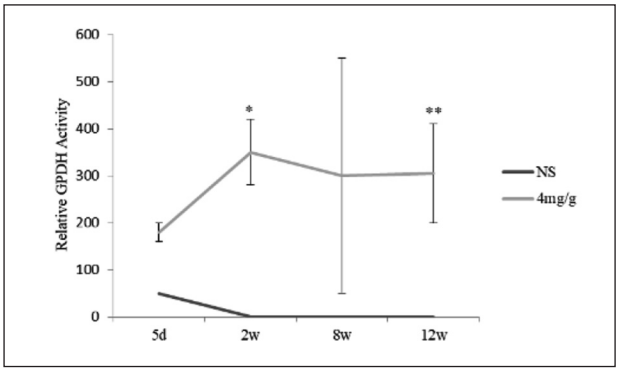

Figure 2: Detection of 3-phosphoglycerate dehydrogenase (GPDH) activity of surviving adipocytes in adipose grafts.
*Notes: Compared with the control group at the same time point, *P<0.05; **P<0.01.*

Expression of PPAPγ in adipose grafts

At 2 weeks after transplantation, the level of PPAPγ in 4 mg/g FBP injection group was significantly higher than that in control group (P<0.05), but there was no significant difference between the two groups after 8 weeks (P>0.05). The results were shown in figure 3.

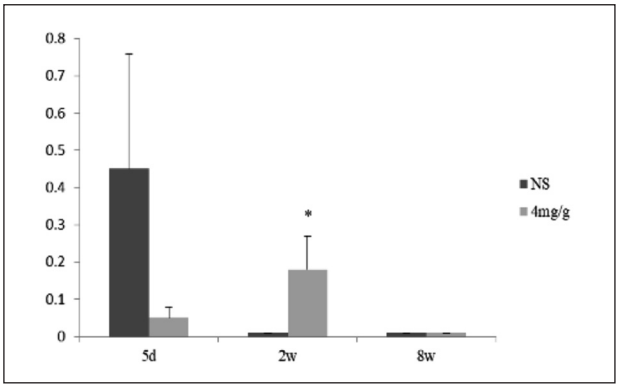

Figure 3: Expression of PPAPγ in adipose grafts.
*Notes: Compared with the control group at the same time point, *P<0.05; Compared with the control group at the same time point, **P>0.05.*

Results of blood vessel staining in adipose grafts

At 2 weeks after transplantation, the number of blood vessels in 4 mg / g FBP injection group was remarkably higher than that in control group (P 0.05), but there was no significant difference in the number of blood vessels among the groups at 12 weeks after transplantation (P>0.05). The results were shown in figure 4 and figure 5.

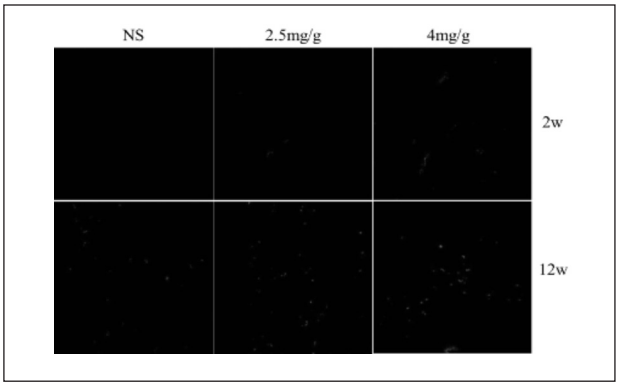

Figure 4: Results of blood vessel staining in adipose grafts.

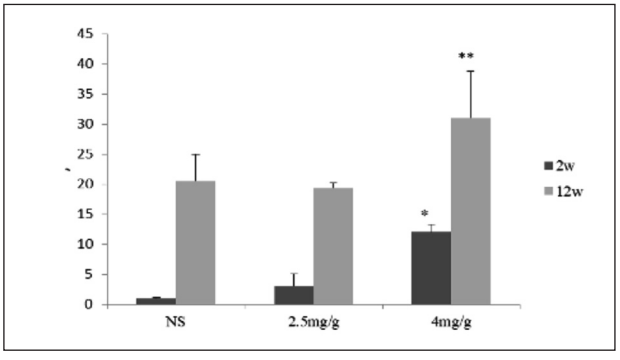

Figure 5: Results of blood vessel staining in adipose grafts.
*Notes: Compared with the control group at the same time point, *P>0.05; **P<0.01.*

Expression of VEGF in adipose grafts

At 2 weeks after transplantation, the expression of vascular endothelial cells VEGF in 4 mg / g FBP injection group was markedly higher than that in control group (P<0.05). The results were shown in figure 6.

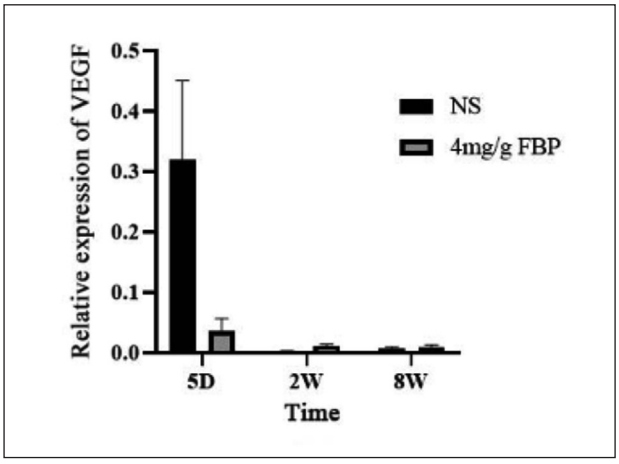

Figure 6: Expression of VEGF in adipose grafts.
*Notes: Compared with the control group at 2 weeks, *P<0.05.*

Discussion

FBP is an important intermediate product of energy metabolism, which can promote glycolysis and increase the release of adenosine triphosphate (ATP). Since the 1980s, it has been widely used in clinical practice as a commercial drug[9]. AFP, as an engineering free tissue transplantation, the normal structure and function of each cell is an indispensable condition for fat graft to maintain structural integrity[10]. However, in the process of sampling, the integrity of adipose tissue is easily destroyed, and a large number of adipocytes and vascular networks are seriously damaged.

After AFP, if not intervened in time, the cellular nutrients and oxygen supply in fat particles were seriously deficient, which led to the further destruction of graft structure and activity, and finally led to the failure of transplantation[11]. It has been reported in the clinical literature that FBP plays an important role in the process of glycolipid metabolism, which can affect and regulate the activity of some important enzymes and promote the progress of decomposable glycolysis reaction.

In addition, FBP can regulate the enzymes involved in sugar regeneration, and then inhibit the process of sugar regeneration[12]. Therefore, the final function of FBP is to positively promote the glycolysis process, improve the utilization rate of glucose, and then accelerate the production of ATP and maintain the normal biological activity of tissues and cells[13]. Slater[14] et al. found that FBP can regulate lipid metabolism and promote lipid synthesis in liver and other models, but it can promote lipid decomposition in adipose tissue. However, there are few reports about the effect of FBP on human adipocytes. In this study, the activity of the adipose cells in the fat graft was tested by HE staining. The results showed that, for 2 weeks, 12 weeks after transplantation, the number of survival adipocytes in 2.5 mg/g, 4mg/g FBP injection group was significantly higher than that in control group (P<0.05). The intercellular profile was clear and full in the FBP injection group, but the cells in control group had more damage. GPDH is a key enzyme that specifically reflects the glycolipid metabolism in adipocytes. In this study, GPDH was detected and the results indicated that, from 2 weeks after transplantation, GPDH in 4mg/g FBP injection group was significantly higher than that in control group (P<0.05).

Finally, the time point of 5 days was added to analyze the effect of FBP on adipocytes in the early stage of transplantation. The results showed that at 2 weeks after transplantation, the level of PPAPγ in 4 mg/g FBP injection group was significantly higher than that in control group (P<0.05), but there was no significant difference between the two groups after 8 weeks (P>0.05). Complete vascular network is an essential condition for tissues and organs to maintain normal structure and activity, and can provide rich blood supply for tissues and organs[15].

The rich nutrients and oxygen in the blood are beneficial to the aerobic metabolism and survival of the cells, and can also take away the metabolic waste in the blood and discharge it out of vitro[16]. It takes a long time for fat graft to enter the recipient area and form a complete vascular network and connect to the recipient vascular system. During this period, the living environment of tissue cells in the graft is poor, which is easy to lead to many adverse reactions[17].

Therefore, it has become a hot topic in clinical research to find an effective method to promote graft angiogenesis. The results of this study indicated that at 2 weeks after transplantation, the number of blood vessels in 4 mg/g FBP injection group was remarkably higher than that in control group (P<0.05), but there was no significant difference in the number of blood vessels among the groups at 12 weeks after transplantation (P>0.05). At 2 weeks after transplantation, the expression of vascular endothelial cells VEGF in 4 mg/g FBP injection group was markedly higher than that in control group (P<0.05). In con-

clusion, FBP can protect the key enzymes of glycolipid metabolism of adipocytes, maintain the normal function of cells, and promote the formation of blood vessels.

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