Short-Term Fasting Induces Cell Cycle Arrest in Immature Hematopoietic Cells and Increases the Number of Naïve T Cells in the Bone Marrow of Mice

Takakuwa T., Nakashima Y., Koh H., Nakane T., Nakamae H., Hino M.

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Teruhito Takakuwa, Yasuhiro Nakashima, Hideo Koh, Takahiko Nakane, Hirohisa Nakamae, Masayuki Hino

Department of Hematology, Graduate School of Medicine, Osaka City University, Osaka, Japan

Corresponding author: Dr. Yasuhiro Nakashima, M.D., Ph.D., Department of Hematology, Graduate School of Medicine, Osaka City University, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585 JAPAN

E mail: yakkun414@med.osaka-cu.ac.jp

Phone number: +81666453881

Fax number: +81666453880

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●Abstract

Calorie restriction (CR) has been studied as a way to prolong longevity, and CR before chemotherapy can reduce hematological toxicity in cancer patients. We investigated the influence of fasting on immune cells and immature hematopoietic cells. In fasted mice, there was a significant reduction in the hematopoietic stem cell count but no significant difference for progenitor cells. Colony assays showed no difference and the rates of early and late apoptosis were almost identical when comparing fasted and control mice. DNA cell cycle analysis of immature bone marrow (BM) cells showed that CR caused a significant increase in the percentage in G0/G1 phase and decreases in S and G2/M phases. We detected a remarkable increase of T cells in the BM of fasted mice. $CD44^{-}$ naive $CD8^{+}$ T cells were more numerous in fasted BM, as were naive $CD4^{+}$ T cells and part of those T cells showed less tendency in G0/G1 phase. Immature hematopoietic cells remained in a relatively quiescent state and retention of colony forming capacity during CR. The number of naïve T cells in the BM of fasted mice increased. These

findings imply immature hematopoietic cells and some lymphoid cells can survive starvation, whilst maintaining their function.

●Introduction

Prolonged lifespan in rats subjected to calorie restriction (CR) was initially observed in the 1930s [1], and life extension caused by reducing calorie intake has been widely reported in several organisms [2-10]. CR also reduces incidence of malignancy and aging-related diseases such as cardiovascular disorders and diabetes, contributing to extended lifespan [11, 12]. These effects of CR are mainly derived from hypercatabolism of fat, which produces ketone bodies accompanied by hepatic glycogen depletion and regulation of protein synthesis [13].

CR has attracted widespread attention as an approach for cancer prevention and treatment. Fasting for 48 hours protected mice from various chemotherapy drugs and other harmful agents and enhanced the efficacy of chemotherapy in cancer cells [14]. In addition, fasting for 48–140 hours before chemotherapy administration in human patients diagnosed with various types of cancer reduced adverse effects including fatigue, weakness, and gastrointestinal symptoms [15]. The reduction of hematological toxicity following short-term fasting in breast cancer patients [16] suggests that CR has

a remarkably positive influence on the hematopoietic system, but the detailed mechanism through which it weakens the adverse events of anticancer drugs is not fully understood.

In this study, we examined murine immune cells, hematopoietic stem cells, and progenitor cells in the BM and spleen in order to elucidate the hematologic effect of CR. We observed that progenitor cells and naïve T cells in the BM survived starvation whilst preserving their function, via cell cycle arrest and migration into the BM, respectively.

●Materials and Methods

＜Animals＞

C57Bl/6 mice were purchased from Japan SLC Incorporated (Shizuoka, Japan). Six- to 8-week old mice were used for colony assay and we used 10- to 12-week old mice for flow cytometry analyses. They had free access to food and water until the start of the intervention. We kept them on a 12:12 light-dark cycle (8:00–20:00 light, 20:00–8:00 dark) in a specific pathogen-free animal facility keeping room temperature at 21–25°C with a humidity of 50%–60%. They were divided into two groups: normal control (NC) mice had a free diet, and short-term fasted (SF) mice had access to only water for 48 hours. All experimental procedures were approved by an institutional review committee

of the Osaka City University.

＜Colony assay＞

BM cells were collected from femurs of NC and SF mice, and the number of cells was counted. The cells were added to culture medium (Methocult GF M3534; Stem cell Technologies) in a ratio of 1 part cells to 9 parts medium to give final concentrations of 1×10^5 cells/ml. The media and cells were vortexed until completely mixed, followed by 5 minutes settling time to allow for air bubbles to surface. Each sample was divided into three, and plated in 35 × 10 mm petri-dishes with grids. The cultures were incubated in a humidified environment at 37°C with 5% CO_2. The colony numbers were counted using an inverted microscope after 7–9 days. Each dish was then washed 3 times with 2 ml of RPMI1640 media, for the secondary or tertiary colony assays.

＜FACS＞

Mice spleen was minced in RPMI1640 media and passed through a wire-mesh in order to remove connective tissue. BM and spleen cells from mice of the two groups were divided into two groups respectively. Group1 contained 1×10^6 cells to analyze cell lineages, and Group2 contained 5×10^6 cells to analyze the proportion of hematopoietic

stem cells and progenitor cells. The cells were incubated for 15 minutes on ice with 2.4G2 mAb to block nonspecific FcγR binding, then incubated with antibodies as described for 15 minutes on ice in the dark. Flow cytometric analyses were performed using a Beckman Coulter Gallios. Antibodies specific for mouse surface markers were as follows: CD11b-PE-Cy5, CD3-APC, Gr-1-Alexa700, and CD19-Pacific Blue were added to Group 1; CD4-FITC, CD8-FITC, CD11b-FITC, CD19-FITC, NK1.1-FITC, Ter119-FITC, Sca-1-APC, and c-kit-Pacific Blue were added to Group 2, in which the lineage set includes CD4, CD8, CD19, NK1.1, and Ter119. We defined hematopoietic stem cells expressing $Lin^{-}c\text{-}kit^{+}Sca\text{-}1^{+}$, which are so called KSL cells, and progenitor cells expressing $Lin^{-}c\text{-}kit^{+}Sca\text{-}1^{-}$, as it has been well known [17]. Apoptosis analysis was performed using a PE annexin V apoptosis detection kit with 7-AAD (BioLegend, Tokyo, Japan) as per the manufacturer's instructions. In T cell subtype analyses, we used CD62L-FITC, CD44-PE, CD8-PE-Cy7, CD3-APC, and CD4-Pacific Blue. All antibodies were purchased from BioLegend.

＜DNA cell cycle analysis＞

We performed cell cycle analysis using propidium iodide (PI) staining. 5×10^{6} BM cells from NC and SF mice were stained with specific antibodies for a lineage set, c-kit,

and Sca-1. $CD4^+$ and $CD8^+$ T cells in BM were each sorted using MojoSort Mouse CD4 and CD8 T Cell Isolation Kit (BioLegend, Tokyo, Japan), respectively, according to the manufacturer's instructions. Sorted T cells were co-stained with CD3-APC and CD4-FITC or CD8-FITC antibodies. After washing, these cells were transferred to 15 ml polypropylene tubes and 5 ml cold 70% ethanol was added drop wise while gently vortexing. After fixation for at least 1 hour at 4ºC, 50 μl of 10 μg/ml RNase A stock solution was directly added to the cell pellet and 1 ml of 50 μg/ml PI staining solution was added. Samples were incubated overnight at 4ºC and analyzed using a Beckman Coulter Gallios.

＜Statistical analysis＞

All statistical analyses between control and fasting mice were performed using the unpaired 2-tailed Student *t* test with SPSS ver. 22.0.0.0. P values less than 0.05 were considered to be statistically significant.

●Results

After 48 hours of fasting, body weight significantly decreased in the SF group by an average of 24.1% (18.15% − 30.84%), while in the NC mice there was almost no

change in body weight (−3.12% to 2.55%) (Fig 1A).

48 hours of fasting caused a significant decrease in peripheral white blood cell count (Fig 1B) by an average of 48.3% (from −82.05% to +27.91%). In contrast, hemoglobin level and platelet count were less affected by calorie restriction. After 48 hours of fasting, hemoglobin level in SF mice was slightly decreased by an average of 11.9% ($P = 0.34$). The platelet count tended to decrease (−23% on average) compared with NC mice ($P = 0.078$).

Colony assays were performed using BM cells from NC and SF mice (Fig 1C). Cells cultured for a week were collected to conduct the secondary or tertiary assays. The average colony number after the first assay was 173 (121–259) for NC mice and 180 (120–242) for SF mice, with no statistical difference. The total number of cells in the cultured plate of SF mice was notably lower than one of NC mice (3.64×10^6 versus 5.08×10^6, $P < 0.05$, data not shown) in the first colony assay. We detected no significant differences not only in colony number, but also total cell number between the two groups in the secondary and tertiary rounds (Fig 1C).

To assess the effect of fasting on the immune and hematopoietic systems, we analyzed T cells, B cells, granulocytes, monocytes, hematopoietic stem cells, and progenitor cells in the BM and spleen of NC and SF mice by flow cytometry. The average number of

total BM cells was significantly reduced in the SF group compared to the NC group (2.31×10^7 versus 3.05×10^7, $P < 0.01$) (Table 1A, Fig 2A). The proportion of Lin^- c-$kit^+$$Sca\text{-}1^+$ (KSL) cells in the BM was the same in the two groups, although the number of KSL cells in SF BM was significantly lower than in NC (0.82×10^5 versus 1.16×10^5, $P < 0.05$). In contrast, the proportion of progenitor cells in SF BM was significantly higher than that in NC (2.98% versus 2.55%, $P < 0.01$), and the number was almost in the same range in the two groups. The number of B cells and granulocytes in the BM was significantly lower in the SF group than in the NC group and the difference in monocytes was not so pronounced. To our surprise, we detected a remarkable increase in the number of T cells in the BM of fasted mice (1.25×10^6 versus 0.91×10^6, $P < 0.01$). We performed similar analyses on the spleen cells and found that short-term fasting caused a striking decrease in not only total cell number (1.19×10^7 versus 5.36×10^7, $P < 0.01$) but also in the number of every cell type.

In order to further evaluate the increase of BM T cell number in SF mice, we examined the subtypes in the population. Of greatest significance, $CD62L^+$ $CD44^-$ naïve $CD8^+$ T cells were dramatically increased in number in fasted BM (1.74×10^6 versus 0.47×10^6, $P < 0.01$), as were naive $CD4^+$ T cells (0.23×10^6 versus 0.07×10^6, $P < 0.05$). In contrast, the numbers of $CD62L^-$ $CD44^+$ effector memory and $CD62L^+$ $CD44^+$ central

memory T cells were not substantially changed after starvation, excepting central memory $CD4^+$ T cells (Table 1B, Fig 2B). Reflecting the large decrease in T cells after fasting, all three subsets in the spleen were extremely decreased.

To evaluate the mechanisms producing these changes, we examined the ratio of apoptosis of cells in NC and SF mice using Annexin V and 7-AAD. Based on the presence of the two markers, Annexin V^+ 7-AAD^+ cells were defined as late apoptotic cells and Annexin V^+ 7-AAD^- as early apoptotic. There was no notable difference in late or early apoptotic proportions in progenitor cells, B cells, granulocytes, or monocytes (Table 2). We detected a tendency towards increased apoptosis in KSL cells of fasted mice. In contrast, the proportion of early apoptotic T cells decreased significantly in the SF group (4.53% versus 11.4%, $P < 0.01$) with a corresponding trend for late apoptotic cells.

We performed further experiments to evaluate the effect of fasting on DNA cell cycle in both immature hematopoietic cells and T cells in the BM. The percentage of cells in G0/G1 phase, indicted by the leftmost peak, was significantly higher in SF mice than in NC mice (83.1% versus 70.7%, $P = 0.023$) (Fig 3). Conversely, the proportion of cells in S and G2/M phase was lower in SF mice than in NC mice (12.6% versus 25.4%, $P = 0.015$). To the contrary, $CD4^+$ T cells in the BM in SF mice were significantly less in

G0/G1 phase than in NC mice (82.7% versus 93.8%, $P = 0.035$) and $CD8^+$ T cells in the BM in SF mice showed less tendency in G0/G1 phase than in NC mice (81.8% versus 92.4%, $P = 0.068$) (Fig 3).

●Discussion

The effects of CR include not only extended lifespan and prevention of physiological changes associated with aging, but also regulation of the hematopoietic system and an improved immune system. Here we examined the influence of fasting on murine hematopoietic cells, from the BM as a primary hematopoietic organ, and the spleen as a secondary lymphatic organ.

In our study, fasting caused a significant decrease in the number of total cells, both in the BM and the spleen, with the most pronounced effect on B cells. This result is congruent with previous reports [18, 19] and one possible reason for B cell decrease is inhibition of B cell differentiation in the BM during fasting [18].

The immunological influence of CR on T cells has previously been reported in various species. T cell development is initiated by Notch signaling in pluripotent hematopoietic stem cells in the BM. 'Double negative' $CD4^-$ $CD8^-$ T cells then progress to naive T cells in the thymus, where they can be directed to produce an effector T cell, or a

memory T cell, following an immune response in lymphoid or other organs. It has been reported that fasting reduces T cells including double negative cells and double positive cells in the thymus, B cells in BM, and peripheral lymphocytes, caused by an increase in plasma corticosterone levels and inhibited by injection of leptin [20-22]. The relationship between CR and the mTOR pathway is also well known. The mechanistic target rapamycin (mTOR), a protein kinase regulating cell growth and promoting aging, forms signaling complexes, which reduces the number of T cells, represses cell development [23], and is suppressed by fasting. In experiments on mice and monkeys, it was shown that CR protected naive T cells in the thymus and peripheral blood, which were reduced in number by aging, from immune senescence. They also retained a higher diversity of TCR repertoire, and production of inflammatory cytokines such as INF-γ and TNF-α by central memory T cells was inhibited [19, 24]. In our study, fasting caused T cells to increase in number in the BM and decrease in the spleen, as previously reported. Interestingly, the percentage of apoptotic cells was reduced in SF mice compared with NC mice, suggesting that the number of death-resistant T cells increased in the BM after fasting. In addition, our analysis of T cell subsets showed that 48 hours fasting caused naïve T cells to significantly increase in number in the BM and decrease in the spleen. $CD4^+$ or $CD8^+$ T lymphocytes, which are educated in the thymus, can

recirculate through the BM [25, 26], and Notch signaling is necessary for memory T cells to survive for a prolonged period [27]. Experiments involving adoptive transfer of CFSE-labeled memory T cells showed that $CD8^+$ T lymphocytes tend to migrate to the BM [28]. In considering the above, our results could indicate that fasting caused inhibition of cell activity and recirculation of naïve T cells, not memory phenotypes as previously reported, from secondary lymphoid tissue, including the spleen, to the BM. It should be mentioned that not only $CD8^+$ but also $CD4^+$ naive T cell populations were increased in the BM of SF mice. It seems that T cells are recirculated to the BM under starvation conditions, which aids survival and maintains functionality in preparation for future unfavorable conditions.

It was reported that fasting-mimicking diet increased lymphoid progenitors in BM and that cytotoxic $CD8^+$ T cells in breast cancer and melanoma tissues were increased, resulting in the delay of tumor progression [29]. Moreover Lu Z et al. recently reported that fasting alone effectively inhibited the acute lymphoblastic leukemia progression [30]. We identified that both $CD4^+$ and $CD8^+$ T cells in the BM in SF mice existed much more in S and G2/M phase than in NC mice. Over 80% of T cells in the BM in SF mice were naïve phenotype in both $CD4^+$ and $CD8^+$ T cells, therefore they didn't actively develop cellular division and should be ready for proliferation. Considering with past

reports, accumulated T cells in BM in SF mice could locally play a role as anti-leukemic immunosurveillance. Serum level of inflammatory cytokines such as TNFa and IL-1b was not upregulated even in SF group [21].

There have been many reports discussing the effect of fasting on the hematopoietic system, whereas there are few reports regarding the influence of starvation on hematopoietic stem cells. The number of hematopoietic stem cells in BM was changed with aging and the function of repopulation as stem cells in vivo diminished when hematopoietic stem cells form aged mice were transplanted along with competitor cells. Diet restriction restored not only the number of stem cells but also the repopulation capacity [31, 32]. In this study, the number of colonies was not different between SF and NC mice in the first, secondary, and tertiary courses. However, the total number of cells in the plate from NC mice was statistically higher than those from SF mice, which indicated that the average size of colonies in the plate obtained from the NC mice was higher. The colony assay is an indication of the number of progenitor cells that have the capacity to produce daughter cells and form colonies within a week. The results of the colony assay were comparable because the initial numbers of progenitor cells in the BM were almost equal between SF and NC mice. DNA cell cycle analyses showed G0/G1 arrest in the SF group, which could explain the total number of cells in the plate from

SF mice being significantly lower, while there was no difference in the number of colonies. Progenitor cells from SF mice were slow to respond to growth factors in colony assays, and the capacity for producing daughter cells was retained as the numbers of proliferative cells from colonies in the secondary and tertiary courses were nearly identical between SF and NC mice, as were the numbers of colonies. In the fasting state, mice make an effort to minimize their energy consumption and metabolism as demonstrated by low blood pressure, low body temperature, hypothyroidism, sex hormone decrease, and weight loss in each organ [33]. The protective effects of fasting on the hematopoietic system, especially on immature cells, were induced by a reduction in IGF-1 levels and subsequent inhibition of the PKA pathway [34]. However it is not certain whether such effects of fasting on hematopoietic immature cells directly reduce the adverse events of chemotherapy in human patients with various malignancies. The decrease of hemoglobin level by chemotherapy with previous short-term fasting was less in some cases [15] and hematological toxicity was well tolerated following short-term fasting in breast cancer patients [16]. Together with the reduction of gastrointestinal adverse effects of chemotherapy by short-term fasting such as oral inflammation, nausea, and diarrhea, CR should also have a significantly positive effect on the hematopoietic system.

As to limitations of this study, we have not yet elucidated the detailed mechanisms by which naïve T lymphoid cells promptly accumulate in the BM during starvation. We suppose that a chemokine would be crucial for the recruitment of naïve T cells into BM. Niches for naïve T cells in case of critical incidents may exist in the BM, similar to those reported for memory T cells [25, 26]. In addition whether T cells in the BM in SF mice have the ability of anti-leukemic effect or not should be carefully elucidated. The mechanisms of migration to the BM and survival capability of naïve T cells during fasting are still under investigation.

In our experiments, 48-hour fasting caused body weight loss in mice by an average of 24%. It is not recommended for cancer patients to reduce their body weight to a less than ideal weight by taking fewer calories than they would with a healthy diet for a long time [35]. However, it is not harmful for cancer patients to fast for 2–5 days before chemotherapy, which can reduce chemotherapy-associated adverse effects such as fatigue, weakness, and gastrointestinal disorders [15]. Moreover, further experiments to disclose the influence of fasting on hematopoietic stem cells and other normal tissues may enable patients to create a pseudo-fasting state with minimally invasive procedures.

In summary, short-term fasting decreased the number of hematopoietic stem cells, but progenitor cells retained their colony-forming capabilities with cell cycle arrest. The

number of naïve T cells in the BM after starvation also increased significantly and part of those T cells were in cell division phase. These results show that progenitor cells and naïve T cells in the BM endured harsh conditions whilst maintaining their functions.

●Disclosure Statement

The authors indicated no potential conflict of interest.

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Table 1A) FCM analysis of KSL, progenitor and lineage cells in the bone marrow and spleen

	BM		P value	spleen		P value
	control	fasting		control	fasting	
TNC($\times 10^7$)	3.05(2.19-3.83)	2.31(1.75-2.90)	0.003	5.36(1.33-11.37)	1.19(0.35-3.36)	0.001
KSL(%)	0.39(0.23-0.65)	0.36(0.20-0.50)	0.64	0.04(0.01-0.08)	0.04(0.01-0.07)	0.77
KSL abs($\times 10^5$)	1.16(0.73-1.82)	0.82(0.58-1.12)	0.012	0.17(0.05-0.30)	0.03(0.02-0.05)	0.001
progenitor(%)	2.55(2.16-3.01)	2.98(2.60-3.44)	0.007	0.10(0.05-0.15)	0.14(0.06-0.22)	0.18
progenitor abs($\times 10^5$)	7.75(5.42-9.53)	6.81(5.28-8.62)	0.11	0.54(0.15-1.39)	0.16(0.09-0.25)	0.012
Tcell (%)	2.95(2.12-3.94)	5.46(4.58-6.58)	<0.001	26.7(21.6-36.8)	33.0(25.5-43.9)	0.027
T abs ($\times 10^6$)	0.91(0.51-1.24)	1.25(0.94-1.69)	0.009	14.5(3.53-32.5)	3.91(1.39-11.5)	0.005
Bcell (%)	26.1(20.3-37.3)	22.5(15.7-31.6)	0.18	61.9(54.2-68.0)	54.7(36.0-65.0)	0.033
B cell abs ($\times 10^6$)	8.10(4.97-14.3)	5.38(3.37-8.73)	0.035	33.0(8.56-73.6)	6.65(1.84-18.6)	<0.001
Granulocyte (%)	36.8(27.1-44.2)	34.4(26.8-40.5)	0.31	3.22(1.26-6.34)	6.34(1.82-10.4)	0.019
Granulocyte abs ($\times 10^6$)	11.1(8.74-14.0)	7.91(5.43-11.2)	0.002	2.03(0.17-6.98)	0.72(0.10-1.57)	0.087
Monocyte (%)	20.1(17.2-22.0)	24.5(18.7-28.8)	0.003	5.21(3.08-9.12)	3.02(1.04-6.58)	0.024
Monocyte abs ($\times 10^6$)	6.11(4.82-7.80)	5.58(4.35-7.09)	0.23	2.91(0.62-8.27)	0.47(0.07-2.21)	0.008

BM: bone marrow, TNC: total nucleated cell count, abs: absolute value control n = 7, fasting n = 7

Table 1B) T cell subtype

	BM		P value	spleen		P value
	control	fasting		control	fasting	
$CD8^+$ T cell						
naïve($\times 10^6$)	0.47(0.29-0.60)	1.74(1.48-2.15)	0.005	7.19(5.09-8.31)	1.40(1.01-2.12)	0.006
central memory($\times 10^5$)	0.92(0.72-1.31)	1.92(1.18-2.70)	0.10	4.90(4.29-5.52)	0.63(0.26-1.14)	<0.001
effector memory($\times 10^5$)	0.15(0.06-0.29)	0.11(0.06-0.18)	0.64	0.30(0.24-0.38)	0.04(0.03-0.05)	0.003
$CD4^+$ T cell						
naïve($\times 10^6$)	0.07(0.02-0.13)	0.23(0.15-0.29)	0.043	6.67(5.30-8.02)	1.23(0.71-2.02)	0.003
central memory($\times 10^5$)	0.10(0.09-0.12)	0.20(0.14-0.24)	0.042	1.33(1.07-1.51)	0.31(0.14-0.49)	0.004
effector memory($\times 10^5$)	0.88(0.24-2.00)	0.31(0.18-0.56)	0.376	5.69(5.05-6.79)	1.03(0.82-1.37)	0.001

control n = 3, fasting n = 3

Table 2. Flow cytometry analysis of apoptosis by annexin V and 7-AAD

	control	fasting	P value
KSL			
late apoptosis(%)	0.15	0.2	0.029
early apoptosis(%)	16.1	25.5	0.12
progenitor			
late apoptosis(%)	1.44	1.27	0.28
early apoptosis(%)	8.65	12.5	0.13
T cell			
late apoptosis(%)	4.43	1.84	0.13
early apoptosis(%)	11.4	4.53	0.004
B cell			
late apoptosis(%)	1.13	1.69	0.27
early apoptosis(%)	6.94	5.56	0.31
Granulocyte			
late apoptosis(%)	0.89	0.45	0.47
early apoptosis(%)	12.5	9.39	0.21
Monocyte			
late apoptosis(%)	5.18	2.18	0.18
early apoptosis(%)	20.5	18.3	0.46

control n = 3, fasting n = 3

●Figure legends

Fig1. A) The body weight decreased by an average of 24.1% after 48 hours fasting ($P < 0.01$). Both groups included 10 mice. B) The peripheral white blood cells decreased by an average of 48.3% after 48 hours fasting ($P < 0.01$). Both groups included 7 mice. C) Colony assay was performed three times in separated experiments and total 7 mice of each group were assessed. The means of triplicate plates from each mouse are shown and there was no statistical difference of numbers of colonies between normal control and short-term fasted group.

Fig2. A) Representative FACS profiles and the percentage of KSL, progenitor and T cell, B cell, granulocyte and monocyote from 7 mice of each group. KSL and progenitor cells were observed in the fraction gated with Lineage negative. Flow cytometric analysis showed that two days fasting caused a decrease in KSL cells and increase of T cells. B) T cell subpopulations in bone marrow and spleen were checked in 3 mice of each group. Among increased T cells, naïve T cells in the BM of fasted mice were especially increased and there were not remarkable differences of central memory and effector memory T cells between two groups.

Fig3. A) Representative DNA cell cycle analyses of BM from 3 mice of each group are shown. $Lin^{-}c\text{-}kit^{+}$ immature BM cells of SF mice showed higher peak levels in G0/G1

phase and lower in G2/M phase than NC mice. $CD4^+$ and $CD8^+$ T cells in the BM of SF mice were much more in S and G2/M phase than NC mice. B) Date are the means and SD of 3 mice of each group.

Fig 1. Weight loss and PB WBC decrease after 48 hours of fasting

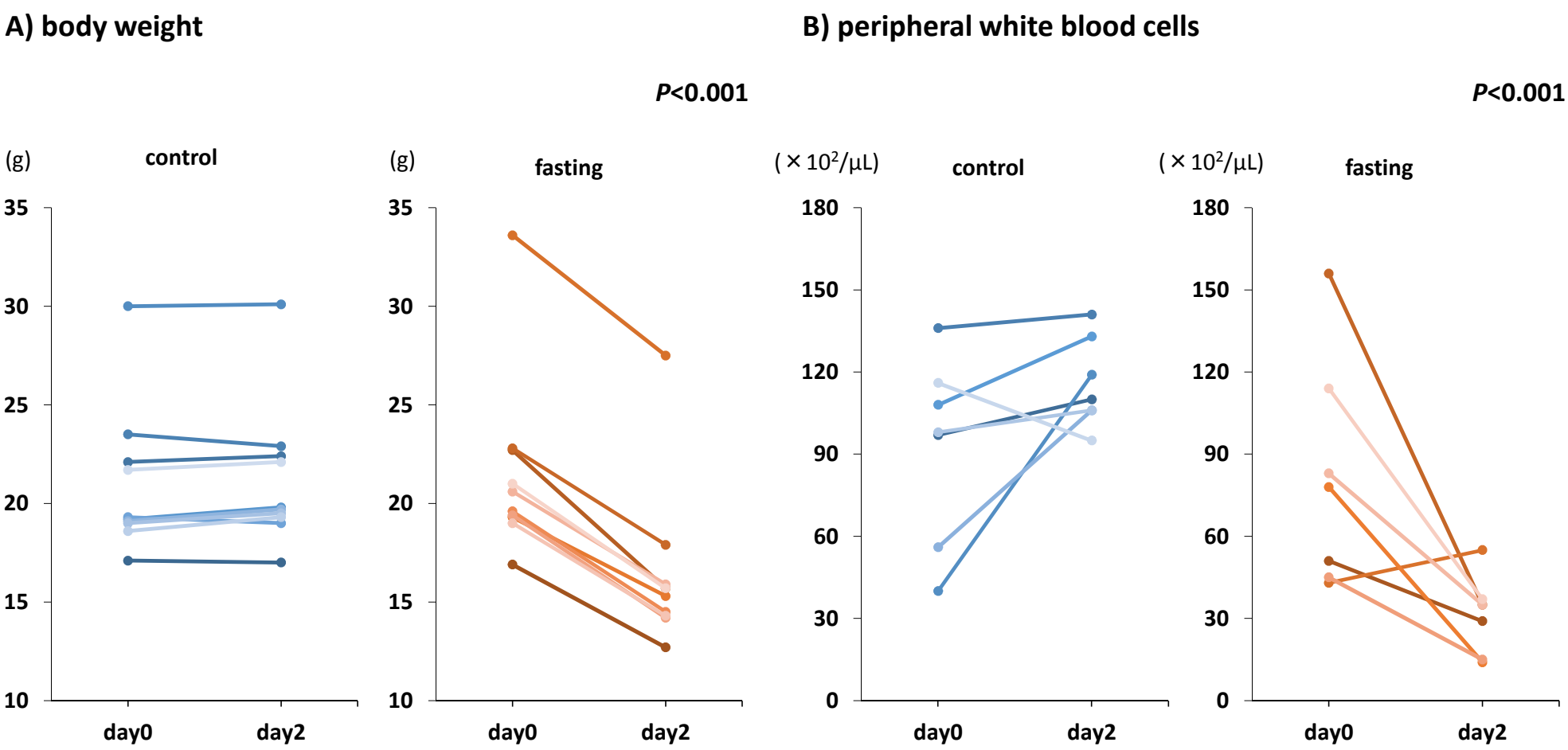


Fig 1. C) Colony assays of progenitor cells from bone marrow

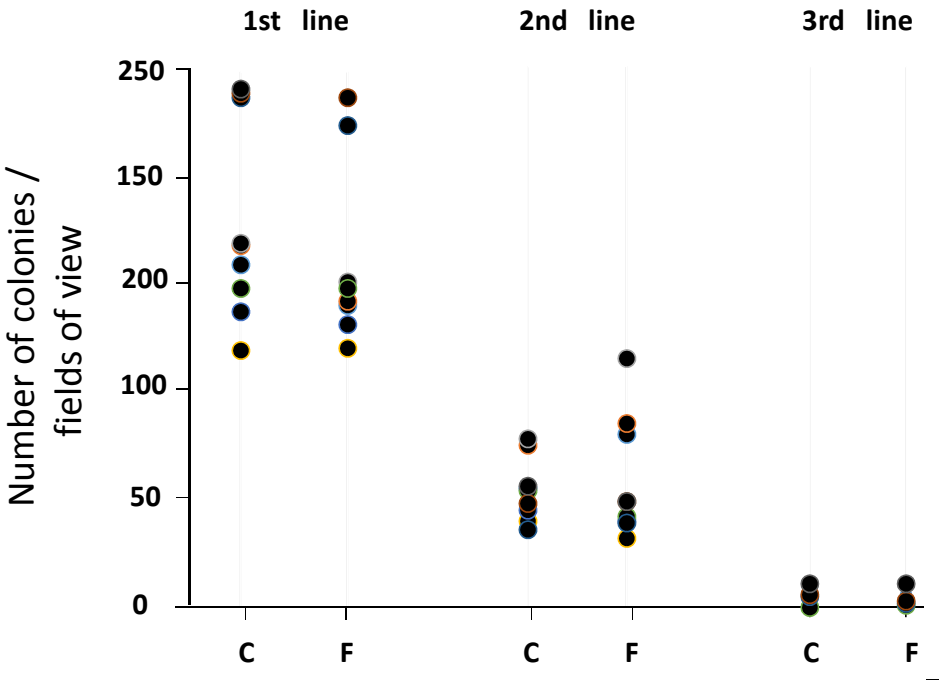


Fig 2. A) FCM analysis of KSL, progenitor and lineage cells in the bone marrow

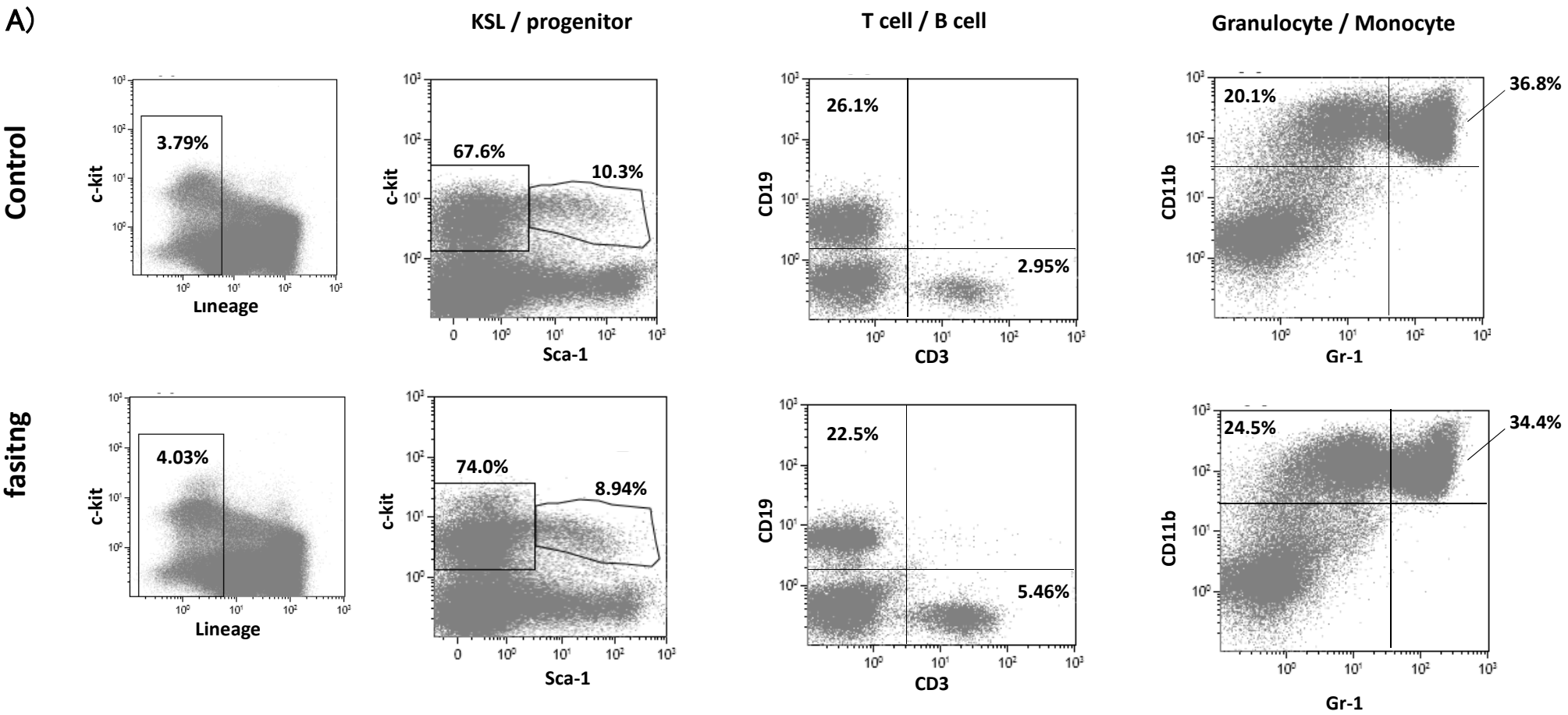


Fig 2. B) FCM analysis of T cell subtype

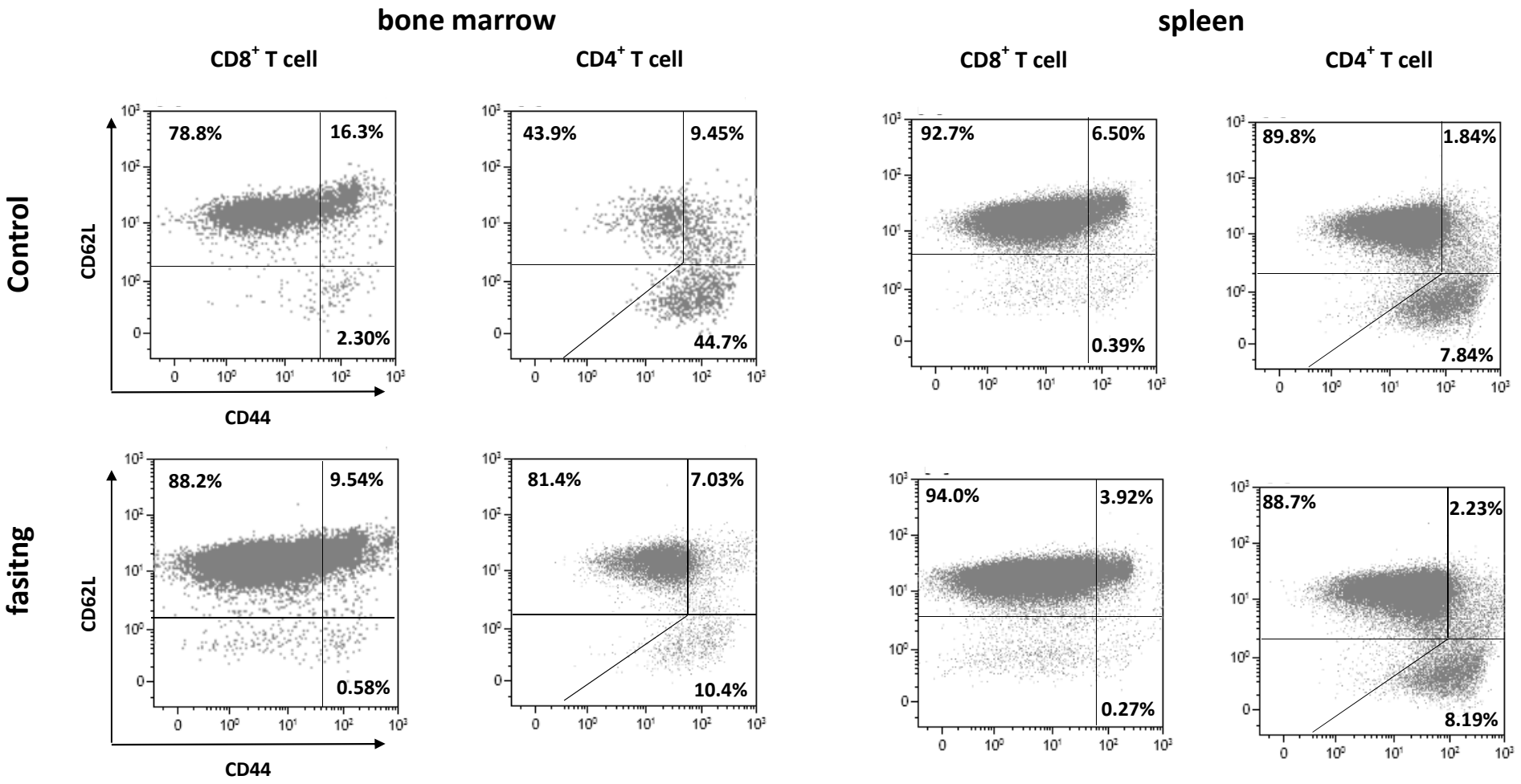
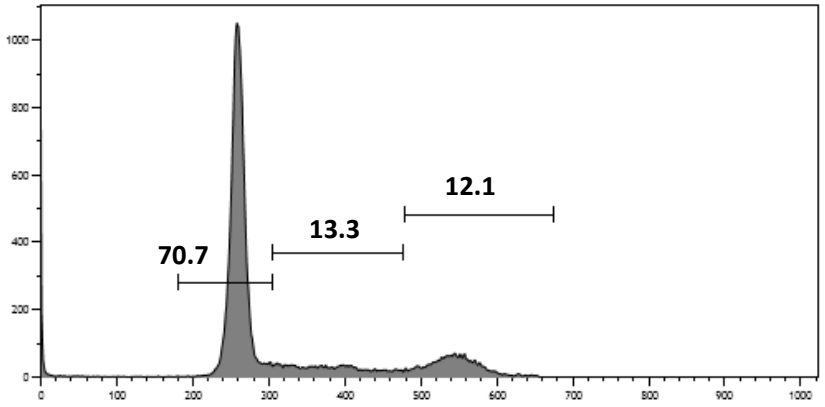


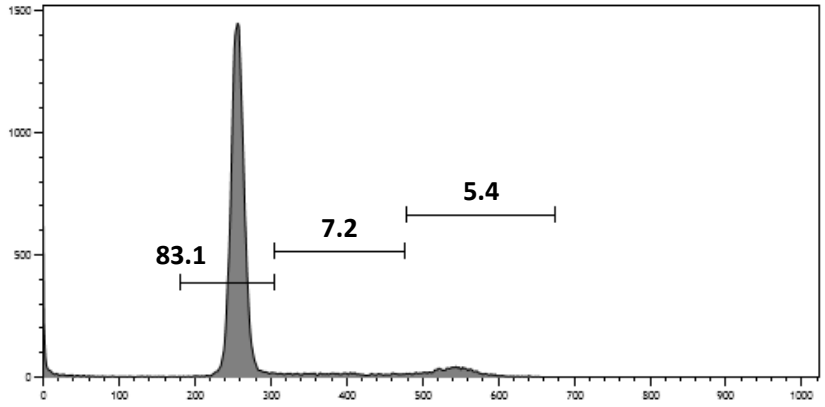
Fig 3. A) DNA cell cycle analysis with Propidium Iodide

control

fasting

immature BM cells

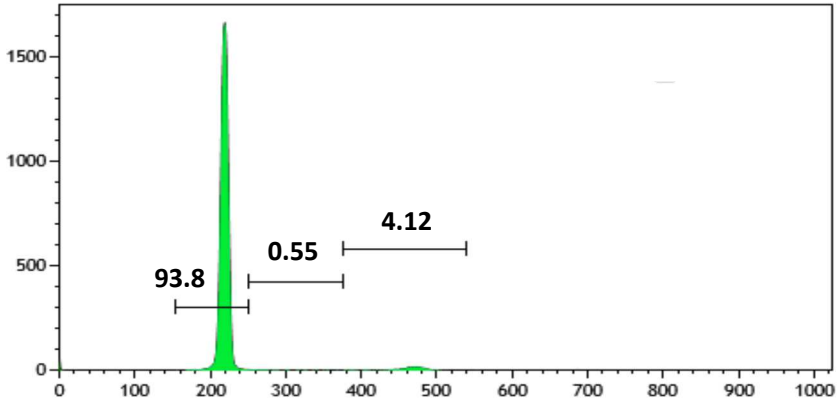


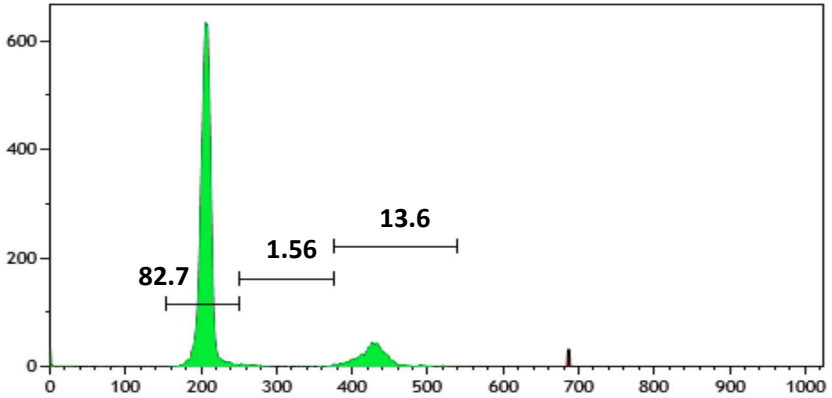


control

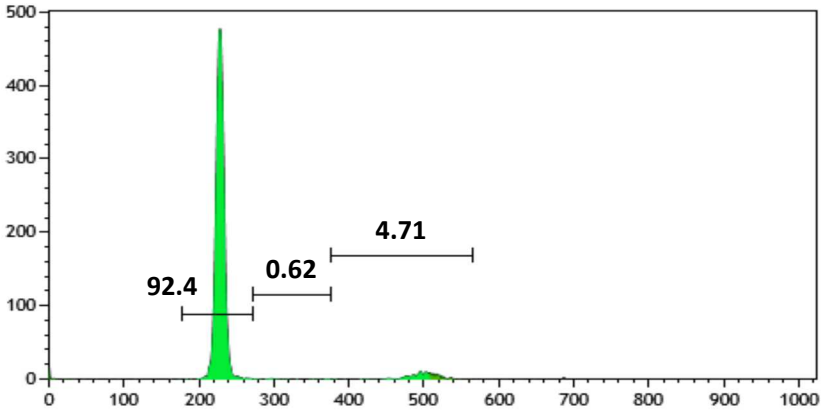
fasting

CD4





CD8



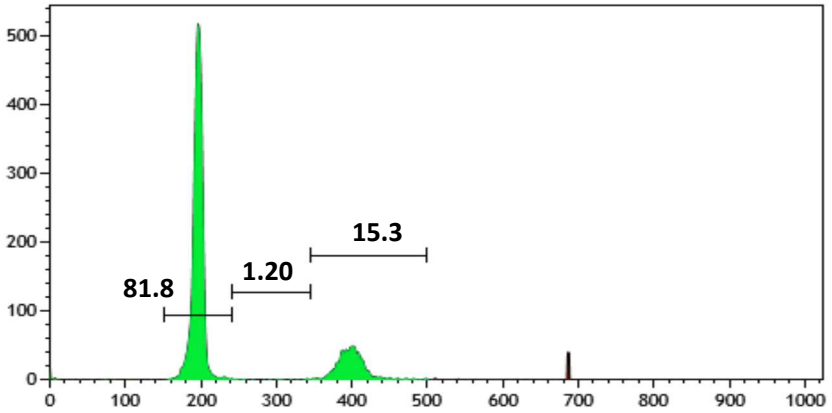
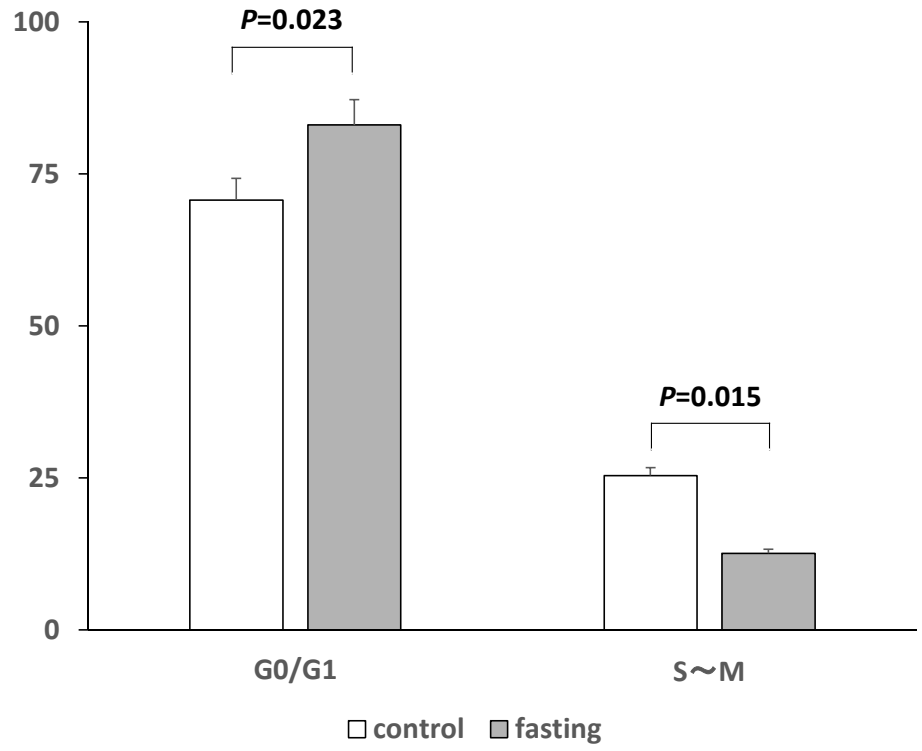
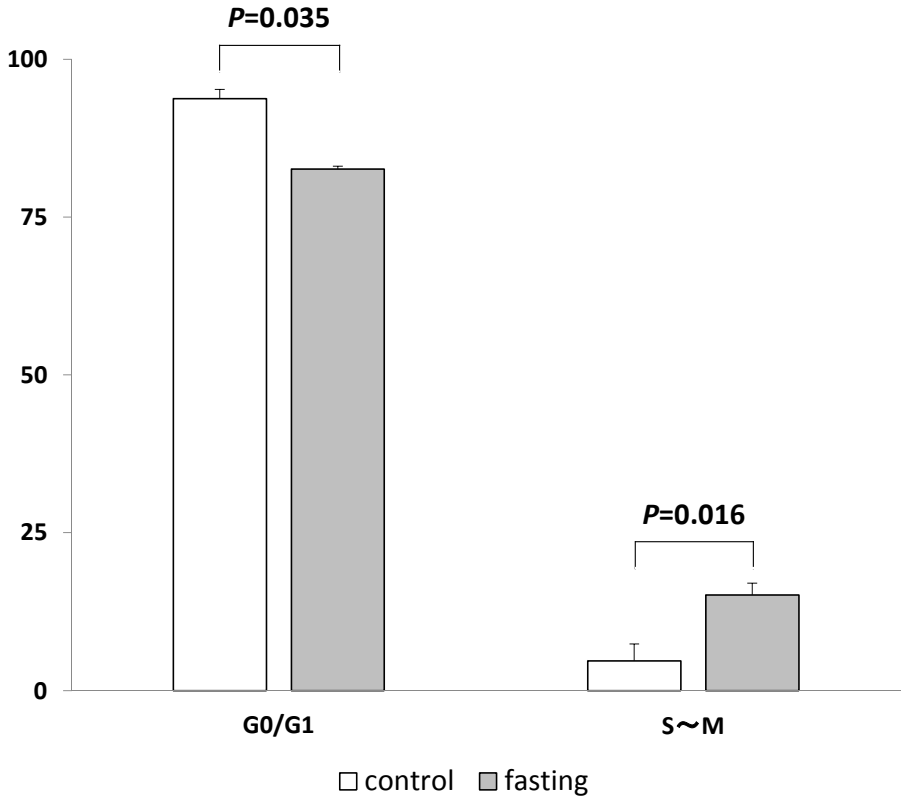


Fig 3. B) DNA cell cycle analysis with Propidium Iodide



immature BM cells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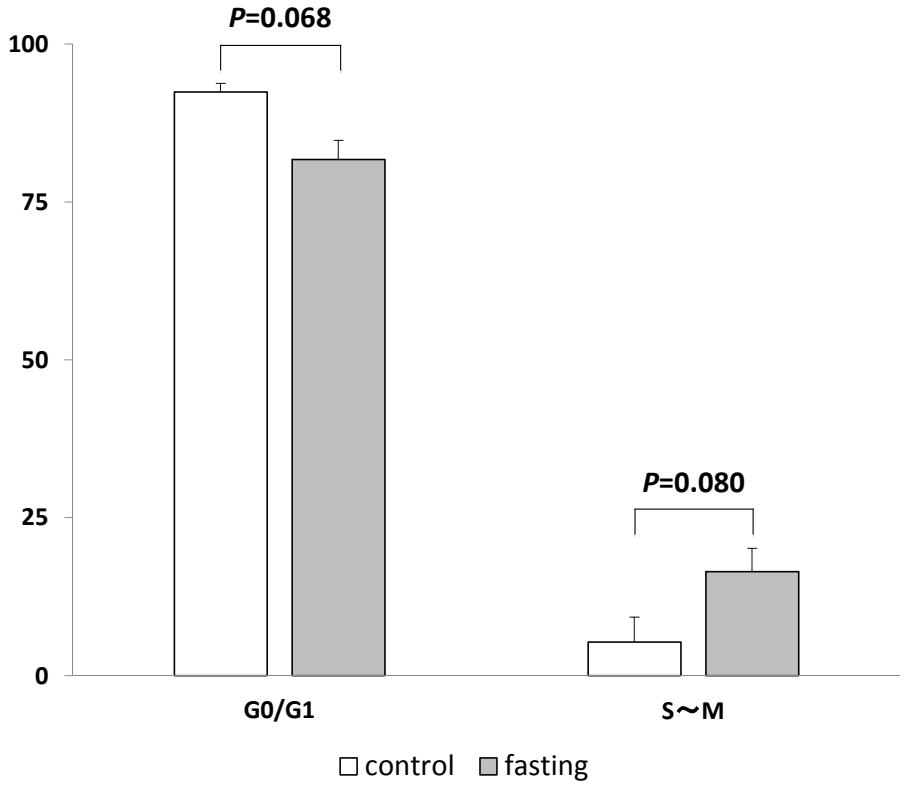
P=0.016
100
75
50
25
0
G0/G1
S～M
control
fasting

CD4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P=0.080
100
75
50
25
0
G0/G1
S～M
control
fasting

CD8