



Peripheral invariant natural killer T cell deficiency in metabolically unhealthy but normal weight versus metabolically healthy but obese individuals

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Abstract

Objective: To investigate the proportion of circulating invariant natural killer T (iNKT) cells in four body health types.

Methods: In this cross-sectional study, participants were classified into four body health types according to the body mass index and metabolic status: metabolically healthy and normal weight (MHNW), metabolically unhealthy but normal weight (MUNW), metabolically healthy but obese (MHO), or metabolically unhealthy and obese (MUO). Demographic and clinical characteristics were measured, and the homeostasis model assessment of insulin resistance (HOMA-IR) and visceral adiposity index (VAI) were calculated. The proportion of circulating iNKT cells was also evaluated by flow cytometry.

Results: The study enrolled 41 MHNW, 37 MUNW, 30 MHO, and 43 MUO participants. Compared with the MHNW group, the MUNW, MHO, and MUO groups had significantly lower iNKT cell proportions. The iNKT cell proportion was significantly higher in the MHO group than the MUNW and MUO groups. The iNKT cell proportion was inversely correlated with high-sensitivity C-reactive protein, HOMA-IR, and VAI values.

Conclusion: The proportion of iNKT cells was lower in people (lean or obese) with excessive visceral fat accumulation, suggesting that iNKT cell deficiency may be involved in the pathophysiology of obesity-related metabolic disorders.

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Keywords

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Introduction

Obesity is characterized by chronic inflammation, immune dysregulation, and insulin resistance, and is associated with high mortality.¹ However, obesity is not a homogeneous condition. Some patients who have a normal weight may also present with metabolic disabilities and insulin resistance.² These individuals are classified as metabolically unhealthy but normal weight (MUNW),² and the prevalence of MUNW has been recently reported to be 19.98% among the general population.³ In contrast, a subgroup of obese individuals is protected against the development of insulin resistance, chronic inflammation, or metabolic abnormalities associated with obesity; and these individuals are classified as metabolically healthy but obese (MHO).⁴ From 9% to 41% of obese individuals are classified as MHO depending on the definition of metabolically healthy.^{5,6} Nevertheless, the aetiologies underlying the heterogeneous metabolic phenotypes in individuals with normal weight or obesity are poorly understood.⁷ The immunometabolic interaction in obese individuals is currently considered as a key factor in chronic low degree inflammation.⁸ However, the link between different obese phenotypes and immune status remains unclear.

Invariant natural killer T (iNKT) cells are a specialized subset of innate T lymphocytes.⁹ Unlike conventional $\alpha\beta$ T cells that recognize the major histocompatibility complex-peptide complex, iNKT cells selectively recognize lipid ligands on CD1d through their semi-invariant T-cell receptor.⁹ The most important feature of iNKT cells is that

they can produce various types of cytokines, both rapidly and at high level, which suggests that they can potently transactivate other immune cells.¹⁰ Thus, the iNKT cells act as an important bridge between innate and adaptive immunity. Although iNKT cells only comprise a small proportion of T cells, their roles have been described in various diseases, including tumours, autoimmune diseases, and metabolic diseases.^{11–13} Studies have identified a role for iNKT cells in the regulation of weight and metabolism in adult obesity.^{14,15} Moreover, the depletion of iNKT cells in mice receiving a high-fat diet is associated with the proliferation of M1 macrophages, enhanced weight gain, larger adipocytes, fatty liver, and insulin resistance.¹⁴

To the best of our knowledge, only limited research has evaluated the relationship between circulating iNKT cells and different metabolic phenotypes in individuals with normal weight or obesity.¹⁶ The present study aimed to assess the frequency of circulating iNKT cells and related confounding factors in participants who were metabolically healthy normal weight (MHNW), MUNW, MHO, or metabolically unhealthy and obese (MUO).

Patients and methods

Study participants

This cross-sectional study evaluated individuals who visited the First Affiliated Hospital, School of Medicine, Shihezi University, Xinjiang, China for routine medical examinations between June 2014 and May 2015. All participants provided written informed consent, and the study design was approved by

the institutional review board of the First Affiliated Hospital (No.2014-121-01). Exclusion criteria included the following: (i) individuals with cancer; (ii) a history of cancer in the last 5 years; (iii) pregnancy or lactation; (iv) immunodeficiency; (v) chronic organ disease; (vi) infectious disease; (vii) individuals who were receiving immunosuppressive or hormone-containing drugs.

Anthropometric and biochemical measurements

All participants underwent anthropometric and body composition measurements, which were performed before breakfast. The height and weight were obtained while the participants wore light clothing without shoes. Body mass index (BMI) was calculated as kg/m^2 . Waist circumference (WC) was measured at the midpoint between the costal margin and the iliac crest at the end of a normal expiration. Blood samples (5 ml) were withdrawn from the cubital vein after an overnight fast, collected into BD Vacutainer sample tubes (Becton, Dickinson & Co., Franklin Lakes, NJ, USA) containing heparin (95 USP Units) for biochemical measurements. Sample tubes were centrifuged at 1200 g for 10 min at 4°C. The plasma was subsequently analysed at the Central Laboratory of the First Affiliated Hospital, School of Medicine, Shihezi University, Xinjiang, China.

The levels of fasting plasma total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), high-sensitivity C-reactive protein (hs-CRP), and fasting plasma glucose (FPG) were analysed using an Hitachi 7180 clinical autoanalyser (Hitachi, Tokyo, Japan). Fasting insulin (FINS) levels were measured using an electrochemiluminescence immunoassay kit and a modular analytics E170 analyser (Roche, Basel, Switzerland). The homeostatic model

assessment of insulin resistance (HOMA-IR) was used to evaluate insulin resistance, and was calculated as follows: $\text{HOMA-IR} = (\text{FINS } [\mu\text{U/ml}] \times \text{FPG } [\text{mmol/l}]) / 22.5$. The visceral adiposity index (VAI) was calculated by using the published formula,¹⁷ as follows:

$$\text{Males: VAI} = \left(\frac{\text{WC}}{39.68 + (1.88 \times \text{BMI})} \right) \times \left(\frac{\text{TG}}{1.03} \right) \times \left(\frac{1.31}{\text{HDL} - \text{C}} \right)$$

$$\text{Females: VAI} = \left(\frac{\text{WC}}{36.58 + (1.89 \times \text{BMI})} \right) \times \left(\frac{\text{TG}}{0.81} \right) \times \left(\frac{1.52}{\text{HDL} - \text{C}} \right)$$

Defining the study groups

The participants were classified as overweight or obese ($\text{BMI} \geq 25 \text{ kg/m}^2$) or normal weight ($18.5 \text{ kg/m}^2 \leq \text{BMI} < 25 \text{ kg/m}^2$) based on the BMI values, as per the World Health Organization Western Pacific Region definitions.¹⁸ Thereafter, each body weight group was further subdivided as metabolically healthy or metabolically unhealthy according to the criteria for the Adult Treatment Panel III (ATP-III) components.¹⁹ However, the WC criterion was not used in that classification due to its collinearity with BMI. The individuals who fulfilled < 2 of the following criteria were considered metabolically healthy:⁶ (i) TG levels $\geq 1.7 \text{ mmol/l}$ or the use of lipid-lowering drugs; (ii) glucose levels $\geq 5.6 \text{ mmol/l}$ or the use of diabetes medication; (iii) systolic blood pressure $\geq 130 \text{ mmHg}$, diastolic blood pressure $\geq 85 \text{ mmHg}$, or the use of antihypertensive drugs; (iv) HDL-C levels $< 1.29 \text{ mmol/l}$ for women or $< 1.03 \text{ mmol/l}$ for men. By using these criteria, individuals were classified as MHNW, MUNW, MHO, or MUO.

Preparation of PBMC and flow cytometric analysis

Following an overnight fast, venous blood (10 ml) was collected into BD Vacutainer tubes (Becton, Dickinson & Co.) containing 1.8 mg/ml ethylenediaminetetra-acetic acid and stored at 4°C. Within 2 h of collection, peripheral blood mononuclear cells (PBMC) were isolated using Ficoll-Hypaque (Lymphoprep; Haoyang Biological Technology Co Ltd, Tianjin, China) density gradient centrifugation at 400 *g* for 25 min. Cells were washed twice in 0.01 M phosphate-buffered saline (pH 7.2), and the resulting cell pellet was suspended in 100 μ l of complete RPMI 1640 medium. The PBMC suspension was incubated with a combination of monoclonal antibodies against CD3PerCP (final concentration 1 μ g/100 μ l) and V α 24-J α 18 FITC (final concentration 1 μ g/100 μ l) (eBioscience, San Diego, CA, USA) for 20 min at room temperature in the dark. The resultant cell suspension was then subjected to flow cytometry (CyFlow[®] flow cytometer; Partec, Nuremberg, Germany) using FloMax[®] software (Partec). The lymphocytes were identified by using the forward scatter and side scatter methods. The cells were electronically gated based on their density and granularity, and were assessed for the markers of interest. The results are expressed as the percentage of total lymphocytes.

Statistical analyses

All statistical analyses were performed using the SPSS[®] statistical package, version 17.0 (SPSS Inc., Chicago, IL, USA) for Windows[®]. The data are presented as mean \pm SD or median (interquartile range), as appropriate. Groups were compared using one-way analysis of variance or Mann–Whitney *U*-test, as appropriate. Categorical variables are presented as number (%), and the χ^2 -test was used to evaluate the distributions of the categorical

variables. Spearman's correlation coefficient was used to examine the relationship between iNKT cell level and biochemical parameters. Linear regression was also performed to adjust for the confounding factors for iNKT cell level. A *P*-value of <0.05 was considered statistically significant.

Results

A total of 151 study participants (80 men and 71 women) with a mean age of 54.5 years (range, 35–71 years) were enrolled in the study. Of these, 78 were non-obese and 73 were obese. Of the non-obese subjects, 41 were classified as MHNW (20 men and 21 women; mean \pm SD age, 53.0 \pm 9.2 years) and 37 were classified as MUNW (22 men and 15 women; mean \pm SD age, 57.0 \pm 10.9 years). Of the obese subjects, 30 were classified as MHO (17 men and 13 women; mean \pm SD age, 53.9 \pm 12.1 years) and 43 were classified as MUO (21 men and 22 women; mean \pm SD age, 54.2 \pm 8.7 years). Age and sex distribution did not significantly differ among the four groups.

The patient characteristics and biochemical parameters in the four groups are presented in Table 1. The MHNW and MUNW groups had similar BMIs and WCs, although the MUNW group had significantly higher systolic blood pressure, TG, LDL-C, hs-CRP, FPG, FINS, HOMA-IR, and VAI levels, and a significantly lower HDL-C level, compared with the MHNW group (*P* < 0.05 for all comparisons). Compared with the MUNW group, the MHO group had a significantly larger BMI and WC and higher HDL-C, but significantly lower hs-CRP, HOMA-IR, and VAI values (*P* < 0.05 for all comparisons). The MHO and MUO groups had similar BMIs, whereas the MUO group had a significantly larger WC, and significantly higher systolic blood pressure, diastolic blood pressure, TG, hs-CRP, FPG, FINS, HOMA-IR, and VAI values, and significantly lower HDL-C

Table 1. Patient characteristics and biochemical parameters of the study population ($n = 151$) categorized according to their metabolic and body mass index status.

Characteristics	MHNW $n = 41$	MUNW $n = 37$	MHO $n = 30$	MUO $n = 43$	Statistical significance
Body mass index, kg/m^2	22.5 \pm 1.4	22.9 \pm 1.3	28.2 \pm 3.2 ^{ab}	28.9 \pm 4.2 ^{ab}	$P < 0.001$
Waist circumference, cm	82.4 \pm 5.3	85.7 \pm 5.8	93.5 \pm 8.4 ^{ab}	99.9 \pm 10.5 ^{ab,c}	$P < 0.001$
Systolic blood pressure, mmHg	124.2 \pm 6.8	130.4 \pm 9.1 ^a	125.9 \pm 6.9	134.9 \pm 11.7 ^{a,c}	$P < 0.001$
Diastolic blood pressure, mmHg	75.7 \pm 6.6	79.6 \pm 6.7	76.7 \pm 7.8	82.2 \pm 8.6 ^{a,c}	$P < 0.001$
Total cholesterol, mmol/l	4.55 \pm 0.84	4.97 \pm 1.17	4.71 \pm 0.82	5.14 \pm 0.80 ^a	$P = 0.020$
Triglycerides, mmol/l	1.00 \pm 0.30	1.51 \pm 0.43 ^a	1.17 \pm 0.33	2.34 \pm 0.93 ^{ab,c}	$P < 0.001$
LDL-C, mmol/l	2.64 \pm 0.60	3.10 \pm 0.83 ^a	2.75 \pm 0.64	3.19 \pm 0.80 ^a	$P = 0.002$
HDL-C, mmol/l	1.46 \pm 0.31	1.07 \pm 0.25 ^a	1.35 \pm 0.22 ^b	0.97 \pm 0.26 ^{a,c}	$P < 0.001$
Hs C-reactive protein, mg/l	0.56 (0.27–1.00)	1.27 (0.53–2.01) ^a	0.68 (0.29–1.05) ^b	1.68 (0.76–2.65) ^{a,c}	$P < 0.001$
Fasting plasma glucose, mmol/l	5.28 \pm 0.83	7.31 \pm 2.52 ^a	5.40 \pm 1.15	8.53 \pm 2.90 ^{a,c}	$P < 0.001$
Fasting insulin, $\mu\text{U}/\text{ml}$	45.10 (35.83–58.14)	59.59 (50.08–74.68) ^a	45.35 (39.38–66.38)	70.24 (49.46–97.35) ^{a,c}	$P < 0.001$
HOMA-IR	1.4 (1.2–1.9)	2.6 (1.9–3.3) ^a	1.5 (1.2–2.3) ^b	3.6 (2.9–4.2) ^{a,c}	$P < 0.001$
Visceral adiposity index	1.2 (0.7–1.5)	2.2 (1.7–3.1) ^a	1.3 (1.0–1.7) ^b	4.0 (3.0–5.4) ^{ab,c}	$P < 0.001$
Absolute white blood cell count, $\times 10^9$	6.02 \pm 0.96	6.25 \pm 1.52	6.11 \pm 1.29	6.75 \pm 1.40	NS
Neutrophil count, %	57.44 \pm 4.30	58.05 \pm 3.61	57.66 \pm 4.02	59.15 \pm 3.69	NS
Lymphocyte count, %	31.20 \pm 4.23	31.68 \pm 3.84	31.94 \pm 3.35	30.71 \pm 3.62	NS

Data are reported as mean \pm SD or median (interquartile range).

^aCompared with the MHNW group, $P < 0.05$; one-way analysis of variance or Mann-Whitney U-test.

^bCompared with the MUNW group, $P < 0.05$; one-way analysis of variance or Mann-Whitney U-test.

^cCompared with the MHO group, $P < 0.05$; one-way analysis of variance or Mann-Whitney U-test.

MHNW, metabolically healthy and normal weight; MUNW, metabolically unhealthy but normal weight; MHO, metabolically healthy but obese; MUO, metabolically unhealthy and obese; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Hs, high sensitivity; HOMA-IR, homeostatic model assessment of insulin resistance; NS, no statistically significant difference ($P \geq 0.05$).

levels, compared with the MHO group ($P < 0.05$ for all comparisons). Almost all the parameters were similar among the two metabolically healthy groups (MHNW and MHO groups), except for BMI and WC ($P < 0.05$ for both comparisons). Moreover, among the two metabolically unhealthy groups (MUNW and MUO), the MUO group had significantly higher BMI, TG, and VAI values as well as a significantly larger WC compared with the MUNW group ($P < 0.05$ for all comparisons). The other parameters were not significantly different between the two metabolically unhealthy groups.

The absolute white blood cell count and the proportion of neutrophils and lymphocytes did not significantly differ among the four groups (Table 1). The proportions of iNKT cells among the four groups are presented in Figure 1. The MHNW group had the highest proportion of iNKT cells (mean \pm SD, 0.65 ± 0.21) among the four groups ($P < 0.05$ for all comparisons). The proportion of iNKT cells in the MHO group (mean \pm SD, 0.52 ± 0.24) was significantly higher than that in the MUNW group (mean \pm SD, 0.40 ± 0.24) and MUO group (mean \pm SD, 0.31 ± 0.22) ($P < 0.05$ for both comparisons), although the value did not significantly differ between the MUNW and MUO groups.

The results of univariate analysis between the proportion of iNKT cells and metabolic parameters of total, non-obese, and obese participants are described in Table 2. The proportion of iNKT cells of the total study population was inversely correlated with the BMI, WC, TC, TG, FPG, hs-CRP, HOMA-IR, and VAI values ($P < 0.05$ for all analyses). In the analysis limited to non-obese participants, the proportion of iNKT cells was found to be inversely correlated with the TG, LDL-C, hs-CRP, HOMA-IR, and VAI values ($P < 0.05$ for all analyses). However, in obese participants, the proportion of iNKT cells was inversely correlated

with the FPG, hs-CRP, HOMA-IR, and VAI values ($P < 0.05$ for all analyses). Furthermore, the proportion of iNKT cells was positively correlated with HDL-C levels in non-obese, obese, or total subjects ($P < 0.05$ for all analyses).

In the multivariate linear regression model, the metabolic parameters that were found to be associated with the proportion of iNKT cells on univariate analysis were assessed (Table 3). The proportion of iNKT cells was selected as the dependent variable, whereas the TC, LDL-C, hs-CRP, HOMA-IR, and VAI values were selected as independent variables. To reduce the potential collinearity between the related variables (e.g. BMI, WC, TG, HDL-C and VAI, or FPG and HOMA-IR), BMI, WC, TG, HDL-C, and FPG were not selected in constructing the model. In this model, R^2 was 0.369 ($P < 0.001$). Moreover, the proportion of iNKT cells remained correlated with hs-CRP, HOMA-IR, and VAI values ($P < 0.05$ for all analyses).

Discussion

Recent advances in our understanding of the pathophysiology of obesity have indicated that iNKT cells are key players in the immune regulation of metabolism.¹¹ In the present study, the proportion of iNKT cells was decreased in the MUNW, MHO and MUO groups compared with MHNW individuals. The decreased proportion of iNKT cells was correlated with increased BMI and WC, as well as TC, TG, FPG, hs-CRP, HOMA-IR, and VAI values in the overall study population in the present study. The associations between the proportion of iNKT cells and metabolic parameters were slightly different between non-obese and obese individuals. On multivariate linear regression analysis, the proportion of iNKT cells was found to be inversely correlated with the hs-CRP, HOMA-IR, and VAI values. These data highlight the

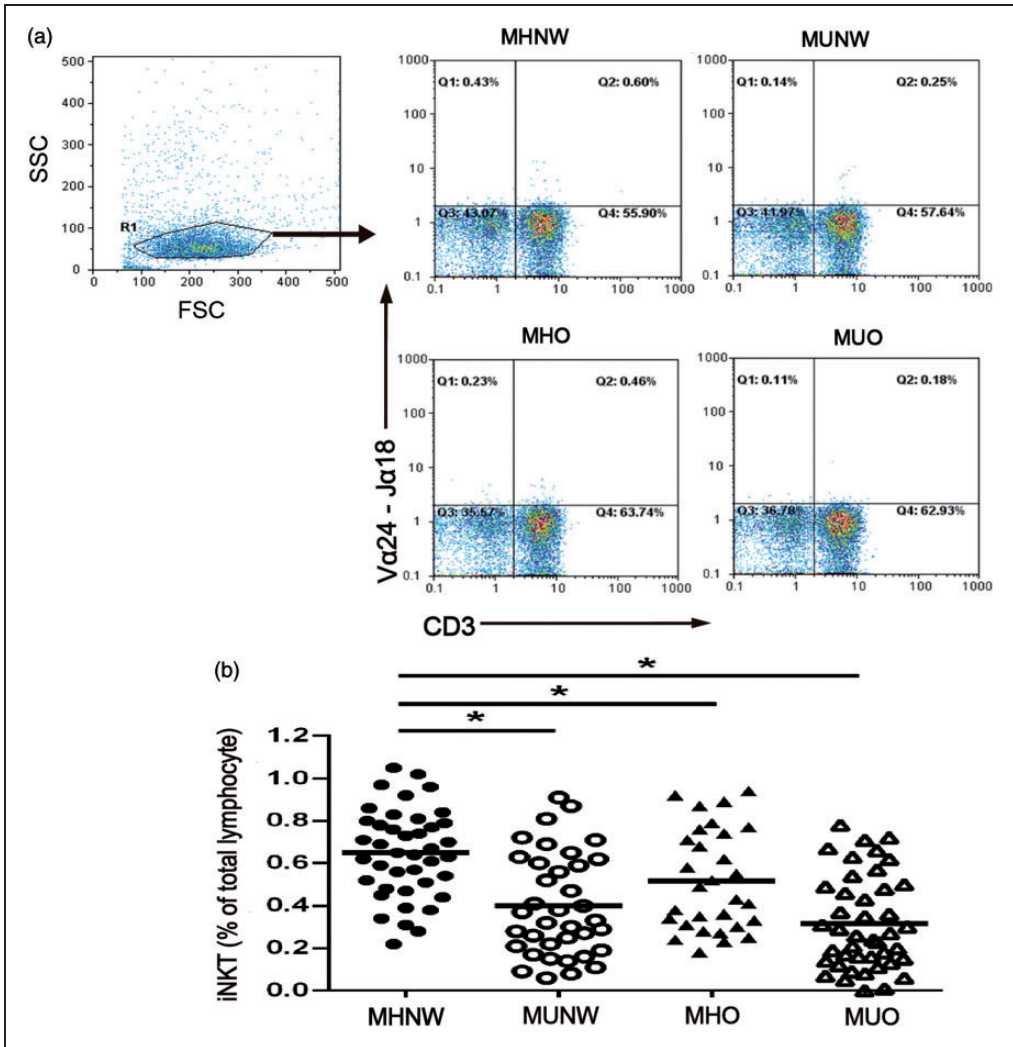


Figure 1. (a) Representative flow cytometry analysis plots showing invariant natural killer T (iNKT) cell frequencies (percentage of the total lymphocyte population) in each group. Quadrant 2 (Q2) shows iNKT cell plots (i.e. cells that were double-positive for Vα24-Jα18 and CD3). (b) Comparisons of iNKT cell frequencies among the four groups. The solid line shows the mean value. *P < 0.05; one-way analysis of variance. MHNW, metabolically healthy and normal weight; MUNW, metabolically unhealthy but normal weight; MHO, metabolically healthy but obese; MUO, metabolically unhealthy and obese. The colour version of this figure is available at: <http://imr.sagepub.com>.

potential role of iNKT cells in obese and obesity-related metabolic disorders.

With regard to BMI and metabolic phenotypes, the overall population can be divided into four subtypes: MHNW,

MUNW, MHO, and MUO.^{20,21} However, there is currently no standardized method for identifying these subtypes in research protocols or clinical practice. In the present study, the widely accepted ATP-III criteria

Table 2. Univariate associations between the proportion of invariant natural killer T cells and metabolic parameters of total, non-obese, or obese patients who participated in the study.

Metabolic parameters	Total study population n = 151		Non-obese patients n = 78		Obese patients n = 73	
	r	P-value	r	P-value	r	P-value
Age, years	0.022	NS	-0.051	NS	0.117	NS
Systolic blood pressure, mmHg	-0.151	NS	-0.180	NS	-0.025	NS
Diastolic blood pressure, mmHg	-0.056	NS	-0.021	NS	-0.013	NS
Body mass index, kg/m ²	-0.223	P = 0.006	-0.022	NS	0.087	NS
Waist circumference, cm	-0.321	P < 0.001	-0.192	NS	-0.061	NS
Total cholesterol, mmol/l	-0.179	P = 0.029	-0.182	NS	-0.083	NS
Triglycerides, mmol/l	-0.450	P < 0.001	-0.276	P = 0.015	-0.083	NS
LDL-C, mmol/l	-0.142	NS	-0.281	P = 0.013	-0.079	NS
HDL-C, mmol/l	0.453	P < 0.001	0.460	P < 0.001	0.396	P = 0.001
Fasting plasma glucose, mmol/l	-0.313	P < 0.001	-0.052	NS	-0.302	P = 0.011
Hs C-reactive protein, mg/l	-0.469	P < 0.001	-0.483	P < 0.001	-0.417	P < 0.001
HOMA-IR	-0.523	P < 0.001	-0.365	P = 0.001	-0.545	P < 0.001
Visceral adiposity index	-0.503	P < 0.001	-0.368	P = 0.001	-0.599	P < 0.001

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Hs, high sensitivity; HOMA-IR, homeostatic model assessment of insulin resistance; NS, not statistically significant ($P \geq 0.05$).

Table 3. Multivariate linear regression model of metabolic parameters related to the proportion of invariant natural killer T cells.

Metabolic parameters	B	Standard error	P-value
Total cholesterol, mmol/l	-0.025	0.027	NS
LDL-C, mmol/l	0.018	0.033	NS
Hs C-reactive protein, mg/l	-0.050	0.016	P = 0.002
HOMA-IR	-0.057	0.014	P < 0.001
Visceral adiposity index	-0.036	0.012	P = 0.003

Overall model: $R^2 = 0.369$, $P < 0.001$.

B, regression coefficient; LDL-C, low-density lipoprotein cholesterol; Hs, high sensitivity; HOMA-IR, homeostatic model assessment of insulin resistance; NS, not statistically significant ($P \geq 0.05$).

and BMI were used to categorize the participants.^{18,19} The MUNW group exhibited higher blood pressure, and significantly higher levels of FPG, the inflammatory

marker hs-CRP, and significantly higher insulin resistance (HOMA-IR), as well as a worse lipid profile, compared with the MHNW group. Despite similar BMI values, the MUNW group had more visceral fat (i.e. a higher VAI) compared with the MHNW group. VAI, an index estimated using both anthropometric (BMI and WC) and metabolic (TG and HDL-C) parameters, is a reliable marker of central lipid accumulation.^{17,22} In contrast, individuals classified as MHO exhibited excessive body fat; lower WC, lower blood pressure, TG, hs-CRP, FPG, FINS, HOMA-IR and VAI values; and higher HDL-C levels, compared with those classified as MUO.

Inflammation has been confirmed to play an important role in obesity and metabolic disorders.²³ Many immunometabolic studies have demonstrated that several immune cells, including macrophages, lymphocytes, and neutrophils, play certain roles in either the development of or protection from chronic inflammation that drives

obesity-induced metabolic disorders.²⁴⁻²⁶ Recently, iNKT cells were also found to be one of the key players involved in obesity-related disorders. For example, iNKT cells induce an anti-inflammatory phenotype in macrophages through the production of certain cytokines.^{27,28} Previous studies have reported that iNKT cells are depleted in the early stages of human and mouse obesity.^{16,29,30} However, studies involving humans have not evaluated the individuals' metabolic status. The present study reported on the proportion of iNKT cells in different obese and metabolic phenotypes. The current results demonstrated reduced proportions of iNKT cells not only in obese individuals, but also in non-obese individuals with metabolic abnormalities. To the best of our knowledge, this is the first report about the proportion of iNKT cells in non-obese individuals classified as MUNW, who are prone to visceral fat accumulation and insulin resistance despite being generally less obese. In contrast, individuals classified as MHO presented with obesity, good metabolic health, and lower VAI. The proportion of iNKT cells in the MHO group was higher than that in the MUO and MUNW groups, but lower than that in the MHNW group. This suggests that the circulating iNKT cells were decreased in people (non-obese or obese) with excessive fat accumulation, particularly among those with visceral fat accumulation.

A series of studies indicated that iNKT cells in adipose tissue play a protective role against obesity-induced inflammation, glucose intolerance, and weight gain through regulatory cytokine production.^{29,31,32} Hence, the reduction of iNKT cells usually results in a state of chronic inflammation that contributes to metabolic disease. The adoptive transfer of iNKT cells into obese mice led to decreases in body fat, TG levels, leptin levels, and improved insulin sensitivity.¹⁴ Nevertheless, some contrasting results have also been observed. In particular, a

study in obese mice found that iNKT cells progressively increased the production of proinflammatory cytokines.³³ However, other studies reported that, under obese conditions, the inflammatory cytokine expression in iNKT cell-deficient mice did not differ from that in wild-type mice.^{34,35} These animal experiments indicate that the functions of iNKT cells in the development of obesity-associated disorders are complicated and controversial. The possible contributing factors include differences in the genetic backgrounds of the animals, diet compositions, and feeding durations. To the best of our knowledge, the evidence from human studies has been relatively limited thus far. These current data showed that decreased levels of circulating iNKT cells appear to play important roles in systemic chronic inflammation and insulin resistance. Moreover, the reduction of iNKT cells was found to be associated with visceral fat accumulation. We believe that iNKT cells may be used as a potential therapeutic target for obesity in the near future. Nevertheless, further studies are needed to determine methods for restoring iNKT cell numbers in both obese and non-obese individuals with excessive visceral fat.

This study had several limitations that should be considered when interpreting the results. First, it did not assess the proportion of iNKT cells in the subcutaneous or omental adipose tissue, as this would involve an invasive sampling process. Nevertheless, circulating iNKT cells can be considered to partially reflect the systemic conditions. Secondly, this study had a small sample size, which made it difficult to detect the difference in the correlation of the parameters when evaluating individuals of only one sex. Thirdly, the cross-sectional design cannot confirm the causality of the relationship between decreased iNKT cell numbers and metabolic disorders. Hence, clinical trials of the effect of iNKT agonists on obesity and related metabolic disease

therapy are warranted. Finally, the study did not employ the use of TCR-specific CD1d tetramers and some additional gatings (CD45⁺, exclusion of dead cells) for the flow cytometric analysis of iNKT cells.

In conclusion, these current data suggest that decreased levels of circulating iNKT cells play an important role in systemic chronic inflammation, insulin resistance and visceral fat accumulation. As a consequence, iNKT cells might become therapeutic targets for obesity in the future.

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Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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