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High-free Fatty Acid Treatment Induced Anti-inflammatory Changes in a Natural Killer (NK) Cell Line

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ABSTRACT

Background: Natural killer (NK) cells play a role in the pathogenesis of various metabolic diseases related to obesity. While our initial findings have indicated a potential involvement of NK cells in the pathogenesis of type 2 diabetes mellitus, the precise mechanism underlying NK cell-mediated development of this form of diabetes remains inadequately comprehended.

Objective: To investigate the impact and the underlying mechanism of high glucose and elevated levels of free fatty acids (FFAs) on immune and inflammatory responses and oxidative stress in NK92 cells.

Methods: In this experiment, the CCK8 cytotoxicity assay was used to select the 44.4 mM and 1.5 mM concentrations of high glucose and high FFAs, respectively, to treat NK92 cells for 4 days. The concentrations of superoxide dismutase (SOD) and glutathione (GSH) were determined using a biochemical analyzer. Intracellular reactive oxygen species (ROS) levels, cytokines concentrations (TNF-α, IFN-γ, IL-6, and IL-10), and the expression levels of intracellular molecules (perforin and granzyme B) were assessed by flow cytometry.

Results: The number of NK92 cell clumps was significantly reduced in the high-FFA (HF) group. In addition, the production of ROS and levels of cytokines (TNF-α, IFN-γ, IL-6, and IL-10) significantly decreased in the HF group but showed no significant change in the high-glucose (HG) group. This observation was consistent with the expression levels of perforin and granzyme B that decreased in the HF group.

Conclusion: High FFAs induced morphological changes and serious damage to oxidative stress and inflammatory response in NK92 cells.

Keywords: Cytokines, High FFA, Intracellular Molecules, NK92 Cells, Oxidative Stress

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INTRODUCTION

Diabetes mellitus is a type of metabolic disorder characterized by the disturbance in glucose, fat, and protein metabolism caused by defects in insulin secretion or insulin resistance (1, 2). In addition to high glucose, the majority of type 2 diabetic mellitus (T2DM) patients exhibit high blood lipid levels. High plasma-free fatty acids (FFAs) resulting from advanced diabetes mellitus have been associated with insulin resistance and inflammation. (3, 4). Therefore, hyperglycemia accompanied by high FFAs can promote the occurrence and deterioration of T2DM and mediate a chronic inflammatory response in the body.

Natural killer (NK) cells are important sentinels of the immune system that differentiate and develop from hematopoietic stem cells (5, 6). NK cells are effector lymphocytes with cytolytic functions and can secrete immunoregulatory factors. These cells also serve as the interface between innate and adaptive immunity. NK cells are known to play a key role in various immune responses, including apoptosis of tumor cells and virus and bacterium-infected cells (6-8).

Studies have demonstrated that NK cells are an important component of the inflammatory microenvironment, and could play a role in the pathogenesis of various metabolic diseases related to obesity (9, 10). Inflammation caused by obesity is generally presumed to be the main reason for the occurrence and deterioration of insulin resistance and diabetes (11). Although traditional research has shown that the polarization of adipose tissue macrophages (ATM) from anti-inflammatory macrophages (M2) to pro-inflammatory macrophages (M1) was a key process in the insulin resistance caused by obesity (12), the emerging research also suggested that the proliferation and activation of NK cells in visceral tissue cells of obese individuals can also exhibit a significant role in insulin resistance and T2DM (9, 10). Recent studies also have demonstrated that NK cells in obese patients could trigger and amplify the secretion of inflammatory cytokines such as tumor necrosis factor (TNF)-α by macrophages (13, 14). Although our preliminary results revealed that the change of NK cells ratio in peripheral blood lymphocytes and some cytokines (IL-12p70, IL-6, IL-10, and IFN-γ) in serum may be related to the development of type 2 diabetes and pre-diabetes population (15), the mechanism underlying the NK cell-mediated regulation of chronic inflammatory response in diabetic mellitus is not fully understood.

In the present study, we focused on the effects of high glucose and high FFAs on the oxidative stress, inflammatory response, and intracellular molecular alterations in NK92 cells and investigated the underlying mechanisms.

MATERIALS AND METHODS

Cell Lines

The human NK cell line, NK92, was obtained from the Type Culture Collection of the Chinese Academy of Sciences (Wuhan, China). The culture conditions for cells were alpha-minimum essential medium (MEM) (C11095500BT, Gibco) supplemented with 100 U/mL recombinant IL-2 (200-02, PeproTech Asia), 12.5% horse serum (SBJ-SE-HO014, Nanjing SenBeiJia Biological Technology, China), 12.5% fetal bovine serum (SH30396.03; HyClone), 0.2 mM inositol, 0.1 mM β-mercaptoethanol, and 0.02 mM folic acid at 37°C in a 5% CO_2 humidified incubator. In accordance with the proliferation characteristics of NK cells, it was necessary to refresh the cell culture every three days.

Cytotoxicity Assay

NK92 cells were plated in 96-well plates at a density of 5×10^3 cells/well, and incubated with 5.55 or 44.4 mM glucose and various concentrations of FFAs (0.25, 0.5, 0.75,

1.0, 1.25, 1.5, and 1.75 mM) prepared in the presence of 1% bovine serum albumin (BSA) for 4 days at 37 °C. FFAs comprise sodium oleate and sodium palmitate, both of which are long-chain fatty acids. At the end of treatment, 10 μL of Cell Counting Kit 8 (CCK8) solution (GK10001, GLPBIO) was added to each well. The plate was incubated for 4 h at 37 °C, and the absorbance was tested by a microplate reader (RT-6000, Rayto, Shenzhen, China) at 450 nm wavelength. Before the experiment, the cells treated with various concentrations of FFAs were subjected to cytotoxicity analysis; the data showed that FFAs had no significant toxicity to cell proliferation.

Cell Treatment and Experimental Groups

NK92 cells were treated for 4 days under the following four conditions: the control group (5.5 mM glucose+1% BSA), the high-glucose (HG) group (44.4 mM glucose+1% BSA), the high-FFA (HF) group (5.5 mM glucose+1.5 mM FFA), and the high-glucose and high-FFA (HGHF) group (44.4 mM glucose+1.5 mM FFA).

Morphological Observation

NK92 cells were treated under the abovementioned four conditions, and their morphology was directly observed under a TE-300 Nikon microscope (Nikon, Tokyo, Japan).

Detection of Oxidative Stress (Reactive Oxygen Species [ROS], Superoxide Dismutase [SOD], and Glutathione [GSH]) Analysis of Intracellular ROS Levels

NK92 cells were incubated under four different conditions in 12-well plates for 4 days and then treated with dichlorodihydrofluorescein diacetate (DCF-DA), a molecular probe for the detection of ROS. Intracellular ROS level was detected using a ROS assay kit (S0033; Beyotime) according to the manufacturer's instructions on the FL-1 channel of a flow cytometer (Cytomics FC 500, Beckman Coulter, USA).

Detection of SOD and GSH Levels

NK92 cells were treated under four different conditions in 12-well plates for 4 days. After centrifugation for 10 min at 1,000 ×*g* and 4 °C, the supernatant was obtained for the detection of SOD concentrations (10804A11; Lecheng Bio, China) and GSH concentrations (20211102; Lecheng Bio, China) on a biochemical analyzer (Autolas FX8 Series, Autobio, China).

The SOD level was detected by the pyrogallol autoxidation method. The GSH level was detected by the method based on the enzyme cycle method and ultraviolet-visible absorption (UV-VIS).

Detection of Cytokines

NK92 cells were treated under four different conditions in 12-well plates for 4 days. After centrifugation for 10 min at 1,000 ×g and 4°C, the supernatant was obtained for the detection of cytokines concentrations(TNF-α, IFN-γ, IL-6, and IL-10) using a commercially available, human assay kit (20211201, Saiji Bio, China) by flow cytometry (Cytomics FC 500, Beckman Coulter, USA) based on the Cytometric bead array (CBA) technology. The fundamental procedures of the experiment closely resemble those of a capture sandwich immunoassay. The specific operation was executed in accordance with the methods outlined in the prior publication (16).

Intracellular Perforin and Granzyme B Levels

NK92 cells were subjected to the four treatment conditions in 12-well plates for 4 days. After incubation, the cells were treated with fluorescent antibody-labeling reagents containing CD45 (200259, Beckman), CD3 (200138, Beckman), CD8 (200052, Beckman), CD16 (200109, Beckman), and CD56 (200072, Beckman) for 20 min in the dark. After fixation and membrane permeabilization, the cells were labeled with a granzyme/perforin antibody (220202, Ruisikaier Bio, China), and the expression of perforin and granzyme B was detected using a flow cytometer (Cytomics FC

500, Beckman Coulter, USA) and analyzed using FlowJo version 10.5.3 software after incubation for 20 min. in the dark.

Statistical Analysis

All experiments were carried on six times to guarantee the reliability of results. The data were presented as the mean±standard deviation. The data were normalized based on the readings reported for the control group. Statistical analyses were executed by the Statistical Package for Social Sciences (SPSS) 11.5 software (SPSS, Inc., Chicago, IL, USA). Oneway analysis of variance was used to detect statistical differences in this experiment. $P<0.05$ indicated statistically significant differences.

RESULTS

High-glucose or/and High-FFA Treatment Altered Cellular Viability

NK92 cells were treated with increasing concentrations of FFAs (0-1.75 mM) and 5.5 mM or 44.4 mM glucose solution for 4 days. While the treatment with 1.75 mM FFAs led to a significant decrease in cellular viability ($p<0.05$), 1.5 mM or lower concentrations had only a mild effect on cellular viability (Fig. 1). Based on the above results, we chose 44.4 mM and 1.5 mM concentrations of high glucose and high FFAs, respectively, for further experiments.

Effects of the High-glucose or/and the High-FFA Treatment on the Morphology of NK92 Cells

The NK92 cells derived from the control group displayed suspension growth and the formation of cellular aggregates. In contrast to the control group, the High Glucose (HG) group's cellular morphology remained largely unaltered, showing no significant deviations. The clumping of NK92 cells significantly decreased in the HF group, which was consistent with cellular deformation and atrophy (Fig. 2). The NK92 cells from the HGHF group showed a more obvious decrease in clumping along with serious morphological changes in terms of atrophy and deformation (Fig. 2).

Effects of the High-glucose or/and the High-FFA Treatment on Oxidative Stress in NK92 Cells

In comparison with the control group (1.00±0.00), the HF group showed a significant decrease in the production of ROS (0.76±0.09) ($p<0.05$); no significant change in ROS level was observed in the HG group (1.05±0.12) ($p>0.05$).

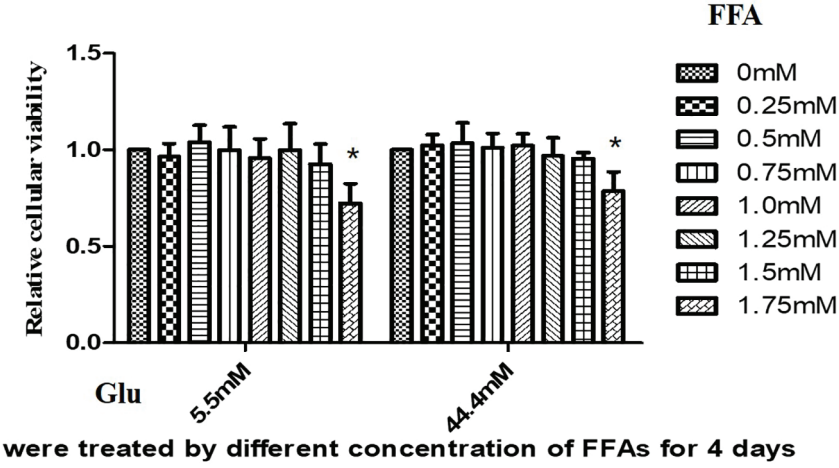

Fig. 1. High-glucose or/and high-FFA treatment altered cellular viability. The graph on the left shows cellular viability in the presence of 5.5 mM glucose and that on the right shows cellular viability in the presence of 44.4 mM glucose. Treatment with 1.5 mM or lower concentrations of FFAs had only mild effects on cellular viability, but 1.75 mM FFAs significantly reduced cellular viability ($p<0.05$). $^{*}p<0.05$ vs. the groups treated without FFAs; FFAs: plasma free fatty acids

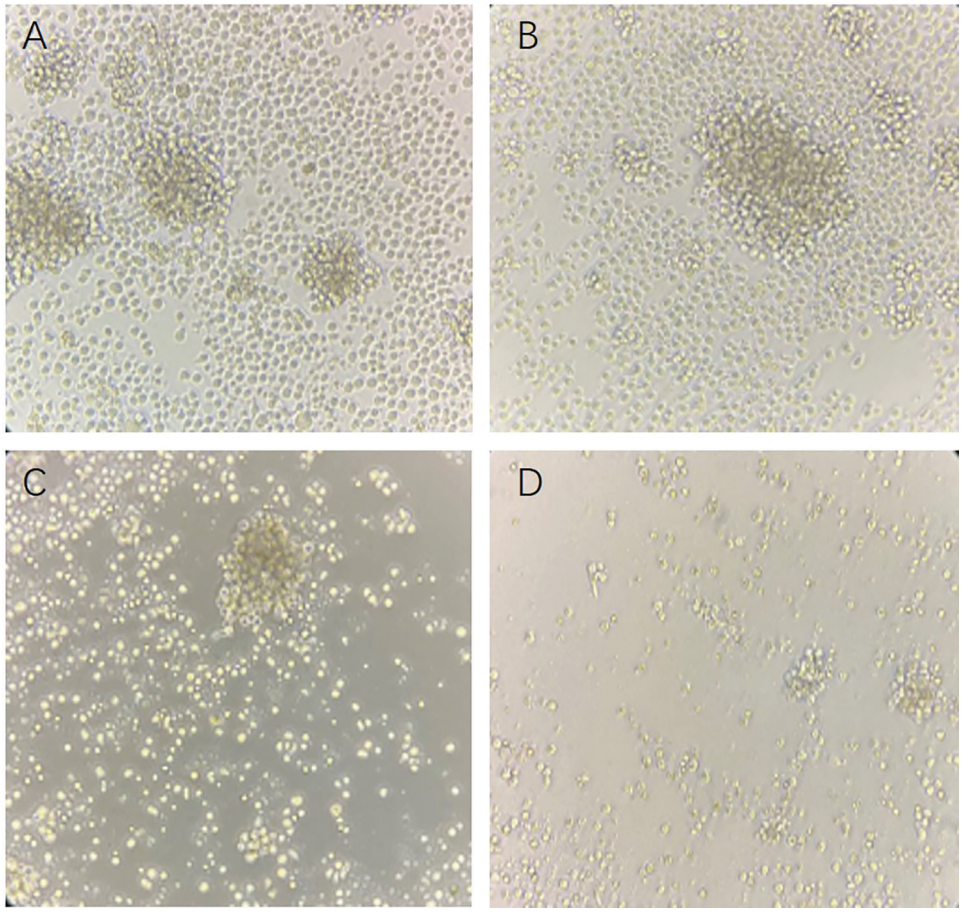

Fig. 2. Morphology of NK92 cells treated with the high glucose or/and the high FFAs (magnification: 10×20). Photographs A, B, C, and D show the morphology of NK92 cells from the control, HG, HF, and HGHF treatment groups, respectively. In comparison with the control group, the HG group showed no significant change in cellular morphology. The clumping of NK92 cells significantly reduced in the HF group, consistent with morphological changes such as atrophy and deformation.

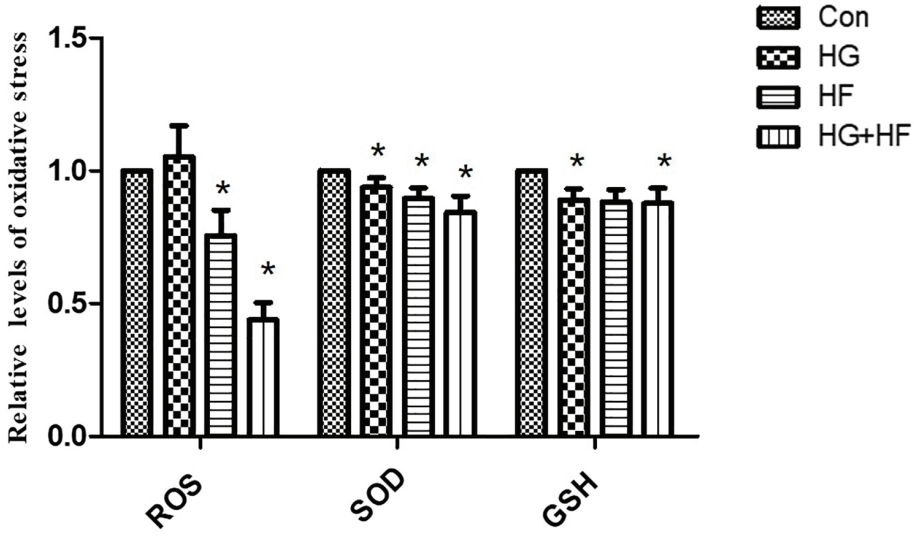

Fig. 3. Effects of the high-glucose or/and the high-FFA treatment on oxidative stress in NK92 cells. The levels of ROS in control, HG, HF, and the HGHF groups were 1.00±0.00, 1.05±0.12, 0.76±0.09, and 0.44±0.06, respectively. The contents of SOD and GSH in control, HG, HF, and HGHF groups were 1.00±0.00, 0.94±0.04 and 0.89±0.04, 0.90±0.04 and 0.88±0.05, and 0.84±0.06 and 0.88±0.06, respectively. $^{*}p<0.05$ vs. the control group. ROS: reactive oxygen species; SOD: superoxide dismutase; GSH: glutathione; Con: control; HG: high-glucose; HF: high-FFA

The expression levels of SOD and GSH significantly decreased in both the HG (0.94±0.04 and 0.89±0.04, respectively) and the HF (0.90±0.04 and 0.88±0.05, respectively) groups (p<0.05) compared with those in the control group (1.00±0.00) (Fig. 3).

The Effects of High-glucose or/and the High-FFA Treatment on Inflammatory Cytokine Levels in NK92 Cells

The levels of inflammatory cytokines (TNF-α, IFN-γ, IL-6, IL-10) significantly decreased in the HF group (0.78±0.09, 0.31±0.05, 0.55±0.05, 0.14±0.02, respectively) (1.00±0.00) (p<0.05), but not in the HG group (0.91±0.08, 0.94±0.07, 1.06±0.08, 0.94±0.09, respectively) (p>0.05) compared with those in the control group (Fig. 4).

The Effects of High-glucose or/and the High-FFA Treatment on Intracellular Molecules (Perforin and Granzyme B) in NK92 Cells

In comparison with the control group (1.00±0.00), the HF group showed significant downregulation in the expression of perforin and granzyme B (0.36±0.02 and 0.20±0.02, respectively) (p<0.05). No significant changes were observed in perforin and granzyme B levels in the HG group (0.99±0.01 and 0.99±0.01, respectively) (p>0.05, Fig. 5).

DISCUSSION

Although there are many mechanisms leading to T2DM, more and more evidence shows that chronic low-grade inflammation may participate in the pathogenesis and progress of T2DM (17). NK cells hold a pivotal role within the innate immune system due to their capacity for direct lysis of infected cells without prior sensitization. Additionally, NK cells possess the capability to stimulate the production of cytokines and growth factors, thereby influencing diverse immune responses, such as anti-tumor and anti-inflammatory functions (5, 6, 18).

A previous study found that the upregulated expression of the P2X7 receptor in monocytes in response to inflammatory cytokine production was likely to participate in the pathogenesis and progress of T2DM (19). The results indicated that immune cells and cytokines participate in mediating chronic inflammation associated with T2DM. Our preliminary study results demonstrated that the change of NK cell ratio in peripheral blood lymphocytes and some cytokines (IL-12p70, IL-6, IL-10, and IFN-γ) in serum may be related to the development of type 2 diabetes and pre-diabetes population (15). This result suggested that NK cells along with inflammatory cytokines were involved in the pathophysiological process of T2DM. In

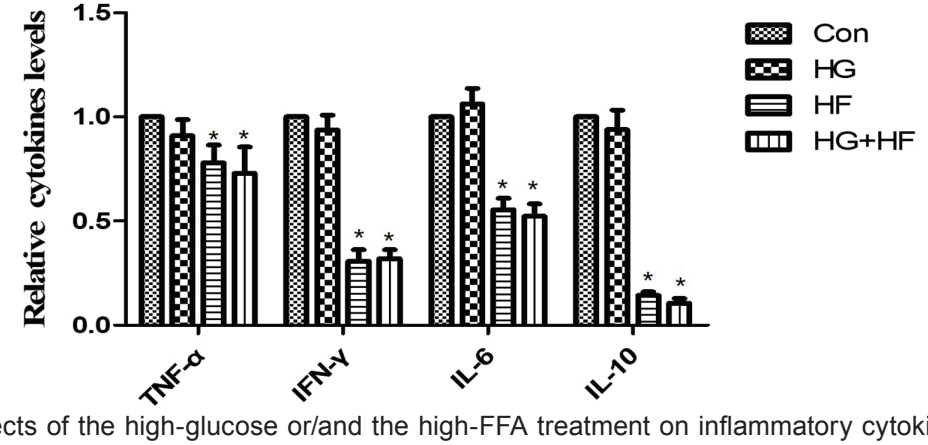

Fig. 4. Effects of the high-glucose or/and the high-FFA treatment on inflammatory cytokine levels in NK92 cells. The levels of TNF-α, IFN-γ, IL-6, and IL-10 were as follows: control group, 1.00±0.00; HG group, 0.91±0.08, 0.94±0.07, 1.06±0.08, and 0.94±0.09, respectively; HF group, 0.78±0.09, 0.31±0.05, 0.55±0.05, and 0.14±0.02, respectively; the HGHF group, 0.73±0.13, 0.32±0.04, 0.52±0.06, and 0.11±0.02, respectively. *p<0.05 vs. the control group. TNF-α: tumor necrosis factor-alpha; IFN-γ: interferon gamma; IL: interleukin; Con: control; HG: high-glucose; HF: high-FFA

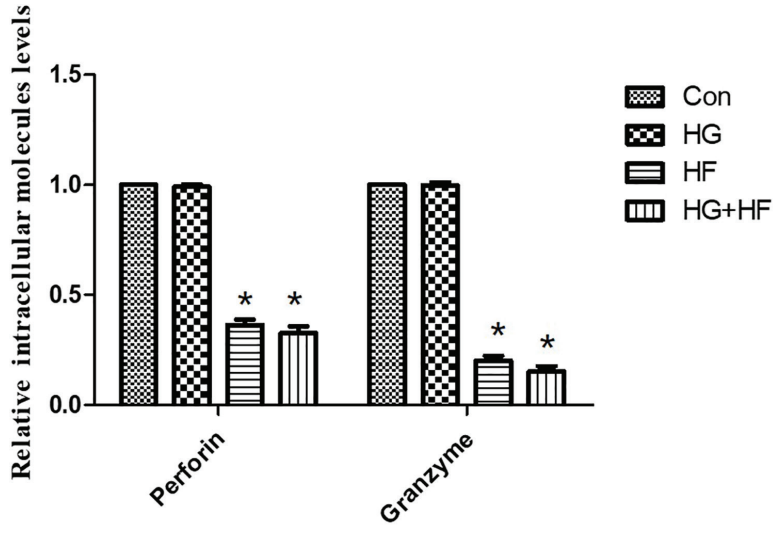

Fig. 5. Effects of the high-glucose or/and the high-FFA treatment on intracellular molecules (perforin and granzyme B) in NK92 cells. The expression levels of perforin and granzyme B in control, HG, HF, and HGHF groups were 1.00±0.00, 0.99±0.01 and 0.99±0.01, 0.36±0.02 and 0.20±0.02, 0.33±0.03 and 0.15±0.02, respectively. $^{*}p<0.05$ vs. the control group. Con: control; HG: high-glucose; HF: high-FFA

this study, we investigated the effect and the mechanism of action of high glucose and high FFA on immune and inflammatory responses, oxidative stress, and intracellular molecular alterations in NK92 cells.

NK92 is an established NK cell line derived from the peripheral blood of a 50-year-old male non-Hodgkin's lymphoma patient (20). NK92 cells provide an opportunity for the development of next-generation off-the-shelf cell therapy platforms (21). For *in vitro* experiments, NK cells are mostly derived from human peripheral blood (22, 23). However, peripheral blood-derived NK cells often exhibit variations in their state owing to individual differences. Therefore, the NK92 cell line has been commonly used in preclinical research because of its high-intensity and broad-spectrum cytotoxic effects (24, 25).

In the present study, we established the model of NK cells' injury *in vitro* induced by high glucose and high FFA using the NK92 cell line. Based on the principle of inducing high glucose and high FFAs in NK92 cells without significant cytotoxicity to cell proliferation, we chose 44.4 mM and 1.5 mM concentrations of the high glucose and the high FFAs, respectively in the experiment (Fig. 1).

As we all know, the maintenance of normal cell morphology requires definite cell culture conditions. In this experiment, we found that the morphology of NK cells cultured in a high-glucose medium was hardly compromised compared with that of cells from the control group; however, the high-FFA treatment seriously affected NK cell morphology, which was evident from cellular atrophy and deformation as well as the formation of clumps (Fig. 2). Thus, NK cells cultured in a high-FFA medium had compromised morphology, which may adversely affect their functions.

The imbalance between an increased generation of ROS and their removal by antioxidant defenses could cause oxidative stress. Intracellular antioxidant defenses mainly consist of low-molecular-weight thiols (mainly GSH) and various antioxidant enzymes (such as SOD). Oxidative stress-induced damage is associated with the occurrence and development of several chronic diseases (26). Innate immune cells as the main factor of the innate immune defense system could have an important effect in controlling the damage caused by oxidative stress (27). Research has shown that the activation of NK cells was achieved through various effector and regulatory functions via

metabolic programming, which was based on glucose-driven glycolysis and upregulation of glutamine metabolism (28, 29). The increase in ROS and GSH might promote the upregulation of NK cell proliferation and cytotoxic function. On the contrary, the downregulation of ROS and GSH led to functional impairment and recruitment of inflammatory sites (28-30). We found that the levels of three oxidative stress indicators ROS, SOD, and GSH significantly decreased in NK92 cells after the high-FFA treatment in this study; on the other hand, only GSH and SOD concentrations significantly decreased after the high-glucose treatment. These findings reflect that high-FFA treatment was more likely to stimulate NK92 cells to undergo oxidative stress-induced damage than the high-glucose treatment. Our research results are consistent with the above-mentioned literature (28, 30), indicating that high-FFA treatment could induce NK92 cells to be in an inflammatory state, leading to a decrease in the oxidative stress indicators and triggering changes in various other functions of NK92 cells.

Next, we detected the cytokines produced by NK92 cells after the high-glucose and the high-FFA treatment. Our preliminary results revealed that the change of NK cell ratio in peripheral blood lymphocytes and some cytokines (IL-12p70, IL-6, IL-10, and IFN-γ) in serum may be related to the development of type 2 diabetes and pre-diabetes population (15). In a study of NK primary cells isolated from the mice's spleen, it was found that the expression of inflammatory cytokines (IFN-γ and IL-17) was not significantly different from the control group in the high glucose culture. If the serum of diabetes mice were used for culture, it would be found that the expression of inflammatory cytokines (IFN-γ and IL-17) significantly reduced (31). In this study, we witnessed no obvious change in levels of various cytokines from NK92 cells cultured in the high-glucose medium; however, these cytokine levels significantly decreased in cells cultured in the high-FFA medium (Fig. 4). Our findings are consistent with the previously cited literature (31), suggesting that high-FFA treatment was crucial for the altered inflammatory state of NK92 cells in diabetes rather than hyperglycemia. Interestingly, high FFA-treated NK92 cells had reduced concentrations of pro-inflammatory cytokines (TNF-α, INF-γ, and IL-6) as well as the anti-inflammatory cytokine IL-10. This observation suggests that these pro-inflammatory and anti-inflammatory cytokines showed a similar trend in their expression patterns. One might wonder if this is the way for the body to maintain homeostasis to avoid excessive promotion or inhibition of inflammatory responses.

NK cells can release perforin and granzyme. Perforin can perforate the surface of target cells allowing the entry of granzyme B (32, 33). Perforin and granzyme B act directly or by further activating other types of immune cells to induce apoptosis, therefore they may be considered cytotoxic modulators (33, 34). Another research project in our laboratory found that perforin and granzyme B levels of NK cells significantly increased in the vaccinated group compared with the unvaccinated group in the study on the influence of Omicron BA.2 infection after COVID-19 vaccination *in vitro* (16). According to the literature (31), diabetic condition (the serum of diabetes mice was used for culture) attenuated the expression and production of cytolytic molecules (perforin and granzyme B) in splenic NK cells, however only the expression of perforin decreased in high glucose culture compared with the control group. These studies showed that perforin and granzyme were important for regulating the immune function of NK cells, meanwhile, perforin and granzyme could also participate in the chronic inflammatory response induced by diabetes. Our experiments show that high-FFA treatment decreased the expression of cytolytic molecules (perforin and granzyme B) in NK92 cells (Fig. 5). Our results were basically consistent with the above report (31),

suggesting that the cytotoxicity of NK cells could decrease under the high-FFA treatment instead of high-glucose treatment, leading to a decrease in cytokine secretion and inhibition of oxidative stress response-ability.

The present study has some limitations. The specific signaling pathways involved in the oxidative stress, inflammatory response, and intracellular molecular alterations in NK92 cells were not elucidated. Effective interventions to rescue NK92 cells from the damage caused by the high-FFA treatment are yet unknown, which warrants further studies. In the next step, we will try to improve the cytotoxic effects of NK cells through the study of NK cell receptors or by modifying the function of NK cells. Then we will still study how to rescue the NK cells' injury caused by diabetes through specific signal pathways, providing a new perspective for the future application of NK cells in diabetes.

In summary, our findings confirm that high FFAs induced various alterations, including morphology changes, oxidative stress, inflammatory response, and intracellular molecular damage, in NK92 cells, suggesting that the high adipose state of diabetic patients may affect the functions of NK cells and mediate an adverse state in the body.

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AUTHOR'S CONTRIBUTION

YN and HW were responsible for the conception and design of the study. YF, YJ, JL acquired the data. HW drafted the manuscript and YL revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Glovaci D, Fan W, Wong ND. Epidemiology of Diabetes Mellitus and Cardiovascular Disease. Curr Cardiol Rep. 2019,21(4):21.
2. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of Type 2 Diabetes Mellitus. Int J Mol Sci. 2020,21(17):6275.
3. Hirano T. Pathophysiology of Diabetic Dyslipidemia. J Atheroscler Thromb. 2018,25(9):771-782.
4. Mitri J, Tomah S, Furtado J, Tasabehji MW, Hamdy O. Plasma Free Fatty Acids and Metabolic Effect in Type 2 Diabetes, an Ancillary Study from a Randomized Clinical Trial. Nutrients. 2021,13(4):1145.
5. Yilmaz A, Cui H, Caligiuri MA, Yu J. Chimeric antigen receptor-engineered natural killer cells for cancer immunotherapy. J Hematol Oncol. 2020,13(1):168.
6. Kundu S, Gurney M, O'Dwyer M. Generating natural killer cells for adoptive transfer: expanding horizons. Cytotherapy. 2021,23(7):559-66.
7. Sivori S, Pende D, Quatrini L, Pietra G, Della Chiesa M, Vacca P, et al. NK cells and ILCs in tumor immunotherapy. Mol Aspects Med. 2021,80(8):100870.
8. Kucuksezer UC, Aktas Cetin E, Esen F, Tahrali I, Akdeniz N, Gelmez MY, et al. The Role of Natural Killer Cells in Autoimmune Diseases. Front Immunol. 2021,25(2):622306.
9. Li Y, Wang F, Imani S, Tao L, Deng Y, Cai Y. Natural Killer Cells: Friend or Foe in Metabolic Diseases? Front Immunol. 2021,24(2):614429.
10. Bonamichi BDSF, Lee J. Unusual Suspects in the Development of Obesity-Induced Inflammation and Insulin Resistance: NK cells, iNKT cells, and ILCs. Diabetes Metab J. 2017,41(4):229-50.
11. Saltiel AR, Olefsky JM. Inflammatory mechanisms linking obesity and metabolic disease. J Clin Invest. 2017,127(1):1-4.
12. Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, Neels JG. Ablation of CD11c-positive cells

normalizes insulin sensitivity in obese insulin resistant animals. Cell Metab. 2008,8(4):301-9.
13. Wang H, Cao K, Liu S, Xu Y, Tang L. Tim-3 Expression Causes NK Cell Dysfunction in Type 2 Diabetes Patients. Front Immunol. 2022,13(4):852436.
14. Fernø J, Strand K, Mellgren G, Stiglund N, Björkström NK. Natural Killer Cells as Sensors of Adipose Tissue Stress. Trends Endocrinol Metab. 2020,31(1):3-12.
15. Wu H, Nie Y. Proportion of natural killer cells in peripheral blood lymphocytes is correlated with cytokine levels in patients with type 2 diabetes mellitus and prediabetes: a preliminary report. Int J Diabetes Dev Ctries.2022, 42(4):781–786.
16. Peng H, Xiang T, Xu F, Jiang Y, Zhong L, Peng Y, et al. Redistribution and Activation of CD16 brightCD56dim NK Cell Subset to Fight against Omicron Subvariant BA.2 after COVID-19 Vaccination. Microorganisms. 2023,11(4):940.
17. Donath MY, Dinarello CA, Mandrup-Poulsen T. Targeting innate immune mediators in type 1 and type 2 diabetes. Nat Rev Immunol. 2019,19(12):734-746.
18. Abel AM, Yang C, Thakar MS, Malarkannan S. Natural Killer Cells: Development, Maturation, and Clinical Utilization. Front Immunol. 2018,13(8):1869.
19. Wu H, Nie Y, Xiong H, Liu S, Li G, Huang A, et al. $P2X_7$ Receptor Expression in Peripheral Blood Monocytes Is Correlated With Plasma C-Reactive Protein and Cytokine Levels in Patients With Type 2 Diabetes Mellitus: a Preliminary Report. Inflammation. 2015, 38(6):2076-81.
20. Gong JH, Maki G, Klingemann HG. Characterization of a human cell line (NK-92) with phenotypical and functional characteristics of activated natural killer cells. Leukemia. 1994,8(4):652-8.
21. Fabian KP, Hodge JW. The emerging role of off-the-shelf engineered natural killer cells in targeted cancer immunotherapy. Mol Ther Oncolytics. 2021,23(10):266-76.
22. Kim JH, Park K, Lee SB, Kang S, Park JS, Ahn CW, et al. Relationship between natural killer cell activity and glucose control in patients with type 2 diabetes and prediabetes. J Diabetes Investig. 2019,10(5):1223-228.
23. Lv X, Gao Y, Dong T, Yang L. Role of Natural Killer T (NKT) Cells in Type II Diabetes-Induced Vascular Injuries. Med Sci Monit. 2018, 24(11):8322-332.
24. Bergman H, Lindqvist C. Human IL-15 Inhibits NK Cells Specific for Human NK-92 Cells. Anticancer Res. 2021,41(7):3281-285.
25. Wang X, Liang C, Xia W, Guo C, Niu Z, Zhu W, et al. VEGF165b augments NK92 cytolytic activity against human K562 leukemia cells by upregulating the levels of perforin and granzyme B via the VEGR1-PLC pathway. Mol Immunol. 2020,128(12):41-6.
26. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018, 13(4):757-72.
27. Laforge M, Elbim C, Frère C, Hémadi M, Massaad C, Nuss P, et al. Tissue damage from neutrophil-induced oxidative stress in COVID-19. Nat Rev Immunol. 2020,20(9):515-16.
28. Morris G, Gevezova M, Sarafian V, Maes M. Redox regulation of the immune response. Cell Mol Immunol. 2022,19(10):1079-101.
29. Assmann N, O'Brien KL, Donnelly RP, Dyck L, Zaiatz-Bittencourt V, Loftus RM, et al. Srebp-controlled glucose metabolism is essential for NK cell functional responses. Nat Immunol. 2017,18(11):1197-206.
30. Millman AC, Salman M, Dayaram YK, Connell ND, Venketaraman V. Natural killer cells, glutathione, cytokines, and innate immunity against Mycobacterium tuberculosis. J Interferon Cytokine Res. 2008,28(3):153-65.
31. Gajovic N, Jurisevic M, Pantic J, Radosavljevic G, Arsenijevic N, Lukic ML, et al. Attenuation of NK cells facilitates mammary tumor growth in streptozotocin-induced diabetes in mice. Endocr Relat Cancer. 2018,25(4):493-507.
32. Tuomela K, Ambrose AR, Davis DM. Escaping Death: How Cancer Cells and Infected Cells Resist Cell-Mediated Cytotoxicity. Front Immunol. 2022,13(3):867098.
33. Prager I, Liesche C, van Ooijen H, Urlaub D, Verron Q, Sandström N, et al. NK cells switch from granzyme B to death receptor-mediated cytotoxicity during serial killing. J Exp Med. 2019,216(9):2113-127.
34. Sordo-Bahamonde C, Lorenzo-Herrero S, Payer ÁR, Gonzalez S, López-Soto A. Mechanisms of Apoptosis Resistance to NK Cell-Mediated Cytotoxicity in Cancer. Int J Mol Sci. 2020,21(10):3726.