Received: 30 September 2022 | Revised: 12 January 2023 | Accepted: 7 February 2023

DOI: 10.1002/oby.23772

ORIGINAL ARTICLE

Obesity Biology and Integrated Physiology

Obesity A Research Journal | THE OBESITY SOCIETY | WILEY

Glucagon-like peptide-1 therapy in people with obesity restores natural killer cell metabolism and effector function

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Funding information
Health Research Board, Grant/Award Number: ILP-POR-2019-110; Maynooth University, Grant/Award Number: 16/RI/3399

Abstract

Objective: People with obesity (PWO) have functionally defective natural killer (NK) cells, with a decreased capacity to produce cytokines and kill target cells, underpinned by defective cellular metabolism. It is plausible that the changes in peripheral NK cell activity are contributing to the multimorbidity in PWO, which includes an increased risk of cancer. This study investigated whether therapy with long-acting glucagon-like peptide-1 (GLP-1) analogues, which are an effective treatment for obesity, could restore NK cell functionality in PWO.

Methods: In a cohort of 20 PWO, this study investigated whether 6 months of once weekly GLP-1 therapy (semaglutide) could restore human NK cell function and metabolism using multicolor flow cytometry, enzyme-linked immunosorbent assays, and cytotoxicity assays.

Results: These data demonstrate that PWO who received GLP-1 therapy have improved NK cell function, as measured by cytotoxicity and interferon-γ/granzyme B production. In addition, the study demonstrates increases in a CD98-mTOR-glycolysis metabolic axis, which is critical for NK cell cytokine production. Finally, it shows that the reported improvements in NK cell function appear to be independent of weight loss.

Conclusions: The restoration, by GLP-1 therapy, of NK cell functionality in PWO may be contributing to the overall benefits being seen with this class of medication.

INTRODUCTION

Obesity is a major global health care issue, with more than 600 million adults living with obesity worldwide [1]. Obesity is a disease defined by the accumulation of excess adipose tissue, which is harmful to an individual's health [2]. A major contributor to obesity's burden on health is its striking and strong association with numerous chronic diseases, including type 2 diabetes mellitus (T2DM), cardiovascular disease, and many types of cancer [3, 4]. Obesity is also associated with poor outcomes following infections such as influenza and SARS-CoV-2 [5, 6]. Significant immune dysregulation, which has been described in people with obesity (PWO), is associated with both the chronic disease burden and poor outcomes in acute infections [7–10].

Natural killer (NK) cells are a critical front line immune population tasked with protecting the host from invading pathogens and the development of malignancies [11]. They represent approximately 10% of circulating lymphocytes. NK cells are able to rapidly kill infected or transformed malignant cells without prior sensitization [12, 13]. NK

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cells are also potent cytokine producers and they can direct other immune populations via their early production of cytokines such as interferon-γ (IFN-γ) [14–16]. Rapid production of IFN-γ by NK cells is also important for their antitumor activities [17, 18]. The importance of NK cells for antitumor immunity is highlighted by the increased prevalence of cancer in humans deficient in NK cells [19].

Cellular metabolism has emerged as a critical regulator of NK cell responses. On activation, NK cells are shaped and instructed by intrinsic metabolic processes and nutrient availability [20]. Glycolysis is one of the key metabolic pathways required for NK cell activity, in particular their ability to produce IFN-γ [21, 22]. Another critical factor for NK cell metabolism is the metabolic master regulator mammalian target of rapamycin (mTOR) [21, 23]. The serine/threonine-protein kinase mTOR also functions as a nutrient sensor and it is activated/sustained by amino acid transport via the surface expressed transporter SLC7A5 [24].

Our group and others have previously reported the detrimental impact of obesity on circulating NK cells, with reduced frequencies and defective functions, including cytotoxicity and IFN-γ production [25–28]. We have provided mechanistic evidence that dysregulated cellular metabolism underpinned the defective circulating NK cell functionality in obesity [27, 28]. Furthermore, NK cells with obesity-related defects were limited in their control of tumor progression in murine models of cancer [28]. Therefore, it would be of potential clinical significance to identify therapeutic approaches that could restore peripheral NK cell activity in PWO.

Glucagon-like peptide-1 (GLP-1) is a multifaceted gut hormone that facilitates glucose-dependent stimulation of insulin secretion and regulation of satiety [29]. Because of these metabolic actions, GLP-1 has been developed into a pharmacological agent to treat both T2DM and obesity [30]. In addition to its classical metabolic effects, GLP-1 has been reported to be neuroprotective and immunomodulatory [30]. We have previously demonstrated the modulation of invariant natural killer T (iNKT) cells by GLP-1 in mice and humans, including restoration of obesity-associated defects in frequencies [31, 32].

In this study, we investigated whether GLP-1 therapy could restore circulating NK cells in PWO. We show that 6 months of GLP-1 analogue therapy in PWO is associated with improved NK cell cytokine production and cytotoxicity, underpinned by increased cellular metabolism. We also show that the observed improvements in NK cells were independent of weight loss and were mimicked by GLP-1 treatment of NK cells from PWO *in vitro*.

Study Importance

What is already known?

- Obesity is strongly associated with increased mortality from cancer and viral infection.
- Peripheral blood natural killer (NK) cells are defective in people with obesity.
- Dysregulated NK cell metabolism underpins the defective functionality in obesity.

What does this study add?

- Following glucagon-like peptide-1 analogue treatment, NK cell functionality is restored in people with obesity.
- Glucagon-like peptide-1 analogue treatment boosts the cellular metabolism of NK cells from people with obesity.
- Glucagon-like peptide-1 analogue treatment-related improvements in NK cells are independent of weight loss.

How might these results change the direction of research or the focus of clinical practice?

- Our study demonstrates that glucagon-like peptide-1 analogue treatment improves NK cell functionality, which may contribute to the benefits reported with these medications in people with obesity.

METHODS

Study approval

Ethical approval was granted by the Medical Research Ethics Committees at St Vincent's University Hospital and by Maynooth University Ethics Committee. All patients gave written informed consent prior to partaking in the study.

Study participants

In total, we recruited a cohort of 20 PWO who were due to commence GLP-1 analogue therapy (once weekly 0.25 mg semaglutide with standard dose escalation to 1 mg weekly) for weight management from the St Columcille's Hospital, Dublin, Ireland. Inclusion criteria included the following: aged between 18 and 55 years old, ability to give informed consent, body mass index (BMI) > 30, and no previous use of GLP-1 therapies. Exclusion criteria included the following: recent infection (<2 weeks), history of T2DM, or use of immunomodulatory medications. Patient characteristics are outlined in Supporting Information Table S1.

Cell culture

Peripheral blood mononuclear cell (PBMC) samples were isolated by density centrifugation over Ficoll from fresh peripheral blood samples and biobanked for batch analysis after completion of 6 months of therapy. When necessary, primary NK cells were isolated via negative selection using an NK cell purification kit (Miltenyi Biotec). PBMCs or NK cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 Medium GlutaMax (Gibco) supplemented with 10% fetal bovine

serum (FBS) (Gibco) and 100 U/mL of penicillin and 100 µg/mL of streptomycin (Sigma). Cells were then incubated at 37 °C and 5% CO_2. For cytokine stimulation, NK cells or PBMCs were plated at 1×10^6 cells/mL and left untreated or stimulated with interleukin (IL)-15 (50 ng/mL) and IL-12 (30 ng/mL; both BioLegend) for 18 hours.

Flow cytometry

NK cell staining was performed using specific surface monoclonal antibodies (all Miltenyi Biotec), namely, CD3 and CD56. Cell populations were acquired using a Attune NxT Flow Cytometer (Life Technologies) and analyzed using FlowJo software (Treestar). Results are expressed as a percentage of the parent population as indicated and determined using fluorescence minus one and unstained controls IFN-γ, granzyme B, CD98, hexokinase-2 (HKII), and pS6.

NK cell cytokine production

NK cells were isolated from PBMCs using a negative selection NK cell isolation kit (Miltenyi Biotec). NK cell purity was confirmed via flow cytometry and was shown to be >93%. NK cells were stimulated as before, and supernatants were analyzed for IFN-γ and granzyme B concentration using DuoSet ELISA kits (R&D Systems). For direct stimulation with GLP-1, isolated NK cells from PWO were pretreated with GLP-1 analogues (1 µg/mL) for 60 minutes before stimulation for 18 hours as outlined previously. Supernatants were assessed by enzyme-linked immunosorbent assay (ELISA).

NK cell cytotoxicity

K562s were washed three times in 10% FBS in phosphate-buffered saline (PBS) and counted. K562s were resuspended in serum free IMDM (Gibco) at a concentration of 1×10^6 cell/mL and were treated with Calcein-AM (BioLegend) at a concentration of 20 µM for 30 minutes. K562s were washed again three times in 10% FBS in PBS and resuspended in IMDM with 10% FBS. K562s were added to a round bottom 96-well plate at a concentration of 2×10^5 cells/mL. Effector cells were added at a 40:1 effector to K562 ratio and incubated at 37 °C, 5% CO_2 for 3 hours. After incubation the plate was spun at 300*g* for 5 minutes and 75 µL of the supernatant was removed and added to a black walled 96-well plate. The plate was read for fluorescent intensity at 530 nm. Killing percentage was measured using the following formula:

$$\frac{(\text{Sample} - \text{spontaneous})}{(\text{Max} - \text{spontaneous})} \times 100$$

NK cell metabolic inhibitor experiments

Purified NK cells from healthy donors were stimulated as before in the absence or presence of 2-deoxy-glucose (2 mM, Sigma) or rapamycin (20 nM, Sigma). For SLC7A5 inhibition experiments, the concentration of amino acids in RPMI was diluted twofold using Hank's Balanced Salt Solution (Invitrogen) in the presence or absence of 2-aminobicyclo-(2,2,1)heptane-carboxylic acid) (BCH) (50 mM, Sigma).

Statistics

Statistical analysis was completed using GraphPad Prism 6 software. Data are expressed as SEM. We determined differences between two groups using Student *t* test and Mann-Whitney U test where appropriate. Analysis across three or more groups was performed using ANOVA. Correlations were determined using linear regression models and expressed using Pearson or Spearman rank correlation coefficient, as appropriate. *P* values were expressed with significance set at <0.05.

RESULTS

NK cell frequencies and cytokine production are defective in PWO

Several previously published studies have demonstrated reduced circulating NK cell frequencies in PWO [27, 33]. We first confirmed this in our cohort of PWO before they commenced GLP-1 therapy (Figure 1A–C). A critical function of NK cells is their ability to produce effector molecules such as IFN-γ. Previous studies from our group and others have demonstrated a severe defect in NK cell production of IFN-γ in PWO [28]. Again, we confirmed this in our cohort of PWO before they commenced GLP-1 therapy (Figure 1D). In addition to defective cytokine production, we also demonstrated reduced NK cell cytotoxicity in PWO (Figure 1E).

GLP-1 therapy results in weight loss and improved glycemic control in PWO

GLP-1 analogue therapy, the gold standard for pharmacological treatment of obesity, can result in clinically significant (>5%) reductions in body weight. In our cohort of patients (mean BMI = 46.6), we observed reduced BMI in the cohort overall, with clinically significant weight loss in 9 of our 20 patients (Supporting Information Table S1 and Figure S1A). Although none of our patients had abnormal glycated hemoglobin, we did see a reduction in glycated hemoglobin in 12 of our patients (Supporting Information Figure S1B). We did not observe any change in the lipid profile of our patients with GLP-1 therapy (Supporting Information Table S1 and Figure S1C,D).

GLP-1 analogue therapy does not restore NK cell frequencies in PWO

To investigate the impact of GLP-1 therapy on NK cells we first investigated peripheral blood NK cell frequencies in PWO before and after

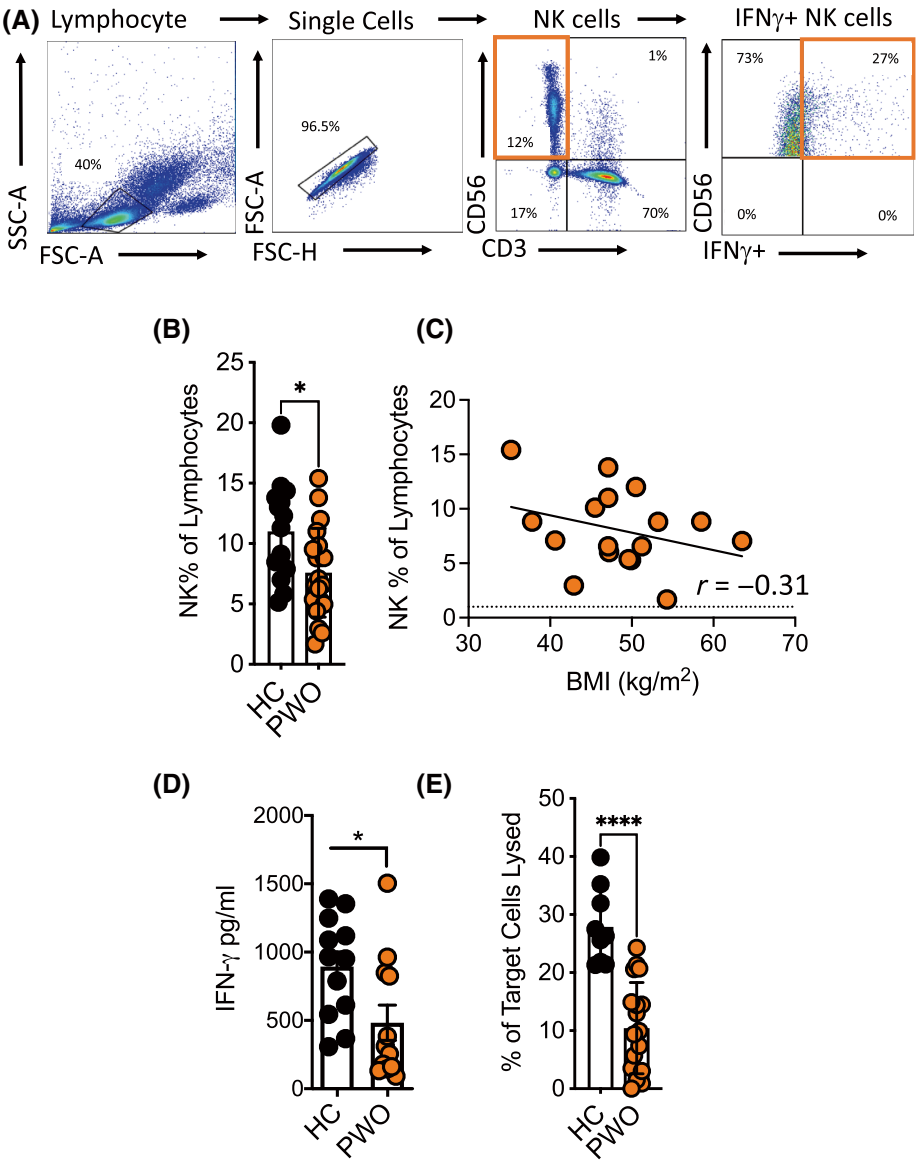

FIGURE 1 NK cells are altered in PWO. (A) Representative flow cytometry dot plots detailing the gating strategy used to identify NK cells in peripheral blood of PWO. (B) Scatterplot showing the NK cell frequencies in cohort of HC individuals and PWO. (C) XY graph showing the relationship between NK cell frequencies and BMI in PWO. (D) Scatterplot showing the levels of IFN-γ produced by activated (18 hours with IL-12 and IL-15) NK cells isolated from either HC individuals or PWO. (E) Scatterplot showing the percentage lysis of K562 target cells over 3 hours by either HC individuals or PWO. FSC-A, Forward Scatter-Area; FSC-H, Forward Scatter-Height; HC, healthy control; NK, natural killer; PWO, people with obesity; SSC-A, Side Scatter-Area. *$p < 0.05$; ****$p < 0.0001$ [Color figure can be viewed at wileyonlinelibrary.com]

commencing therapy. We observed no change in total NK cell frequencies after 6-month GLP-1 analogue therapy (Figure 2A). We next investigated whether either $CD56^{BRIGHT}$ or $CD56^{DIM}$ NK cell subsets were changed post GLP-1 and again noted no difference (Figure 2B,C). GLP-1 analogue therapy was used as a weight loss agent in this cohort, so we next investigated whether a clinical response in body weight (>5% weight loss) was associated with changes in NK cell frequencies and observed no difference in responders versus nonresponders (Figure 2D). Next, we assessed phenotypic changes after GLP-1 and noted no difference in CD25, CD57, CD69, CD95, CD158b, NKG2A, or NKG2D expression on NK cells after GLP-1 therapy (Supporting Information Figure S2).

GLP-1 analogue therapy increases NK cell effector function

We next investigated whether GLP-1 therapy impacted NK cell IFN-γ production and noted a robust increase in NK cells producing IFN-γ (Figure 3A–C). To confirm this finding, we isolated NK cells from patients before and after GLP-1 therapy and again found elevated IFN-γ production post GLP-1 via ELISA (Figure 3D). Cytokine production by NK cells is concentrated in the $CD56^{BRIGHT}$ population, so we next investigated whether GLP-1 therapy increased the IFN-γ production from $CD56^{BRIGHT}$ and/or $CD56^{DIM}$ NK cells and demonstrated a significant increase in both bright and dim populations (Figure 3E,F).

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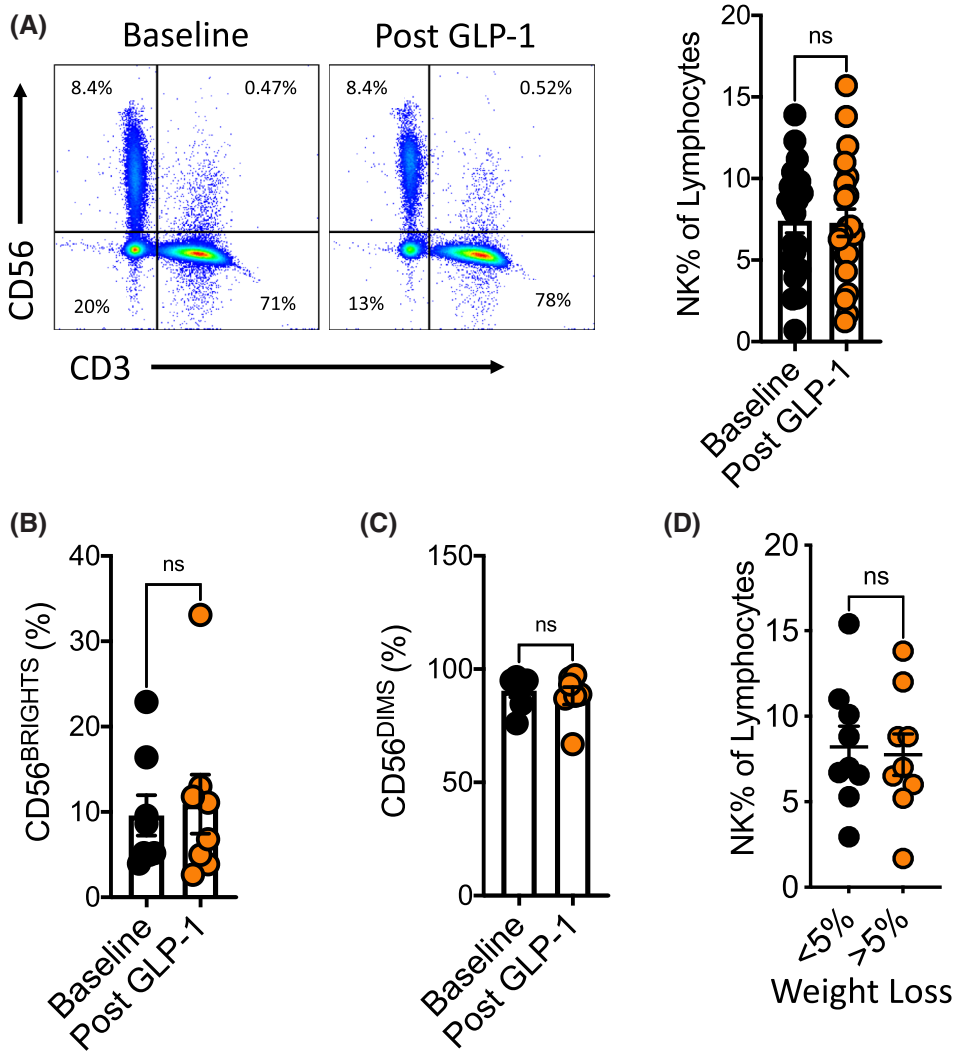


FIGURE 2 No change in NK cell frequencies in PWO treated with GLP-1 analogues. (A) Representative dot plot and graph showing the NK cell frequencies in cohort of 20 PWO before and after 6 months of GLP-1 analogue therapy. (B,C) Scatterplots showing the frequencies of $CD56^{BRIGHT}$ and $CD56^{DIM}$ NK cell subsets in cohort of 20 PWO before and after 6 months of GLP-1 analogue therapy. (D) Scatterplot showing the NK cell frequencies in PWO who lost or did not lose a clinically significant amount of weight (>5% of initial body weight). GLP-1, glucagon-like peptide-1; NK, natural killer; ns, no significance; PWO, people with obesity [Color figure can be viewed at wileyonlinelibrary.com]

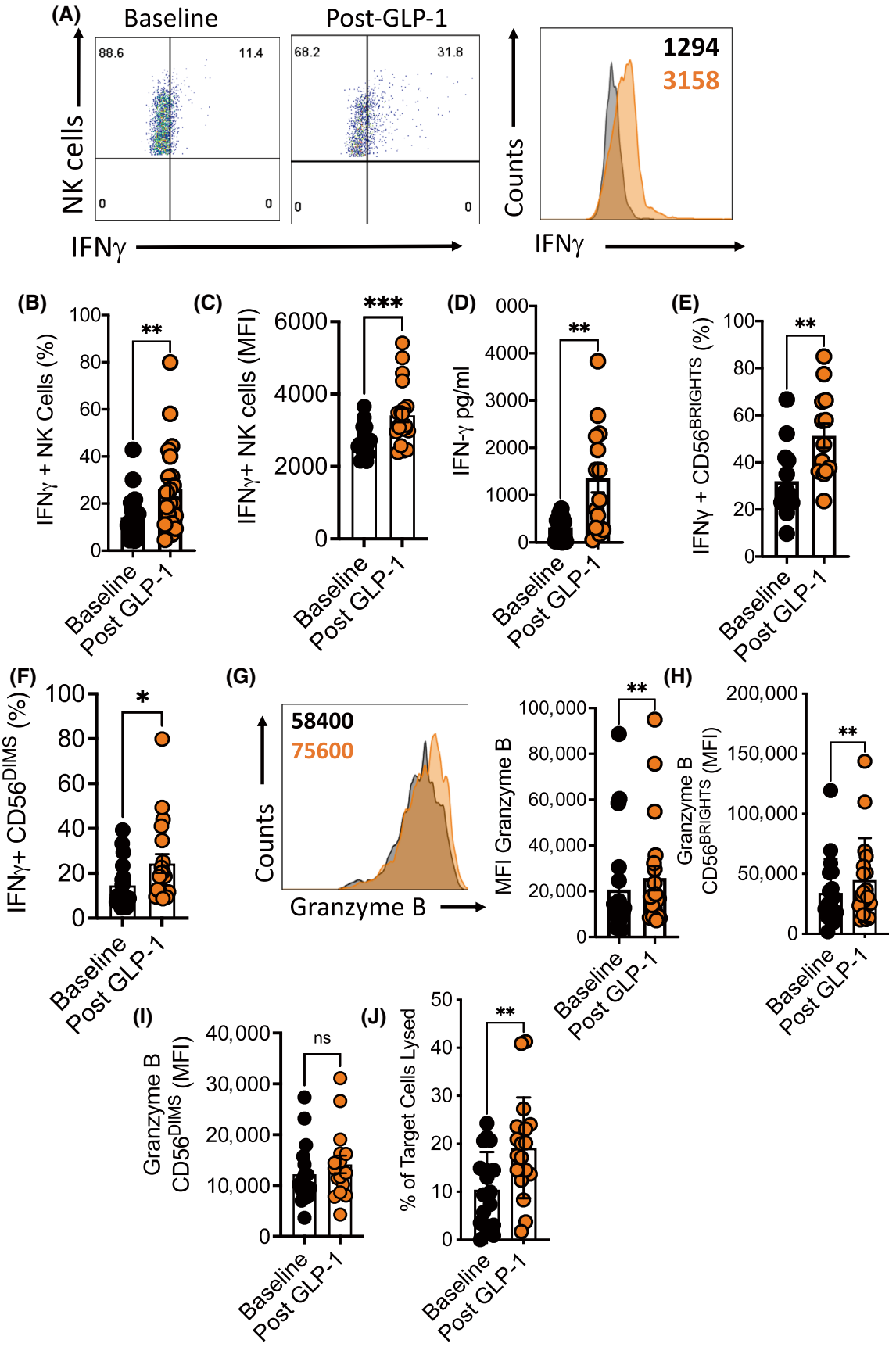
In addition to IFN-γ we investigated granzyme B production and noted increased expression post GLP-1 therapy (Figure 3G,H). Similarly, we observed a significant increase in the granzyme B expression in $CD56^{BRIGHT}$ NK cells but not $CD56^{DIM}$ NK cells post GLP-1 therapy (Figure 3H,I). Finally, we demonstrated increased lysis of the NK cell sensitive line K562 after GLP-1 treatment (Figure 3J).

GLP-1 therapy increases a metabolic axis of SLC7A5, mTORC1, and glycolysis in NK cells from PWO

As highlighted, NK cell cytokine production is critically dependent on intrinsic cellular metabolism, so we investigated a SLC7A5-mTOR-glycolysis axis [20] in NK cells from PWO before and after GLP-1 therapy. We found that GLP-1 therapy increased the expression of the amino acid transporter CD98 on NK cells from PWO, and we demonstrated, using a CD98 specific inhibitor BCH, that it is critical for NK cell production of IFN-γ in NK cells from healthy control individuals (Figure 4A–C). Next, we demonstrated that GLP-1 therapy increases mTOR activity (as measured by pS6) in NK cells from PWO, and again we demonstrated that mTOR is critical for NK cell production of IFN-γ using the specific inhibitor rapamycin (Figure 4D–F). Finally, we showed that GLP-1 therapy increased HKII expression (suggesting elevated glycolysis) in NK cells from PWO and, using the specific inhibitor 2-deoxy-glucose, showed that it was essential for NK cell production of IFN-γ (Figure 4G–I).

GLP-1 therapy-induced restoration of NK cell cytokines and metabolism is independent of weight loss

Having observed robust restoration of NK cell cytokine production and cellular metabolism in patients treated with GLP-1, we next



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investigated whether these improvements were due to GLP-1-mediated weight loss. We interrogated our data for statistical associations between weight loss and changes in IFN-γ, CD98, pS6, or HKII expression and found no significant associations (Figure 5A–D). To explore the possibility of a direct effect of GLP-1 on NK cells, we isolated NK cells from PWO and treated them with GLP-1 *in vitro*, and we noted significant increases in both IFN-γ and granzyme B (Figure 5E,F).

DISCUSSION

Numerous studies have investigated the impact of obesity on peripheral blood NK cell frequencies and function [25–28, 33–36], with the majority reporting altered frequencies and defective effector function (cytokine production and/or cytotoxicity). It is worth noting that the impact of obesity on NK cells in the periphery is very different to adipose tissue, in which NK cell frequency and cytokine production are increased, with strong links to metabolic dysregulation reported [14, 15, 37, 38]. Additional work from our group has highlighted obesity-associated defects in peripheral blood NK cell metabolism as a mechanism underpinning defective cytokine production [27, 28, 36]. In murine models of cancer, these obesity-associated defects in NK cells have been directly linked to poorer outcomes [25, 28]. Exploring therapeutic strategies that restore NK cell activity in PWO may lead to improved outcomes across the range of multimorbidities that are associated with this disease. In the current study, we report that 6-month GLP-1 therapy restores NK cell metabolism and effector function (cytokine production and cytotoxicity) in a cohort of PWO without T2DM. This effect is independent of any weight loss that occurred.

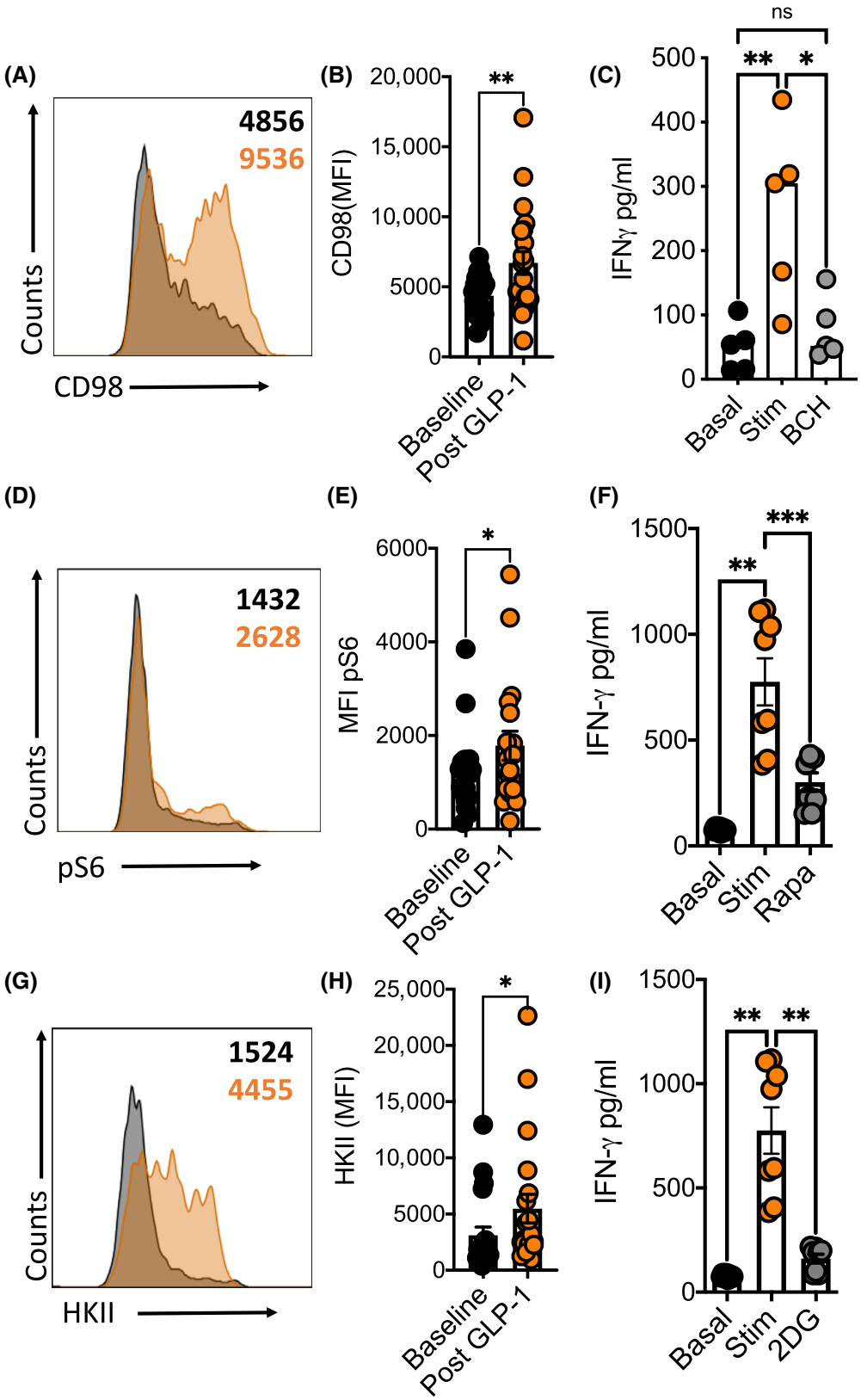
GLP-1 therapy is currently the most effective pharmacological intervention for obesity [39]. The primary mechanisms of action are delayed gastric emptying and increased central satiety [29]. We have previously demonstrated that GLP-1 also modulates the immune system and that this effect is required for optimal weight loss [32]. Several studies have demonstrated that GLP-1 can directly impact different immune cell populations, including macrophages and T cells [31, 40, 41]. In this study, we demonstrate that GLP-1 therapy also modulates NK cells. After 6 months of GLP-1 analogue therapy we did not observe any change in NK cell frequencies in PWO. A previous study from Moulin and colleagues investigated the impact of metabolic surgery on NK cells in PWO and, in line with our data, observed no change in frequencies 6 months after surgery [42]. In the current study, we show that GLP-1 therapy results in increased NK cell cytotoxicity, along with increased IFN-γ and granzyme B production by NK cells, suggesting more functional NK cells.

Cellular metabolism is a critical requirement for NK cell functionality and it can dictate the magnitude of responses [20]. A series of studies have revealed a central mTOR-glycolysis axis as among the most important for NK cell cytokine production [21, 23, 43]. In this study, we again demonstrate the importance of this axis for NK cell cytokine production, adding an additional node, CD98. CD98 is a heterodimer comprising SLC3A2 and SLC7A5 and it functions as the large neutral amino acid transporter (LAT1). CD98, and in particular SLC7A5, is critical for mTOR activity and glycolysis in T cells [44]. In a study by Loftus et al., amino acid transport via SLC7A5 was shown to be critical for NK cell metabolism and function [24]. Previously, we have linked defective NK cell functionality in obesity to dysregulated cellular metabolism [27, 28]. We have also demonstrated obesity-associated defects in the proposed SLC7A5-mTOR-glycolysis axis in mucosal-associated invariant T cells, another effector population of immune cells [45]. Therefore, we investigated the impact of GLP-1 therapy on NK cell metabolism in our cohort of PWO and demonstrated a significant increase in the SLC7A5-mTOR-glycolysis axis. Using specific inhibitors for each component of this axis, we demonstrate its importance for NK cell cytokine production and propose that it is likely that increased cellular metabolism with GLP-1 supports the increased IFN-γ production reported.

Metabolic surgery results in major metabolic changes, including a 30% reduction in body weight, and this is proposed as the mechanism for restoration of NK cell activity [42]. GLP-1 therapy results in more modest weight loss, ≈5% to 15%. In our study, the increases in IFN-γ production and cellular metabolism were not associated with weight loss, suggesting an independent mechanism. This finding is in line with our previous observations on the reduction of inflammation with GLP-1 treatment, in which the effect was also independent of the impact on weight [41]. We show that direct *in vitro* treatment of NK cells from PWO with GLP-1 increases both IFN-γ and granzyme B

FIGURE 3 GLP-1 therapy increases NK cell effector function in PWO. (A) Representative flow cytometry dot plots and histogram showing the frequencies or MFI of IFN-γ-producing NK cells (after 18 hours of cytokine [IL-12/15] stimulation) in cohort of 20 PWO before and after 6 months of GLP-1 analogue therapy. (B,C) Scatterplots showing the frequencies or MFI of IFN-γ-producing NK cells (after 18 hours of cytokine [IL-12/15] stimulation) in cohort of 20 PWO before and after 6 months of GLP-1 analogue therapy. (D) Scatterplot showing the quantities of IFN-γ produced by NK cells (after 18 hours of cytokine [IL-12/15] stimulation) isolated from PWO before and after GLP-1 therapy. (E,F) Scatterplots showing the frequencies of either $CD56^{BRIGHT}$ or $CD56^{DIM}$ NK cells producing IFN-γ in cohort of 20 PWO before and after 6 months of GLP-1 analogue therapy. (G) Representative flow cytometry histogram and scatterplot showing the MFI of granzyme B expression in NK cells (after 18 hours of cytokine [IL-12/15] stimulation) in cohort of 20 PWO before and after 6 months of GLP-1 analogue therapy. (H,I) Scatterplots showing the MFI of granzyme B expression by $CD56^{BRIGHT}$ or $CD56^{DIM}$ NK cells in PWO before and after GLP-1 analogue therapy. (J) Scatterplot showing the percentage lysis of K562 target cells over 3 hours by PWO before and after GLP-1 therapy. GLP-1, glucagon-like peptide-1; MFI, mean fluorescence intensity; NK, natural killer; ns, no significance; PWO, people with obesity. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ [Color figure can be viewed at wileyonlinelibrary.com]

production, supporting the concept of a direct effect. Interestingly, analysis of existing data sets (proteomics and RNA sequencing) on NK cells has suggested that they do not express the GLP-1 receptor [24, 46]. Previously, we have demonstrated that GLP-1 could also activate murine iNKT cells *in vitro*; subsequent RNA sequencing analysis again has suggested that iNKT cells do not express the GLP-1 receptor [32,



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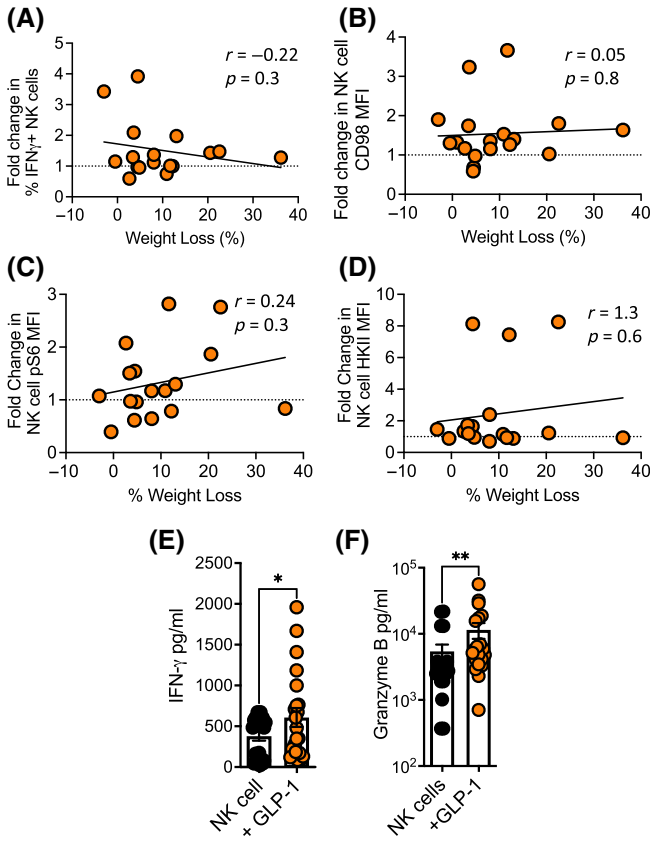

FIGURE 5 GLP-1 therapy-induced restoration of NK cell cytokines and metabolism is independent of weight loss. (A) Relationship between fold change in IFN-γ-producing NK cell frequencies and percentage weight loss in PWO after 6-month GLP-1 analogue therapy. (B–D) Relationships between fold change in CD98, pS6, or HKII expression (MFI) in NK cells and percentage weight loss in PWO after GLP-1 therapy. (E,F) IFN-γ or granzyme B produced by activated (18 hours with IL-12 and IL-15) NK cells isolated from PWO pretreated for 60 minutes with GLP-1 (1 μg) *in vitro*. GLP-1, glucagon-like peptide 1; HKII, hexokinase-2; MFI, mean fluorescence intensity; NK, natural killer; ns, no significance; PWO, people with obesity. $^{*}p < 0.05$; $^{**}p < 0.01$ [Color figure can be viewed at wileyonlinelibrary.com]

47]. In addition to our findings, numerous studies have demonstrated GLP-1 activity in cells not expressing the classical GLP-1 receptor or using a truncated GLP-1 peptide, perhaps indicating an alternative receptor [48–50].

Collectively, to our knowledge, our data demonstrate for the first time the restoration of peripheral blood NK cell cytokine production and cytotoxicity in PWO treated with GLP-1 analogues. The restoration appears to be in a weight loss-independent manner. We provide evidence that the restoration in cytokine production is linked to improved cellular metabolism. The direct restoration, by GLP-1 therapy, of NK cell cytokine production and metabolism in PWO may be contributing to the overall benefits being seen with this class of medication.O

AUTHOR CONTRIBUTIONS

Conor De Barra and Kiva Brennan performed the experiments, carried out analysis, and approved the final manuscript as submitted. Mohammed Khalil, Arimin Mat, Ferrah Shaamile, and Cliona O'Donnell enrolled participants, collected and analyzed clinical data, and approved the final manuscript as submitted. Andrew E. Hogan and Donal O'Shea conceptualized and designed the study, analyzed the data, drafted the manuscript, and approved the final manuscript as submitted.

ACKNOWLEDGMENTS

The authors would like to thank all the participants of the study, as well as the Irish Coalition for People living with Obesity for support and guidance. Open access funding provided by IReL.

FUNDING INFORMATION

This study is supported by the Health Research Board (ILP-POR-2019-110). Conor De Barra is supported by a fellowship from Irish Research Council. Financial support for the Attune NxT was provided to Maynooth University Department of Biology by Science Foundation Ireland (16/RI/3399).

CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interest.

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FIGURE 4 GLP-1 therapy increases a metabolic axis of SLC7A5, mTORC1, and glycolysis in NK cells from PWO. (A,B) Representative flow cytometry histogram and scatterplot showing the MFI of CD98 expression in NK cells (after 18 hours of cytokine [IL-12/15] stimulation) in cohort of 20 PWO before and after 6 months of GLP-1 analogue therapy. (C) Scatterplots showing the IFN-γ production by NK cells isolated from healthy controls after 18-hour stimulation with IL-12/IL-15, in the absence or presence of SLC7A5 specific inhibitor BCH (50 mM). (D,E) Representative flow cytometry histogram and scatterplot showing the MFI of pS6 expression in NK cells (after 18 hours of cytokine [IL-12/15] stimulation) in PWO ($n = 20$) before and after 6 months of GLP-1 analogue therapy. (F) Scatterplot showing the IFN-γ production by NK cells isolated from healthy controls after 18-hour stimulation with IL-12/IL-15, in the absence or presence of mTORC1 specific inhibitor rapamycin (20 nM). (G,H) Representative flow cytometry histogram and scatterplot showing the MFI of HKII expression in NK cells (after 18 hours of cytokine [IL-12/15] stimulation) in PWO ($n = 20$) before and after 6 months of GLP-1 analogue therapy. (I) Scatterplot showing the IFN-γ production by NK cells isolated from healthy controls after 18-hour stimulation with IL-12/IL-15, in the absence or presence of the glycolysis inhibitor 2DG (2 mM). BCH, 2-aminobicyclo-(2,2,1)heptane-carboxylic acid; GLP-1, glucagon-like peptide-1; 2DG, 2-deoxy-glucose; HKII, hexokinase-2; MFI, mean fluorescence intensity; NK, natural killer; ns, no significance PWO, people with obesity. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ [Color figure can be viewed at wileyonlinelibrary.com]

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: De Barra C, Khalil M, Mat A, et al. Glucagon-like peptide-1 therapy in people with obesity restores natural killer cell metabolism and effector function. *Obesity (Silver Spring)*. 2023;31(7):1787-1797. doi:10.1002/oby.23772