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Alterations of oxygen consumption and extracellular acidification rates by glutamine in PBMCs of SLE patients

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ABSTRACT

We evaluated plasma glutamine levels and basal mitochondrial oxygen consumption rate ($mOCR_B$) and basal extracellular acidification rate ($ECAR_B$) of peripheral blood mononuclear cells (PBMCs) of systemic lupus erythematous (SLE) patients and healthy controls (HCs). Lower plasma glutamine levels correlated with higher SLE disease activity indexes ($p = 0.025$). Incubated in DMEM containing 100 mg/dL glucose, SLE-PBMCs displayed lower $mOCR_B$ ($p = 0.018$) but similar $ECAR_B$ ($p = 0.467$) to those of HC-PBMCs, and their $mOCR_B$ got elevated ($p < 0.001$) without altering $ECAR_B$ ($p = 0.239$) by supplementation with 2 or 4 mM glutamine. We conclude that impaired mitochondrial respiration of SLE-PBMCs could be improved by glutamine under euglycemic condition.

1. Introduction

Systemic lupus erythematous (SLE) is characterized by the cascades of tissue/organ damages through the signaling abnormalities in T and B lymphocytes (Kaul et al., 2016). Although the pathogenesis of SLE is multifactorial (Mok and Lau, 2003), the role of mitochondrial dysfunction in immune cells has been discussed recently (Morel, 2017). Previously, we demonstrated that SLE leukocytes exhibited a higher incidence of heteroplasmic pattern in the D310 region of leukocyte mitochondrial DNA (mtDNA) than those of the healthy controls (HCs), and a higher SLE disease activity index (SLEDAI) was correlated with a lower mtDNA copy number in leukocytes (Lee et al., 2012). Besides, SLE leukocytes expressed lower mRNA transcripts of mitochondrial biogenesis-related genes including mitochondrial transcriptional factor A (TFAM), nuclear respiratory factor 1 (NRF-1), pyruvate dehydrogenase (PDH) and mtDNA-encoded polypeptides than HC leukocytes (Lee et al., 2014). Moreover, Perl A and colleagues showed that mitochondrial hyperpolarization with excessive reactive oxygen intermediates (ROIs) production and ATP depletion existed in activated peripheral blood lymphocytes (PBLs) of SLE patients (Gergely Jr et al., 2002). In light of these findings, we hypothesized that dysfunction in mitochondria of immune cells may play an important role in the pathogenesis of SLE.

There are three cooperative pathways of glucose utilization in human immune cells, the glycolysis, pentose phosphate pathway (PPP) and oxidative phosphorylation (Morel, 2017). During glucose metabolism for ATP production, mitochondrial dysfunction with impaired oxidative phosphorylation can be compensated for by an amplified glycolysis with increased lactate production upon triggering of a stabilized hypoxia inducible factor-1 alpha (HIF-1α) and lactate dehydrogenase (LDH) (Masson and Ratcliffe, 2014). This glucose metabolic alteration also has been found in synovial tissues, including fibroblasts,

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dendritic cells, macrophages and T cells, with mitochondrial dysfunction of rheumatoid arthritis (RA) patients (Fearon et al., 2016). Although we speculate mitochondrial dysfunction in SLE leukocytes, one of our previous studies failed to show increased mRNA expression in glycolytic enzymes including hexokinase II, glucose 6-phosphate isomerase, phosphofructokinase, glyceraldehyde 3-phosphate dehydrogenase and lactate dehydrogenase as well as HIF-1α in SLE leukocytes (Lee et al., 2014). The role of mitochondrial dysfunction with amplified glycolysis in immune cells of SLE remained a puzzle.

Because multiple steps are involved in mitochondrial respiration during ATP production, including oxygen consumption, electron transport, mitochondrial membrane potential establishment, proton gradient establishment and then ATP synthesis, several methods are developed to detect the above processes to reflect mitochondrial function (Brand and Nicholls, 2011). Theoretically, oxygen consumption rate is an optimal indicator to reflect mitochondrial function for a given normal human cell.

Peripheral blood mononuclear cells (PBMCs), including T and B cells, are the main cells participating in SLE pathogenesis (Dorner et al., 2011; Mak and Kow, 2014). Until now, the widely accepted methods for immune cell function evaluation is through the overnight CD3/CD28 or phytohemagglutinin (PHA) stimulation of PBMCs, PBLs or T cells (Green, 2014). However, few appraised their native or *de novo* status immediately after blood harvesting. As a result, to analyze the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in freshly isolated PBMCs to reflect the "real-time" metabolic situation seems feasible in SLE patients.

Apart from glucose, glutamine is one of the most common energy sources in human cells (DeBerardinis and Cheng, 2010). In addition to supplying α-ketoglutarate into Krebs cycle in maintaining mitochondrial function in human (Maciver et al., 2013), glutamine serves as an essential amino acid for protein synthesis and *de novo* biosynthesis of glutathione (GSH) to cope with oxidative stress (DeBerardinis and Cheng, 2010). Glutamine homeostasis is regulated in a delicate manner in mitochondria (Mates et al., 2009). Ample glutamine utilization seems an optimal strategy for human cells to deal with mitochondrial dysfunction.

Mitochondrial dysfunction has also been detected in human cancers. Some researchers found glutamine is an important nutrient to rescue the impaired cancer mitochondria (DeBerardinis and Cheng, 2010). It has been demonstrated that hypo-glutaminemia is an adaptive phenomenon leading to bioenergetic failure in skeletal muscle (Krajcova et al., 2015). Therefore, glutamine might be an alternative energy source to keep proper mitochondrial function in immune cells of SLE patients. In order to evaluate the clinical relevance of glutamine in SLE, the differences of plasma glutamine between SLE patients and healthy subjects, and the relationship between SLEDAIs and plasma glutamine levels deserved to be investigated.

In this study, we analyzed the differences in plasma glutamine levels between HCs and SLE patients initially. Besides, we determined the OCR, ECAR and alterations of mtDNA copy number of PBMCs between HCs and SLE patients. Finally, we intended to appraise the effects of glutamine on abnormal OCR or ECAR of SLE-PBMCs. We also proposed a scheme to explain the mitochondrial dysfunction in the pathogenesis of PBMCs of SLE.

2. Materials and methods

2.1. Study protocol

An algorithm summarizing the study protocols is illustrated in Fig. 1.

2.2. Patient recruitment (cohorts 1 and 2)

According to the 1997 ACR updated criteria (Hochberg, 1997) and 2012 SLICC criteria for the classification of SLE (Petri et al., 2012), 93 SLE patients and 50 HCs were enrolled as cohort 1 and the other 30 SLE patients and 25 HCs were enrolled as cohort 2 after the approvals from the Institutional Review Board of Mackay Memorial Hospital (No. 14MMHIS123) and Taipei Veterans General Hospital (No. 2013-04-43B) as well as the signature of the informed consents by patients.

For cohort 1, 93 SLE patients (Women/Men = 80/13) with a mean ± standard deviation (M ± SD) age of 45.1 ± 13.1 years and 50 HCs matches for age (within 10 years) and sex (Women/Men = 42/8) were studied. Partial of their demographic data have been reported previously (Lee et al., 2012; Lee et al., 2014; Lee et al., 2016).

For cohort 2, 30 SLE patients (Women/Men = 24/6) with a M ± SD age of 51.7 ± 17.3 years, including 24 women with a M ± SD age of 50.5 ± 16.5 and 6 men with a M ± SD age of 56.5 ± 21.3, were studied. As controls, 25 HCs (Women/Men = 19/6, $p = 0.721$) with a M ± SD age of 42.9 ± 9.9 years ($p = 0.537$), including 19 women with a M ± SD age of 48.8 ± 9.1 ($p = 0.704$) and 6 men with a M ± SD age of 50.5 ± 12.9 ($p = 0.570$), were studied.

2.3. Blood sample collection

In each cohort 1 subject, approximately 10 mL of peripheral venous blood was collected (VACUETTE® with EDTA, Greiner Bio-One) to harvest plasma (Lee et al., 2012) for determining relative plasma glutamine level.

In each cohort 2 subject, 8 mL venous blood was collected (BD Vacutainer® CPT™) for isolating PBMCs immediately (Ruitenberg et al., 2006), with a yield rate > 10^6 cells/mL (*i.e.*, 8×10^6 cells in total) and viability > 98% as verified by automated cell analyzer (Nucleo-Counter® NC-3000™).

The *de novo* PBMCs without any stimulation were subjected for the analysis of OCR and ECAR immediately. Another aliquot of PBMCs were subjected to extraction of DNA and determination of mtDNA copy number.

2.4. Determination of the mtDNA copy number

The relative mtDNA copy number of the PBMCs were determined by real-time quantitative PCR, with adjustment of copy number of HC-No.1 PBMC as 1.00 (Lee et al., 2012).

2.5. Determination of plasma glutamine levels

The plasma glutamine level was determined by human Glutamine Elisa kit (myBioSource, California, USA) (Luo et al., 2014). Each sample was analyzed in duplicate to get the average value with the mean (M) of the HCs adjusted to 1.00.

2.6. Measurements of bioenergetic parameter

The OCR and ECAR of PBMCs were measured on a Seahorse XF^e-24 Analyzer (Seahorse Bioscience, Billerica, MA, USA) in cohort 2 and are illustrated in Fig. 2 (Lin et al., 2016). In brief, an aliquot of 3.5×10^5 HC-PBMCs or SLE-PBMCs in 100 μL of non-buffered, glucose/glutamine free DMEM medium, were seeded onto the XF^e 24-well culture microplates coated with Cell-Tak™ (Corning, NY, USA) and were centrifugated at 1600 × *g* for 15 min. The pellet was re-suspended in non-buffered DMEM medium containing different concentrations of glucose (100 and 400 mg/dL) for simulating to physiological euglycemia and pathologic hyperglycemia states (Turina et al., 2006) as well as glutamine (0, 2 or 4 mM) (He et al., 2016; Kramer et al., 2015). The mixture was then incubated at 37 °C without CO_2 for 1 h as reported previously. The HC-PBMCs in the glutamine-free medium containing 100 mg/dL glucose were designated "group A" or "$HC_{100/0}$". The HC-PBMCs in the glutamine-free medium containing 400 mg/dL glucose were designated "group E" or "$HC_{400/0}$", and so on for the other groups. Groups B, C, D,

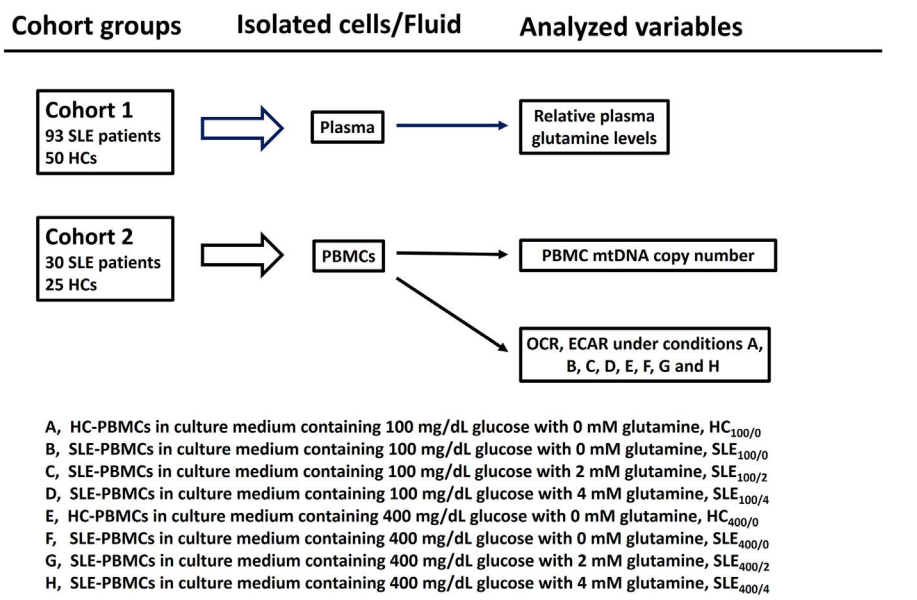
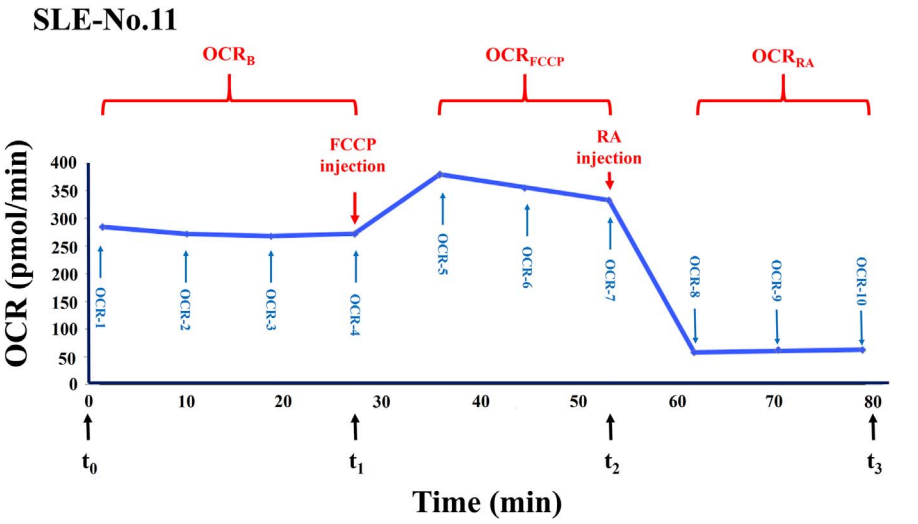


Fig. 1. Illustration of an algorithm to summarize the study protocols.

F, G, and H denote SLE-PBMCs under 100 mg/dL glucose and 0 mM glutamine [group B; $SLE_{100/0}$], 100 mg/dL glucose and 2 mM glutamine [group C; $SLE_{100/2}$], 100 mg/dL glucose and 4 mM glutamine [group D; $SLE_{100/4}$], 400 mg/dL glucose and 0 mM glutamine [group F; $SLE_{400/0}$], 400 mg/dL glucose and 2 mM glutamine [group G; $SLE_{400/2}$], and 400 mg/dL glucose and 4 mM glutamine [group H; $SLE_{400/4}$], respectively (Fig. 1).

The measurements were done at the beginning (t_0) and several time points thereafter as appropriate (t_1, t_2, and t_3, respectively). A representative case (SLE patient No. 11) of OCR determination at each time point is illustrated in Fig. 2. At t_0 to t_1, the OCR-1, OCR-2, OCR-3, and OCR-4 were recorded to calculate the basal OCR (OCR_B) (= [OCR-1 + OCR-2 + OCR-3 + OCR-4]/4). Simultaneously, the ECAR change was also detected serially (data not shown) and basal ECAR ($ECAR_B$) =



SLE-No.11, PBMCs, 3.5x10^5 cells, under culture medium with 100 mg/dL glucose with 0 mM glutamine
Abbreviations: OCR, oxygen consumption rate; t_0, beginning; t_1, FCCP injection; t_2, RA injection; t_3, ending; FCCP, carbonyl cyanide-4-(trifluoromethoxy)-phenylhydrazone; RA, rotenone/antimycin A
OCR_B was detected between t_0 and t_1. OCR_{FCCP} was detected between t_1 and t_2. OCR_{RA} was detected between t_2 and t_3.

Equations
OCR_B = (OCR-1 + OCR-2 + OCR-3 + OCR-4) / 4
OCR_{FCCP} = (OCR-5 + OCR-6 + OCR-7) / 3
OCR_{RA} = (OCR-8 + OCR-9 + OCR-10) / 3

$mOCR_B$ (basal mitochondrial OCR) = OCR_B - OCR_{RA}
$mOCR_{Max}$ (maximal mitochondrial OCR) = OCR_{FCCP} – OCR_{RA}

Each experiment was done in duplicate to get the average and the data were adjusted to per 10^6 cells (OCR as pmol/min/10^6 cells)

Fig. 2. Representative illustration (SLE-No.11 patient) of the time course during the measurements of oxygen consumption rate (OCR) and the ways that were used for data calculation.

[ECAR-1 + ECAR-2 + ECAR-3 + ECAR-4]/4.

At t_1, 0.3 μM of carbonylcyanide-4-(trifluoromethoxy)-phenylhydrazone (FCCP) was added to uncouple mitochondrial respiration and to lead to the maximal respiration rate. From t_1 to t_2, OCR-5, OCR-6, and OCR-7 were recorded to get OCR_{FCCP} (=[OCR-5 + OCR-6 + OCR-7]/3), which represents the oxygen consumption rate during FCCP uncoupling.

At t_2, rotenone/antimycin A (RA, Complex I/III inhibitor, 0.5 μM) was added to inhibit mitochondrial respiration. From t_2 to t_3, OCR-8, OCR-9, and OCR-10 were recorded to get OCR_{RA} (=[OCR-8 + OCR-9 + OCR-10]/3), which represents the oxygen consumption rate during the administration of rotenone/antimycin A (assumed to be non-mitochondrial OCR).

Finally, the basal and maximal mitochondrial OCRs of PBMCs were calculated as follows (Lin et al., 2016):

$mOCR_B$ (basal mitochondrial OCR) = OCR_B - OCR_{RA}.

$mOCR_{Max}$ (maximal mitochondrial OCR) = OCR_{FCCP}-OCR_{RA}.

Each experiment was done in duplicate to get the average and the data were adjusted to the OCR per 10^6 cells (OCR as pmole/min/10^6 cells; ECAR as mpH/min/10^6 cells). The data for all HCs and SLE patients are presented as mean ± standard deviation (M ± SD).

2.7. Statistical analysis

The continuous variables between two groups or paired two/three groups were compared using Mann-Whitney *U* test or Wilcoxon Signed Ranks/Friedman test as appropriate. Relationships between two variables were evaluated by Spearman's ρ. R^2 was shown when a significant association was achieved. Differences or associations are considered significant when *p*-values is < 0.05.

3. Results

3.1. Associations of plasma glutamine levels with lupus activity

As shown in Table 1 for cohort 1, the 93 SLE patients had a higher relative plasma glutamine level than the 50 HCs (1.14 ± 0.83 *vs.* 1.00 ± 0.74, $p = 0.043$). Besides, a higher SLEDAI score was correlated with a lower relative plasma glutamine level ($\rho = -0.232$, $p = 0.025$). Defining SLEDAI = 8 as the cutoff value, 49 patients with SLEDAI ≤ 8 had higher relative plasma glutamine level than the other 44 with SLEDAI > 8 (1.27 ± 0.87 *vs.* 0.99 ± 0.77, $p = 0.018$) and the HCs (1.27 ± 0.87 *vs.* 1.00 ± 0.74, $p = 0.011$), respectively.

3.2. Relative mtDNA copy numbers of HC-PBMCs and SLE-PBMCs in cohort 2

Relative PBMC mtDNA copy numbers in the HCs and SLE patients in cohort 2 were 1.26 ± 0.45 and 1.32 ± 0.60, which was not obviously different ($p = 0.707$).

3.3. Differences in OCRs of HC-PBMCs and SLE-PBMCs at various concentrations of glucose and glutamine

At 100 mg/dL glucose without glutamine, HC-PBMCs (group A, $HC_{100/0}$) exhibited higher $mOCR_B$ (668.5 ± 238.2 in A *vs.* 515.7 ± 165.4 in B, $p = 0.018$) and $mOCR_{Max}$ (1358.7 ± 671.6 in A *vs.* 916.8 ± 421.2 in B, $p = 0.011$) than SLE-PBMCs (group B, $SLE_{100/0}$) (Table 2, Part A; Fig. 3A). In groups B ($SLE_{100/0}$), C ($SLE_{100/2}$), and D ($SLE_{100/4}$) SLE-PBMCs at 100 mg/dL glucose, a steady increase in $mOCR_B$ (515.7 ± 165.4 in B, 586.0 ± 214.9 in C, 630.7 ± 228.7 in D, $p < 0.001$) and $mOCR_{Max}$ (916.8 ± 421.2 in B 1065.3 ± 563.2 in C, 1285.5 ± 639.7 in D, $p < 0.001$) were observed after 0, 2 and 4 mM glutamine administration (Table 2, Part A; Fig. 3A). SLE-PBMCs in either group C ($SLE_{100/2}$) or group D ($SLE_{100/4}$) had similar $mOCR_B$ (586.0 ± 214.9 in C *vs.* 668.5 ± 238.2 in A, $p = 0.335$; 630.7 ± 228.7 in D *vs.* 668.5 ± 238.2 in A, $p = 0.866$) and similar $mOCR_{Max}$ (1065.3 ± 563.2 in C *vs.* 1358.7 ± 671.6 in A, $p = 0.089$; 1285.5 ± 639.7 in D *vs.* 1358.7 ± 671.6 in A, $p = 0.748$) as compared to those of HC-PBMCs in group A ($HC_{100/0}$) (Table 2, Part A; Fig. 3A).

At 400 mg/dL glucose without glutamine, HC-PBMCs (group E, $HC_{400/0}$) exhibited higher $mOCR_B$ (836.9 ± 308.1 in E *vs.* 573.4 ± 165.5 in F, $p = 0.001$) and higher $mOCR_{Max}$ (1694.9 ± 727.9 in E *vs.* 875.2 ± 453.4 in F, $p < 0.001$) than the SLE-PBMCs (group F, $SLE_{400/0}$) (Table 2, Part B; Fig. 3A). The $mOCR_B$ of SLE-PBMCs at 400 mg/dL glucose was decreased from groups F ($SLE_{400/0}$) to G ($SLE_{400/2}$) and then H ($SLE_{400/4}$) in a descending order after 0, 2 and 4 mM glutamine administration (573.4 ± 165.5 in F, 536.9 ± 162.9 in G, and 452.2 ± 152.7 in H, $p < 0.001$) (Table 2, Part B; Fig. 3A). A parabola phenomenon of increase followed by decrease was observed in $mOCR_{Max}$ of SLE-PBMCs among groups F ($SLE_{400/0}$), G ($SLE_{400/2}$) and H ($SLE_{400/4}$) (875.2 ± 453.4 in F *vs.* 1024.1 ± 436.4 in G *vs.* 860.1 ± 361.8 in H, $p = 0.016$) (Table 2, Part B; Fig. 3A).

In HC-PBMCs at 100 or 400 mg/dL glucose without glutamine, group E ($HC_{400/0}$) had higher $mOCR_B$ (836.9 ± 308.1 in E *vs.* 668.5 ± 238.2 in A, $p < 0.001$) and $mOCR_{Max}$ (1694.9 ± 727.9 in E *vs.* 1358.7 ± 671.6 in A, $p = 0.001$) than group A ($HC_{100/0}$) (Table 2,

Table 1
Relative plasma glutamine levels in HCs and SLE patients, and their associations with SLEDAI in SLE patients[f].

Parameters	HCs (*n* = 50, 100.0%)	SLE patients (*n* = 93, 100.0%)		*p*-Value
Relative plasma glutamine level (M ± SD)[e]	1.00 ± 0.74	1.14 ± 0.83		0.043[a]
SLEDAI (M ± SD)	–	9.9 ± 6.6		
Median	–	8.0		
SLEDAI with association to relative plasma glutamine level	–	− 0.232 (Spearman's ρ)[b]		0.025[b]
Parameters	HCs (n = 50)	SLE with SLEDAI ≤ 8 (*n* = 49)	SLE with SLEDAI > 8 (*n* = 44)	*p*-Value
Relative plasma glutamine level (M ± SD)[f]	1.00 ± 0.74	1.27 ± 0.87	0.99 ± 0.77	0.014[c]
	1.00 ± 0.74	1.27 ± 0.87		0.011[a]
		1.27 ± 0.87	0.99 ± 0.77	0.018[a]
SLEDAI	–	4.9 ± 2.1	15.5 ± 5.2	< 0.00[d]

[a] Compared between HCs and SLE patients, between HCs and SLE patients with SLEDAI ≤ 8 and between SLE patients with SLEDAI ≤ 8 and SLE patients with SLEDAI > 8, Mann-Whitney *U* test.

[b] Among the 93 SLE patients, the distribution of relative plasma glutamine levels with their association to their SLEDAI, Spearman's ρ correlation coefficient.

[c] Compared among HCs, SLE patients with SLEDAI ≤ 8 and SLE patients with SLEDAI > 8, Kruskal-Wallis *H* test.

[d] Compared between SLE patients with SLEDAI ≤ 8 and SLE patients with SLEDAI > 8, *t*-test

[e] the M (mean) of the relative plasma glutamine level of HCs was adjusted to 1.00.

[f] Cohort 1 patients; Abbreviations: HCs = healthy controls; SLEDAI = SLE disease activity index; M = mean; SD = standard deviation.

Table 2
Differences in PBMC $mOCR_B$, $mOCR_{Max}$, $ECAR_B$, and $ECAR_B/mOCR_B$ ratios between HCs-PBMCs and SLE-PBMCs under various concentrations of glucose and glutamine in culture medium.

Part A, Glucose 100 mg/dL											
Subjects/case number	HCs (n = 25)	SLE (n = 30)	SLE (n = 30)	SLE (n = 30)	Groups comparisons, *p*-value						
Culture medium condition											
Glucose (mg/dL)/glutamine (mM)	100/0	100/0	100/2	100/4							
Group/group name	$A/HC_{100/0}$	$B/SLE_{100/0}$	$C/SLE_{100/2}$	$D/SLE_{100/4}$	AB[a]	AC[a]	AD[a]	BC[b]	CD[b]	BD[b]	BCD[c]
Basal mOCR ($mOCR_B$, M ± SD)	668.5 ± 238.2	515.7 ± 165.4	586.0 ± 214.9	630.7 ± 228.7	0.018	0.335	0.866	< 0.001	0.002	< 0.001	< 0.001
Maximal mOCR ($mOCR_{Max}$, M ± SD)	1358.7 ± 671.6	916.8 ± 421.2	1065.3 ± 563.2	1285.5 ± 639.7	0.011	0.089	0.748	0.015	0.002	< 0.001	< 0.001
Basal ECAR ($ECAR_B$, M ± SD)	159.9 ± 56.6	146.7 ± 48.5	151.4 ± 50.3	144.4 ± 52.0	0.467	0.813	0.310	0.280	0.094	0.382	0.239
$ECAR_B/mOCR_B$ ratios (M ± SD)	0.25 ± 0.07	0.30 ± 0.10	0.28 ± 0.11	0.25 ± 0.10	0.032	0.417	0.457	0.052	0.001	< 0.001	< 0.001

Part B, Glucose 400 mg/dL											
Subjects/case number	HCs (n = 25)	SLE (*n* = 22)	SLE (*n* = 22)	SLE (n = 22)	Groups comparisons, *p*-value						
Culture medium condition											
Glucose (mg/dL)/glutamine (mM)	400/0	400/0	400/2	400/4							
Group/group name	$E/HC_{400/0}$	$F/SLE_{400/0}$	$G/SLE_{400/2}$	$H/SLE_{400/4}$	EF[a]	EG[a]	EH[a]	FG[b]	GH[b]	FH[b]	FGH[c]
Basal mOCR ($mOCR_B$, M ± SD)	836.9 ± 308.1	573.4 ± 165.5	536.9 ± 162.9	452.2 ± 152.7	0.001	< 0.001	< 0.001	0.039	< 0.001	< 0.001	< 0.001
Maximal mOCR ($mOCR_{Max}$, M ± SD)	1694.9 ± 727.9	875.2 ± 453.4	1024.1 ± 436.4	860.1 ± 361.8	< 0.001	0.001	< 0.001	0.003	0.009	0.709	0.016
Basal ECAR ($ECAR_B$, M ± SD)	204.4 ± 70.8	164.5 ± 44.5	154.2 ± 46.3	128.3 ± 39.8	0.028	0.006	< 0.001	0.101	< 0.001	< 0.001	< 0.001
$ECAR_B/mOCR_B$ ratios (M ± SD)	0.25 ± 0.07	0.31 ± 0.14	0.31 ± 0.12	0.31 ± 0.12	0.060	0.043	0.042	0.733	0.274	0.689	0.717

Part C, Glucose 100 mg/dL *vs.* Glucose 400 mg/dL					
	Groups comparisons, *p*-value				
	AE[b]	BF[b]	CG[b]	DH[b]	BH[b]
	$HC_{100/0}$ *vs.* $HC_{400/0}$	$SLE_{100/0}$ *vs.* $SLE_{400/0}$	$SLE_{100/2}$ *vs.* $SLE_{400/2}$	$SLE_{100/4}$ *vs.* $SLE_{400/4}$	$SLE_{100/0}$ *vs.* $SLE_{400/4}$
Basal mOCR ($mOCR_B$)	< 0.001	0.001	0.178	< 0.001	0.009
Maximal mOCR ($mOCR_{Max}$)	0.001	0.858	0.615	< 0.001	0.548
Basal ECAR ($ECAR_B$, M ± SD)	< 0.001	0.012	0.306	0.054	0.012
$ECAR_B/mOCR_B$ ratios (M ± SD)	0.427	0.961	0.131	0.001	0.357

OCR = oxygen consumption rate, pmol/min/10^6 cells; OCR_B = basal OCR; $mOCR_B$ = basal mitochondrial OCR; $mOCR_{Max}$ = maximal mitochondrial OCR;
ECAR = extracellular acidification rate, mpH/min/10^6 cells; $ECAR_B$ = basal ECAR;
Abbreviations: HCs = healthy controls; M = mean; SD = standard deviation;
[a] compared between groups AB, AC, AD, EF, EG and EH, Mann-Whitney *U* test.
[b] compared between groups BC, CD, BD,FG, GH and FH, or between groups AE, BF, CG, DH and BD, or between groups BH, Wilcoxon Signed Ranks test.
[c] compared among groups BCD and FGH, Friedman test.

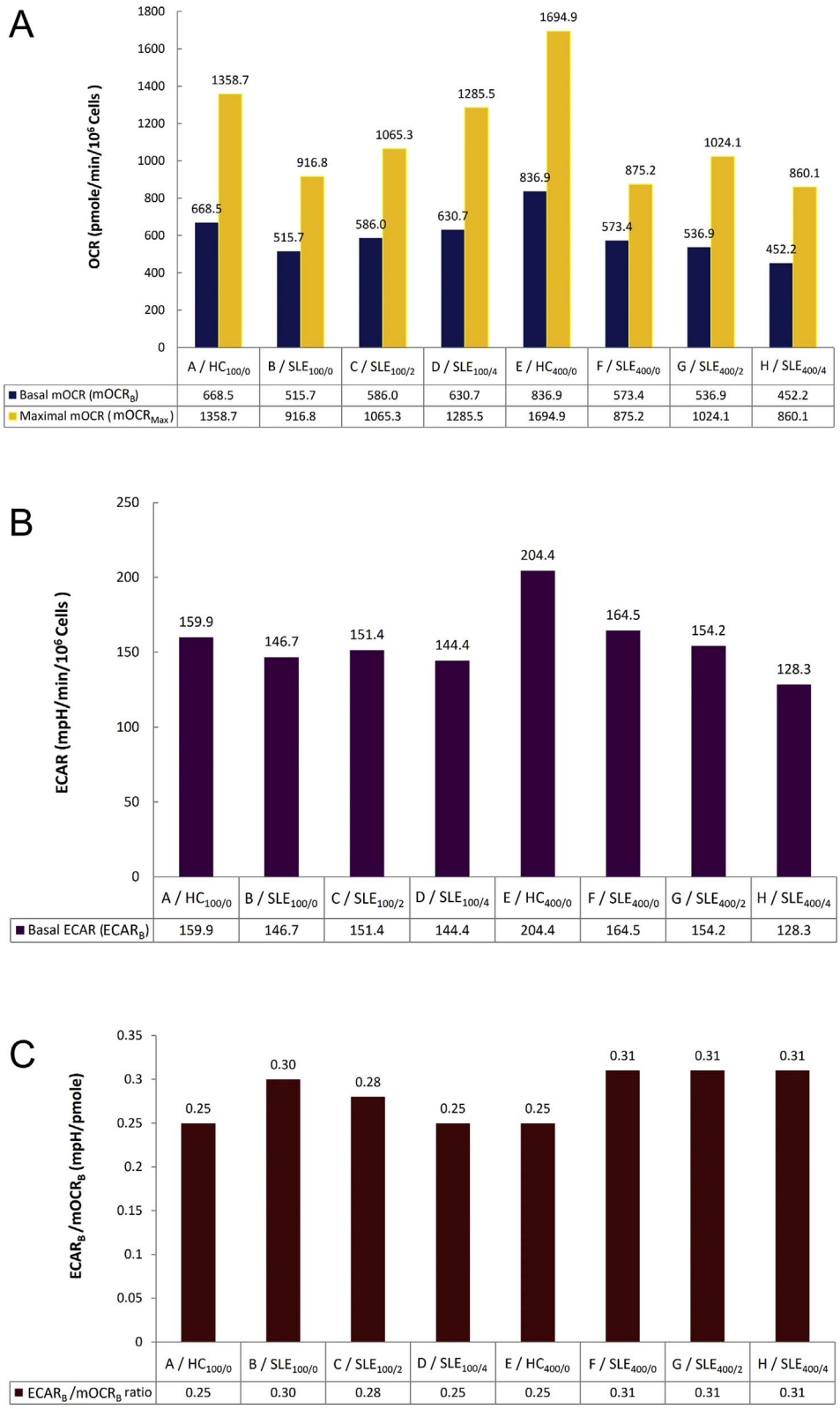


Fig. 3. The distributions and alterations of mitochondrial oxygen consumption rate (mOCR) including basal mOCR ($mOCR_B$) and maximal mOCR ($mOCR_{Max}$), basal extracellular acidification rate ($ECAR_B$) and $ECAR_B/mOCR_B$ ratio in peripheral blood mononuclear cells (PBMCs) of healthy control (HC) and SLE patients in culture medium containing 100 mg/dL or 400 mg/dL glucose supplemented with 0, 2, and 4 mM glutamine.

Part A, B &C; Fig. 3A). In SLE-PBMCs at 100 or 400 mg/dL glucose without glutamine, group F ($SLE_{400/0}$) had higher $mOCR_B$ (573.4 ± 165.5 in F *vs.* 515.7 ± 165.4 in B, $p = 0.001$) but similar $mOCR_{Max}$ (875.2 ± 453.4 in F *vs.* 916.8 ± 421.2 in B, $p = 0.858$) than those of group B ($SLE_{100/0}$) (Table 2, Part A, B &C; Fig. 3A). However, in SLE-PBMCs at 100 or 400 mg/dL glucose with 2 mM glutamine, group G ($SLE_{400/2}$) had similar $mOCR_B$ (536.9 ± 162.9 in G *vs.*586.0 ± 214.9 in C, $p = 0.178$) and $mOCR_{Max}$ (1024.1 ± 436.4 in

G *vs.*1065.3 ± 563.2 in C, $p = 0.615$) to those of group C ($SLE_{100/2}$) (Table 2, Part A, B & C; Fig. 3A). In SLE-PBMCs at 100 or 400 mg/dL glucose with 4 mM glutamine, group H ($SLE_{400/4}$) had lower $mOCR_B$ (452.2 ± 152.7 in H *vs.*630.7 ± 228.7 in D, $p < 0.001$) and lower $mOCR_{Max}$ (860.1 ± 361.8 in H *vs.*1285.5 ± 639.7 in D, $p < 0.001$) than group D ($SLE_{100/4}$) (Table 2, Part A, B & C; Fig. 3A).

Compared to group B ($SLE_{100/0}$), concurrent administration of 400 mg/dL glucose and 4 mM glutamine (group H, $SLE_{400/4}$) led to a decrease of $mOCR_B$ (452.2 ± 152.7in H *vs.* 515.7 ± 165.4 in B, $p = 0.009$) without an improvement of $mOCR_{Max}$ (860.1 ± 361.8 in H *vs.* 916.8 ± 421.2 in H, $p = 0.548$) in SLE-PBMCs (group H, $SLE_{400/4}$) (Table 2, Part A, B &C; Fig. 3A).

3.4. Differences in ECARs of HC-PBMCs and SLE-PBMCs at various concentrations of glucose and glutamine

At 100 mg/dL glucose without glutamine, HC-PBMCs (group A, $HC_{100/0}$) had similar $ECAR_B$ with SLE-PBMCs (group B, $SLE_{100/0}$) (159.9 ± 56.6 in A *vs.* 146.7 ± 48.5 in B, $p = 0.467$) (Table 2, Part A; Fig. 3B). Among group B ($SLE_{100/0}$), C ($SLE_{100/2}$), and D ($SLE_{100/4}$) SLE-PBMCs at 100 mg/dL glucose, the $ECAR_B$ were not obviously different, despite the supplementation of 0, 2, and 4 mM glutamine (146.7 ± 48.5 in B, 151.4 ± 50.3 in C, and 144.4 ± 52.0 in D, $p = 0.239$) (Table 2, Part A; Fig. 3B). $ECAR_B$ of SLE-PBMCs in group C ($SLE_{100/2}$) (151.4 ± 50.3 in C *vs.* 159.9 ± 56.6 in A, $p = 0.813$) or D ($SLE_{100/4}$) (144.4 ± 52.0 in D *vs.*159.9 ± 56.6 in A, $p = 0.310$) were not different from that of HC-PBMCs in group A ($HC_{100/0}$) (Table 2, Part A; Fig. 3B).

At 400 mg/dL glucose, the HC-PBMCs (group E, $HC_{400/0}$) had higher $ECAR_B$ than SLE-PBMCs in groups F ($SLE_{400/0}$), G ($SLE_{400/2}$) and H ($SLE_{400/4}$), respectively, despite the supplementation of 0, 2, or 4 mM glutamine (204.4 ± 70.8 in E *vs.* 164.5 ± 44.5 in F, $p = 0.028$; 204.4 ± 70.8 in E *vs.*154.2 ± 46.3 in G, $p = 0.006$; and 204.4 ± 70.8 in E *vs.* 128.3 ± 39.8 in H, $p < 0.001$) (Table 2, Part B; Fig. 3B). Furthermore, there was a stepwise decrease in $ECAR_B$ from groups F ($SLE_{400/0}$) to G ($SLE_{400/2}$) and further H ($SLE_{400/4}$) among SLE-PBMCs at a concentration of 400 mg/dL glucose after the supplementation of 0, 2, and 4 mM glutamine (164.5 ± 44.5 in F, 154.2 ± 46.3 in G, and 128.3 ± 39.8 in H, $p < 0.001$) (Table 2, Part B; Fig. 3B).

In the medium without glutamine, higher $ECAR_B$ was observed in HC-PBMCs as well as in SLE-PBMCs in the presence of 400 mg/dL glucose as compared to those maintained in 100 mg/dL glucose (204.4 ± 70.8 in E $HC_{400/0}$ *vs.* 159.9 ± 56.6 in A $HC_{100/0}$, $p < 0.001$, for HC-PBMCs; 164.5 ± 44.5 in F $SLE_{400/0}$ *vs.* 146.7 ± 48.5 in B $SLE_{100/0}$, $p = 0.012$, for SLE-PBMCs) (Table 2, Part A, B & C; Fig. 3B). However, when cultured in 2 mM glutamine, SLE-PBMCs in group G ($SLE_{400/2}$) and C ($SLE_{100/2}$) exhibited similar $ECAR_B$ regardless of the glucose concentration of 100 or 400 mg/dL (154.2 ± 46.3 in G *vs.* 151.4 ± 50.3 in C, $p = 0.306$) (Table 2, Part A, B &C; Fig. 3B). On the contrary, with further increase of glutamine to 4 mM, SLE-PBMCs in group H ($SLE_{400/4}$) exhibited a lower $ECAR_B$ than group D ($SLE_{100/4}$) (128.3 ± 39.8 in H *vs.* 144.4 ± 52.0 in D, $p = 0.054$) (Table 2, Part A, B &C; Fig. 3B).

Compared to group B ($SLE_{100/0}$), at high concentrations of 400 mg/dL glucose and 4 mM glutamine, PBMCs in group H ($SLE_{400/4}$) exhibited a significantly lower level of $ECAR_B$ (128.3 ± 39.8 in H *vs.* 146.7 ± 48.5 in B, $p = 0.012$) (Table 2, Part A, B &C; Fig. 3B).

3.5. Difference in $ECAR_B/mOCR_B$ ratio in HC-PBMCs and SLE-PBMCs at various concentrations of glucose and glutamine

To evaluate the trend of metabolic shift from mitochondrial respiration to lactate fermentation in HC-PBMCs or SLE-PBMCs, we measured the $ECAR_B/mOCR_B$ ratio of PBMCs (Lin et al., 2016). As shown in Table 2, in glutamine-free medium, SLE-PBMCs had a higher $ECAR_B/mOCAR_B$ ratio than HC-PBMCs, regardless of the glucose concentration of 100 mg/dL (group B, $SLE_{100/0}$ *vs.* group A, $HC_{100/0}$, 0.30 ± 0.10 *vs.*0.25 ± 0.07, $p = 0.032$; Table 2, Part A; Fig. 3C) or 400 mg/dL (group F, $SLE_{400/0}$ *vs.* group E, $HC_{400/0}$, 0.31 ± 0.14 *vs.* 0.25 ± 0.07, $p = 0.060$; Table 2, Part B; Fig. 3C). At 100 mg/dL glucose, SLE-PBMCs showed a steady decrease in $ECAR_B/mOCR_B$ ratio from group B ($SLE_{100/0}$) to group C ($SLE_{100/2}$) and further to group D ($SLE_{100/4}$) after the supplement of 0, 2, and 4 mM glutamine, respectively (0.30 ± 0.10 in B, 0.28 ± 0.11 in C and 0.25 ± 0.10 in D, $p < 0.001$; Table 2, Part A; Fig. 3C). PBMCs in either group C ($SLE_{100/2}$) or group D ($SLE_{100/4}$) had similar $ECAR_B/mOCR_B$ ratios to that of group A ($HC_{100/0}$) (0.28 ± 0.11 in C *vs.* 0.25 ± 0.07 in A, $p = 0.417$; 0.25 ± 0.10 in D *vs.* 0.25 ± 0.07 in A, $p = 0.457$; Table 2, Part A; Fig. 3C). At a concentration of 400 mg/dL glucose, $ECAR_B/mOCR_B$ ratios of PBMCs in group F ($SLE_{400/0}$), group G ($SLE_{400/2}$), and group H ($SLE_{400/4}$) remained constantly high at about 0.31, which was similar to that in baseline disease control (group B, $SLE_{100/0}$), regardless of the supplementation of glutamine (Table 2, Part A and B; Fig. 3C).

3.6. Difference in $mOCR_B$ of SLE-PBMCs in association with different clinical medication

Among the 30 SLE patients in cohort 2, 22 took oral steroid, 18 hydroxychloroquine, 5 ciclosporin, 4 mycophenolate mofetil and/or their combinations (Table 3). Except for steroid, we found that SLE-PBMCs from patients taking these disease modifying anti-rheumatic drugs (DMARDs) would not have different $mOCR_B$ from those who did not take, under euglycemic condition with 0 (Group B, $SLE_{100/0}$), 2 (Group C, $SLE_{100/2}$) or 4 mM (Group D, $SLE_{100/4}$) glutamine, respectively (Table 3). SLE-PBMCs from 22 SLE patients who took steroid did not have different $mOCR_B$ from the other 8 patients who did not take steroid at euglycemic condition with 0 mM glutamine (489.1 ± 172.6 *vs.* 588.0 ± 125.2, $p = 0.219$, in B, $SLE_{100/0}$). However, PBMCs from SLE patients who took steroid would have lower $mOCR_B$ than the other 8 patients who did not take at euglycemic condition with 2 mM (537.5 ± 213.0 *vs.* 719.3 ± 165.9, $p = 0.026$, in C, $SLE_{100/2}$) and 4 mM glutamine (584.5 ± 233.2 *vs.* 757.6 ± 168.3, $p = 0.039$, in D, $SLE_{100/4}$), respectively (Table 3).

3.7. Correlation between $mOCR_B$ and $ECAR_B$ in HC-PBMCs and in SLE-PBMCs at 100 mg/dL and 400 mg/dL glucose without glutamine

At 100 mg/dL or 400 mg/dL glucose without glutamine (Table 4), a positive correlation between $mOCR_B$ and $ECAR_B$ was found in group A ($HC_{100/0}$, $\rho = 0.722$, $p < 0.001$, $R^2 = 0.379$), group E ($HC_{400/0}$, $\rho = 0.750$, $p < 0.001$, $R^2 = 0.488$) and group B ($SLE_{100/0}$, $\rho = 0.599$, $p < 0.001$, $R^2 = 0.385$), but not in group F ($HC_{400/0}$, $\rho = 0.348$, $p = 0.112$).

3.8. Correlation between OCR and mtDNA copy number in HCs-PBMCs and SLE-PBMCs

For HC-PBMCs at 100 md/dL or 400 mg/dL glucose without glutamine, the $mOCR_B$ and $mOCR_{Max}$ were positively correlated to their mtDNA copy number (group A, $HC_{100/0}$, $mOCR_B$, $\rho = 0.572$, $p = 0.003$, $R^2 = 0.510$, $mOCR_{Max}$, $\rho = 0.452$, $p = 0.023$, $R^2 = 0.440$; group E, $HC_{400/0}$, $mOCR_B$, $\rho = 0.648$, $p < 0.001$, $R^2 = 0.550$, $mOCR_{Max}$, $\rho = 0.645$, $p < 0.001$, $R^2 = 0.541$, Table 5). For SLE-PBMCs at 100 md/dL or 400 mg/dL glucose without glutamine, only the $mOCR_B$ in group B ($SLE_{100/0}$) was positively correlated with the mtDNA copy number (group B, $SLE_{100/0}$, $mOCR_B$, $\rho = 0.478$, $p = 0.008$, $R^2 = 0.196$, Table 5).

4. Discussion

Mitochondrial dysfunction has been observed in SLE patients as a

Table 3
Difference in $mOCR_B$ of SLE-PBMCs in the presence of 0, 2 and 4 mM glutamine under euglycemic conditions according to their clinical medication.

Glucose and glutamine conditions of culture medium/Group/Group name	mOCRB of SLE-PBMCs in different type of medication		*p*-value*
	Steroid		
	No (n = 8)	Yes (n = 22)	
Group B, $SLE_{100/0}$,100 mg/dL Glucose & 0 mM Glutamine	588.0 ± 125.2	489.1 ± 172.6	0.219
Group C, $SLE_{100/2}$,100 mg/dL Glucose & 2 mM Glutamine	719.3 ± 165.9	537.5 ± 213.0	0.026
Group D, $SLE_{100/4}$,100 mg/dL Glucose & 4 mM Glutamine	757.6 ± 168.3	584.5 ± 233.2	0.039
	Hydroxychloroquine		
	No (n = 12)	Yes (n = 18)	
Group B, $SLE_{100/0}$,100 mg/dL Glucose & 0 mM Glutamine	531.6 ± 160.8	505.0 ± 172.2	0.674
Group C, $SLE_{100/2}$,100 mg/dL Glucose & 2 mM Glutamine	625.9 ± 218.1	559.4 ± 214.8	0.416
Group D, $SLE_{100/4}$,100 mg/dL Glucose & 4 mM Glutamine	654.9 ± 229.8	614.5 ± 233.1	0.643
	Ciclosporin		
	No (n = 25)	Yes (n = 5)	
Group B, $SLE_{100/0}$,100 mg/dL Glucose & 0 mM Glutamine	530.3 ± 158.8	442.3 ± 197.4	0.391
Group C, $SLE_{100/2}$,100 mg/dL Glucose & 2 mM Glutamine	607.6 ± 209.7	477.9 ± 231.2	0.294
Group D, $SLE_{100/4}$,100 mg/dL Glucose & 4 mM Glutamine	645.0 ± 228.6	558.9 ± 240.3	0.491
	Mycophenolate Mofetil		
	No (n = 26)	Yes (n = 4)	
Group B, $SLE_{100/0}$,100 mg/dL Glucose & 0 mM Glutamine	512.3 ± 170.9	537.3 ± 142.8	0.784
Group C, $SLE_{100/2}$,100 mg/dL Glucose & 2 mM Glutamine	605.0 ± 217.3	462.6 ± 172.6	0.223
Group D, $SLE_{100/4}$,100 mg/dL Glucose & 4 mM Glutamine	642.4 ± 230.3	554.3 ± 233.7	0.521

* Mann-Whitney *U* test or *t*-test.

Table 4
Correlation between $mOCR_B$ and $ECAR_B$ of HCs-PBMCs and SLE-PBMCs under 100 mg/dL or 400 mg/dL glucose with glutamine-free in the culture medium.

Associations between $mOCR_B$ and $ECAR_B$			
Subjects/Groups	Spearman's ρ	*p*-Value	R^2
HCs			
Groups A; $HC_{100/0}$, n = 25	0.722	< 0.001	0.379
Groups E; $HC_{400/0}$, n = 25	0.750	< 0.001	0.488
SLE patients			
Groups B; $SLE_{100/0}$, n = 30	0.599	< 0.001	0.385
Groups F; $SLE_{400/0}$, n = 22	0.348	0.112	–

Table 5
Correlation between mtDNA copy number and $mOCR_B$, and mtDNA copy number and $mOCR_{Max}$ of HC-PBMCs and SLE-PBMCs.

	Spearman's ρ	*p*-Value	R^2
HCs			
Groups A; $HC_{100/0}$, n = 25			
mtDNA copy number *vs.* $mOCR_B$	0.572	0.003	0.510
mtDNA copy number *vs.* $mOCR_{Max}$	0.452	0.023	0.440
Groups E; $HC_{400/0}$, n = 25			
mtDNA copy number *vs.* $mOCR_B$	0.648	< 0.001	0.550
mtDNA copy number *vs.* $mOCR_{Max}$	0.645	< 0.001	0.541
SLE patients			
Groups B; $SLE_{100/0}$, n = 30			
mtDNA copy number *vs.* $mOCR_B$	0.478	0.008	0.196
mtDNA copy number *vs.* $mOCR_{Max}$	0.163	0.389	–
Groups F; $SLE_{400/0}$, n = 22			
mtDNA copy number *vs.* $mOCR_B$	− 0.078	0.728	–
mtDNA copy number *vs.* $mOCR_{Max}$	− 0.169	0.453	–

result of the alterations in mtDNA copy number (Lee et al., 2012; Lee et al., 2016), mRNA expression of mitochondrial biogenesis-related proteins (Lee et al., 2011; Lee et al., 2014), mitochondrial membrane potential and the capacity of ATP production (Gergely Jr et al., 2002). We further demonstrated that the SLE-PBMCs exhibited lower $mOCR_B$ and lower $mOCR_{Max}$ than HC-PBMCs in a culture condition of 100 or 400 mg/dL glucose without glutamine (Table 2, Part A & B; Fig. 3A) in the current study. Recently, West et al. (West et al., 2015) reported that mitochondrial dysfunction from decreased mtDNA copy number in lymphocytes involved in the triggering innate immunity against virus. Taken together, we speculated that mitochondrial dysfunction in immunocytes played an important role in the pathogenesis of SLE (Morel, 2017).

Glutamine is a non-essential amino acid generated by muscle breakdown (DeBerardinis and Cheng, 2010). However, in rapidly dividing normal cells such as enterocytes, lymphocytes, and macrophages at high metabolic state (*e.g.*, inflammation, infection or sepsis), glutamine becomes the fuel of choice and serves as conditioning essential amino acids. Lower or decreased plasma glutamine levels have been observed in critically ill patients (Rodas et al., 2012), autism (Shimmura et al., 2011), chronic obstructive pulmonary disease (Pouw et al., 1998), coronary artery disease or type I diabetes mellitus (Liu et al., 2016) as well as inflammatory bowel disease (IBD) (Lecleire et al., 2008). Compared to HCs, SLE patients exhibited a parabolic trend of increase followed by decrease in their relative plasma glutamine levels with a peak at the medium SLEDAI of 8 (Table 1, HCs, M = 1.00; SLEDAI ≤ 8, M = 1.27; SLEDAI > 8, M = 0.99, cohort 1). Moreover, a higher SLEDAI score was related to a lower plasma glutamine level among 93 SLE patients in cohort 1 (p = 0.025, Table 1). It seemed that SLE *per se* with different disease activities could affect the relative plasma glutamine levels. Under low disease activity, the increase in plasma glutamine level is sufficient to overcome the impaired mitochondrial function in SLE-PBMCs. Along with the progression of disease to a higher activity, much more glutamine would be required to compensate for the impaired mitochondria in immune cells, and thereby the plasma glutamine become insufficient to replenish. Such a parabolic phenomenon has also been observed in the alterations of mtDNA copy number in SLE leukocytes with different SLEDAI scores or in esophageal cancer, lung tissues or patients with head and neck cancer who have different degrees of cigarette smoking resulting in oxidative stress (Kim et al., 2004; Lee et al., 1998; Lee et al., 2012; Lin et al., 2013). Previous studies have also demonstrated that glutamine supplementation is beneficial in critically ill patients with APACHE score < 15, indicating that glutamine insufficiency in patients with low disease severity could be compensated for (Oldani et al., 2015). However, the effect of glutamine supplementation to improve mitochondrial function in SLE patients with different disease activities

may need further investigations.

Under physiological condition, glucose can provide ATP through oxidative phosphorylation in the mitochondria (major part) and glycolysis in the cytoplasm (minor part) in a constant ratio, depending on the cell types or *in situ* milieu (Kramer et al., 2014). If the glucose supply is sufficient, the total oxygen consumption during mitochondrial respiration would be increased cooperatively with an increase in total lactate generated by glycolysis. Interestingly, for cohort 2, in glutamine-free condition, we found (1) an increase of $mOCR_B$ and $ECAR_B$ in both HC-PBMCs and SLE-PBMCs when glucose concentration was increased from 100 to 400 mg/dL (Table 2, Part A, B&C); and (2) a positive correlation between the $mOCR_B$ and $ECAR_B$ in HC-PBMCs at 100 and 400 mg/dL glucose and in SLE-PBMCs at 100 mg/dl glucose but not at 400 mg/dl glucose (Table 4). This indicated a possible immunometabolic alteration between mitochondrial respiration and anaerobic glycolysis in SLE-PBMCs. Besides, SLE-PBMCs (group B, $SLE_{100/0}$; group F, $SLE_{400/0}$) had higher $ECAR_B/mOCR_B$ ratios (~0.30–0.31) than HC-PBMCs (group A, $HC_{100/0}$; group E, $HC_{400/0}$) (~0.25) (Table 2, Part A, B & C), indicating that SLE-PBMCs would generate more lactate than HCs-PBMCs per glucose utilization. These findings implied that the increased supply of glucose concentration for SLE-PBMCs might lead to attenuation in $mOCR_B$ but enhancement in $ECAR_B$ than HC-PBMCs. The acidic milieu generated by the high glucose concentration might be harmful to SLE-PBMCs because of subsequent oxidative stress and damage (Xiu et al., 2014). At such a disease state, tissue cells would need a substitute fuel for energy production, and thus glutamine becomes a suitable choice.

For cohort 2 SLE-PBMCs at 100 md/dL glucose, the $mOCR_B$ and $mOCR_{Max}$ could be increased steadily by adding 0, 2, and 4 mM glutamine without enhancement of $ECAR_B$ (Table 2, Part A, group B, $SLE_{100/0}$; group C, $SLE_{100/2}$; and group D, $SLE_{100/4}$). The increase could even reach to a level equivalent to normal controls (group A, $HC_{100/0}$). For cohort 2 SLE-PBMCs in group B ($SLE_{100/0}$), C ($SLE_{100/2}$), and D ($SLE_{100/4}$), the mean $ECAR_B/mOCR_B$ ratios decreased from 0.30 to 0.28 and further to 0.25 in a descending order, equivalent to that of HC-PBMCs (Group A, $HC_{100/0}$) of 0.25 (Table 2, Part A). These findings indicate a U-turn of metabolic shift from anaerobic glycolysis to mitochondrial respiration in SLE-PBMCs, which could reach that of the normal levels of HC-PBMCs at 100 mg/dL glucose. It implied that glutamine is essential for the survival of SLE-PBMCs *ex vivo* under physiological glucose concentration. However, patients with autoimmune diseases such as SLE under steroid medication (Shaharir et al., 2015) or critically ill patients with profound septicemia in ICU (Andersen et al., 2004) are frequently protracted with severe hyperglycemia. The therapeutic effect of glutamine for the treatment of these patients under hyperglycemia state warrants further investigations.

A paradoxical and conflicting results showing synchronous decrease of $mOCR_B$ and $ECAR_B$ in the SLE-PBMCs of cohort 2 after administration of glutamine were observed at a hyperglycemia-equivalent environment (Table 2, Part A&B; Fig. 3 A&B). Interestingly, the mean $ECAR_B/mOCR_B$ ratios of SLE-PBMCs in groups F ($SLE_{400/0}$), G ($SLE_{400/2}$), H ($SLE_{400/4}$) were 0.31, 0.31 and 0.31, respectively, similar to group B ($SLE_{100/0}$) of 0.30, which were all higher than that of HCs-PBMCs of 0.25 (Table 2, Part A&B; Fig. 3C). This indicates "no U-turn" of glucose metabolic shift and "low" glucose utilization after supplementation of glutamine at a very high concentration of glucose (400 mg/dL) in the culture medium for SLE-PBMCs. The clinical relevance of glutamine supplementation in SLE patients under hyperglycemia state remains unclear. Previous studies have shown that glutamine supplementation for critically ill patients with profound septicemia is ineffective because the illness is confounded with hyperglycemia (Oldani et al., 2015). The benefit of glutamine in hyperglycemic status deserves further investigation.

Hyperglycemia is common in SLE patients under steroid treatment (Shaharir et al., 2015). Per the present *in vitro* studies, it is intriguing whether steroid-related hyperglycemia would attenuate the glutamine effect in improving mitochondrial function of SLE-PBMCs. As shown in Table 3, the PBMCs of the 22 SLE patients who took steroid had similar $mOCR_B$ to the other 8 who only took DMARDs in an euglycemic condition without glutamine supply (489.1 ± 172.6 *vs.* 588.0 ± 125.2, $p = 0.219$, Group B, $SLE_{100/0}$). However, the PBMCs of the 22 SLE patients who took steroid had lower $mOCR_B$ than the other 8 patients who did not take steroid under an euglycemic condition with 2 or 4 mM glutamine, respectively (537.5 ± 213.0 *vs.* 719.3 ± 165.9, $p = 0.026$, Group C, $SLE_{100/2}$; 584.5 ± 233.2 *vs.* 757.6 ± 168.3, $p = 0.039$, Group D, $SLE_{100/4}$). Although we did not measure the blood sugar levels of 30 SLE patients, all these results did give us an important clue that steroid or steroid-related hyperglycemia may attenuate the glutamine effect in improving mitochondrial dysfunction in SLE-PBMCs. The exact mechanisms underlying this phenomenon need further study.

Measurement of mitochondrial respiration is a tedious and expensive procedure. Determination of mtDNA copy number seems a feasible substitute. We have demonstrated that mtDNA copy number in PBMCs could reliably predict the $mOCR_B$ and may serve as a surrogate biomarker for energy supply by aerobic metabolism as well as oxidative stress in these immunocompetent cells (Table 5), which has also been suggested in previous studies (Lee and Wei, 2005).

However, some controversies and differences existed between our current study and other reports in mitochondrial oxygen consumption. Although Perl A et al., speculated mitochondrial dysfunction in SLE PBL, they demonstrated an elevated mitochondrial oxygen consumption, or hyper-metabolism, in "activated" SLE-T cells stimulated with CD3/CD28. Nevertheless, their ATP productions were decreased (Doherty et al., 2014; Gergely Jr et al., 2002; Morel, 2017). Such an elevated oxygen consumption was supposed mainly spent in the production of ROIs through ETC complex I hyperactivity rather than in ATP production through ATP synthetase. The increased ROI and complex I hyperactivity of "activated" T cells could be reversed by the administration of *N*-acetylcysteine and metformin, respectively (Doherty et al., 2014; Yin et al., 2015). It deserved to mention that the PBMCs in the current study were not stimulated *in vitro* to induce activation and proliferation (*i.e.*, the *de novo* state) and could reflect the "real" condition of these SLE disease status.

We demonstrated that SLE PBMCs had a lower $mOCR_B$ and $mOCR_{Max}$ than HCs PBMC, which could be improved by glutamine supply. Like *N*-acetylcysteine (Doherty et al., 2014), glutamine could reverse the reduced activity of glutathione for counteracting the ROI production (DeBerardinis and Cheng, 2010; Maciver et al., 2013), and we concluded that the increased $mOCR_B$ and $mOCR_{Max}$ after glutamine supplement might be spent mainly in ATP rather than ROIs productions.

The Ficoll-Plaque standard density gradient and Vacutainer cell preparation tube (CPT) are the two major procedures in PBMC isolation, and they have similar yield and viability of ~70% of T cells and ~15% B cells (Corkum et al., 2015). Nevertheless, the CPT protocol is less elaborate and could minimize procedure time and offer a significant operating advantage to detect the "real" and "*de novo*" PBMC oxygen consumption rate immediately. Because both B cells and T cells have different roles in the pathogenesis of SLE, further evaluations of the ORC of B cell and T cells might be indicated in the future.

In conclusion, as compared to HC-PBMCs, SLE-PBMCs did exhibit decreased mitochondrial function with glucose metabolic shift from decrease of mitochondrial respiration to increase of glycolysis. The disease activity (SLEDAI) score was inversely correlated to the relative plasma glutamine level. Glutamine supplementation could improve mitochondrial function and trigger U-turn of glucose metabolic shift from anaerobic metabolism to boosted mitochondrial respiration in SLE-PBMCs. However, high glucose environment or hyperglycemic status may attenuate or inhibit this beneficial effect.

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Conflicts of interest

The authors declare no conflict of interest

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