

# Impact of obesity and glucose tolerance on human circulating iNKT cells

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## 1 Introduction

In addition to induce low-grade inflammation contributing to type 2 diabetes, obesity also causes immune dysregulation by negatively impacting several immune cell populations. This results in a loss of immunosurveillance which has been proposed to contribute to increased susceptibility of obese patients to develop some cancers. Peripheral invariant Natural Killer T (iNKT) cells are unconventional T cells that express semi-invariant TCR. iNKT cells recognize through their TCR lipid antigens presented by CD1d on antigen presenting cells, causing the rapid release of Th1 and/or Th2 and/or Th17 cytokines. iNKT cells play an important role in tissue homeostasis, defense against infection and tumor immunosurveillance.

We previously demonstrated that PBMCs from obese patients with dysglycemia (OBDysG) present phospholipidome alteration in comparison to normoglycemic obese (OBNG) and lean individuals<sup>[1]</sup> (Figure 1). Based on these results and because the frequency of iNKT cells is downmodulated in obesity, we hypothesized that an excessive activation of iNKT cells could result from a different lipid antigen presentation (quantitative or qualitative) via CD1d on monocytes of obese patients. This chronic and/or robust activation of peripheral iNKT cells could lead to iNKT cells disruption and contribute to the loss of immunosurveillance.

[1] Wilkin C et al. New Insights on the PBMCs Phospholipidome in Obesity Demonstrate Modulations Associated with Insulin Resistance and Glycemic Status. *Nutrients*. 2021;13(10):3461. doi:10.3390/nu13103461

## 3 Results

### 1. Characterization of peripheral iNKT cells in OBDysG compared to OBNG and lean individuals

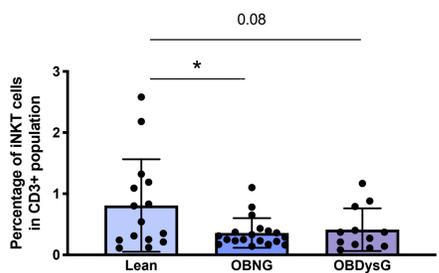


Figure 3. Frequency of iNKT cells in Lean, OBNG and OBDysG patients relative to CD3+ population

The frequency of peripheral iNKT cells is decreased in OBNG and OBDysG compared to lean patients

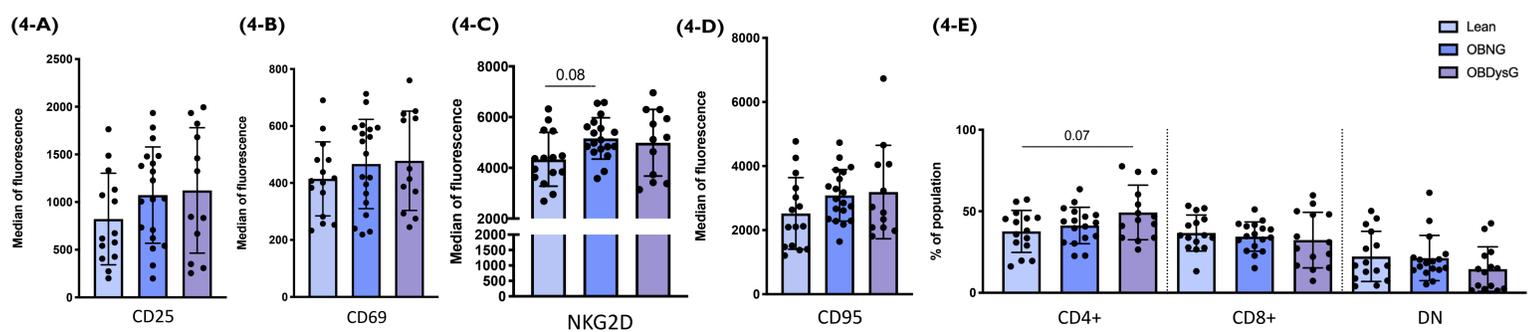


Figure 4. Median of fluorescence of (A) CD25, (B) CD69, (C) NKG2D, (D) CD95 at iNKT cells surface and (E) iNKT cells subpopulation expressed as the percentage of total iNKT cells population

The expression of several activation (CD25, CD69 and NKG2D) and apoptosis cell markers (CD95) tends to be upregulated in both obese phenotypes. Moreover, the CD4+ subpopulation tends to be increased in OBDysG patients compared to lean individuals

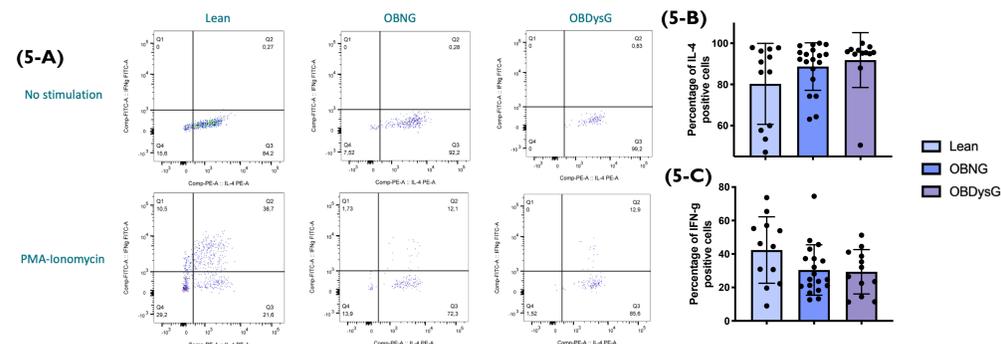


Figure 5. (A) Dot plot of IL-4 (x-axis) and IFN- $\gamma$  (y-axis) production by iNKT cells following no stimulation (top graph) or PMA-ionomycin stimulation (bottom graph) in Lean, OBNG and OBDysG patients. Frequency of iNKT cells producing (B) IL-4 and (C) IFN- $\gamma$  following PMA-ionomycin stimulation

The percentage of IL-4-producing iNKT cells tends to increase whereas IFN- $\gamma$ -producing cells tends to decrease following PMA-ionomycin stimulation in both obese phenotypes compared to lean individuals

## 2 Materials and Methods

A total of 60 individuals (aged between 18-65 years) will be recruited on a voluntary basis. The study protocol was approved by the ethics Committee of the Liège University Hospital, and all patients provided written informed consent. Subjects with inflammatory or malignant diseases were excluded. The participants were categorized in three groups based on the BMI and glycemic status (Figure 2): (i) lean with normoglycemia (Lean), (ii) obese with normoglycemia (OBNG) and (iii) obese with dysglycemia (OBDysG). So far, 45/60 patients have been recruited (Lean: n=14, OBNG: n=19, OBDysG: n=12).

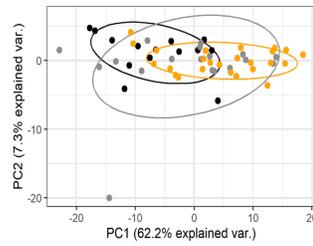


Figure 1. PBMCs phospholipidome allows to distinguish OBDysG from lean patients. Principal component analysis score plot of the phospholipid profiles (PC, PE, PI, PS, SM) obtained on PBMCs from lean (n=14), OBNG (n=18) and OBDysG (n=25) patients.

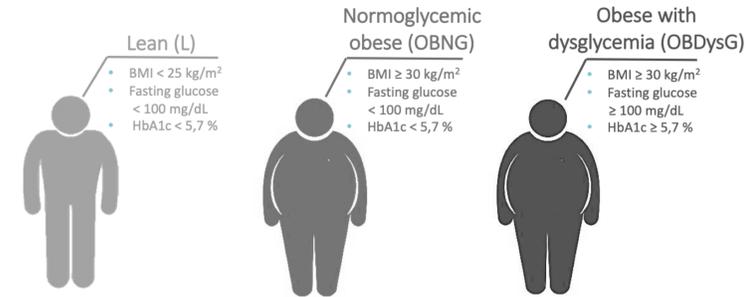
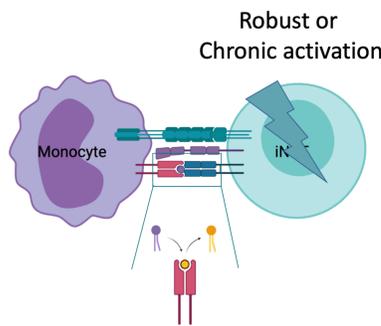


Figure 2. Patient recruitment criteria

### 2. Characterization of peripheral monocytes in OBDysG compared to OBNG and lean individuals

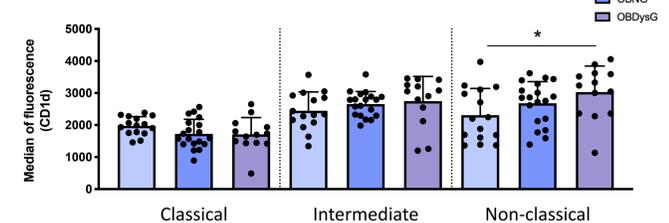
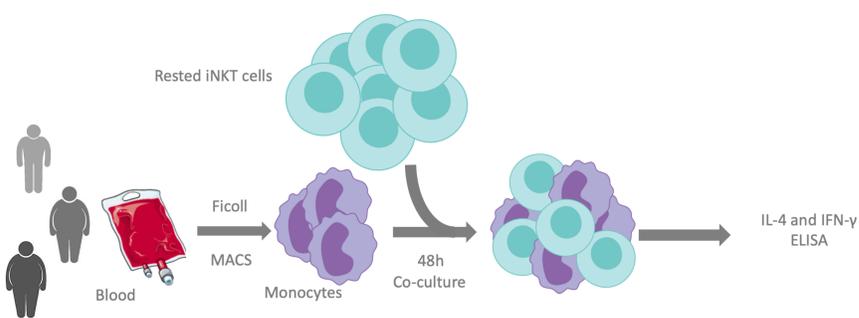


Figure 7. Median of fluorescence of CD1d at surface of monocytes subpopulation (i.e., classical, intermediate and non-classical monocytes)

The expression of CD1d at the monocytes cell surface is upregulated in Non-classical monocyte population

### 3. Assessment of ability of patient's monocytes to activate healthy iNKT cells



First experiments on healthy donors are promising. Experiment on obese subjects will started in June/July 2022

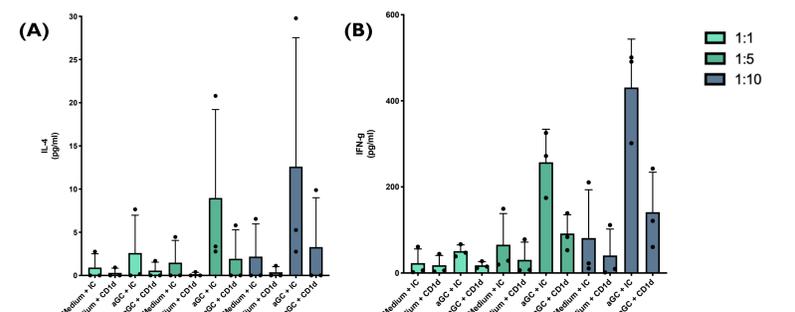


Figure 8. (A) IL-4 and (B) IFN- $\gamma$  production following 48h of iNKT cells and monocytes co-culture with CD1d-blocking antibody (IC) and/or  $\alpha$ -GalactosylCeramide (aGC). Different iNKT cells-Monocytes ratios (1:1; 1:5 and 1:10)

## 4 Discussion

Although these results are incomplete, we observed a disruption of peripheral circulating iNKT cells in both obese phenotypes compared to lean patients. Indeed, our data demonstrate a strong depletion of circulating iNKT cells with obesity. Moreover, it seems that iNKT cells in obese patients switch to Th2 phenotype as demonstrated by an increased % of CD4+ iNKT cells and a decreased ratio of IFN-g/IL-4 production in response to PMA-ionomycin stimulation. Thus, iNKT cells of obese patient could be less efficient in tumor immunosurveillance.